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50TH ISSUE

JKIMSU

JOURNAL OF KRISHNA INSTITUTE OF MEDICAL SCIENCES UNIVERSITY

An Official Publication of
Krishna Vishwa Vidyapeeth (Deemed to be University), Karad

Dr. Suresh Bhosale
Hon'ble Chancellor,
Krishna Vishwa Vidyapeeth (Deemed to be University), Karad

Message

It is with great satisfaction that I convey my greetings to the editorial team of the Journal of Krishna Institute of Medical Sciences University (JKIMSU) on the occasion of the publication of its 50th issue.

Since its inaugural release in June 2012, JKIMSU has steadily contributed to the advancement of biomedical knowledge under the auspices of Krishna Vishwa Vidyapeeth (Deemed to be University). The journal's sustained publication over more than a decade reflects institutional commitment to academic integrity, peer-review discipline, and responsible scientific communication.

The progress of a university is measured not only by its teaching programmes but also by the quality and continuity of its scholarly output. Indexed in SCOPUS and demonstrating consistent academic engagement, JKIMSU represents the University's dedication to fostering research culture and evidence-based practice.

This milestone is a tribute to the collective efforts of editors, reviewers, authors, and administrators who have upheld standards of rigor and ethical scholarship.

On this commemorative occasion, I extend my congratulations to all associated with JKIMSU and offer my best wishes for its continued contribution to medical science and society.

Dr. Pravin H. Shingare
Principal Advisor, Academics & Accreditation
Krishna Vishwa Vidyapeeth (Deemed to be University), Karad

Message

It is a privilege to convey my congratulations to the editorial team of the Journal of Krishna Institute of Medical Sciences University (JKIMSU) on the publication of its 50th issue. Since its inaugural issue in June 2012 under Krishna Vishwa Vidyapeeth (Deemed to be University), the journal has reflected the University's sustained commitment to academic rigor and research excellence.

From an academic governance perspective, the continuity of a peer-reviewed journal represents an essential component of institutional quality frameworks. It strengthens research culture, supports faculty scholarship, and provides structured opportunities for postgraduate and doctoral engagement in scientific writing and critical appraisal.

JKIMSU's indexing in SCOPUS, its Q3 positioning, and an H-index of 13 demonstrate measurable scholarly presence while underscoring the importance of maintaining robust peer-review standards, ethical compliance, and methodological integrity.

As higher education increasingly integrates interdisciplinary research, digital scholarship, and outcome-based academic evaluation, journals assume a strategic role in aligning institutional research output with national and global benchmarks.

The publication of fifty issues stands as evidence of sustained editorial stewardship and collaborative academic effort. I extend my appreciation to the editors, reviewers, and contributors whose dedication has enabled this milestone, and I wish JKIMSU continued progress in strengthening the University's academic and research ecosystem.

Dr. Vedprakash Mishra
Chief Advisor to Chancellor & KVV, Karad

Message

It is indeed gratifying and satisfying to note that Journal of Krishna Institute of Medical Sciences shall be publishing its commemorative 50th Issue, which is a genuine and bonafide landmark in its own right in as much as, it is a tall reflection of passion, commitment, prudence, pursuance and persistence in unison as a result of which it gained strength in an incremental manner openendedly.

Ever since its launch in terms of actualization of the vision harbored by the Founder Chancellor Late Shri Appaji Bhosale to begin with and pursued as a legacy passionately by the present Hon. Chancellor Dr. Suresh Bhosale that the pursuit not only grew but developed and matured in an exemplary manner over a period of time in the domain of quality centricity, innovative creativity and resultant usability by the targeted beneficiaries which turns out to be an emulative example of rarity.

The attributable profile to the journal in terms of its augmented level of indexing over a period of time stands out as an effective scalable measure of its fulfilment of the set out objectives of incessantly catering to the cause of scientific community specially in the biomedical research domain and promotion of the purposive focus of passionate original path breaking research by the inquisitive and innovative ignited minds of researchers borne out by unfathomable efforts and placing them into public domain through publications within its fold.

Truly speaking, the respect and honor that the journal has been able to evoke for itself over a period of time amongst the entire spectrum of its end users is a genuine testimony to the efforts that have been put by all concerned through a collective team spirit which stands as a mother repository of this landmark achievement.

The yeomen efforts selflessly put in by Late Dr. Arun Patil, Professor of Biochemistry at Krishna Institute of Medical Sciences, Karad shall always stand out inerasably, because it is his sweat poured into the initiative that is yielding fruits in such a visible manner. My humble salutations to the departed soul with utmost respect and gratitude.

Let this journey be on with same vigor, vitality, purposive focus, enthusiasm, gusto and commitment ensuring that newer benchmark shall stand trampled upon and resulting in scaling of still higher heights in an alround manner, which would be a genuine salutation to this notable landmark of commemorative 50th issue of JKIMSU the prestigious journal.

I wish the entire team presently led by Dr. (Brig.) G. Himashree, Director Research of the University a warmest best to successfully carry the mantle of responsibility on the futuristic path that stand set out in an exemplary manner. Recording my diligent salutations to all the contributors ever since the inception and launch of the journal till date as a mark of payable respect with gratitude.

Prof. Dr. Mrs. Neelam Mishra
Hon'ble Vice Chancellor,
Krishna Vishwa Vidyapeeth (Deemed to be University), Karad

Message

It is a matter of pride to extend my congratulations to the Journal of Krishna Institute of Medical Sciences University (JKIMSU) on the publication of its 50th issue. Since its inaugural issue in June 2012, JKIMSU has grown steadily under the aegis of Krishna Vishwa Vidyapeeth (Deemed to be University), reflecting our institutional commitment to rigorous scholarship and responsible scientific inquiry.

The sustained publication of fifty consecutive issues represents more than a chronological milestone; it affirms editorial discipline, peer-review integrity, and collaborative academic effort. Indexed in SCOPUS, currently positioned in the Q3 quartile, and with an H-index of 13, the journal demonstrates consistent scholarly engagement while maintaining methodological rigor and regional relevance.

Over the past decade, biomedical science has undergone significant transformation—advances in molecular medicine, immunotherapy, digital health technologies, and translational research have reshaped clinical practice. In such a dynamic environment, academic journals play a critical role in ensuring that innovation is carefully evaluated, ethically grounded, and responsibly disseminated.

At Krishna Vishwa Vidyapeeth, we believe that research must serve both scientific advancement and societal need. JKIMSU has contributed meaningfully to this vision by providing a credible platform for original research, clinical scholarship, and interdisciplinary dialogue.

I commend the editorial leadership, reviewers, authors, and institutional stakeholders whose sustained efforts have enabled this milestone. As we look ahead, I am confident that JKIMSU will continue to strengthen its academic impact and uphold the highest standards of biomedical publishing.

I convey my best wishes to the entire editorial team on this significant achievement.

Prof. Kusal K. Das
Professor of Physiology
BLDEA's Shri B. M. Patil Medical College,
Bijapur – 586108, Karnataka, India

Message

The publication of the 50th issue of the Journal of Krishna Institute of Medical Sciences University (JKIMSU) marks a significant academic milestone. Since its inception in June 2012 under Krishna Vishwa Vidyapeeth (Deemed to be University), the journal has sustained a culture of methodological rigor, disciplined peer review, and scholarly continuity.

In the contemporary research environment—characterized by rapid data generation, translational acceleration, and expanding publication ecosystems—the responsibility of a scientific journal extends beyond dissemination. It involves safeguarding reproducibility, ensuring statistical robustness, reinforcing ethical compliance, and promoting transparent reporting standards. Editorial policy, therefore, becomes an instrument of research governance.

JKIMSU's indexing in SCOPUS, its Q3 quartile position, and an H-index of 13 indicate measurable academic engagement. More importantly, they reflect sustained adherence to quality benchmarks in a competitive scholarly landscape.

Biomedical science today spans molecular mechanisms, systems integration, digital analytics, and clinical translation. Such breadth demands editorial structures that preserve depth while encouraging interdisciplinary scholarship.

As we mark fifty issues, the imperative is to consolidate research quality, strengthen policy-aligned publication standards, and foster globally relevant yet regionally grounded scholarship.

I commend the editorial leadership and contributors for sustaining this platform of rigorous and responsible biomedical inquiry.

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	Indian Science Abstract, (CSIR), Goverment of India
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Scopus	Scopus
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iCi Indian Citation Index	Indian Citation Index
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NATIONAL LIBRARY OF MEDICINE	United States National Library of Medicine (US-NLM)
IMSEAR Index Medicus for South-East Asia Region	Index Medicus for WHO South-East Asia Region
DOAJ DIRECTORY OF OPEN ACCESS JOURNALS	Directory of Open Access Journals
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CAS	Chemical Abstracts Service (A division of the American Chemical Society)
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Universal Impact Factor {UIF}	Universal Impact Factor
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EDITORIAL

Milestone Reflections: 50 Issues of JKIMSU

G. Himashree

Editor-in-chief, Journal of Krishna Institute of Medical Sciences University,

Krishna Vishwa Vidyapeeth (Deemed to be University), Malkapur, Karad-415339 (Maharashtra) India

Milestone Reflections: 50 Issues of JKIMSU

Launched as a bi-annual journal in 2012, JKIMSU celebrates its 50th issue after 14 years, featuring nearly 1000 peer-reviewed articles on clinical advancements, public health, and biomedical innovation amid global challenges such as pandemics and antimicrobial resistance. This enduring journey owes its strength to collective dedication-innovative submissions from authors, rigorous evaluations by reviewers, and steadfast institutional support.

Milestones are not merely numerical markers; they clarify continuity of purpose and invite reconsideration of foundational ideas. Among the most enduring in biomedicine is the question of how the immune system distinguishes self from non-self.

This editorial seeks to engage the academic community with contemporary advances in immunology—particularly the evolving understanding of self and non-self-while placing them in disciplined dialogue with the age-old sanātana conception of non-dual unity.

The Molecular Governance of Immune Identity

Immune recognition is structured through peptide presentation on Major Histocompatibility Complex (MHC) molecules, deletion of strongly autoreactive lymphocyte clones during thymic maturation, and peripheral regulatory circuits that constrain residual autoreactivity. Because negative selection cannot eliminate all self-reactive clones, active peripheral control mechanisms are indispensable for maintaining physiological equilibrium [1].

In 2023, the Nobel Assembly recognized discoveries demonstrating that innate immune activation by synthetic Messenger Ribonucleic Acid (mRNA) depends critically on nucleoside composition. Substitution with modified nucleosides such as pseudouridine attenuates Toll-like receptor signaling while preserving protein translation—a principle that enabled clinically viable mRNA-based vaccination during the COVID-19 pandemic [2].

In 2025, the Prize acknowledged work defining the cellular and molecular basis of peripheral immune tolerance. The description of $CD4^+CD25^+$ suppressive T lymphocytes, together with the identification of FOXP3 mutations as the cause of Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) syndrome, established regulatory T cells (Tregs) as a distinct lineage essential for immune restraint [1,3-5]. $FOXP3^+$ Tregs exert control through CTLA-4–dependent modulation of antigen-presenting cells, secretion of IL-10 and TGF-β, metabolic competition, and tissue-repair mediators [5].

Autoimmune pathology demonstrates the clinical implications of regulatory failure. In rheumatoid arthritis, defects in Treg number and suppressive capacity contribute to sustained synovial inflammation and persistence of pathogenic effector T cells. Comparable pertur-bations are observed across several organ-specific autoimmune disorders [6].

Collectively, these advances recalibrate the classical self/non-self-framework. Immune identity is not merely recognition; it is the emergent outcome of continuously regulated equilibrium.

Non-Duality in the Sanātana Tradition: An Epistemological Analogy

Early immunological models relied heavily on binary distinction: self-versus non-self. Contemporary understanding emphasizes modulation, context, and feedback. Distinction remains biologically necessary; absolutism does not.

Advaita Vedanta advances a philosophical critique of rigid duality. The *Brihadaranyaka Upanishad* speaks of a realization in which multiplicity is no longer apprehended as ultimate (IV.3.23) [7]. The *Chandogya Upanishad* articulates "Tat Tvam Asi" (VI.8.7), dissolving the apparent separation between knower and known [7, 8]. These statements are metaphysical, not biological. Yet as epistemological metaphors, they illuminate an important caution: conceptual binaries may organize experience without fully capturing underlying coherence.

Autoimmunity is not metaphysical error; it is dysregulated biology. Yet it offers a striking illustration of imbalance-when defensive discrimination proceeds without adequate restraint. Immune tolerance does not abolish recognition; it calibrates it. Regulatory T cells do not negate immunity; they preserve organismal integrity by preventing self-directed injury [1, 5].

Understood in this limited analogical sense, Advaita does not collapse science into philosophy. It instead underscores a broader intellectual pattern: mature systems evolve from rigid opposition toward dynamic integration. The trajectory of immunology-from clonal deletion models to active regulatory networks-reflects that deepening sophistication.

Continuing the Discipline

As we mark our 50th issue, gratitude is accompanied by responsibility. Scientific paradigms evolve; editorial standards must remain exacting. The maturation of the self/non-self-concept-from deletion alone to regulated equilibriumdemonstrates how medical science advances: through incremental molecular insight, methodological rigor, critical scrutiny, and disciplined interpretation.

Our charge remains unchanged: to publish work that withstands methodological examination, to encourage inquiry that is innovative yet precise, and to sustain a forum where science serves clinical and societal good.

Issue 50 signals continuity, not conclusion. We proceed with clarity of purpose toward the work ahead.

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GUEST EDITORIAL

Chronic stress as a catalyst for non-communicable diseases

Asha K. Pratinidhi

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Ex-Director Research, Directorate of Research, Krishna Vishwa Vidyapeeth

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The World Health Organization (WHO) defines stress as a state of worry or mental tension caused by a difficult situation. It is a natural human response that prompts individuals to address challenges and threats in their lives, with its impact on well-being largely depending on how one responds to it [1].

Stress is the body and mind's natural, automatic response to any demand, threat, or pressure-known as a stressor [1]. It causes physical and mental tension, triggering chemical changes like increased heart rate and adrenaline [2]. While short-term stress can boost performance (eustress) [3], chronic, long-term stress can severely harm physical and mental health [4].

Stress means a mental tension caused by a situation that is difficult, dangerous, worrying, frightening, frustrating, confusing, and makes one feel worthless [1]. Stress is one of the most important modifiable risk factors for causation of chronic Non-Communicable Diseases (NCDs) like myocardial infarction, stroke, atherosclerosis, hypertension, diabetes mellitus, obesity, metabolic syndrome, chronic kidney disease, and various psychiatric and mental ailments [5]. According to the WHO, NCDs kill at least 43 million people annually, equivalent to 74% of all deaths globally [6]. Often stress is not identified and mentioned as a cause of death in NCDs, though it is an important contributing factor triggering the pathophysiological changes in a person experiencing chronic stress for a prolonged period, ultimately accelerating these disease conditions and death.

Acute stress activates the sympathetic adrenomedullary system often cited typically by Walter Bradford Cannon (1915) as the 'fight and flight response' [7]. Chronic stress on the other hand is predominantly mediated through sustained activation of the Hypothalamic–Pituitary–Adrenal (HPA) axis [4]. This neuroendocrine system serves as the principal interface between central neural processing of stress and peripheral glucocorticoid secretion [5]. Corticotropin-releasing hormone released from the hypothalamus stimulates adrenocorticotropic hormone secretion from the anterior pituitary, which in turn promotes cortisol synthesis in the adrenal cortex [8]. This critical neurohumoral link was identified by researchers like Geoffrey Harris in 1950 [9].

During prehistoric times humans dealt with brief periods of acute stress, contrary to the modern period, where human experience chronic stress of a psychosocial nature [10]. Early stress responses were acute and self-limiting, resolving once the physical threat subsided [4]. In contrast to those, occupational demands, socioeconomic pressures, and constant cognitive rumination result in chronic stress. Stress is related mainly to socioeconomic pressures, conflicts at work and at home, major life changes, loss of near and dear ones, illness in the family. To these conventional causes are added modern environmental causes related to urbanization and population explosion. The traditional and conventional social support systems are disrupted due to changing family composition like breaking up of joint family, changing concepts of marriage,

live in relationships and break ups. Such situations resulting in low grade constant activation of HPA axis [5] and elevated adrenergic hormonal levels which adversely affect every organ of the body.

Screening and diagnostic tests for various NCDs are based on objective criteria that are sensitive, specific, affordable, acceptable, and reliable, allowing them to be performed rapidly and easily [11]. In contrast, the diagnosis of stress remains a predominantly clinical process based on subjective assessments of symptoms and face-to-face medical interviews to evaluate physical and psychological indicators. Current assessment tools, including medical history, reviews and questionnaires, are often viewed as tedious, non-specific, and time-consuming [12]. Furthermore, laboratory tests measuring cortisol levels in blood, saliva, urine, and hair, do face significant limitations; there is no single universally accepted marker, and results are frequently confounded by diurnal fluctuations or external factors such as infection and medication [13].

Except during natural or man-made disasters, acute stress is relatively infrequent, whereas chronic low-grade stress has become an inevitable part of modern life. Physiological changes namely elevated blood pressure, sustained activation of the sympathetic nervous system, proinflammatory response, and metabolic syndrome occur as a result [5,14]. The contribution of stress in the causation of diseases is often not recognized due to its gradual onset and the complexity of modern lifestyle variables. Often, individuals under stress do not recognize it themselves, and the physicians frequently fail to diagnose the condition due to a lack of well-defined, objective criteria. Whether diagnosed or undiagnosed, existing chronic stress adversely affects the body stealthily by altering pathophysiology by persistent activation of HPA axis, as emotional part of the brain responds and reacts to stress.

Evolutionarily the primitive brain is responsible for survival instincts like hunger, self-defence and propagation of species. It is present across all animal species [15]. As complexity increased, the emotional and intellectual brain structures emerged in higher animals. In humans, this evolution culminated in a highly developed prefrontal cortex, often associated with a 'spiritual' or moral consciousness [16]. The emotional centre (hypothalamus), along with the nervous, endocrine, and immune systems, forms a continuous psychosomatic network. This mind-body communication is mediated by approximately 60 to 70 distinct neuropeptides [17]. These peptides act as biochemical markers, translating complex emotions —such as fear, joy, and anger—into physiological signals felt throughout the entire network.

It is not possible to have completely stress-free life but we can reduce the stress and learn to manage it [3]. Stress often arises from the heavy burden of responsibility we carry for ourselves, our families, and the world at large. Due to competition, we feel that 'he has it, why not me?' This mindset can drive people to pursue money, power and material comforts by any means [17]. We strive to get all worldly comforts and yet remain dissatisfied [18]. It's a mirage, an illusion... therefore it is important to recognise chronic stress in timely manner and avoid its long- term adverse effects. Along with regular yearly medical check-ups and preventive health practices, it is important to focus on a balanced diet, regular exercise, maintenance of healthy weight and adequate sleep. Relaxation and mindful techniques such as chanting, yoga, meditation, pursuing sports and hobbies can be very helpful [19]. One should seek counselling for stress relief, if it is uncontrolled. Along with the stress relief measures, if we can develop an attitude of little detachment from the worldly desires maybe we can beat the stress of life! [20].

Bhagavan Shree Krishna advises Arjuna
(Bhagwat Gita 2.71)
विहाय कामान्यः सर्वान्पुमांश्चरति निःस्पृहः।
निर्ममो निरहंकारः स शान्तिमधिगच्छति॥

Meaning: "A person who has given up all desires for sense gratification, who lives free from desires, who has given up all sense of proprietorship (mine-ness), and is devoid of false ego—he alone can attain real peace!"

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REVIEW ARTICLE

Competency based dental education curriculum proposed by Dental Council of India as compared to USA and UK dental curriculum: A systematic review

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Abstract

Background: The proposed Bachelor of Dental Surgery (BDS) curriculum draft by Dental Council of India (DCI) adapted to the new University Grants Commission and National Education Policy 2020 requirements, building on competency-based education to maintain the global standards. Till date insufficient evidence is available to compare the proposed DCI draft with USA and UK competency based dental curriculum. This systematic review aims to address this existing gap. The aim of this study was to compare Competency Based Dental Education curriculum (CBDE) proposed by DCI with the USA and UK competency based dental curriculum. *Material and Methods:* The primary goal of this systematic review was conducted to address the research question, 'How does the CBDE proposed by the DCI compare with the competency-based dental curricula of the USA and the UK?' This systematic review was conducted as per the guidelines of PRISMA. A systematic electronic search of PubMed was performed, limited to English-language articles. Emphasis was placed on the studies published in past 15 years and articles reporting on choice based credit system in dentistry, CBDE, dental curriculum, and comparison of dental education worldwide. Previous studies were referenced to present the historical background. Data were retrieved from the database- Medline, PubMed, ERIC, Web of Science, Scopus, Cochrane Library, Google Scholar, Research gate, and Cross-references. MeSH terms and keywords were used to develop the search strategy. A modified PICO search was defined for Population/Participants, Intervention/Method, Comparison/Control and Outcome/Interest. *Results:* Seventy-nine articles were deciphered through PubMed Database and 17 more additional record through other database searches. A total of 96 articles were included. After thorough screening nine studies were finalised for data extraction and quality assessment which was done by Joanna Briggs Institute Critical Appraisal Tool. Three of the nine studies contribute the most compelling evidence with well-executed student perception. All other articles were highly susceptible to bias regarding empirical outcomes due to insufficient data, absence of formal methodologies, or being exclusively conceptual in nature. *Conclusion:* This systematic review emphasizes that in order to assure clinical competency, revamping dental education in India entails both structural curriculum mod-ifications and a review of evaluation techniques. Successful reform will require concerted initiatives that are aligned with national policies including curriculum redesign, faculty development, infra-structure investment, and stakeholder engagement.

Keywords: Competency, dental curriculum, dental education, global standard

Introduction

Dentistry was first practiced in India in 600 BC at Kashi. Students from Taxila and Nalanda universities studied dentistry across Asia. Since the first fully self-governing dental college was established in Calcutta in 1920, this new field of medicine has gradually evolved. As of July 11, 2022

data available on Dental Council of India (DCI) portal, there are 323 Bachelor of Dental Surgery (BDS) colleges offering dental education, with an annual intake of 28,088 graduates [1]. The DCI was established as a Statutory Body on March 29, 1948, in compliance with the Dentist Act, 1948 (XVI of 1948), and it became operational on April 12, 1949 with amendments in 1993, 2016, and 2019. Its goal is to regulate dental education and the dental profession throughout India [2]. Both the Lok Sabha and the Rajya Sabha approved the 'National Dental Commission Bill, 2023' on July 28 and August 11, respectively. According to this Act, a Commission will be established to oversee the cooperative development of high-quality, reasonably priced dental education and the upkeep of universally accessible, high-quality oral healthcare services to all the needy people of society [3].

Recent statistics as on January 2024 on Ministry of External Affairs, 'Global Indian Students Portal' reveals that approximately 1.5 million (1335319) Indian students are studying in universities/higher education institutions abroad with major places of study being USA, UK, Canada and Australia [4]. A competency-based education program is 'a set of skills and abilities intended to suit the needs of a population or individual'. To promote an individual's overall growth, this not only gives opportunities and methods for learning major courses, but also investigates other learning paths beyond them. USA and UK implemented Competency Based Dental Education (CBDE) since long whereas in India, 'Bachelor of Dental Surgery Regulations, 2022' draft was recently proposed by DCI to adopt these competencies in Indian dental education system.

American Dental Education Association (ADEA) and European Dental Education Association (EDEA) competencies for the general dentist includes 'critical thinking, professionalism, interpersonal and communication skills, health promotion, practice management and informatics and patient care.' Retaining Indian students within the country and attracting international students to India for dental education will be achievable by aligning with global standards through the proper implementation of CBDE [5-6].

As part of the University Grant Commission's (UGC) new initiatives under the Eleventh Five-Year Plan, a ′Action Plan for Academic and Administrative Reforms' has been created. The Choice Based Credit System (CBCS) is one of these improvements. It provides a learner-centred approach, self-paced learning, outcome based and course equivalency to suit the demands of the contemporary society and era. The suggested BDS Curriculum by DCI is adapted to the new UGC and National Education Policy (NEP) 2020 requirements for CBCS, building on competency-based education to maintain the global standards [7-10]. Similar strategies have not yet been adopted in dentistry prior to the introduction of CBCS, which necessitates meticulous planning and active involvement of stakeholders. However, several studies conducted in other countries have proposed strategies for implementing CBDE [11].

To date, there is insufficient literature comparing the proposed DCI draft curriculum with the competency-based dental curricula of the USA and the UK. This systematic review seeks to address this gap. The findings of this review have the potential to evaluate the proposed competency-based undergraduate dental curriculum in comparison with those of the USA and the UK, and may inform future modifications to ensure that dental education in India meets global standards.

Therefore, the aim of this study was to compare the CBDE curriculum proposed by the DCI with the competency-based dental curricula of the USA and the UK. The primary objective of this systematic review was to provide a comprehensive answer to the research question: 'How does the CBDE curriculum proposed by the DCI compare with the competency-based dental curricula of the USA and the UK?'

Material and Methods

A systematic review was applied to explore the proposed CBDE in India compared to USA and UK competency based dental education.

Eligibility criteria (Inclusion/Exclusion)

A modified PICO search was defined for population/participants, intervention/method, comparison/control and outcome/interest. Exclusion criteria included studies that were irrelevant to the research question, had inadequate abstracts, or showed poor methodological quality. Table 1 shows PICO framework with inclusion and exclusion criteria.

Table 1: PICO Framework: Inclusion and Exclusion Criteria

Criteria	Inclusion	Exclusion
Population	Undergraduate Dental Students, All Stakeholders and Dental Institutions in India, USA and UK	Postgraduate or Other Health Professions Students
Intervention	Competency Based Dental Education Curriculum recommended by *Dental Council of India*	Traditional Contemporary Indian Dental Curriculum
Comparison	USA and UK Competency Based Dental Curriculum	Dental Curriculum of all the countries excluding USA and UK.
Outcomes	Academic/ Program Outcome- Core Competencies achieved by students (Competent Dental Practitioners with holistic development)	Non-academic outcomes only
Study Design	Quantitative, Qualitative, mixed-methods, Systematic Reviews, Comparative Reviews, Descriptive/Opinion studies, Conference Proceedings	Editorials, Books and Document and Clinical Trials
Publication range	2010-2024 (15 years)	Pre-2010 publications
Language	English studies	Non-English Studies

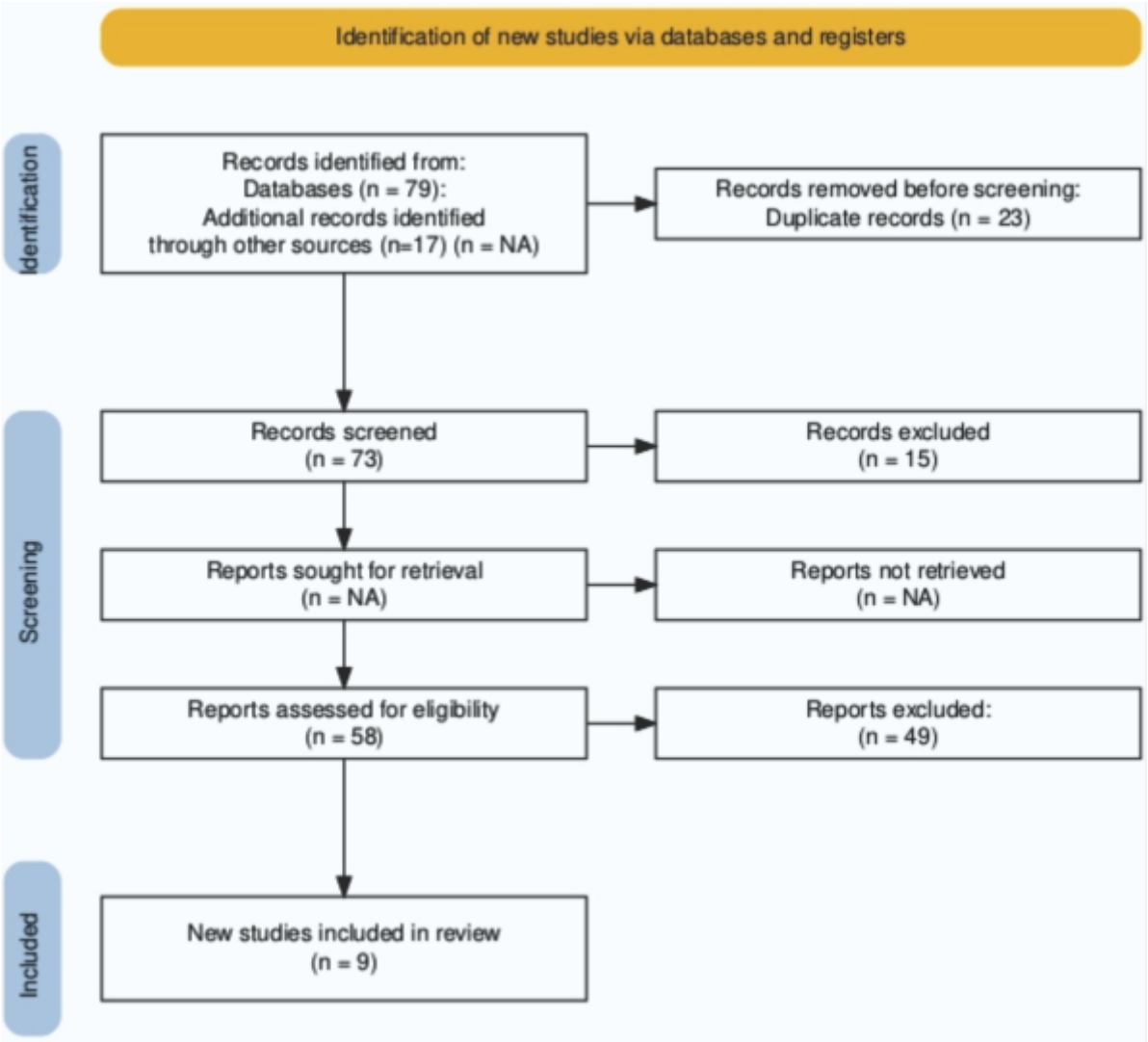

Figure 1: PRISMA Chart

Joanna Briggs Institute (JBI) levels of evidence for meaningfulness

To segregate the studies according to the JBI levels of evidence [12] for meaningfulness, need to focus the type and strength of its methodology, particularly as it pertains to qualitative research and studies of experience or perceptions.

JBI levels of evidence for meaningfulness scale 1-5 are as below: -

1. Qualitative or mixed-methods systematic review
2. Qualitative or mixed-methods synthesis
3. Single qualitative study
4. Systematic review of expert opinion
5. Expert opinion

Search strategy and searching databases

This systematic review was prepared as per the guidelines of Preferred Reporting Items of

Systematic Reviews and Meta-Analyses (PRISMA) [13]. A systematic electronic search of Pub Med was performed, limited to English-language articles. The studies were prioritized which published in past 15 years between January 2010-Decmber 2024 and articles reporting on any one or more on choice based credit system in dentistry, CBDE, dental curriculum, comparison of dental education worldwide. Earlier studies were considered to describe the historical context. The search was conducted using the database- Medline, PubMed, ERIC, Web of Science, Scopus, Cochrane Library, Google Scholar, Research gate, and Cross-references. The search was structured around relevant MeSH terms and Keywords.

Boolean Operators- AND, OR used to combine terms effectively.

The search syntax used with was:

(Choice Based Credit System in Dentistry[MeSH Terms]) OR (Competency-Based Education [MeSH Terms]) OR (Competency-Based Dental Education [MeSH Terms]) OR (Competency-Based health profession Education [MeSH Terms]) AND (Comparison of dental education worldwide [MeSH Terms]) OR (dental education [MeSH Terms]) AND (Dental Curriculum [MeSH Terms]) OR (Dental syllabus [MeSH Terms]) OR (Indian dental curriculum [MeSH Terms]) AND (Critical appraisal of dental education [MeSH Terms]) AND (Dental Education Reforms [MeSH Terms]) AND (Dental student's assessments [MeSH Terms]) AND (Electives [MeSH Terms]) AND (Evidence-Based Dental Education [MeSH Terms]) AND (Learning outcome [MeSH Terms]) AND (National Education Policy [MeSH Terms]) OR (Indian National Education Policy [MeSH Terms]) AND (Student's Feedback [MeSH Terms]) OR (Student Feedback [MeSH Terms])

Study selection and screening

All studies identified in the search underwent meticulous screening. Screening process was two-step. The initial review of titles/abstracts of studies was carried out independently by two reviewers, then full texts articles screening for relevance particularly comparative data. The first step included eliminating any duplication and examining titles. The two writers carefully examined each study in light of the second stage's criteria to ascertain which research met the requirements for inclusion.

The disagreements were discussed in order to come to an agreement. Disagreements about the appropriateness of studies were addressed collaboratively, and unresolved issues were referred to a third reviewer.

Result of searches

Seventy-nine articles were deciphered through PubMed database and 17 more additional record through other database searches. As such, total 96 articles were included.

Data extraction

Nine studies were finalized for further data analysis [14-22]. A structured form was designed to extract data and pilot testing was done and refined for extraction process. Two reviewers independently extracted the data and any disagreement observed were resolve by third investigator. Table 2 presents the data extraction framework, which includes the study author and year, aims/objectives, study design, population, intervention or exposure, outcomes, results, discussion, limitations, and comments. These elements were used to evaluate CBDE in India, the USA, and the UK.

Table 2: Data Extraction Table

Study	Aim/ Objectives	Study Design	Population	Intervention/ Exposure	Outcomes	Results	Discussion	Limitations	Comments
Virdi (2011) [14]	To reform dental education in India by introducing credits and semester system	Descriptive / conceptual	Indian dental students and educators	Credit and semester system introduction	Curriculum structure, acceptance, implementation feasibility	Reported improved flexibility and modularity; positive feedback from stakeholders	Credits & semester system modernizes curriculum; recommended phased implementation	No empirical data; conceptual only	Sets foundational reform ideas
Rao *et al.* (2014) [15]	Compare Indian dental curriculum with developed countries	Comparative review	Curriculum documents from India & developed countries	Curricula comparison	Differences in content, duration, evaluation	Indian curriculum less aligned with competency-based education; lacks integration	Highlights need for curriculum modernization and alignment with global standards. Need to introduce Early clinical exposure, intercalated degrees and Electives	Lacks primary data; expert opinion	Useful gap analysis
Manivasakan *et al.* (2016) [16]	Propose BDS syllabus framework consistent with Choice Based Credit System (CBCS)	Conceptual framework	N/A	Proposed CBCS syllabus framework	Feasibility and alignment with CBCS principles	Framework supports flexibility and student choice; promotes interdisciplinary learning	CBCS framework suits Indian dental education; needs pilot testing	No implementation data	Provides syllabus template
Manivasakan *et al.* (2016) [17]	Assess acceptability and feasibility of CBCS in BDS syllabus	Cross-sectional survey	Dental faculty & students (N=~100)	CBCS adoption	Acceptability, feasibility	Majority (>70%) supported CBCS adoption; noted challenges in assessment & resource allocation	Positive attitude towards CBCS, but need for faculty training and infrastructure, choice to take electives	Limited sample size; regional bias	Important initial acceptability data

Continued...

Terry *et al.* (2017) [18]	Systematic review: Do summative coursework assessments predict clinical performance?	Systematic review	Multiple studies (N=18 studies)	Coursework assessment types	Correlation with clinical skills	Mixed results; some studies showed moderate prediction, others weak/no correlation	Assessment should combine multiple methods; summative exams alone insufficient, Objective Structured Clinical Examination may be the most appropriate summative assessment	Heterogeneity of included studies	Informs assessment strategies
Tonni *et al.* (2020) [20]	Assessment in competency-based dental education: ways forward	Expert consensus/ review	Experts in dental education	Competency-based assessment methods	Best practices in assessment	Emphasizes multi-modal assessment: portfolios, EPA's, OSCEs, workplace-based assessments	Competency-based education needs valid, reliable, continuous assessment methods,	Expert opinion; lacks empirical data	Guides future assessment frameworks
Kakodkar & Manivasakan (2022) [21]	Roadmap for NEP 2020 compliant multidisciplinary education & research universities for dental education	Conceptual / policy review	Policy documents	NEP 2020 framework alignment	Strategies for multidisciplinary dental education	Advocates integrated, research-intensive multidisciplinary universities; policy supportive	Potential to transform dental education quality and research output	Conceptual only; no empirical evidence	Strategic direction aligned with national policy
Biswas (2018) [19]	Analytical study on Choice Based Credit System (CBCS)	Analytical study/ review	N/A	CBCS in higher education	Strengths, weaknesses, implementation challenges The implementation of CBCS system beneficial for Student-centric approach of education Creates interest and applicability in the scope of study	CBCS enhances flexibility and learning autonomy; challenges include assessment standardization	Calls for clear guidelines and faculty development for successful implementation introducing critical thinking and analysis, which leads to creativity and innovation in the educational system	No empirical data; analytical only	General higher education focus, transferable insights

Continued...

Kabra *et al.* (2023) [22]	Explore dental undergraduates' awareness and perspectives on the CBCS in Belagavi City, India	Cross-sectional survey	Undergraduate dental students (N=~150)	Newly proposed CBCS Syllabus	Awareness, perception, acceptance	Moderate awareness (~60%); mixed perceptions on CBCS benefits	Need for better communication and orientation programs for students	Single institution; limited generalizability	Important for stakeholder engagement

Quality assessment

The authors used JBI Critical Appraisal Tool (CAT). This tool is specialised for each type of study design. Table 3 shows the quality assessment of the nine studies included in this review by using this tool.

JBI Critical Appraisal Tool:

The JBI Critical Appraisal Checklist for Cross-Sectional Studies (8 items) was applied to the studies by Kabra *et al*. (2023) and Manivasakan *et al*. (2016) [17, 22]. The JBI Critical Appraisal Checklist for Systematic Reviews (11 items) was used to assess the study by Terry *et al*. (2017) [18] Additionally, the JBI Critical Appraisal Checklist for Text and Opinion Papers (6 items) was utilized for the works of Virdi (2011), Rao *et al*. (2014), Manivasakan *et al*. (2016), Tonni *et al*. (2020), Kakodkar (2022), and Biswas (2018) [14-16,19-21].

Risk of bias assessment

A relevant quality evaluation tool was applied to assess the risk of bias in the studies. The levels mentioned in Table 4 describes the risk of bias key and Table 5 shows risk of bias of comprised studies.

Table 3: Quality assessment by using JBI critical appraisal tool

Study	Design	JBI Tool	Q 1	Q 2	Q 3	Q 4	Q 5	Q 6	Q 7	Q 8	Q9 11	Score (%)	Quality
Kabra *et al.* (2023) [22]	Cross-sectional	Analytical	√	√	√	√	X	X	√	√	–	6/8 (75%)	Moderate
Manivasakan *et al*. (2016) [17]	Cross-sectional	Analytical	√	√	√	X	X	X	√	√	–	5/8 (62.5%)	Moderate
Terry *et al.* (2017) [18]	Systematic Review	SR Checklist	√	√	√	√	√	√	√	√	√	11/11 (100%)	High
Virdi (2011) [14]	Opinion	Textual	√	√	√	X	√	X	–	–	–	4/6 (67%)	Moderate
Rao *et al*. (2014) [15]	Opinion	Textual	√	√	√	X	X	X	–	–	–	3/6 (50%)	Low
Manivasakan et al. (2016) [16]	Opinion	Textual	√	√	√	X	X	X	–	–	–	3/6 (50%)	Low

Continued...

Tonni *et al*. (2020) [20]	Opinion	Textual	√	√	√	√	√	√	–	–	–	6/6 (100%)	High (for opinion)
Kakodkar *et al*. (2022) [21]	Opinion	Textual	√	√	√	X	X	X	–	–	–	3/6 (50%)	Low
Biswas (2018) [19]	Opinion	Textual	√	√	√	X	X	X	–	–	–	3/6 (50%)	Low

Table 4: Risk of bias key

Level	Meaning
Low	Minimal risk of bias; well-conducted empirical study
Moderate	Acceptable but has some limitations (e.g., design clarity, sampling)
High	Conceptual, opinion-based, or lacks methodological rigor

Table 5: Risk of bias table for included studies

Study	Type	Risk of Bias	Reasoning
Virdi (2011) [14]	Theoretical proposal / expert opinion	High	No empirical data; conceptual framework only
Rao et al. (2014) [15]	Narrative review	High	No formal methodology; lacks systematic search or appraisal
Manivasakan et al. (2016) [16]	Theoretical framework	High	No data collection; conceptual proposal only
Manivasakan et al. (2016) [17]	Survey / cross-sectional	Moder ate	Uses survey data but lacks detailed sampling strategy and analysis depth
Terry et al. (2017) [18]	Systematic Review	Low	High-quality SR, transparent methods, PRISMA-compliant
Tonni et al. (2020) [20]	Expert opinion / policy paper	High	Consensus report; no data collection or analysis
Kakodkar & Manivasakan (2022) [21]	Roadmap / expert opinion	High	Descriptive article without primary data
Biswas (2018) [19]	Analytical commentary / policy analysis	High	No formal empirical methodology; largely narrative
Kabra et al. (2023) [22]	Cross-sectional survey	Low	Validated questionnaire, good sample size, robust analysis; risk minimized

Results

Among the nine studies, the strongest evidence comes from Terry *et al.* (2017) [18] with high-quality systematic review, Kabra *et al.* (2023) [22] with well-executed student perception study and Manivasakan *et al.* (2016) [16-17].

All other articles were highly susceptible to bias regarding empirical outcomes due to insufficient data, absence of formal methodologies, or being exclusively conceptual in nature.

Discussion

In order to educate graduates for clinical competency and lifelong learning, the changing landscape of dentistry education necessitates significant curricular modifications and efficient evaluation mechanisms. A wealth of information about this intricate problem may be gained from the studied literature, which includes conceptual frameworks, curriculum comparisons, stakeholder perceptions, and systematic reviews.

Curriculum reforms and adoption of CBCS

Virdi (2011) [14], Manivasakan *et al*. (2016) [17] and Kabra *et al*. (2023) [22] are among the research that highlight the pressing need to update India's traditional dentistry curriculum. Important initiatives toward bringing Indian dentistry education to comply with global benchmark include the implementation of a credits and semester system and suggested CBCS framework.

CBCS model

Numerous drawbacks of strict curricula are addressed by the CBCS approach, which encourages adaptability, interdisciplinary learning, and student autonomy. Overall, teacher and student adoption of CBCS seems to be favourable [17, 22], however issues with infrastructure, faculty development, and uniform evaluation techniques still exist. Rao *et al*. (2014) highlight the importance of these reforms, noting that Indian curricula lag behind those of English speaking nations in incorporating competency-based education [15].

Clinical competence evaluation: Insights from the systematic review

The systematic review by Terry *et al*., 2017, [18] offers important proof about the connection between clinical performance and summative coursework evaluations, which is a key concern for dental educators. The reality is that; no single evaluation technique is enough to completely capture clinical competence is further supported by their mixed predictive validity finding.

The necessity of competency-aligned, multi-method assessment systems including Objective Structured Clinical Examination (OSCEs), portfolios, various case scenarios and workplace-based evaluations are emphasized by Tonni *et al*. (2020) [20] similar to the study conducted by Ozdemir-Ozenen *et al*. (2020); El-Kishawi *et al*. (2020) and Pushpalatha *et al*. (2023) [23-25]. This data backs up reformers' demands to move beyond traditional tests and incorporate continuous and varied assessment methods into CBCS and competency frameworks. Alkhodary *et al*. (2020) suggested that the use of CBDE enabled students to score higher than the conventional education [26].

CBDE and future directions

The more general objectives of the NEP 2020 reforms, which support research-driven, interdisciplinary educational paradigms similar to the study conducted by Meshram *et al.* (2023), incorporated blended learning model used in competency based medical education to improve theoretical knowledge of medical students [17, 21, 27].

India's new DCI draft is explicitly competency-based, integrating modern educational methods, structured assessments, flexible electives, and

alignment with global standards. The USA exemplifies a mature, holistic CBDE model, heavily structured around competencies from ADEA and innovative assessments. The UK adopts competency principles indirectly through regulation and practice-based training rather than explicit CBDE frameworks. The focus on real-world, workplace-based evaluations reflects international best practices and aims to generate graduates capable of meeting workplace expectations as per the suggestion by Ganganahalli & Udgiri (2023), to implement community based education to manage community health issues [28]. The review highlighted shortcomings in summative assessments, suggesting that these can be mitigated through the use of formative assessments and continuous feedback.

Challenges and implementation considerations

Despite the presence of several frameworks at the conceptual and policy levels, there is little empirical data on their implementation. To properly implement new curricula and assessments, faculty development and training are necessary, according to numerous research. Limitations in infrastructure and resources, particularly in Indian contexts, could make the shift to competency-based models like CBCS more difficult. Kabra *et al.* (2023), [22] have identified knowledge gaps among students and professors, which implies that communication and conducting orientation sessions are vital for gaining support. Gugapriya *et al*. (2024) suggested faculty development program for successful implementation of competency based medical curriculum [29]. Spielman (2024) investigated past changes and contemporary patterns in dental practice and education in an effort to forecast the future [30].

Limitations

With few longitudinal or intervention studies, most of the evidence about CBCS and competency-based reforms is still conceptual or survey-based. According to the systematic review and other studies, in Indian dentistry education limited data exists on the effectiveness of assessments and clinical results in specific regions. Implementation tactics, impact assessment, and local context adaptability require more study.

Conclusion

Overall, the evidences emphasize that in order to assure clinical competency, revamping dental education in India entails both structural curriculum modifications, like the introduction of CBCS, and a review of evaluation techniques. Successful reform will require concerted initiatives that are consistent with national policies like NEP 2020 and include curriculum redesign, faculty development, infrastructure investment, and stakeholder engagement.

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ORIGINAL ARTICLE

Evaluation of broth disk elution method for detecting synergy between ceftazidime-avibactam and aztreonam against NDM-producing carbapenem-resistant Gram-negative bacteria

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Abstract

Background: The emergence of Gram-negative bacteria that produce carbapenemase has drastically restricted the available treatment options. The ceftazidime/avibactam-aztreonam combination is a good colistin-sparing strategy in such infections. *Material and Methods*: In total 135 carbapenem-resistant isolates of Enterobacterales and *Pseudomonas aeruginosa* were checked for NDM and OXA-48 genes by conventional multiplex Polymerase Chain Reaction (PCR). Broth disc elution method, a simple and cost-effective phenotypic test, was used to assess the synergistic effect of ceftazidime-avibactam combined aztreonam, against NDM-producing isolates. *Results: Klebsiella pneumoniae* was the most common carbapenem resistant organism (55%) isolated. NDM (37.0%) was the most prevalent carbapenemase gene detected followed by coproduction of NDM and OXA-48 (23.0%). A total of 80 isolates positive for NDM were subjected to synergy testing (ceftazidime + avibactam combined with aztreonam) by broth disc elution method of which 72 (90.0%) demonstrated a positive synergistic effect, while 8 (10.0%) were negative. *Conclusion:* Conventional multiplex PCR may serve as a useful screening tool for the detection of common carbapenemase genes. Broth disk elution method of synergy testing is a reliable method in resource limited settings. Thus, our study emphasizes the significance of using a feasible diagnostic test and monitoring the common genes that are responsible for carbapenem resistance.

Keywords: Carbapenemase, Ceftazidime-Avibactam, Enterobacterales, NDM, *Pseudomonas aeruginosa*

Introduction

Morbidity and death linked to bacterial infections are remarkably reduced by using antibiotics. Nonetheless it's over usage has led to increase in antibiotic resistance [1]. Carbapenems are potent antimicrobials for treatment of infections due to drug-resistant bacteria. Carbapenem resistance could be a result of the ability to resist antibiotic activity through a variety of mechanisms, including improper specific target or a variation in the cytoplasmatic membrane constitution or unable to pass the outer membrane [2]. In Enterobacterales, carbapenem resistance is mainly due to the carbapenemase [3]. The Metallo-Beta-Lactamases (MBL) have been found largely in *Pseudomonas aeruginosa*; with reports of global rise amongst Enterobaterales as well [4].

Presently, there are fewer Antimicrobial Susceptibility Testing (AST) techniques accessible for assessing the effectiveness of Aztreonam plus Ceftazidime/Avibactam (ATM + CZA) combination which is colistin sparing approach. A previous study showed that the ATM-CZA susceptibility testing in vitro can be done by using disk-based methods. Broth Disk Elution (BDE) and strip methods employing Minimum Inhibitory Concentration (MIC) test turned out to be the most suitable and less

complicated procedures. BDE method is especially useful in resource limited settings. As a result, BDE, a newer approach for MIC measurement has the convenience of disc diffusion and the dependability of broth elution method. The BDE technique performs exceptionally well, with 100 percent sensitivity and specificity [5]. Hence, the study aimed to explore for a simple lab synergy testing for MBL-producing Enterobacterales and *Pseudomonas aeruginosa* in this setting. There is a dearth of studies reporting the detection of synergy between ceftazidime-avibactam and aztreonam using BDE method [5].

Material and Methods

Study design and study setting: The project was initiated after receiving approval from the Institutional Ethics Committee of Kasturba Medical College, Mangalore (IEC/KMC/MLR06/2022/268). The microbiology lab at a tertiary care facility was the site of this prospective investigation in South India, spanning a period of 11 months (January 2023 to July 2023).

Sample size calculation: The formula used to determine the proportion of the sample: $n = Z^2pq/d^2$; Z = 1.96 is a standard normal value at 5.0% level of significance, p (prevalence) = 14.2% [6], q = 1-p, d (absolute precession) = 6.0%, n = 130 = minimum number of samples that are resistant to either imipenem or meropenem or resistant to both.

Inclusion criteria: Clinically significant, non-repetitive carbapenem resistant Enterobacterales and Pseudomonas aeruginosa strains [7] from exudate, blood, and urine samples.

Exclusion criteria: Carbapenem sensitive Enterobacterales and Pseudomonas aeruginosa strains from exudate, blood culture and urine and all isolates other than Enterobacterales and *Pseudomonas aeruginosa* were left out of the investigation. The estimated proportion was calculated based on the number of patients (n = 130). However, during the study period, some clinical samples exhibited polymicrobial growth, leading to the isolation of more than one organism from a single patient. Hence although 130 patients were included in the study a total of 135 Carbapenem Resistant Organisms (CRO) were obtained and subjected to analysis. Gram staining, colony morphology, and an automated technique (VITEK® 2) were used to identify every organism. Antibiotic susceptibility reports were taken from VITEK® 2 system. The OXA-48 and NDM were identified molecularly by conventional multiplex Polymerase Chain Reaction (PCR). Using the boiling approach, DNA was extracted, from a 24 hr old culture of the organism grown on 5.0 % sheep blood agar plates. The extracted DNA was kept at -20°C if it wasn't used right away. The primer sequence used were as follows:

Primer set NDM (984bp) [8]:

NDM: Forward sequence (5′ CACCTCATGTTT GAATTCGCC 3′)

NDM: Reverse sequence (5′ CTCTGTCACATC GAAATCGC 3′)

Primer set OXA - 48 (438bp) [9]:

OXA – 48: Forward sequence (5′ GCGTGGTTAA GGATGAACAC 3′)

OXA – 48: Reverse sequence (5′ CATCAAGTTCA ACCCAACCG 3′)

A total volume of the reaction mixture was 25 µl containing 12.5 µl of ready-to-use master mix, 0.625 µl of each primer (forward and reverse), 7.5 µl of nuclease free water and 2.5 µl of DNA template. The multiplex PCR was performed with the following conditions: 10 min in 94°C to initial denature, 30 cycles of 1 min in 94°C for denaturation, 30 secs in 55°C for annealing and 1min 30 secs in 72°C for

extension and 5mins in 72°C for complete extension. Gel electrophoresis was used for 45 minutes in 120 V to separate the DNA fragments and imaged using gel documentation system.

In vitro synergy test of CZA + ATM by BDE method was done on NDM positive isolates. Cation adjusted Muller Hinton Broth (MHB) measuring 2ml was taken in 5 sterile borosilicate tubes. One tube was kept uninoculated, as a sterile broth (clear) control, one for growth of test organism (turbid) as growth control and the rest of the 3 tubes were used for synergy testing, with 1 disk of ATM, 1 disk of CZA and 1ATM+1CZA discs added respectively. The tubes with antibiotic discs were kept for 30 mins at ambient temperature so the antimicrobials get eluted from the discs. Sheep blood agar plate colonies were suspended in normal saline to bring the turbidity equal to 0.5 McFarland. Twelve µl of this inoculum suspension was inoculated to the tubes except the sterile broth control tube and vortexed. For 16-20 hours inoculated tubes were incubated in 37°C. ATCC E. coli 25922 was utilized as strain control. The result was interpreted by visual observation for turbidity of the broth in comparison with clear control broth (Figure 1). Synergy was seen favorably when the test tube having both ATM and CZA discs was clear [5].

The study proformas and medical records were noted for demographic and other clinical data of patients. Statistical analysis was carried out with Minitab for windows. Chi-square test was employed to compare the groups. Statistical significance was defined as a p-value of less than 0.05.

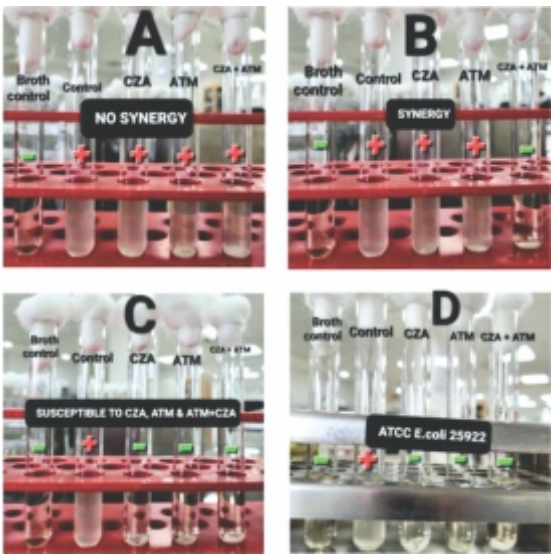

Figure 1: Broth disk elution synergy method

**A – No Synergy as the tubes with antibiotic discs are turbid, B – Synergy positive as the tube with both the discs is clear but tubes with individual discs are turbid, C – Susceptibly to all the tested antibiotic agents as the tubes containing individual discs and the tube with both the discs are clear, D – ATCC E. coli 25922 a negative control for synergy test.*

Results

The present study included samples of exudate, urine and blood from 130 patients. The mean age of patients was 61 years (60.8%), amongst which 88 (67.7%) were males and rest were female 42(32.3%). Up to 41 (32%) CRO were isolated from patients having urinary tract infection, 31 (24%) from respiratory tract infection, 30 (23%) from skin and soft tissue infection, 21 (16%) from blood stream infection and 7 (5%) from sterile body fluid infection. Among 135 CRO isolated, 74 (55%) were *Klebsiella pneumoniae*, 28 (21%) were *Pseudomonas aeruginosa*, 22 (16%) were *E. coli*, 7 (5%) were *Enterobacter spp*, 3 (2%) were *Proteus spp* and 1 (1%) was *Providencia rettgeri*.

Antibiotic resistance pattern of carbapenem non-susceptible Enterobacterales and *Pseudomonas aeruginosa* is as shown in Figure 2.

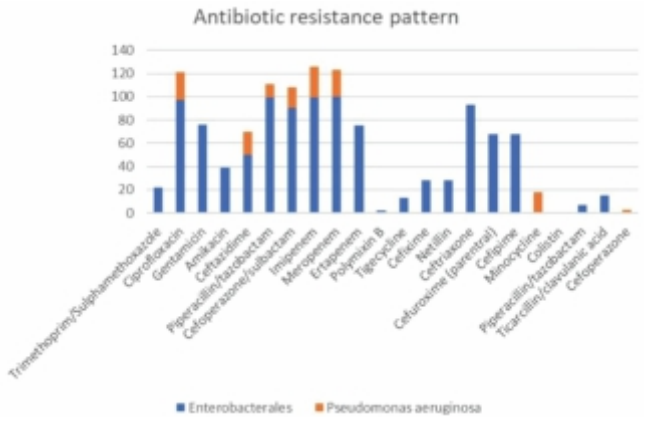

Figure 2: Antibiotic resistance pattern

The molecular detection of NDM and/ OXA-48 was done by PCR as shown in Figure 3. Among the 135 isolates, 50 (37.0%) were NDM, 31 (23.0%) were NDM and OXA-48 co-producers and 18 (13.3%) were OXA-48 producers. A total of 36 (26.7%) were negative for NDM and/OXA-48 production. Out of 50 NDM isolates, 11 (22.0%) were *E. coli*, 27 (54.0%) were *Klebsiella pneumoniae*, 7 (14.0%) were *Pseudomonas aeruginosa,* 4 (8.0%) were *Enterobacter spp*. and 1 (2.0%) was *Providencia retggeri.* Among the 31 isolates positive for both NDM and OXA-48, 2 (6.5%) were *E. coli*, 27 (87.1%) were *Klebsiella Pneumoniae*, 1 (3.2%) was *P. aeruginosa* and 1 (3.2%) was *Enterobacter aerogenes*. Amongst 18 OXA-48 producing isolates 2 (11.1%) were *E. coli* and 16 (88.9%) were *Klebsiella pneumoniae*.

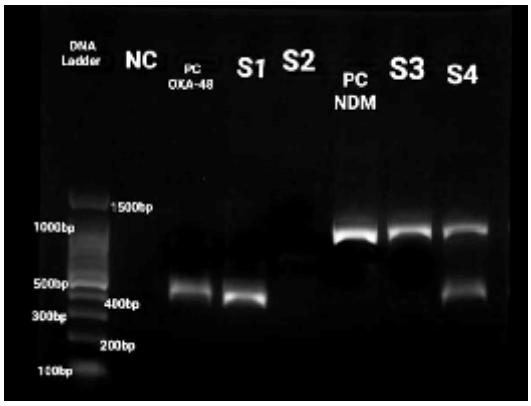

Figure 3: Detection of NDM and OXA-48 by multiplex PCR

**NC – negative control; PC OXA-48 - positive control for OXA-48; PC NDM positive control for NDM; S1, S2, S3, S4 – sample 1, 2, 3 and 4. S1-positive for OXA-48, S2-negative, S3-positive for NDM and S4-positive for NDM and OXA-48*

Statistical analysis of the type of carbapenemase detected revealed that association of *Klebsiella pneumoniae* with coproduction of NDM+OXA-48 and OXA-48 production was significant (p= 0.00 and 0.002 respectively). The infection of urinary tract was substantially associated with NDM (p = 0.05) production. Respiratory infections, and skin and soft tissue infections were significantly associated with NDM+OXA-48 coproduction (p = 0.004 and 0.023 respectively).

Majority of patients had diabetes mellitus (39.0%) and hypertension (36.0%). Twenty-three patients were immunocompromised. The mean duration of hospital stays, and Intensive Care Unit (ICU) stay in individuals afflicted with microbial producing NDM and/ OXA-48 was as shown in Table 1.

Out of the 130 individuals infected with carbapenem resistant Enterobacterales and *Pseudomonas aeruginosa*, the overall mortality rate was 22.3% (n = 29). The mortality rate amongst the patients infected with NDM/OXA-48 producing organisms was as shown in Table 1.

A total of 80 NDM producers were subjected to synergy test among which 72 (90.0%) were positive and 8 (10.0%) were negative.

Table 1: Duration of hospital stay and mortality amongst patients infected in NDM and OXA-48 producing organism infected patients

Enzymes	Duration of hospital stay (days)		Duration of ICU stay (days)	
	Mean ± SD	Median	Mean ± SD	Median
NDM	13.30 ± 8.70	11	3.386 ± 5.027	2
NDM and OXA48	19.47 ± 13.44	16	6.71 ± 6.29	7
OXA48	17.04 ± 8.78	17	5.35 ± 6.91	3
Enzymes	**Mortality rate**			
	Yes (Dead)	**No (Alive)**	***p***	χ^2
NDM (n=50)	9	36	0.294	1.101
NDM and OXA48 (n=31)	11	17	0.05*	3.692
OXA48 (n=18)	5	12	0.683	0.167

SD – Standard deviation; ICU – intensive care unit

**Association of ndm+oxa-48 coproduction with mortality was statistically significant (p-value = 0.05)*

Discussion

Clinical isolates that produce carbapenemase are increasing worldwide causing serious threat to public health, particularly in developing nations like India [10, 11]. During this study period the commonest carbapenem resistant bug recovered was *Klebsiella pneumoniae* (56.9%) followed by *Pseudomonas aeruginosa* (21.5%). A study done in Mahabubnagar, India, showed that *Klebsiella species* (56.7%), *E. coli* (17.7%), and *Pseudomonas species* (10.9%) were predominant [12]. A study from Thailand in the year 2022 showed that *Klebsiella spp.* (76.0%), E. coli (17.0%), and other Enterobacterales (6.0%), were the most common species of carbapenemase producing Carbapenem-Resistant Enterobacterales (CRE) infections [13]. The multiplex PCR revealed NDM (38.5%) gene was more prevalent than OXA-48 (13.8%), the coproduction of NDM plus OXA-48 was 23.8%. The previous study in 2020 from the same set up, showed similar results with 17% of NDM, 7% of OXA-48 and 5% of NDM plus OXA-48 genes identified from a total of 101 isolates of Gram-negative bacilli [14]. Similarly, a study from North India revealed that 44 out of the 164 CRE isolates (26.8%) showed presence of both the NDM and OXA-48 genes. The NDM gene alone tested positive in 94 out of 164 (57.3%) CRE isolates, following OXA-48 in 62 out of 164 (37.8%) isolates [15]. However, the findings of our study is in contrast to the findings from Hungary, in which out of the total 18 carbapenemase genes from 50 isolates, NDM was less prevalent (n = 2) compared to OXA-48 (n = 6) [16].

A study from Turkey also revealed that, NDM (1.3%) was less prevalent than OXA-48 (71.9%) [17]. In our study, 36 (26.7%) out of 135 isolates were negative for NDM and OXA-48 production but were carbapenem resistant. The mechanism of resistance could be through synthesis of additional carbapenemase (such as KPC, IMP, VIM, and OXA-23/24) or by efflux pump/porin loss [18-22]. Statistically substantial correlation was discovered between *Klebsiella pneumoniae* and coproduction of NDM+OXA-48 and OXA-48 production (p-value 0.00 and 0.002 respectively). Similar relevance was found in a study done on carbapenem resistant *Klebsiella pneumoniae* where most isolates co-harbored NDM + OXA-48 (64.5%) followed by OXA-48-like (33.3%) [23].

Distribution of NDM and OXA-48 in isolates from urinary tract infections, respiratory infections and skin and soft tissue infections were statistically significant (p = 0.05, 0.004 and 0.023 respectively). The outcome is comparable to the research conducted by Pudpong *et al*. (2022) where most of the patients infected with carbapenemase producing organisms were diagnosed with urinary tract infections (28.1%) [13]. A study done in a Spanish hospital showed that 42% of the cases with urinary tract infections were due to carbapenemase producing organisms [24]. NDM+OXA-48 (41.5%) coproduction in this study was significantly associated with higher rate of mortality (p = 0.05) and these patients had a longer hospital (18 days) and ICU stay (7 days). Study by Jean *et al.* (2022) showed varying case-fatality rates among patients with CRE (22.0 to 72.0%) and *Pseudomonas aeruginosa* (44.7 to 64.0%) infections [25]. This increased mortality among NDM and OXA-48 co-producers could serve as a critical alert for clinicians. Study by Priyendu *et al.* (2014) showed that median length of hospital stay in carbapenem-sensitive patients was lower (9 days) compared to

that in case of carbapenem-resistant patients (23.5 days) [26]. Another study from Thailand showed that the duration of ICU stays (10 days) was longer in the patients infected with NDM+OXA-48 co-producers [13]. In the present study, DM (n = 75, 57.8%) was the commonest co-morbid condition, followed by hypertension (n = 69, 53.0%) and immunocompromised state (n = 44, 33.8%). Literature quote other risk factors like mechanical ventilation, presence of multiple indwelling devices, surgical procedures in the event of sepsis or a localized infection which attribute to the risk of CRO acquisition [27].

Therapies to CRE that produce metallo-β-lactamase (like NDM) + OXA-48 in India include a 3-hour prolonged infusion of ceftazidime, avibactam, and aztreonam, or polymyxins plus other agents to which the organism has shown to be susceptible MIC, such as tigecycline, amino-glycosides, or intravenous fosfomycin. If the MIC is ≤ 16µg/ml, high-dose carbapenems are an approved treatment option, tigecycline (permitted for skin-soft tissue infections and intra-abdominal infections) or aminoglycosides. If susceptible *in vitro*, *Pseudomonas aeruginosa* resistant to carba-penems can be treated with antibiotics with β-lactam (ceftazidime/cefepime) or β-lactam-β-lactamase inhibitor combos (piperacillintazo-bactam/cefoperazone sulbactam). For infections for which there are no other options, amino-glycosides or polymyxins can be used [18].

In our study, a combination of CZA+ATM was administered to 10 patients who were NDM producers, with 8 surviving (80.0% survival rate). The effectiveness of ATM in combination with CZA aligns with studies conducted by Radha *et al.* (2023) (20.9%) and Swaminathan *et al.* (2022) (17.2%), both of which demonstrated a reduction in mortality [28, 29]. However, the observed clinical response in our study to CZA+ATM combination therapy is to be interpreted with caution as the data was available for small subset of patients (n = 10). Hence these observations are only exploratory and do not allow generalization or definitive conclusion regarding clinical effectiveness.

A simple and reliable phenotypic method, the BDE test, was adopted to evaluate the synergy between CZA and ATM. Furthermore, recent Clinical and Laboratory Standards Institute (CLSI) guidelines have recognized this in-vitro susceptibility testing method using BDE as a dependable approach for synergy testing. Our results revealed, synergy was positive amongst majority of NDM (88.0%, n = 44) and NDM plus OXA-48 (93.0%, n = 28) producing CRO. The results align with the research conducted by Taha *et al.* (2023) where 98.0% of the NDM producers were positive for synergy testing [30]. The results of this simple and cost-effective synergy test can assist clinicians in initiating the CZA + ATM combination therapy with greater confidence, minimizing concerns about treatment failure due to the rapid turnaround time of the BDE method. Aztreonam + ceftazidime-avibactam together are only useful in treating Enterobacterales that express MBL. Avibactam is not able to reverse ATM activity in *P. aeruginosa* and *Acinetobacter spp*. that express MBL, where efflux resistance mechanisms make ATM less efficient. However, our study had certain limitations. Convenience sampling and testing was employed which restricts the findings' potential to be applied generally. Presence of other carbapenemase genes (KPC, IMP or VIM) was not assessed in our study, because molecular characteri-zation of carbapenemase resistance was restricted to NDM and OXA-48.

Conclusion

In the current research, out of the total 135 CRO, majority were from urinary tract infection (32.0%) and the most common carbapenem resistant *Enterobacterale* isolated was *Klebsiella pneumoniae* (55.0%). NDM (37.0%) was most common following by NDM and OXA-48 (23.0%) coproduction. Conventional multiplex PCR may serve as useful screening tool for the detection of common carbapenemase genes. BDE method of synergy testing is a reliable method in resource limited settings. As a result, our research highlights the significance of monitoring common carbapenemase genes alongside the judicious use of feasible diagnostic methods.

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ORIGINAL ARTICLE

Correlation of mucin1 expression with various grades and stages of colorectal carcinomas

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Abstract

Background: Carcinoma of the colon and rectum is one of the commonest causes of cancer-related deaths globally. In India, the incidence of carcinoma of the colon and rectum is high due to genetic factors, lifestyle, and environmental factors. Over 50% of cases are diagnosed in the late stage, which leads to limitations in treatment and worsens prognosis. Biomarkers such as Carcinoembryonic Antigen and Mucin-1 (MUC1) play a crucial role as prognostic markers in Colorectal Carcinoma (CRC). Aim and Objectives: To evaluate and correlate MUC1 expression with the grading and staging of CRC. *Material and Methods:* A hospital-based cross-sectional study was done on 40 specimens received in the histopathology section of the department of pathology. Tumor tissue blocks on which diagnosis of CRC was made were evaluated for MUC1 expression and were correlated with grading and staging of CRC. *Results:* MUC1 expression showed score 3+ positivity in 70% cases of moderately differentiated adenocarcinoma, 50% cases of well-differentiated adenocarcinoma and in all cases of poorly differentiated adenocarcinoma. In 78.57% of T3 cases and 75% of T4 cases, score 3 MUC1 expression was observed. Score 3+ MUC1 expression was noted in 60% of N0, 82% of N1 and 100% of N2. The maximum number of cases showing the depth of invasion in the subserosa showed the highest score, that is, score 3, amounting to 85.71%. *Conclusion:* Overexpression of MUC1 is associated with the aggressiveness and progression of the tumor, and it can aid clinicians in intensifying chemotherapy in cases of CRC with high MUC1 expression.

Keywords: Colorectal Carcinoma, Immunohistochemistry, MUC1 Expression, Staging, Grading, Prognosis

Introduction

Around two million new cases of Colorectal Cancer (CRC), with approximately one million deaths, have been reported [1]. The National Cancer Registry data from 1982 to 2010 revealed a steady rise in the annual percentage change, ranging from 0.9% to 5.8% for carcinoma colon and 2.7% to 9.8% for carcinoma rectum. The latest report, based on 27 population-based cancer registries, indicated an annual incidence rate of 5.36 per one lakh population for carcinoma of the colon and 5.17 per one lakh for carcinoma of the rectum in men and in women [2].

The northeastern and southern regions of India have a much higher prevalence of colorectal cancer, potentially attributable to variations in genetic, lifestyle, and environmental variables. Notably, over 50% of colorectal carcinoma cases present at an advanced stage, which severely reduces the range of available treatments and deteriorates prognosis, ultimately resulting in high death rates [3]. Early detection and correct staging are important for improving patient outcomes. Present diagnostic modalities frequently fail to provide reliable prognostic insights [4]. Various biomarkers in CRC detection include Carcinoembryonic Antigen (CEA), CD133 and epithelial membrane antigen Mucin1 (MUC1). The glycoprotein CEA

has been widely recognized as a biomarker for colorectal cancer [5].

Among the potential biomarkers mentioned in the recent studies of CRC was MUC1. MUC1 is a transmembrane glycoprotein. It has been recognized for its significant aberrant expression in CRC. Also, it is associated with aggressive tumor behavior [6,7]. MUC1 expression studies have also been conducted on various malignant tumours, including ovarian, bladder, and thyroid tumours, by several authors [8,9,10]. A study on MUC1 expression in malignant ovarian tumors indicated that MUC1 is implicated in cancer progression and is also related to poor prognosis [8].

A study on bladder cancer indicated that MUC1 is crucial in preserving the mucosal integrity of the urothelium, and its abnormal expression contributes to the growth and spread of malignant bladder tumors [9]. In a study done on MUC1 expression in colorectal carcinoma, it was mentioned that MUC1 is indicative of poor prognosis. It also plays a role in epithelial-mesenchymal transition [11]. In some studies, a positive correlation between MUC1 and tumor grading was observed [12, 13]. The identification and evaluation of biomarkers such as MUC1 can help to plan treatment for CRC.

Material and Methods

Source of data

This hospital-based cross-sectional study was conducted on resected specimens of colorectal carcinoma received in the histopathology section of the Department of Pathology from 2019 to 2024. The study was approved by the Institutional Ethics Committee (BLDE (DU)/IEC/932/2023-24 dated 10.04.2023). All resected colorectal specimens diagnosed as carcinoma on histopathological examination were included in the study. Cases of colorectal carcinoma in which the tissue was inadequate for further immunohistochemical processing were excluded.

Methods of collection of data

Resected colorectal specimens, which were diagnosed as carcinoma on histopathology, were evaluated for grading and staging. Clinical details were collected from the patient's records. Evaluation of study cases was done under the headings of age, sex of the patient, tumor location, histological grading and staging of the tumor, depth of invasion, lymphovascular invasion and lymph node metastasis. Tumor tissue blocks on which diagnosis of carcinoma was done were evaluated for MUC1 expression. Then correlation of MUC1 expression was done with the grading and staging of CRC. For the immunohistochemistry study of MUC1, tissue sections 3 μm thick were placed on charged slides and incubated at 70°C for 20 minutes. Deparaffinization of the sections was done by two changes of xylene for 10 minutes each. Hydration of the sections was done by passing through absolute alcohol in 100%, 70%, 50% for 3 minutes each. Then, in distilled water for 3 minutes each. Sections were stored in Tris EDTA (pH 8.5 to 9.0) for antigen retrieval. Then, washed in distilled water for 3 minutes. After that slide was kept in 3% hydrogen peroxide for 10 minutes. Then, the sections were washed using a 0.05 mM Tris-buffered saline solution. Diluted mouse monoclonal antibodies targeting MUC1 were used as primary antibodies. Primary antibodies were applied to these sections and incubated for 45 minutes in a moist chamber. Tris-buffered saline solution (0.05mM) was used for washing. Then the target binder was added and incubated for 10 minutes. Then again, washed in Tris-buffered saline for 2 minutes. The sections were incubated with Polyexcel HRP for 10 minutes. Sections were treated with 0.5mg/ml 3, 3'-

diaminobenzidine solution. Hematoxylin was used for counterstaining. Slide mounting was completed after sections were cleaned in xylene and dried in ethanol. MUC1 Immunohistochemical (IHC) expression was evaluated and scored as follows: a score of 0 was assigned when no tumor cells exhibited MUC1 positivity. A score of 1+ was given when fewer than 10% of tumor cells showed positive staining. Cases in which 10–50% of tumor cells demonstrated MUC1 positivity were assigned a score of 2+, while a score of 3+ was recorded when more than 50% of tumor cells showed positive staining for MUC1 [14].

Sample size

The sample size was calculated using the formula described in the study by Kasprzak *et al*. (2018) [15]. Based on an anticipated correlation of 0.602 between the MUC1 expression levels of the two analyzed mucin transcripts, with a 95% confidence level and 98% power, the required sample size for the study was determined to be 40. Statistical analysis was performed using the Statistical Package for the Social Sciences (Version 20). Results were presented in a Microsoft Excel sheet. Categorical variables were compared using the Chi-square test. A p-value less than 0.05 was considered statistically significant.

Results

A total of 40 cases were evaluated for MUC1 expression in CRC. The age of the youngest patient was 28, and the oldest patient was 80 years old. The mean age of the study participants was 58 ± 13. The highest number of study participants was between 41 and 50 years, accounting for 27.5%, followed by those between 61 and 70 years and 71-80 years, which accounted for 22.5% of cases each. Gender wise distribution of CRC cases showed a mild female preponderance amounting to 57.5%.

The commonest clinical manifestation was of a detectable mass in the abdomen, summing up to 55%, followed by per rectal bleeding (20%), obstructive symptoms (15%) and abdominal pain (10%). The resected specimens of the study participants showed the most common gross presentation, with ulcero-proliferative growth accounting for 65%, followed by circumferential growth at 20%, exophytic growth at 10%, and polypoidal growth at 5%. The most common site involved was the rectum, accounting for 42.5%, followed by the colon at 35%, and the caecum at 22.5%. Adenocarcinoma was the predominant histological subtype, with 92.5%, followed by 5% of mucinous adenocarcinoma and 2.5% of signet ring cell carcinoma.

The grading of CRC showed that 82.5% of cases were moderately differentiated carcinomas, followed by 10% of cases that were well differentiated, and 7.5% of cases that were poorly differentiated. In poorly differentiated CRC, 2 cases were mucinous adenocarcinomas, and 1 case was a signet ring cell carcinoma. The maximum number of CRC cases were of T2 staging, amounting to 52.5%, followed by T3 (35%), T4 (10%) and T1 (2.5%). Out of 40 cases of CRC, 27.5% of cases showed metastasis in lymph nodes with N1 status, which is defined as one to three regional lymph node involvements. In 10% of cases, N2 status was noted, indicating involvement of more than four lymph nodes. When the association of grading was examined in conjunction with tumour staging, T3 staging was noted in all cases of poorly differentiated CRC. In contrast, the association between grading and staging was 25% and 30.30%, respectively, for well-differentiated and moderately differentiated CRC. When the correlation between the grading of the tumor and metastasis of lymph node was done, the highest percentage of metastasis in the N2 category was

noted in poorly differentiated CRC as compared to moderate and well-differentiated CRC, and the difference was statistically significant with a p-value of 0.007 (Table 1).

When the association of grading was done with depth of invasion, it was observed that in poorly differentiated CRC, the highest percentage of cases showed extension up to serosa and beyond serosa as compared to well differentiated CRC and moderately differentiated CRC, but the difference was statistically not significant (Table 2).

When the correlation of lymphovascular invasion was done with grading, it was observed that, as compared to well-differentiated and moderately differentiated CRC, the maximum number of cases showed lymphovascular invasion in poorly differentiated CRC. However, the difference was statistically not significant. When the correlation between metastasis in the lymph node and staging of CRC was done, stage 3 and stage 4 showed the highest percentage of metastasis compared to stage 1 and stage 2. However, the difference was not statistically significant. (Table 1).

When the correlation between staging and depth of invasion was studied, in stage T3 and T4, the highest number of cases showed the depth of invasion into serosa and subserosa compared to stage T1 and T2, and the difference was statistically significant (Table 2).

The correlation between staging and lymphovascular invasion showed that a larger number of cases in the T4 stage exhibited lymphovascular invasion compared to stages T1 to T3; however, the difference was statistically not significant (Table 2).

Overall, the evaluation of MUC1 expression in CRC cases showed a score of 1 in 3 cases (7.5%), a score of 2 in 9 cases, followed by a score of 3 in 28 cases (70%). In all cases of poorly differentiated CRC, score 3 MUC1 expression was noted. Score 3 MUC 1 expression was noted in a higher number of cases of moderately differentiated CRC as compared to well differentiated CRC, but the difference was statistically not significant. (Table 3, Figure 1-4 A&B)

Correlation of tumor staging with MUC1 expression showed a maximum number of cases showing score 3 expression in stage T3 and T4 amounting to 78.57 % and 75% respectively. All cases of stage T1 showed score 1 MUC1 expression and the difference between various tumor staging with MUC1 expression score was statistically significant (Table 3).

The score 3 MUC1 expression was highest in CRC cases with N2 category lymph node metastasis, amounting to 100%, and lowest in the N0 category, amounting to 60%. However, the difference was statistically not significant (Table 3).

When the association of depth of invasion and MUC1 expression was done, a maximum number of cases showing the depth of invasion in subserosa showed the highest score that is score 3, amounting to 85.71%. Score 3 MUC1 expression was not observed in cases of CRC limited to submucosa and difference between depth of invasion and MUC1 expression score was statistically significant. (Table 3)

When the association between lymphovascular invasion and MUC1 expression was done, score 3 MUC1 expression was higher in cases showing lymphovascular invasion as compared to CRC cases without lymphovascular invasion, but the difference was not statistically significant. (Table 3)

When the association between the site of CRC and MUC1 expression was studied, score 3 expression of MUC1 was noted in 88.8% of CRC cases of the caecum, followed by 71.4% of colon and 58.8% of rectum. However, the difference was not significant

statistically. The association of gross morphology in CRC and MUC1 expression showed, score 3 expression of MUC1 in 100% of CRC cases presented with polypoidal growth followed by 73.07% ulceroproliferative growth and 62.5% circumferential growth, but the difference was statistically not significant.

Discussion

A distinguishing feature of CRC is its capacity to secrete mucin. Usually, the mucin protects epithelial surfaces by lubricating the surface of the epithelium [12]. MUC1 is crucial in preserving the mucosal integrity, and its abnormal expression can contribute to the progression and spread of malignant tumors [9]. MUC1 expression studies done on various malignant tumors mentioned that MUC1 is implicated in cancer progression [8-10]. The maximum number of study participants was within the age group of 41 to 50 years. These findings in the present study are in concordance with the results of Kesari *et al*. (2015) [12]. The incidence of CRC was slightly higher in males as compared to females. It was mentioned that sex steroid hormones and microbiota of the gastrointestinal tract may be the cause of it [12]. However, in the present study, a mild female preponderance was noted, amounting to 42.5% males and 57.5% females.

The clinical symptoms of CRC vary across different anatomical regions in the colon and rectum, due to their distinct anatomical and physiological functions. Typically, abdominal pain and systemic symptoms are prevalent in right colon cancer, while hematochezia and obstruction are more frequent in left colon cancer, and alterations in defecation habits are more characteristic of rectal cancer [16]. Kesari *et al*. (2015) [12] noted blood in stool in 66%, pain in the abdomen and a mass in the abdomen in 48% of cases, and changed bowel habits in 43% of cases of CRC. It was also mentioned that ulceroproliferative growth was found in 48% of cases of CRC.

The most common site involved in our study was the rectum, accounting for 42.5%, followed by the colon (35%) and the caecum (22.5%). In the same way, Kesari *et al*. (2015) [12] found that CRC most

Table 1: Association of grading and staging of CRC with lymph node metastasis

Grading	**N0**	**N1**	**N2**	**Chi-square**	***p***
WD CRC (n=4)	4 (100%)	0 (0.0%)	0 (0.0%)	14.206	*0.007
MD CRC (n=33)	20(60.61%)	11(33.33%)	2 (6.06%)		
PD CRC (n=3)	1 (33.33%)	0 (0.0%)	2(66.67%)		
Staging					
T1 (n=01)	1 (100%)	0 (0.0%)	0 (0.0%)	3.5	0.73
T2 (n=21)	16(76.19%)	4 (19.04%)	1 (4.76%)		
T3 (n= 14)	7 (50%)	5 (35.71%)	2(14.28%)		
T4 (n= 4)	1 (25%)	2 (50%)	1 (25%)		

Table 2: Association of grading of CRC with depth of invasion & association of staging of CRC with depth of invasion and lymphovascular invasion

Grading	**Depth of invasion**				**Chi-square**	***p***
	Subserosa (n=7)	**Serosa (n=12)**	**Muscularis propria (n=20)**	**Submucosa (n=1)**		
WD CRC (n=4)	0 (0.0%)	2 (50%)	2 (50%)	0 (0.0%)	5.229	0.51
MD CRC(n=33)	6 (18.19%)	8 (24.24%)	18 (54.54%)	1 (3.03%)		
PD CRC(n=3)	1 (33.33%)	2 (66.67%)	0 (0.0%)	0 (0.0%)		
Staging	**Depth of invasion**					
T1(n=1)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100%)	20.85	*0.013
T2(n=21)	2 (9.52%)	3 (14.29%)	16 (76.19%)	0 (0.0%)		
T3(n=14)	5 (35.71%)	6 (42.86%)	3 (21.43%)	0 (0.0%)		
T4 (n= 4)	0 (0.0%)	3 (75%)	1 (25%)	0 (0.0%)		
Staging	**Lymphovascular Invasion**					
	Yes (n=14)		No (n=26)			
T1(n=1)	0(0.0%)		1 (100%)		1.63	0.65
T2(n=21)	6(28.57%)		15(71.43%)			
T3(n=14)	5(35.71%)		9(64.29%)			
T4 (n= 4)	3(75%)		1(25%)			

Table 3: Association of grading, staging, lymph node metastasis, depth of invasion & lymphovascular invasion of CRC with MUC1 expression

Grading	**Score 1**	**Score 2**	**Score 3**	**Chi- square**	***p***
WD CRC (n=4)	1(25%)	1(25%)	2(50%)	3.29	0.51
MD CRC (n=33)	2(6.06%)	8(24.24%)	23(69.70%)		
PD CRC (n=3)	0(0.0%)	0(0.0%)	3(100%)		

Continued...

Tumor Staging					
T1 (n=1)	1(100%)	0(0.0%)	0(0.0%)	13.81	*0.032
T2(n=21)	1(4.76%)	6(28.57%)	14(66.67%)		
T3 (n=14)	1(7.14%)	2(14.29%)	11(78.57%)		
T4 (n=4)	0(0.0%)	1(25%)	3(75%)		
Lymph Node Metastasis					
N0 (n=25)	3(12%)	7(28%)	15(60%)	4.218	0.377
N1 (n=11)	0(0.0%)	2(18.18%)	9(81.82%)		
N2 (n=4)	0(0.0%)	0(0.0%)	4(100%)		
Depth of invasion					
Subserosa (n=7)	0(0.0%)	1(14.29%)	6(85.71%)	13.601	*0.034
Serosa (n=12)	1(8.33%)	3(25%)	8(66.67%)		
Muscularis propria (n=20)	1(5%)	5(25%)	14(70%)		
Submucosa (n=1)	1(100%)	0(0.0%)	0(0.0%)		
Lymphovascular invasion					
Yes (n=14)	1(7.14%)	2(14.29%)	11(78.57%)	0.876	0.645
No (n=26)	2(7.70%)	7(26.92%)	17(65.38%)		

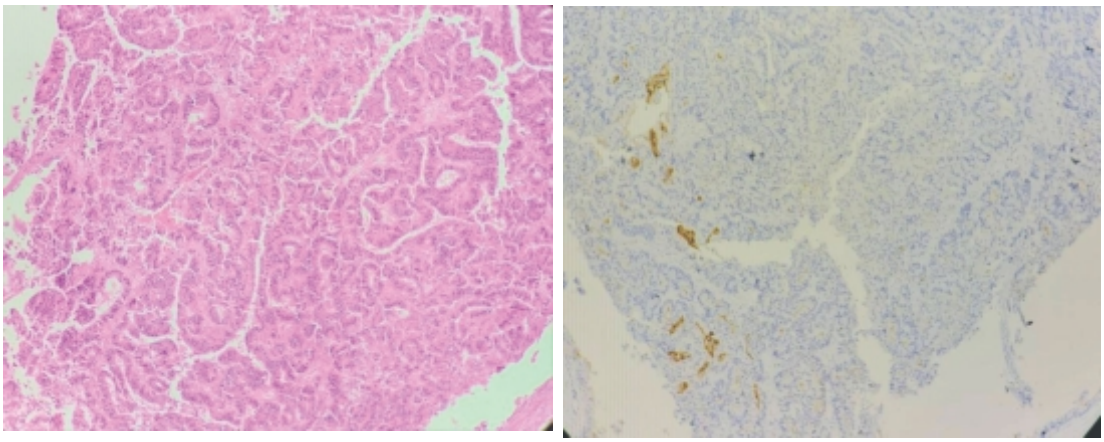

Figure 1: Photomicrograph showing well differentiated adenocarcinoma of MUC1 expression, score 1 (AH & E, B- IHC, 100×)

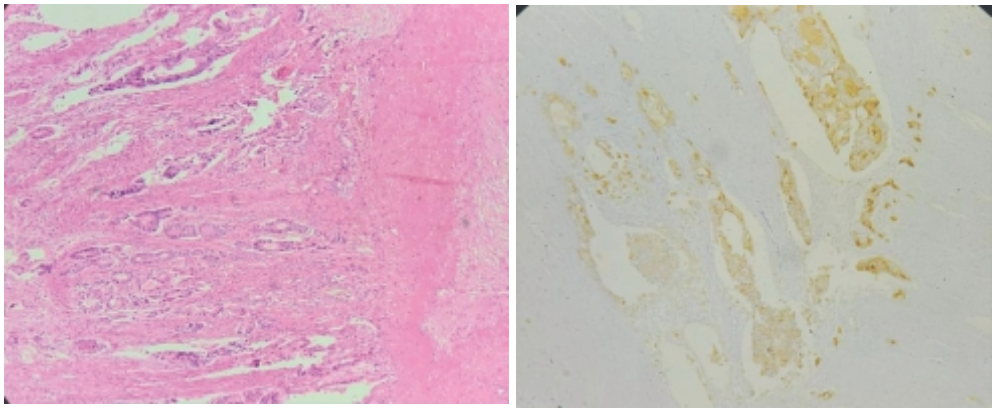

Figure 2: **Photomicrograph showing moderately differentiated adenocarcinoma of MUC1 expression, score 2 (A – H & E, B- IHC, 100×)**

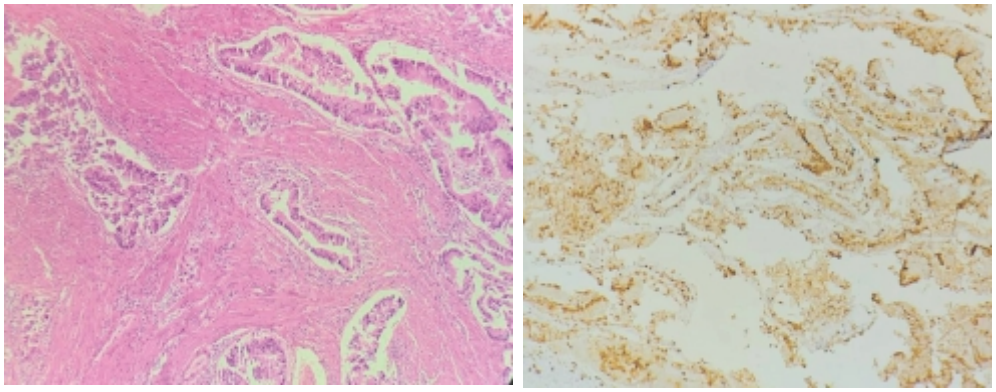

Figure 3: **Photomicrograph showing moderately differentiated adenocarcinoma of MUC1 expression score 3 (A – H & E, B - IHC, 100×)**

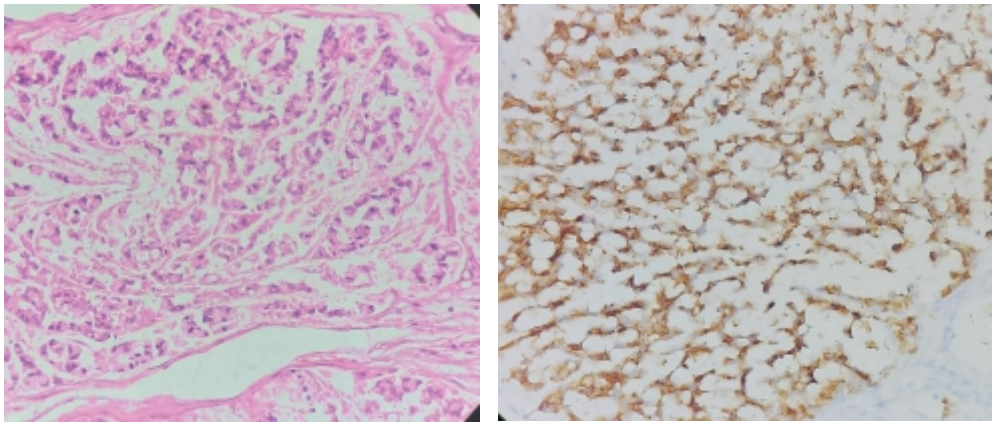

Figure 4: **Photomicrograph showing poorly differentiated Signet ring cell carcinoma of MUC1 expression score 3 (A – H & E, B - IHC, 100×)**

often occurred in the rectum and ascending colon, accounting for 30% each. This was followed by the sigmoid colon in 26%, the descending colon in 8%, and the transverse colon in 6% of cases. In the present study, adenocarcinoma was the predominant histological subtype, accounting for 92.5%, followed by 5% of mucinous adenocarcinoma and 2.5% of signet ring cell carcinoma.

Similar observations were noted in a study by Debbarma *et al*. (2023) [17] and Duncan *et al*. (2007) [18], and Ekta *et al*. (2007) [19] which showed that adenocarcinoma was the predominant histological subtype. The majority of the cases in the present study were moderately differentiated, followed by well-differentiated and poorly differentiated CRC. Similarly, Debbarma *et al*. (2023) [17] also noted that the majority of the tumours were moderately differentiated, followed by poorly differentiated.

The maximum number of cases were of pT stage T2, amounting to 52.5% followed by T3 (35%), T4 (10%) and T1 (2.5%). Similarly, in a study by Kesari *et al*. (2015) [12], 46% of cases were in stage pT2, followed by the pT3 stage in 44 % of cases, pT stage 1 in 6 % of cases, and stage 4 in 4 % of cases of CRC. These findings are similar to the findings of the present study, with the maximum number of cases in the pT2 stage. In the indexed study, out of the 40 cases studied, 62.5% did not show any lymph node involvement, followed by N1 cases, amounting to 27.5%, followed by N2 cases (10%). On the contrary, Díaz *et al*. (2018) [13] noted that lymph node metastases occurred in 39.6% of the cases with N1 status, in 22.1% of % N1 cases and N2 status in 14.8% of % N2 cases.

Four patients in our study with well-differentiated CRC showed N0 lymph node involvement. Out of 33 patients with moderately differentiated CRC, 20 (60.6%) showed N0 lymph node involvement, 11 (33.3%) showed N1 lymph node involvement and two patients (6.06%) showed N2 involvement. Out of 3 patients with poorly differentiated CRC, 1 (33.3%) showed N0 lymph node involvement, and two patients (66.6%) showed N2 involvement ($p = 0.007$). In a study done by Derwinger *et al*. (2010) [20] on 1239 patients who underwent surgical resection for colorectal cancer, a substantial correlation was demonstrated between tumor grading and T-stage, as well as the risk of lymph node metastasis ($p < 0.001$). The higher grade correlated with an increased positive lymph node count in stage III illness ($p < 0.001$).

Depth of submucosal invasion is considered as an important predictive factor for lymph node metastasis. One patient in present study with T1 stage had submucosal involvement. Out of 21 patients with stage T2, subserosal involvement was seen in two patients (9.52%), three patients (14.28%) showed involvement of serosa, and 16 patients (76.19%) showed muscularis propria involvement. Out of 14 patients with stage T3, subserosal involvement was seen in five patients (35.71%), six patients (42.85%) showed serosa involvement, and three patients (21.42%) showed muscularis propria involvement. Out of 4 patients with T4 stage CRC, 3 patients (75%) showed serosa involvement, and one patient (100%) showed muscularis propria involvement. The association of staging with depth of invasion was statistically significant ($p = 0.013$). Foersch *et al*. (2022) [21] observed that tumors with pT3b, which have an infiltration depth of more than 3 mm, showed a worse prognosis compared to pT3a tumors in which invasion of tumor tissue into the adipose tissue was 3 mm or less. The association of tumor staging of CRC and MUC1 expression in the present study showed score 3 MUC1 expression in

more cases of CRC with T3 and T4 stages, as compared to T1 and T2 stages, with a statistically significant difference (p = 0.03). Similar observations were noted in a study done by Yu *et al*. (2007) [22]. In the present study, association of grading with MUC1 expression showed score 3 MUC1 expression in all cases of poorly differentiated adenocarcinoma, but the difference was statistically insignificant. These observations were similar to Khemeri *et al*. (2019) [23] study, which showed that on comparing low grade (G1) with high grade G2/G3 tumors, the high grade-tumors showed significantly stronger MUC1 expression. In the present study, when the association between lymph node metastasis and MUC1 expression was done, the highest number of CRC cases, with N2 and N1, showed a higher percentage of CRC with a score of 3 for MUC1 expression. However, the difference was statistically not significant. A study by Aisawa *et al*. (2024) [24] revealed that 38.8% of MUC1-positive colorectal tumors demonstrated lymph node metastasis in contrast to MUC1-negative tumors. Increased frequency of lymph node metastases in MUC1-positive cases suggests that MUC1-mediated pathways may promote the migration of carcinoma cells to lymph nodes through stromal lymphatic channels [24]. There was a positive association between depth of invasion and MUC1 expression, with a statistically significant difference, (p = 0.03). Similarly, in the study done by Aisawa *et al*. (2024) [23], 26 cases showed positive MUC1 expression, among which highest number of cases showed depth of invasion beyond serosa and the difference was statistically significant. In a study by Betge *et al.* (2016) [25], 13% of cases with high MUC1 expression showed lymphovascular invasion, while 56% of cases with low MUC1 expression exhibited lymphovascular invasion. The association between lymphovascular invasion and MUC1 expression was not significant in our study.

Conclusion

MUC1 expression was high in cases of CRC with advanced stages and in cases with a depth of invasion extending beyond the serosa and subserosa. Also, score 3 MUC1 expression was more in cases of CRC showing poorly differentiated carcinoma and in CRC cases with lymph node metastasis and lymphovascular invasion. These findings suggest that a high MUC1 score leads to the interruption of cell adhesion, which in turn facilitates metastasis and invasion. Based on this, we conclude that MUC1 expression may be upregulated in CRC. This effect of MUC1 may cause the progression of tumor and the aggressiveness of CRC, and it can help in guiding clinicians to intensify chemotherapy in cases of CRC with high MUC1 expression. In cases of metastatic or unresectable CRC, MUC1-based immunotherapy can also be developed to overcome the limited treatment options. It also opens avenue for MUC1-specific CAR-T cell therapy and specific anti MUC1 antibody probes for targeted therapy. However, further multicenter studies are needed with a larger sample size to validate the study's observations.

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ORIGINAL ARTICLE

A study of serum procalcitonin albumin ratio in predicting urosepsis from febrile urinary tract infection

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Abstract

Background: Urinary Tract Infection (UTI) is a common clinical entity and may progress to urosepsis with poor outcomes. Reliable biomarkers are essential for identifying high risk patients. *Aim and Objectives*: To determine the efficacy of the serum procalcitonin to albumin ratio as a predictor of urosepsis in patients with febrile urinary tract infection. *Material and Methods*: This one-year cross-sectional study at a tertiary healthcare institution in Tamil Nadu included 84 adults (≥18 years) with febrile UTI. Serum procalcitonin and albumin levels were measured, and their ratio calculated. SPSS v24 software was used to analyse the data, with $p < 0.05$ considered significant. Continuous data were summarised using Mean ± SD and categorical variables as frequencies and percentages. Receiver Operating Characteristic (ROC) curve analyzed the predictive value. *Results*: Urosepsis was observed in 41% cases with mean age of 61.7 ± 18.3 years. Hypotension, elevated serum urea, creatinine, procalcitonin, procalcitonin/albumin ratio, and leukocyte count ($p < 0.05$) were significantly associated with urosepsis. ROC analysis showed excellent discrimination for procalcitonin and procalcitonin/albumin ratio, with area under the curve of 0.914 and 0.915, respectively ($p < 0.001$). The procalcitonin/albumin cut-off of 0.405 yielded 67.8% sensitivity and 96.2% specificity, highlighting it as a novel marker. *Conclusion*: The pro-calcitonin/albumin ratio shows strong potential as a marker for distinguishing febrile urinary tract infections from urosepsis. Incorporating this can enhance diagnostic accuracy.

Keywords: Biomarkers, procalcitonin, procalcitonin-to-albumin ratio, serum albumin, septic shock, urinary tract infections, complications

Introduction

Urinary Tract Infection (UTI) has a wide clinical spectrum that may lead to infections from the urethra to the kidneys. Clinical presentations [1-2] like fever, rigors, chills, nausea, vomiting, and renal angle tenderness, along with laboratory evidence of urine culture, blood tests substantiate UTI diagnosis. Febrile UTI (fUTI) [3-4] refers specifically to infections accompanied by fever [5], indicating more severe forms such as acute pyelonephritis or acute prostatitis. Acute Pyelonephritis (AP) is a condition where a UTI involves the kidney [6-7] whereas, urosepsis [6] is a sepsis in the urinary tract, which is a serious organ dysfunction resulting due to inappropriate host immune responses to infections. As the pathogens causing AP are increasingly becoming resistant to current therapies, there is a growing need for clinical trials and epidemiological studies to evaluate the risk factors linked with resistant strains and to develop effective prevention strategies-particularly among individuals with a history of AP [8-13]. The prognosis of septic syndromes is influenced by the underlying diseases and severity of inflammatory response, reflected in the extent of organ dysfunction [14].

The prevalence of sepsis among Intensive Care Unit (ICU) patients [15] is significantly high, with

the majority presenting clinically or microbiologically documented infections, although certain subgroups may present without identifiable sources. Previous researches have implied 2 blood cultures detect approximately 80% of bloodstream infections, while three blood cultures detected 96% of episodes [16]. However, as the cultures take a longer time to report, new biomarkers [17] were essential for improving sepsis management. Procalcitonin was first reported to be elevated in infections [18-21] and a reliable predictor of bloodstream infections. Procalcitonin and C-Reactive Protein (CRP) [22] used in clinical practice have limited ability to differentiate bacterial sepsis from other inflammatory conditions. Serum albumin reduces during acute-phase infections, making it a marker of sepsis [23-24]. Patients with sepsis or severe bacterial or fungal infections have high procalcitonin levels. However, procalcitonin levels can vary in the initial course of the disease. However, researchers have suggested that the predictive performance of the test becomes relevant only when the illness advances [18]. To strengthen the diagnostic work-up, the procalcitonin-to-albumin ratio has been investigated as a potential early and reliable biomarker. According to a study by Luo et al. [25], this ratio can serve as a promising marker in discriminating fUTIs and urosepsis. Furthermore, elevated procalcitonin /albumin ratios could indicate an increased risk of progression to uroseptic shock and can reflect the severity. Early differentiation between them is crucial to prevent organ failure and reduce mortality. The study aimed to evaluate the effectiveness of procalcitonin-to-albumin ratio as a promising biomarker for discriminating fUTIs and urosepsis.

Material and Methods

The present study adopted a cross-sectional observational methodology over one year at a specialised care center in Tamil Nadu, India. This study enrolled 84 adult patients diagnosed with fUTI. fUTI was defined as an infection of the upper or lower urinary tract accompanied by fever and chills. Patients who met the inclusion criteria were consecutively recruited from both outpatient and inpatient departments. Individuals aged 18 years or older who presented with fever and had a UTI confirmed by urine culture were eligible for inclusion. Patients younger than 18 years, as well as those with decompensated chronic liver disease or chronic kidney disease, were excluded from the study. Individuals with fUTI was diagnosed to have urosepsis when Quick Sequential Organ Failure Assessment (qSOFA) score was ≥2 [26].

Ethical approval for the study was secured from the Institutional Ethics Committee (Reference Number 23/304) prior to commencement. All study participants gave a written informed consent. Comprehensive history elicitation, physical examination, and laboratory investigations were performed. Serum procalcitonin and albumin levels were measured, and the procalcitonin-to-albumin ratio was computed. Efforts were made to reduce bias by careful sample selection and by considering potential confounders such as age, comorbidities, lactate levels, and qSOFA scores. The formula $n = 4pq/d^2$ was used to calculate the required sample size assuming a sensitivity of 84% for the procalcitonin-to-albumin ratio in predicting urosepsis and expecting 10% non-response, the final sample size consisted of 84 patients. Descriptive statistical analysis was employed to describe patient characteristics and laboratory values. Continuous parameters were summarised as means along with

Standard Deviation (SD), and categorical variables as frequency and percentages. Quantitative data were expressed as median or mean along with standard deviations depending on their distribution. Data were tabulated in Microsoft Excel and analyzed using the Statistical Package for the Social Sciences version 24. Receiver Operating Curve (ROC) analysis was performed to assess the predictive validity of the procalcitonin-to-albumin ratio. Statistically significant p-value was < 0.05.

Results

The study included 90 cases of UTI, of which 37 were diagnosed with urosepsis and 53 with fUTI. Clinical profile and baseline characteristics are detailed in Table 1 including laboratory test results, and clinical presentations. It also presents the overall mean ± SD values of serum procalcitonin, albumin, and their ratio across the study population. Among the clinical presentations, most of them exhibited common UTI symptoms, such as fever (74.4%) and burning micturition (71.1%). Laboratory parameters, including serum urea, creatinine, procalcitonin, procalcitonin/albumin ratio, and leukocyte count, demonstrated higher mean values in urosepsis patients. The baseline characteristics indicate that predominant proportion belonged to the geriatric age group, with a slightly higher proportion of female participants. Collectively, the study findings highlight the role of laboratory markers in distinguishing fUTI from urosepsis, with fever and burning micturition being the commonly reported clinical features at presentation. The study participants were evaluated using the qSOFA score (≥ 2), 29% were diagnosed with urosepsis while 71% had fUTI as depicted in Figure 1.

Based on sepsis cut-off values for various laboratory parameters, Table 2 summarizes the distribution of patients with values in normal and sepsis-related ranges. Elevated serum procalcitonin levels (> 0.5 ng/mL) were observed in 41.1% of patients, which is suggestive of a systemic inflammatory response. Low serum albumin levels ($\leq$ 2.45 g/dL) were noted in only 2.2% of patients. A notable proportion of patients (80%) demonstrated urine pus cells greater than 11/µL, indicating inflammatory response. Additionally, 30% of

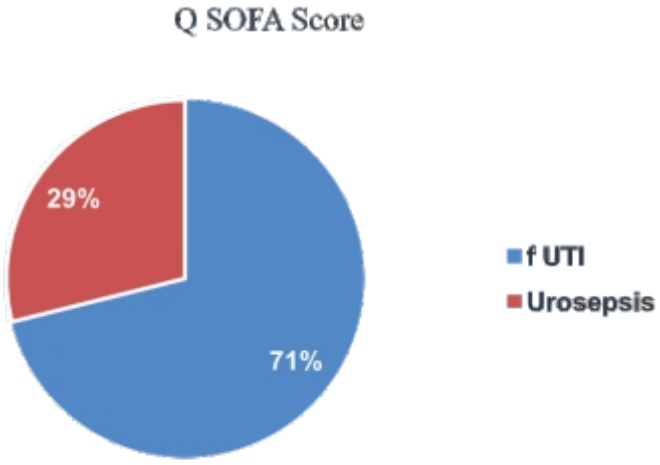

Figure 1: Distribution of study patients based on quick SOFA Score (n=90)

qSOFA – Quick sepsis related organ failure assessment; fUTI – febrile urinary tract infection

Table 1 : Clinico-laboratory profile of study participants (n=90)

Characteristics	fUTI n = 53	Urosepsis n = 37	Total n = 90
Age (Mean ± SD)	60.5 ± 20.2	63.4 ± 15.3	61.7 ± 18.3
Male [n (%)]	26 (49.1)	17 (45.9)	43 (47.8)
Female [n (%)]	27 (50.9)	20 (54.1)	47 (52.2)
Laboratory tests (Mean ± SD)			
Urea (mg/dL)	27.6 ± 13.2	46.1 ± 19.8	35.1 ± 18.5
Creatinine (mg/dL)	1.1 ± 0.4	1.56 ± 0.5	1.36 ± 0.82
Albumin (g/dL)	3.7 ± 0.5	3.2 ± 0.4	3.51± 0.54
Procalcitonin (ng/mL)	0.3 ± 0.42	13.9 ± 33.6	5.94 ± 22.4
Procalcitonin/albumin ratio	0.09 ± 0.12	4.6 ±10.8	1.95 ± 7.27
Leucocyte count ($\times 10^{3/\mu L}$)	11.7 ± 4.3	16 ± 6.2	13.5 ± 5.6
Clinical presentations [n (%)]			
Fever	53 (100)	31 (83.8)	67 (74.4)
Burning micturition	42 (79.2)	22 (59.5)	64 (71.1)
Vomiting	19 (35.8)	15 (40.5)	34 (37.8)
Lower Quadrant pain	17 (32.1)	26 (70.3)	43 (47.7)
Abdominal signs	19 (35.8)	26 (70.3)	45 (50)

Values are expressed as Mean ± standard deviation (SD)
fUTI – Febrile urinary tract infection

patients exhibited an elevated procalcitonin -to-albumin ratio (> 0.44), supporting its potential role as a useful biomarker in diagnosing urosepsis. These laboratory cut-offs may aid in early risk stratification and clinical decision-making in patients with suspected urosepsis.

A univariate analysis of different clinical and laboratory variables is summarized in Table 3. It demonstrates that variables including hypotension, serum urea, creatinine, procalcitonin, the procalcitonin/albumin ratio, leukocyte count, and organ dysfunction were significantly associated with urosepsis ($p < 0.05$) and hence may be considered as important indicators of urosepsis. In contrast, gender, high blood pressure, serum albumin levels, and urine routine showed no statistical significance. The diagnostic performance of various clinical and laboratory parameters in predicting urosepsis among fUTI participants have been tabulated in Table 4.

On ROC curve analysis, procalcitonin (AUC = 0.914, 95% CI: 0.839–0.988) and the procalcitonin/albumin ratio (AUC = 0.915, 95% CI: 0.842–0.988) showed excellent diagnostic performance in predicting urosepsis among fUTI patients. Both markers demonstrated high specificity (94.3%

Table 2 : Laboratory parameters (n=90)

Laboratory tests	Sepsis cutoff values	Normal level n (%)	Sepsis level n (%)
Serum Procalcitonin (ng/ml)	> 0.5	53 (58.9)	37 (41.1)
Serum Albumin (g/dL)	≤ 2.45	88 (97.8)	2 (2.2)
Serum Procalcitonin /Albumin ratio	> 0.44	63 (70)	27 (30)
Urine – pus cells(µl)	> 11	18 (20)	72 (80)

Table 3: Univariate analysis for discriminating urosepsis from fUTI (n=90)

Variables	Univariate OR with 95% CI	*p*
Gender	0.8 (0.38 – 2.04)	0.770
Hypotension < 90/60 mmHg	67.8 (3.8 – 1191.9)	<0.001***
Hypertension > 140/90 mmHg	1.18 (0.3 – 4.4)	0.801
Urea (mg/dL)	11.5 (3.9–33.6)	<0.001***
Creatinine (mg/dL)	8.6 (3.1 – 24.1)	<0.001***
Procalcitonin (ng/mL)	61.4 (16.4 – 229.5)	<0.001***
Albumin (g/dL)	1.44 (0.8 – 23.8)	0.796
Procalcitonin/Albumin ratio	53.1(11.03 –255.7)	<0.001***
Leucocyte count ($\times 10^{3/\mu L}$)	3.3 (1.2 – 8.8)	0.015*
Urine – pus cells	2.96 (0.88 – 9.87)	0.069
Organ dysfunction	41.9 (8.8 – 119.6)	<0.001***

**p <0.05, ** p < 0.01, ***p <0.001*

Table 4 : Various characteristics in predicting urosepsis in febrile UTI patients (n=90)

Characteristics	AUC (95% CI)	*p*	Cut off	Sensitivity	Specificity
Temperature	0.638 (0.519 - 0.757)	0.026*	99.9	54.1 %	68.9 %
Pulse Rate	0.666 (0.550 - 0.783)	0.007**	99	56.8 %	73.6 %
Leucocyte count ($\times 10^{3/\mu L}$)	0.732 (0.626 - 0.837)	<0.001***	11.05	81.1 %	47.2 %
Urea (mg/dL)	0.796 (0.695 - 0.897)	<0.001***	40.5	59.5 %	90.6 %
Creatinine (mg/dL)	0.768 (0.657 - 0.879)	<0.001***	1.14	78.4 %	69.8 %
Procalcitonin (ng/mL)	0.914 (0.839 - 0.988)	<0.001***	1.07	75.7 %	94.3 %
Albumin (g/dl)	0.189 (0.099 - 0.280)	<0.001***	3.15	59.5 %	13.2 %
Procalcitonin /Albumin ratio	0.915 (0.842 - 0.988)	<0.001***	0.405	67.8 %	96.2 %

**p <0.05, ** p < 0.01, ***p <0.001*

and 96.2% respectively), with procalcitonin showing higher sensitivity (75.7%). Among other parameters, serum creatinine (AUC = 0.768) and urea (AUC = 0.796) also exhibited good predictive value, while leucocyte count had higher sensitivity (81.1%) but lower specificity (47.2%). In contrast, albumin alone had poor discriminatory power (AUC = 0.189) but with low specificity (13.2%).

Temperature and pulse rate provided limited discrimination (AUC 0.638 and 0.666) respectively. Overall, procalcitonin and the procalcitonin/albumin ratio were accurate and reliable markers for identifying urosepsis in this study. The clinical parameters along with laboratory predictors were analysed by ROC and illustrated in Figure 2, where pulse rate, temperature, leukocyte count, serum urea, creatinine, procalcitonin, albumin, and the procalcitonin/albumin ratio revealed statistically significant predictive ability ($p < 0.05$) for urosepsis among fUTI patients, however only procalcitonin and procalcitonin/albumin ratio showed excellent diagnostic performance. Overall, these findings highlight the procalcitonin/albumin ratio as a novel and highly effective predictor of urosepsis in this population. Patient outcome studied where majority of patients (72.2%) were discharged after recovery, while 26.7% required ICU discharge for procedures such as stenting or dialysis. Only one death (1.1%) was observed, and the serum procalcitonin/albumin ratio in this patient was 12.7.

Discussion

By using procalcitonin and albumin ratio, the study aimed to develop an early diagnostic predictor which discriminates patients with fUTI and those with urosepsis. Conventional diagnostic methods like inflammatory markers and blood cultures are restricted by some limitations. The prolonged turnaround time for obtaining results [27–28] limits timely initial treatment decisions, highlighting the need for a reliable biomarker. The demographic characteristics of the participants predominantly belong to the geriatric population, with 52.2% females and 47.8% males. Fever was the most common clinical presentation in UTI cases. Similar findings were reported by Luo et al. (2018) [25], where the mean age was 58.47 ± 16.99 years, 32.86% were males, and 72.86% presented with

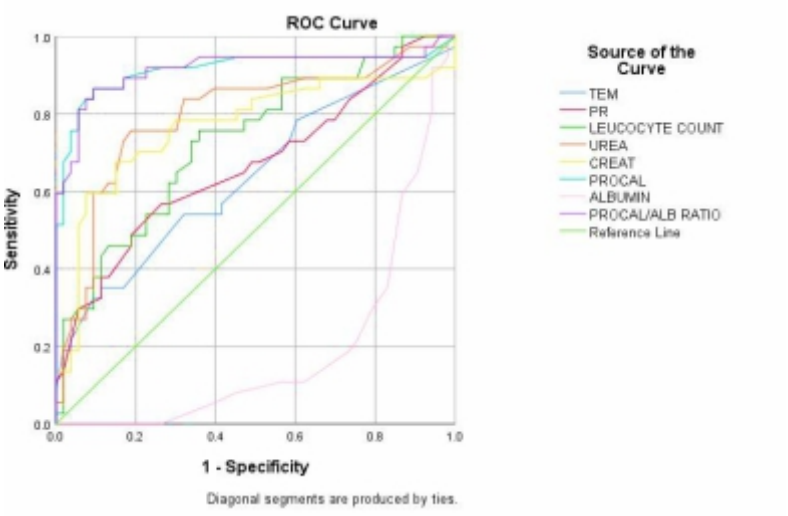

Figure 2: ROC curve of variables in predicting urosepsis in febrile UTI patients

fever or chills. Additionally, 84.29% had flank pain or dysuria, and 21.43% had hypotension.

In contrast, Sahin *et al*. (2023) [29] reported a male predominance (53.85% males, 46.15% females), as did Rout *et al*. (2022) [30] with 57% males and 43% females, yielding a male-to-female ratio of 1.35:1. The sepsis incidence in our study was 41%, lower than the 62.5% reported by Rout *et al*. (2022) [30] However, their findings on procalcitonin levels and serum albumin were consistent with our observations. The qSOFA score was used as an initial screening tool to identify high risk patients. It takes into account altered mental activity, a systolic blood pressure of 100mmHg or less and respiratory rate > 22/min or greater. A score of more than or equal to two of these three clinical conditions indicates urosepsis in fUTI patients. Similar scoring tool was utilized in our study to indicate urosepsis among the study patients.

In the study conducted by Rout *et al*. (2022) [30], the AUC for the procalcitonin/albumin ratio at admission was 84.62% and 96.00%, respectively. CRP demonstrated a sensitivity of 87.50% and a specificity of 50.77%, whereas leukocyte count cut-off values were less specific, with a sensitivity of 64.62% and a specificity of 74.32%. Comparable results were observed in the present study, where the serum procalcitonin/albumin ratio showed a sensitivity of 67.8% and a specificity of 96.2%. In contrast, the leukocyte count cut-off value demonstrated a sensitivity of 81% and a specificity of 47.2%.

In our study, serum albumin demonstrated poor specificity (13.2%) for predicting urosepsis among patients with fUTI probably because sepsis could have caused increased breakdown or loss of albumin from kidney or gastrointestinal tract. However, newer evidence links hypoalbuminemia to severity of the disease and mortality, making it a biomarker rather than a specific diagnostic marker. In contrast, procalcitonin reliably reflects bacterial infection and burden. The combined procalcitonin /albumin ratio improved diagnostic accuracy, with a cutoff value > 0.44 effectively differentiating urosepsis from fUTI. Similar to previous studies, CRP and leukocyte count at admission were associated with urosepsis[25] significantly.

Overall, this study highlights the procalcitonin /albumin ratio for early identification and risk stratification of urosepsis in fUTI patients. Elevated procalcitonin, low albumin, and a high procalcitonin/albumin ratio alongside significant pyuria form a critical diagnostic panel for sepsis. Incorporating these parameters into clinical assessments forms may facilitate diagnosis, guide timely interventions, and ultimately reduce urosepsis-related mortality.

In this study, urosepsis constituted a substantial proportion of fUTI case, predominantly affecting elderly population. Parameters like hypotension, leukocyte count, renal dysfunction, procalcitonin, and the procalcitonin/albumin ratio demonstrated an association that was significant. Among these, the procalcitonin-to-albumin ratio demonstrated strong discriminatory performance, with a cutoff value of 0.405 showing high specificity for urosepsis. A procalcitonin/albumin ratio greater than 0.44 effectively differentiated urosepsis from fUTI and appeared to outperform conventional inflammatory markers such as CRP and leukocyte count.

Conclusion

Given its rapid availability and cost-effectiveness, the procalcitonin/albumin ratio may serve as a valuable adjunctive tool for early identification of urosepsis, facilitating timely clinical decision-making and potentially improving patient outcomes.

Recommendations

A serum procalcitonin/albumin ratio ≥ 0.405 on day 1 of admission, with 67.8% sensitivity and 96.2% specificity, may serve as a promising marker in diagnosing urosepsis in fUTI patients. Incorporation of this marker into early clinical assessment may potentially help with prompt diagnosis and treatment, thereby reducing the mortality.

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ORIGINAL ARTICLE

Association between Vitamin D, calcium and phosphorous deficiency with increased glycosylated fibronectin levels and susceptibility of early trimester maternal gestational diabetes mellitus

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Abstract

Background: Gestational Diabetes Mellitus (GDM) is a more often seen long standing disease in pregnancy. It is expected that maternal deficiencies in important micronutrients can have ill effects on fetal development and pregnancy outcome. Glycosylated Fibronectin (GlyFn) is a positive biomarker that has been extensively studied in the context of pre-eclampsia and GDM over the past decade. *Aim and Objectives:* The present study aimed to evaluate the relation between GlyFn and serum levels of calcium, phosphorous, and Vitamin D in pregnant women, to understand potential metabolic links relevant to the pathogenesis of GDM. *Material and Methods:* This case control study was conducted at Rajarajeshwari Medical College and Hospital, Bengaluru. Eighty-eight pregnant women were recruited from the out-patient department. The Oral Glucose Tolerance Test (OGTT) of participants was done at 24 to 28 weeks. Based on OGTT value, participants were divided into GDM group and control group. Blood sample was collected to estimate GlyFn, calcium, phosphorous and Vitamin D. *Results:* The mean value of phosphorous, calcium and vitamin D was lowered while GlyFn was raised in GDM group compared to control group. *Conclusion:* A negative corelations observed in GDM group in this study imply that calcium, phosphorus and vitamin D metabolism has direct influence on variations in GlyFn levels.

Keywords: Gestational diabetes mellitus, oral glucose tolerance test, glycosylated fibronectin, micronutrients

Introduction

Millions of gestating mothers worldwide suffer with Gestational Diabetes Mellitus (GDM), a common chronic illness [1, 2]. Hyperglycemia initially identified in the period of gestation is known as GDM. [3]. Women experienced remarkable inflammatory reactions that results in insulin resistance and macrosomia, or excessive fetal growth. [4-6]. Pregnancy is an extremely sensitive time that affects the long-term health of both expectant mothers and their unborn offspring [7]. The foetus's primary organs develop during the first trimester, making it a crucial stage of pregnancy [8]. As a result, the growing fetus depends entirely on the mother's surroundings for sustenance [9]. The

regulation of blood calcium levels and the preservation of strong bones are two of vitamin D's most well-known functions [10]. Vitamin D has been associated with a number of critical pregnancy activities, including glucose homeostasis, placental function, inflammatory response, and infection control [11].

Preeclampsia, GDM, and small-for-gestational-age are among the unfavourable pregnancy outcomes that are linked to vitamin D insufficiency [12, 13]. All the macro and micronutrients needed for healthy fetal growth are provided by the mother's diet and nutrient store, which are very much needed for successful pregnancy [14]. Therefore, it is anticipated that maternal deficits in important micronutrients may have detrimental consequences on the growth of the fetus and the outcome of the pregnancy [15]. Gestating mother with GDM have reduced levels of calcium and vitamin D [16,17]. By impairing insulin signal transduction and reducing the function of the Glucose Transporter-4 (GLUT4), changes in calcium in main insulin target tissues may contribute to peripheral insulin resistance [18].

Over the past ten years, Glycosylated Fibronectin (GlyFn) a promising biomarker has been thoroughly investigated in relation to pre-eclampsia and GDM. In particular, glycosylated variations have been connected to impaired endothelial function and vascular remodelling. GDM and preeclampsia are linked to these alterations, especially during spiral artery remodelling in the first trimester [19]. These pathophysiological events are correlated with elevated levels of circulating GlyFn early in pregnancy, which makes it a useful diagnostic tool. Minerals like calcium, phosphorus and hormone vitamin D play a very important role in the maternal health. So, the altered levels of these might result in GDM. In the present scenario it is very necessary to detect GDM at the earliest and intervene with nutritional supplements. Hence our study aimed to explore the association of vitamin D, calcium, and magnesium levels with the tendency for development of GDM.

Material and Methods

This case-control study was conducted at Raja Rajeswari Medical College and Hospital, Bangalore, Karnataka, India, with institutional ethical clearance no. RRMCH-IEC/220/2023. In the year 2023, on the basis of average patient inflow to the Outpatient Department (OPD), the sample size was calculated using Yamane's formula. The estimated total sample size was 90. Participant recruitment occurred from January 2024 to February 2025.

Women who were less than 45 years of age presenting with healthy singleton pregnancy which was conceived spontaneously were recruited following written informed consent. Subjects with prior bariatric surgery, known pre-existing diabetes or current metformin treatment, chronic infections (e.g., hepatitis, HIV), or chronic kidney/liver/heart disease, history of hypertensive disorders treated with prophylactic aspirin, fetal abnormalities requiring intervention and inability to comprehend participant information were excluded [20].

Between 6th and 7th month of gestation, participants underwent an Oral Glucose Tolerance Test (OGTT). Blood glucose levels $\geq$ 140 mg/dL two hours after consuming 75g of glucose were classified as GDM according to the diabetes in pregnancy study group criteria (DIPSI) [21]. Forty-four women were assigned to the GDM group and 45 to the control group based on the sample size of 90. One GDM participant was removed from the study because she declined to provide a sample. The

laboratory expert collected 5 mL of venous blood in a serum Separator Tube (SST tube), allowed it to clot, and spun it at 3000 rpm for 20 minutes after receiving written consent and filling out a questionnaire with demographic data. Serum was isolated and kept at -80°C. Vitamin D, calcium, and phosphorus quantities were measured using an automated analyzer, while GlyFn was measured using an ELISA method. Glycosylated fibronectin was estimated by ELISA method whereas Vitamin D, Calcium and phosphorus was estimated by Automated analyzer. (Roche Cobas)

Statistical analysis

The control and GDM groups' mean values for several parameters were compared using the unpaired t test. The chi-square test was used to evaluate the relationship between the amounts of GlyFn and the biochemical parameters calcium, phosphorus, and vitamin D. The negative association between GlyFn and calcium, phosphorus, and vitamin D was examined using the Pearson correlation coefficient test.

Maternal characteristics

Mean age of GDM group was 24.61 years, while the control group was 23.82 years. The mean OGTT of GDM group was 166.43 mg/dL, while the control group was 116.57 mg/dL. For both groups, the mean duration of pregnancy was around 25.6 weeks. The GDM group had raised amount of GlyFn ($p < 0.001$). The GDM group had less amount of calcium, phosphorus, and vitamin D than the control group; however, the levels of calcium were not statistically significant ($p = 0.503$), whereas the quantities of vitamin D and phosphorus were statistically significant ($p < 0.001$) (Table 1). Chi-square test was used to find the relationship between the levels of calcium, phosphorus, and vitamin D with normal and increased GlyFn levels (Table 2). The chi square test results with p value, chi square value and degree of freedom are summarized in (Table 2). According to chi square analysis based on chi square value , degree of freedom and p value the association between glycosylated fibronectin and calcium, phosphorus was statistically significant.

Table 1 : Maternal characteristics by GDM status

Parameters	GDM (n = 44)	Control (n = 45)	*p*
Age (years)	24.61	23.82	0.0479
OGTT (mg/dL)	166.43	116.57	0.0001*
Gestational weeks	25.70	25.55	0.5895
GlyFn (ng/mL)	31.65	27.03	< 0.001*
Vitamin D (ng/dl)	20.86	29.66	< 0.001*
Calcium (mg/dL)	1.414	1.570	0.5033
Phosphorus (mg/dL)	2.875	3.945	< 0.001*

**Statistically significant; OGTT – oral glucose tolerance test*

Table 2: Chi-square test and Pearson correlation coefficient between GlyFn, calcium, phosphorous and vitamin D

Glycosylated fibronectin (GlyFn)	Calcium		Phosphorous		Vitamin D		
	Normal	Deficient	Normal	Deficient	Normal	Deficient	Insufficient
Normal	1	44	44	1	25	19	1
Increased	11	33	26	18	36	8	0
Chi-square	9.8947		19.83		4.9976		
df	1		1		2		
p	0.00165		0.0000084		0.0241		
Significance	Significant		Highly significant		Significant		
Interpretation	Strong association with GlyFn		Very strong association with GlyFn		No statistically meaningful association with GlyFn		

Table 3: Pearson correlation coefficient results

GlyFn	Calcium	Phosphorous	Vitamin D
r	-0.12991	-0.187	0.0133
p	0.4039	0.224	0.931

But the association between Glycosylated fibronectin and vitamin D was not statistically significant The negative correlation between Gly Fn and calcium, phosphorus, and vitamin D was examined using the Pearson correlation coefficient test; the r values for the calcium and phosphorus groups were -0.12991 and -0.187, respectively, indicating a strong negative correlation, while the r value for the vitamin D group was 0.0133, indicating no correlation. The calcium and phosphorus group's negative r value indicates that as GlyFn rises, the GDM group's calcium and phosphorus levels fall. However, the vitamin D group did not reveal this type of negative relation, indicating that there was no correlation (Table 3).

Discussion

According to our research, there is a statistically significant ($p < 0.05$) correlation between calcium levels and GlyFn. This implies that fluctuations in GlyFn are strongly correlated with changes in calcium levels. Additionally, a robust correlation between phosphorus levels and GlyFn was found, with a highly statistically significant result ($p < 0.001$). GlyFn and vitamin D levels were significantly correlated negatively ($p < 0.05$). The study shows that compared to healthy controls, those with GDM had noticeably lower levels of calcium, phosphorus, and vitamin D. Elevated levels of GlyFn accompany these decreases, indicating a possible inverse relationship between these crucial

biochemical markers and GlyFn. These results emphasize how crucial it is to keep an eye on and control micronutrient levels in GDM patients in order to potentially enhance outcomes for both the mother and the developing foetus. It has been demonstrated that normal pregnant women have far greater serum vitamin D levels than pregnant women with GDM [22]. Pregnancy-related vitamin D insufficiency may impact insulin secretion and raise insulin resistance, which raises the risk of GDM [23]. After controlling for seasonal variables, Australian cohort research revealed that serum 25-(OH)-D levels < 30 nmol/L in the middle of pregnancy were more prone to develop GDM [24]. According to a study by Khagorika *et al*. (2023) health education and simple access to vitamin D testing and supplementation at various healthcare facilities are essential because vitamin D levels were found to be below normal even in adults [25]. In contrast to pregnant women in good health, women with GDM discovered to have considerably reduced serum levels of both calcium and vitamin D [26, 27]. According to these studies, cosupplementing with calcium and vitamin D might be capable of treating GDM. As demonstrated by the lower levels of fasting plasma glucose, serum insulin, and LDL, a meta-analysis verified that vitamin D-calcium supplementation might enhance the metabolic profile for the treatment of GDM [28]. Phosphorus is an essential micronutrient involved in a number of required biological processes. A study demonstrated a significant association between mineral levels and the risk of GDM, particularly for iron, magnesium, manganese, phosphorus, and zinc. However, folate and phosphorus showed only a negligible impact on GDM risk when evaluated using odds ratios. Additionally, the mean levels of magnesium and phosphorus were not found to be statistically significant [29]. Research conducted by Masoodi *et al*. (2015) further indicated that both low dietary intake of vitamin C and reduced plasma vitamin C concentrations are associated with an increased risk of GDM [30].

Conclusion

To the best of our knowledge, this study simultaneously evaluates vitamin D and micronutrients such as calcium and phosphorus within a single investigation. It also examines the likelihood of developing GDM and highlights the role of micronutrients during pregnancy. Therefore, it provides guidance on nutritional requirements in pregnancy that may help reduce and prevent the incidence of GDM.

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ORIGINAL ARTICLE

Analysis of effectiveness of Leishman-Giemsa twin stain preparation in cytopathology: A novel step in laboratory quality assurance

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Abstract

Background: Cytopathology plays dynamic role with respect to diagnostic laboratories in terms of diagnosing broad disease categories and is the primary screening procedure for any superficial swelling of the body. While being daycare procedure with known advantages and limitations, optimal cytochemical stains and its quality forms vital component of any cytology laboratory. Studies have been conducted to analyze standalone and combined cytological stains and to evaluate their efficacy based on standard quality parameters. *Aim and Objectives*: To undertake a novel analysis of two standard air-dried smears, namely standalone Giemsa stain and the Leishman –Giemsa (LG) cocktail, and to evaluate their impact on the effective diagnosis of cytological smears. *Material and Methods:* This prospective study was designed as a comparative observational study, with standard inclusion and exclusion criteria established. The LG working solution was prepared according to standard protocols and existing literature, and its efficacy was compared with that of the routine standalone Giemsa stain based on smear quality parameters. The collected data were tabulated for analysis and assessment of the Quality Index (QI). *Results:* The study evaluated 80 cases comprising body fluids and cytology aspirates (cystic fluids). Among the fluid samples, pleural fluid accounted for the highest number of cases (24), followed by Bronchoalveolar Lavage (BAL) samples (18) and ascitic fluid (13). The specimens were obtained from a wide age range, spanning from pediatric to adult patients, with the highest incidence observed in the fifth and sixth decades of life. The male-to-female ratio was 3:1. The QI of LG cocktail was 0.95 and that of standalone Giemsa stain was 0.50. *Conclusion:* Effective combination of the LG cocktail proved to be more effective than standalone Giemsa stain air-dried cytology smears, especially for cellular differentiation, background clarity and optimal staining quality parameters.

Keywords: Air-dried cytosmears, Leishman-Giemsa cocktail, Giemsa stain, staining quality, cellular differentiation

Introduction

Cytopathology plays a vital role in effective analysis of body fluids, peripheral blood smears and exfoliative cytology providing early and prompt diagnosis [1, 2]. Being a minimally invasive test, the efficacy in terms of sensitivity and specificity varies based on several attrition factors such as nature of fluid sample, fixation time, technical skills, underlying pathology with confounders, warranting further exploration [2].

Although cytochemical stains remain a cornerstone for the accurate evaluation of cytosmears, they require periodic quality assessment [3]. Several studies have been undertaken to evaluate the efficacy of standalone cytological stains as well as

combination stains in parallel [3]. Among the several staining strategies, Leishman-Giemsa (LG) cocktail and May-Grünwald-Giemsa (MGG) are outstanding techniques routinely utilized for peripheral blood film and cytology smears examination [4, 5]. These stains feature essential equipment for visualizing cell components, diagnosing malignancy, parasitic infections and several other conditions [6, 7].

In the Indian context, the LG cocktail is specifically utilized in identification of parasites on blood films and its role in cytosmears are still sub-optimal [8]. MGG stain is widely used for cytological assessment followed by categorization and blood cellular differentiation [8, 9]. Although each stain plays a crucial role in diagnostic evaluation, there is a need to assess their comparative effectiveness, particularly when applied to air-dried cytology smears [10]. The standalone air-dried smear approach is regularly used for its ease of staining, but it could pose challenges for stain absorption and cellular visualization [11, 12]. This broadens the scope for evaluating the combined cytostain, namely the LG stain, and comparing its performance with the MGG stain in such smears, particularly in terms of lesion identification, cellular morphology, background characteristics, and turnaround time [13, 14]. This study aimed to analyze the nuances of the combined staining strategies and their effectiveness in air-dried cytology smears, offering a deeper understanding of which stain gives better diagnostic effects in this precise putting.

Material and Methods

Study design

This prospective study was conducted for a period of six months after receiving institutional ethical clearance. The effectiveness of cytochemical stain was evaluated primarily based on numerous parameters such as staining quality, differentiation of cells, cytoplasmic and nuclear clarity, and background nature. Standard inclusion and exclusion criteria were fixed wherein fluid sample less than 2 ml and crucial samples were excluded from the study. All the body fluids received were centrifuged and processed with routine standard operating procedure of the laboratory and the reports were dispatched to the respective end users. The patients' age, nature of sample, quantity, and site of aspiration were documented in a structured proforma.

Sampling technique and sample size

The study design being paired comparative observational study, a convenience sample technique was used. Following the application of predetermined inclusion and exclusion criteria, a total of 80 consecutive cytological samples received at the department of pathology during the study period were included. Sixty-three body fluid samples and 17 Fine-Needle Aspiration Cytology (FNAC) samples made up the study material. As part of standard diagnostic procedures, all samples were processed. In order to reduce patient-related bias, the residual sample material that would have been thrown away after diagnostic requirements were met was used for the study. The same specimen was used to create matched air-dried smears for each pair. MGG stain was used to stain one smear and the LG cocktail was used to stain the corresponding paired smear. By using each specimen as its own control, this paired design narrowed the inter-sample variability by enabling direct intra-sample comparison. The analysis did not include samples with poor cellularity, or substandard smear quality. The sample size of 80 cases was determined based on feasibility, specimen availability, and consistency with previously published cytopathology studies evaluating the efficacy of the LG cocktail

staining technique. For instance, Kavya *et al*. (2021) [8] analyzed approximately 70 paired cytological samples, which was taken into consideration while finalizing the sample size. A formal power-based sample size calculation was overlooked as the current study was a laboratory-based method-comparison study that focused on qualitative staining factors rather than outcome estimation or prevalence.

Preparation of working stock solution

The LG cocktail working solution was prepared according to standard protocols and previous literature. As described by Kavya *et al*. (2021) [8], filtered Leishman stain was mixed with an equal volume of Giemsa stain and diluted with distilled water in a 1:1 proportion. The additional air dried slides were prepared and LG cocktail was added and kept for one to two minutes and standard buffer was added and stained for further six minutes and washed and dried for further analysis.

Evaluation parameter and Quality Index (QI)

For calculation of QI, parameters such as overall staining pattern and clarity, cell morphology based on degree of differentiation of cytoplasmic as per prior work done by Kavya *et al.* [8].

For the individual scoring index, each parameter was classified into three levels of quality—satisfactory, good, and excellent—scored 1 to 3, respectively, along with the number of cases in each category. The quality parameters of the cytosmears were evaluated by experienced cytopathologists with reference to standard textbooks. The actual score under each parameter was calculated by adding scores obtained with respect to observed grade with number of cases in reference to prior studies [8] The final QI was calculated by comparing the actual scores obtained for each parameter against the maximum possible score. The data were tabulated for analysis, and the final QI of the MGG stain was compared with that of the LG cocktail.

Results

This prospective study evaluated 80 cases, including body fluids and cytology aspirates (cystic fluids). The comparative analysis of the LG cocktail stain versus the standalone MGG stain was conducted following the methodology outlined by Kavya *et al*. (2021) [8]. Among the body fluid samples, pleural effusion was the most common (24 cases), followed by Bronchoalveolar Lavage (BAL) samples (18) and ascitic fluid (13). Other fluids, including synovial fluid, urine, and sputum, each accounted for one case, as summarized in Table 1. The cystic fluid from thyroid aspirate was commoner followed by degenerative fluid from breast lesions (6) cases. The samples were received from wide range of age group from pediatric to adult with highest range noted in the 5th and 6th decade with male to female ratio of 3:1 as shown in Table 2. Of the 80 cases, 15 lacked sufficient diagnostic material and could not be included in the analytic scoring. The remaining 65 cases, which had adequate material, were evaluated and scored accordingly. The overall staining, cytoplasmic staining and nuclear morphology staining was good with LG cocktail when compared to MGG stain (Table 3). The QI of LG cocktail was 0.95 and that of standalone MGG was 0.51. The differentiation between myoepithelial and ductal epithelial cells in the breast were well made out in the LG stain.

Table 1: Nature of specimens

Type of specimens	Number of cases n (%)
Fluids (n=63)	
Pleural fluid	24 (29.62)
Bronchioalveolar lavage	18 (22.22)
Ascitic fluid	13 (16.04)
Peritoneal fluid	1 (1.23)
Cystic fluid	2 (2.46)
Pericardial effusion	2 (2.46)
Others*	4 (4.93)
FNAC (n=17)	
Thyroid	8 (9.87)
Lymph node	1 (1.23)
Breast	6 (7.40)
Oropharyngeal growth	1 (1.23)
Sub pleural nodule	1 (1.23)
Total	**81 (100)**

**Sputum, synovial fluid & urine each constituted one case*

Table 2: Age and gender wise distribution of study samples

Age group (Year)	Male n (%)	Female n (%)
11-20	1 (1.58)	2 (11.76)
21-30	3 (3.70)	3 (17.64)
31-40	6 (9.52)	4 (23.52)
41-50	10 (15.87)	1 (5.88)
51-60	17 (26.98)	1 (5.88)
61-70	18 (28.57)	3 (17.64)
>70	8 (12.69)	3 (17.64)
Total	**63 (100)**	**17 (100)**

Table 3: Comparison of quality parameters scores and index between standalone MGG and LG cocktail

Parameters	MGG (no. of cases × score)	LG Cocktail (no. of cases × score)
Overall staining		
Satisfactory (1)	2×1=2	0×1=0
Good(2)	61×2=122	64×2=128
Excellent(3)	2×3=6	1×3=3
Score	120	151
Clarity of staining		
Satisfactory (1)	1×1=1	0×1=0
Good(2)	56×2=112	57×2=114

Continued...

Excellent(3)	8×3=24	8×3=24
Score	117	178
Cytoplasmic staining		
Satisfactory (1)	10×1=10	2×1=2
Good(2)	48×2=96	58×2=116
Excellent(3)	7×3=21	5×3=15
Score	105	173
Nuclear staining		
Satisfactory (1)	17×1=17	1×1=1
Good(2)	45×2=90	58×2=116
Excellent(3)	3×3=9	6×3=18
Score	102	155
Background material staining		
Satisfactory (1)	0×1=0	1×1=1
Good(2)	55×2=110	52×2=104
Excellent(3)	10×3=30	12×3=36
Score	115	181
Actual score obtained (Z)	560	880
Maximum possible score (N)	975	975
Quality index (Z/N)	**0.51**	**0.95**

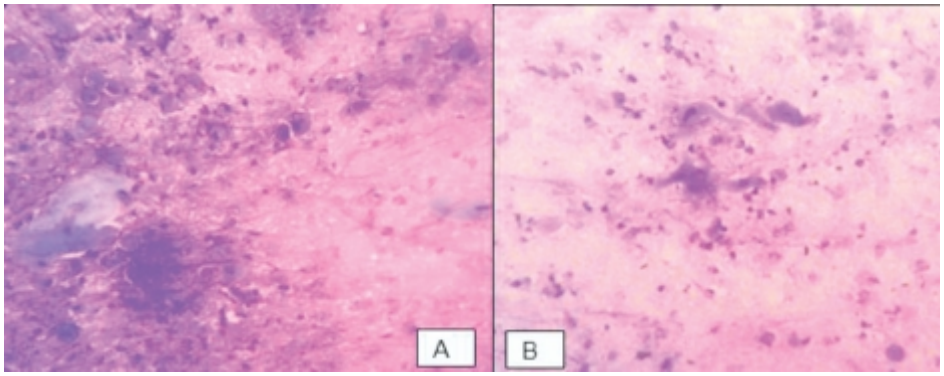

Figure 1A:Giemsa standalone with poor cytoplasmic & nuclear clarity, MGG at 10×

Figure 1B:LG cocktail showing clear background and squamous cell carcinoma pleural fluid, LG stain at 10×

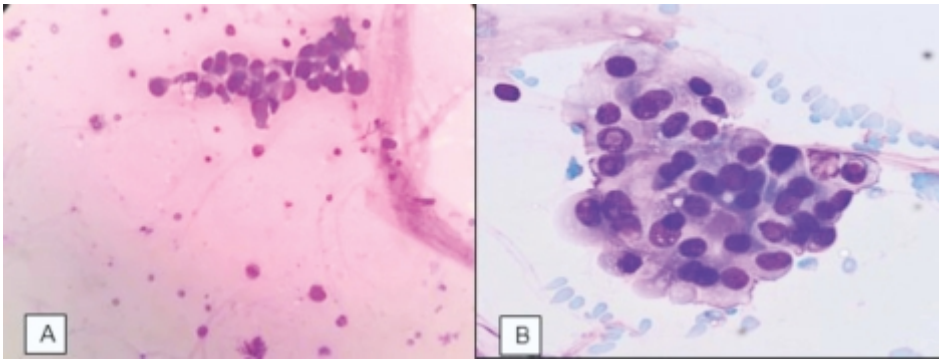

Figure 2A: Giemsa standalone with low clarity of inclusion, MGG at 10×

Figure 2B: LG cocktail showing clear inclusions with distinct cytoplasm of thyroid aspirate, LG stain at 40×

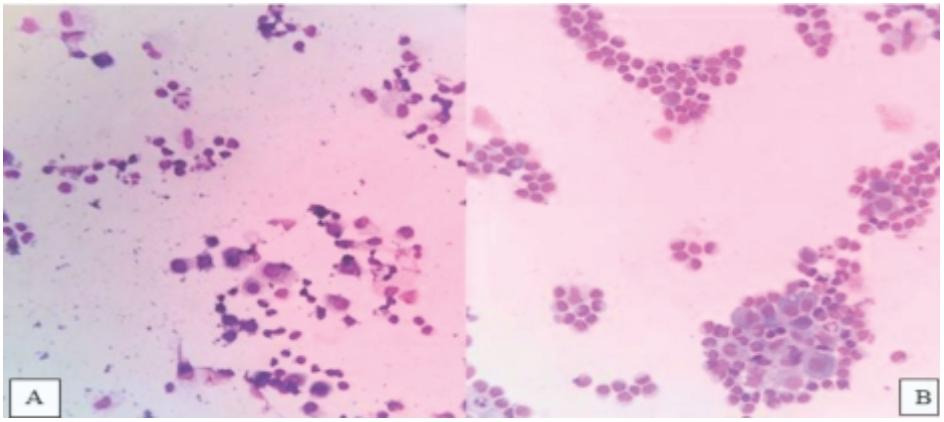

Figure 3A: Giemsa standalone with low clarity on cell morphology, MGG at 10×

Figure 3B: LG cocktail showing clear cells with distinct cytoplasm, LG stain at 40×

Discussion

Cytopathology is a specialized domain in pathology and it is an often preferred technique in sampling owing to its less invasiveness and easy daycare procedure. As a widely used screening technique, it is influenced by several attrition factors, with the quality and efficacy of cytochemical stains playing a key role in achieving an accurate and reliable report [1, 2]. Romanowsky stains encompassing a spectrum of cytochemical stains possess diversified nature of staining characterization when used either as a standalone or with combination of other air-dried dyes[2, 3]. The perennial lacunae with readily available stains is their volatility and variable efficacy which was superseded by conventional stains such as Giemsa, Leishman, and MGM stains [4, 5]. In the Indian context, previous studies assessing the quality and efficacy of cytological stains have employed experimental designs for sampling. In the present study, a convenience sampling technique was used, in line with the approach of Sidhu *et al.* (2018) [6]. As recommended by the National Accreditation Board for

Laboratories (NABL), in any quality assurance analysis during the pre-analytical phase, both the sampling method and the quality of staining are critical factors in determining the outcome and overall efficiency [7-9].

While cytopathologist's expertise plays a vital role in determining the sampling methodology, grading and scoring the QI and its parameters depends on the staining composition and techniques [10-12]. While Leishman stain is a cost-effective stain, it has its own limitation when it comes to cytosmears owing to its nuclear binding and background deposits [13-15]. This limitation was overcome by Giemsa stain wherein the combination of LG cocktail is being used widely in recent times. Ensuring optimal Turnaround Time (TAT) by minimal staining time, LG cocktail enhances QI in hematology as well as in cytosmears as observed by Jani & Satav (2012) [16]. While LG cocktail application on cytosmears is still underrated compared to Wright-Giemsa combination across the laboratories, studies have been conducted to assess its utilities [17-20]. High background intensity obstructing with visualization was another drawback with standalone Giemsa stain which was effectively overcome by LG cocktail as observed in prior studies as well [18-20]. The higher QI observed for the LG cocktail is attributed to its ability to effectively stain cells at different stages of maturation, including polymorphic and mixed reactive cells, as noted by Shilpa *et al*. (2017) [21]. Studies by Kavya *et al*. (2021) [8] and Sahu *et al*. (2024) [25] assessed the QI of the LG cocktail in 90 cases, consistent with our study analysis. Similarly, Joshi *et al*. (2014) [17] evaluated 200 body fluid samples over a five-year period and reported that the LG cocktail was particularly effective in highlighting background material, distinguishing cell types in salivary gland lesions, visualizing granules, and demonstrating metachromasia. Comparable findings were observed in the present study, where the LG cocktail showed a high QI in pleural and ascitic fluids by effectively demonstrating three-dimensional clusters, cellular patterns, and nuclear and chromatin architecture. Thus, LG cocktail was beneficial in assessing primary versus metastatic effusion on cytosmears. Although the Giemsa stain demonstrated substantial scores for cytoplasmic staining, particularly in salivary gland and BAL samples, factors such as background deposits, enlarged nuclear features, and drying artifacts limited its performance, often complicating the diagnosis and classification of malignant effusions.

The major component of analysis in our study was QI which was calculated by the ratio of actual score obtained on the test score stain (LG) with maximum score possible in reference to prior literature [22-24]. The cytology slides were scanned and the scores were awarded based on quality parameters as per literature and studies done by Sahu *et al*. (2024) [25]. Although several authors have reported varying QI scores—typically nuclear features scoring higher than cytoplasmic and background characteristics—with both standalone Giemsa and LG cocktail stains, the present study demonstrated a QI of 0.95 for the LG cocktail compared to 0.5 for the standalone Giemsa stain, indicating nearly a twofold increase in QI. Thus the rationale use of LG cocktail on cytosmears could be implemented across laboratories concurring with the findings of previous studies [26-30].

Another additional observation in the study was enhanced specificity on the International System for Reporting Serous Fluid Cytopathology (ISRSFC) wherein the proportion of Atypia of Undetermined Significance (AUS) in category III was high in

standalone Giemsa stain whereas LG cocktail clearly delineated malignancy versus benign effusions thereby providing a clear management guideline to clinicians. The higher quality index is attributed to the optimal visualization of nuclear parameters and their contents, which facilitates clear delineation of cytomorphology and aids in distinguishing benign from malignant lesions. As category III often warrants repeat sampling or invasive procedures to ascertain the diagnosis, LG cocktail with its enhanced QI had substantially aided in categorizing the fluids as observed in previous studies as well

Limitations

The present study evaluated the LG stain in comparison with Giemsa on air-dried smears and primarily focused on body fluids rather than cystic fluids. Future research may expand the scope to include other air-dried staining techniques, such as Wright's stain, and incorporate a more stratified and larger sample size for comprehensive analysis.

Conclusion

LG cocktail stain is beneficial when compared to standalone Giemsa stain in terms of better QI and parameters aiding enhanced quality assurance for cytology laboratories. The proportion of skeptical diagnostic entities in serous fluids can be minimized by effective staining of LG cocktail. Given its cost-effectiveness, optimal staining efficiency, and reduced staining time, the LG stain can be recommended for use in under-resourced laboratory settings.

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ORIGINAL ARTICLE

Lipoprotein-associated phospholipase-A2 as an inflammatory biomarker for vascular risk in SARS-CoV-2 affected individuals with rheumatoid arthritis

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Abstract

Background: Rheumatoid Arthritis (RA) patients are predisposed to elevated vascular risk, which is exacerbated by SARS-CoV-2 infection. Lipoprotein-associated phospholipase A2 (Lp-PLA2) is an inflammatory biomarker implicated in atherosclerosis and Cardiovascular Diseases (CVD). *Aim & Objectives:* This study aimed to assess the level of Lp-PLA2 in RA patients with and without COVID-19 exposure, and to evaluate its utility as a biomarker for vascular risk in these populations. The objective was to compare Lp-PLA2 levels in RA patients with COVID-19 exposure against those without, and to explore the correlation with other inflammatory markers and lipid parameters. *Material and Methods:* A cross-sectional comparative study was conducted on 100 individuals aged 20-77 years at Sri Ramachandra Institute of Higher Education and Research. The participants were divided into two groups: 50 RA patients with COVID-19 exposure and 50 RA patients without COVID-19 exposure. Lp-PLA2 levels were measured using Sandwich-ELISA, while rheumatoid factor (RF) and C-Reactive Protein (CRP) levels were assessed using IMMAGE® Immunochemistry Systems. Statistical analysis was performed using SPSS version 16, with significance set at $p < 0.05$. *Results:* Lp-PLA2 levels were significantly higher in RA patients with COVID-19 exposure (73.94 ng/ml) compared to those without (10.99 ng/ml) ($p = 0.005$). RF and CRP levels were elevated in both groups but did not show significant differences ($p = 0.059$ and 0.190, respectively). No significant correlations were observed between Lp-PLA2 and traditional lipid profile parameters. *Conclusion:* RA patients exposed to COVID-19 had significantly higher levels of Lp-PLA2, indicating increased vascular inflammation and risk of atherosclerosis. Lp-PLA2 may serve as a sensitive marker for cardiovascular risk assessment in RA patients, independent of traditional lipid profile parameters.

Keywords: Vascular inflammation, cardiovascular risk, atherosclerosis, inflammatory biomarkers, rheumatoid arthritis

Introduction

The single-stranded RNA virus termed SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), fuelled by a unique coronoviridae member, emerged in December 2019 [1]. Mammals can acquire beta coronaviruses, and it is generally accepted that COVID-19 originated in bats with changes in the Receptor-Binding Domain (RBD) and the furin protease cleavage site [2]. The coronavirus disease 2019 (COVID-19) SARS-CoV-2 infection is often caused by inflammation of the airways. Both Gastrointestinal (GI) and Upper Respiratory (UR) tracts of people are infected by the virus. It can also occasionally culminate in severe alveolar disease, which can significantly increase the risk of

pulmonary failure by shortening breath, lowering blood oxygen saturation, and lung infiltration. The main causes of profound lung disease in COVID-19 patients have been recognized as immune hyper-activation and cytokine involvement in alveolar tissues [3].

It is well known that the SARS-CoV-2 virus infected patients exhibits comorbidities such as diabetes mellitus, lung disease, Chronic Kidney Disease (CKD), and Cardiovascular Disease (CVD) [4]. The mortality risk is higher for patients with comorbidities who are elderly (> 65 years) and have weakened immune systems. Due to their weakened immune systems and use of immunosuppressive medications as prescribed, people with autoimmune disorders including lupus and Rheumatoid Arthritis (RA) are prone to infection and this has been reported as one of the most common immune-mediated disorders in COVID-19 patients [5].

These individuals on immunosuppressants may exhibit unusual symptoms; for instance, those taking IL-6 inhibitors might not have an increase in inflammatory markers. The possibility of overlap in symptoms is seen between a RA flare and COVID-19 infections. In both situations, symptoms such as myalgia, arthralgia, fever, and raised inflammatory markers may manifest. In the COVID-19 era, people with rheumatic disorders who are on immunosuppressive medications need to be given extra consideration. RA patients have been documented to have a higher risk of infection than people in general [6]. Many comorbidities such as hypertension, Chronic Obstructive Pulmonary Disease (COPD), and CVD are more prevalent in RA patients and patients with RA were at 25% higher risk of SARS-CoV-2 infection and 35% risk of COVID-19 hospitalization or death [7,8].

Provided the same immunological, genetic, and clinical features between inflammatory diseases and COVID-19, it is possible that SARS-CoV-2 could be a catalyst for the emergence of excessive inflammatory disorders, particularly in susceptible individuals. The risk of COVID-19 in persistent RA is suggested to result from immunological dysregulation and the use of chemical and biological anti-rheumatic medications, both of which normally raise infection risk due to immune system impairment and related comorbidities. Patients with RA particularly experienced thymus dysfunction, a rise in peripheral T cell turnover, and systemic T cells, all of which enhance their susceptibility to infections. Furthermore, ACE2 upregulation is stimulated by Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and increases the vulnerability to SARS-CoV-2 infection. These drugs may be utilized as an additional therapy to relieve arthritic pain but they exacerbate the clinical course or even cover up some signs that help with COVID-19 diagnosis, since both diseases overlap in pathogenesis [9].

RA and COVID-19 in humans are diseases that show similar inflammatory responses. This is caused by immune metabolic suppression and compromising the host immunity. The SARS-CoV-2 infection caused inflammation of the airways and GI and UR tracts. And RA is one of the most common effects of this inflammation [4].

The lipid metabolism and inflammatory response are intertwined at lipoprotein-associated phospholipase A2 (Lp-PLA2). It is secreted due to the binding of LDL with other lipoproteins in the structure of inflammatory cells. The activity of this catalyst conjointly results in alterations in High-Density Lipoprotein (HDL), making it less potent to suppress inflammation. These oxidized lipoprotein molecules promote vascular inflammation and

result in atherosclerotic plaque formation. This may lead to reduced blood flow, especially if the rupture occurs or in spasm, and therefore, a shortage of oxygen to the myocardium [10]. The Lp-PLA2 may play an essential role in the assessment of the development and progression of atherosclerosis [11].

Other pathogenic factors, such as Homocysteine (HCY) promote Insulin Resistance (IR) and impaired function of beta cells through mechanisms, including oxidative stress, systemic inflammation, and endothelial dysfunction. HCY is also an independent risk factor for CVD and accelerated atherosclerosis [12]. It may also help as a risk predictor for coronary and carotid events and is thought to be a promising approach for treating vascular diseases [10]. Both diseases elicit a pro-inflammatory response that could result in vascular risk. Lp-PLA2 is recognized as a marker for vascular endothelium rather than being viewed as a systemic inflammatory marker because it is produced only within atherosclerotic plaque and its levels do not increase during systemic inflammatory events. Individuals with coronary atherosclerosis have higher circulating levels of Lp-PLA2 than those without the disease, which in turn causes higher levels of lysoPC (lysophosphatidylcholine), a major component of biological membranes and a kind of lysophospholipid produced from Phosphatidylcholine (PC).

It is important in cell signaling, inflammation, and metabolic processes. Lp-PLA2 plays a proatherogenic inflammatory marker that is generated mainly by macrophages and lymphocytes, which stimulates atherosclerosis [9]. Increased Lp-PLA2 has already been advised as one of the risk-specific markers in cardiovascular conditions and has also been reorganized as a biomarker in COVID-19. [13] Its validity as a biomarker in COVID-19 is increased by the fact that Lp-PLA2 has already been advised for the evaluation of the risk of specific cardiovascular conditions in otherwise apparently healthy individuals [14]. The purpose of our study was to evaluate the level of Lp-PLA2 in patients with RA and post-COVID-affected RA individuals.

Material and Methods

The Institutional Ethics Committee approved this cross-sectional comparative study on 100 men and women aged 20–77 at the Sri Ramachandra Institute of Higher Education and Research (Ethical Clearance Number: CSP/22/SEP /116/481). All participants were divided into two groups: group 1 contained 50 RA patients exposed to COVID-19, and group 2 included 50 RA patients without exposure to COVID-19.

Exposure to COVID-19 was defined as a previous confirmed SARS-CoV-2 infection, based on RT-PCR or rapid antigen test report. Patients with RA for over five years were included. Those with other autoimmune disorders, juvenile arthritis, myocardial infarction, stroke, or vascular thrombosis were excluded. All subjects gave written informed consent before data collection, and institutional and ethical patient confidentiality requirements were followed.

The sample size was calculated using the formula below, based on hypothesis testing for two

$$n = \frac{2s_p^2[Z_{1-\alpha/2} + Z_{1-\beta}]^2}{\mu^2_d} \qquad S_p^2 = \frac{s^2_1 - s^2_2}{2}$$

independent means:

Where,

$S^1 2$ – Standard deviation in the first group – 38.9

$S^2 2$ – Standard deviation in the second group – 33.5

μ 2 d – Mean difference between the samples –
21 α – Significance level – 5%
1-β – power – 80%
1 or 2-sided – 2
Based on this calculation, each group needed 50 members for statistical power and confidence.

Sample collection and analysis

For each patient, 5 mL of fasting venous blood was collected from each participant into basic red-topped vacutainers at the central laboratory of Sri Ramachandra Medical Centre. The samples were centrifuged at 2000–3000 rpm for 20 minutes after clotting at room temperature for an hour. Immediately following appropriate serum separation, lipid profile, rheumatoid factor (RF), and C-Reactive Protein (CRP) were assessed. Labelled serum aliquots maintained at –80°C until further testing were used in Lp-PLA$_2$ analysis. Lipid profile was done using Beckman AU5800 and AU680 automated clinical chemistry analyzers, which analyzed total cholesterol, triglycerides, and HDL cholesterol. The GPO–POD technique measured triglycerides; the enzymatic CHO–POD endpoint approach computed total cholesterol. Masking non-HDL lipoproteins enabled a selective enzymatic colorimetric HDL cholesterol measurement. The Sampson formula approximated sdLDL-C; the Friedewald formula calculated LDL-C and VLDL. One computed the ratios LDL/HDL and TC/HDL to assess cardiovascular risk. The RF examination was done using rate nephelometry; the IMMAGE® Immunochemistry System measured RF. Light scatter between RF and IgG-coated latex particles quantified immunological complexes. Assay sensitivity was 20 IU/mL, and the linear measurement range was 800 IU/mL.

Statistical analysis

All statistical analyses were performed with Statistical Package for the Social Science, (SPSS Inc., version 16 SPSS Inc., Chicago. IL, USA) a statistical software package for Microsoft windows. The collected data were first tested for normality of distribution. Statistical analysis was then conducted using parametric methods, including the chi-square test to compare two attributes and the independent Student's *t*-test. The results were presented as Mean ± Standard Deviation (SD). Pearson's correlation coefficient analysis (r) was used to evaluate the correlation between the study variables. All tests were considered significant when the p value was less than 0.05.

Results

The study revealed that Lp-PLA2 levels alone showed a statistical significance between both groups (Table 1). There was a significant increase in Lp-PLA2 levels in group 1 (73.94 ng/ml) compared to group 2 (10.99 ng/ml), with a p-value of 0.005. This indicates a strong association between COVID-19 exposure and elevated Lp-PLA2 levels. However, RF levels and CRP were high in both groups but this difference was not statistically significant as p-values were 0.059 and 0.190 respectively. Also, the lipid profile parameters did not show significant differences between the groups, with p-values ranging from 0.116 to 0.833.

Pearson correlation analysis was done to test whether there was an association of Lp-PLA2, RF, and CRP levels with the other parameters of the study, between the two study groups. As in Table 2, RF showed significant correlations with several lipid profile parameters, particularly with TC/HDL and LDL/HDL ratios, suggesting that higher RF levels are associated with an unfavorable lipid

Table 1: Comparison of biochemical parameters in RA exposed and non exposed to COVID-19 study groups

Variables	RA exposed to COVID 19 (N=50) Mean ± SD	RA Non exposed to COVID 19 (N=50) Mean ± SD	Significance (2-tailed)
Age (years)	48.2 ± 12.6	47.5 ± 12.2	0.773
RF (mg/dl)	130.72 ± 154.41	81.142 ± 99.39	0.059
CRP (mg/dl)	1.50 ± 1.68	1.10 ± 1.36	0.190
Lp-PLA2 (ng/ml)	73.94 ± 15.33	10.99 ± 6.58	0.005*
Cholesterol (mg/dl)	180.58 ± 52.58	167.78 ± 34.95	0.155
Triglyceride (mg/dl)	149.34 ± 91.95	133.18 ± 62.08	0.306
HDL (mg/dl)	40.94 ± 12.69	41.40 ± 8.69	0.833
LDL (mg/dl)	109.77 ± 44.96	99.74 ± 32.34	0.203
VLDL (mg/dl)	27.97 ± 12.77	26.8 ± 12.27	0.642
Cholesterol / HDL ratio	4.52 ± 1.20	4.16 ±1.07	0.116
LDL / HDL ratio	2.79 ± 1.03	2.49 ± 0.88	0.131
Non-HDLc	139.64 ± 49.41	126.38 ± 34.24	0.122
Triglyceride/HDL ratio	4.38 ± 4.24	4.24 ± 2.28	0.185
Small dense LDL (mg/dl)	37.29 ± 14.8	33.52 ± 9.75	0.137

*$p < 0.05$ was considered statistically significant, * Indicates statistically significant p-value*
RA – Rheumatoid arthritis; RF – rheumatoid factor; CRP – C reactive protein; Lp-PLA2 – lipoprotein associated phospholipase A2; HDL – high density lipoprotein; LDL – low density lipoprotein; VLDL – very low density lipoprotein

profile. Likewise, CRP was significantly correlated with TG and VLDL levels, indicating that higher CRP levels were associated with higher levels of these lipids. However, Lp-PLA2 did not show significant correlations with any lipid profile parameters in this study, indicating that its association with vascular risk may be independent of traditional lipid profile parameters. These results support the idea that while traditional lipid profile parameters are important, biomarkers like Lp-PLA2 provide additional information about cardiovascular risk, especially in inflammatory conditions like RA.

Table 2 : Correlation between lipid profile parameters with RF, CRP, and LP-PLA

Variables Significance (2-tailed)	TC	TG	HDL	LDL	VLDL	TC/ HDL	LDL/ HDL	Non-HDLc	TG/ HDL	sdLDL
RF	0.362	0.830	**0.000****	0.170	0.089	**0.002****	**0.005****	0.057	**0.039***	0.051
CRP	0.496	**0.042***	0.652	0.921	**0.044***	0.520	0.846	0.407	0.104	0.257
LP-PLA2	0.771	0.371	0.634	0.577	0.385	0.718	0.952	0.854	0.327	0.977

*$p < 0.05$ was considered statistically significant, **Denotes correlation is significant at the 0.01 level (2-tailed), * Denotes correlation is significant at the 0.05 level (1-tailed), RF – Rheumatoid factor; CRP – C reactive protein; Lp-PLA2 – lipoprotein associated phospholipase A2; TC – total cholesterol; TG – triglycerides; HDL – high density lipoprotein; LDL – low density lipoprotein; VLDL – very low density lipoprotein; sdLDL – small dense LDL*

Discussion

In COVID-19 patients, RA inflammation is a high-risk inflammatory disease [4]. These inflammatory responses are intertwined at Lp-PLA2. It yields potent pro-inflammatory products by hydrolyzing oxidized phospholipids and oxidized lipoproteins in the vascular walls that generate pro inflammatory lipid mediators which would act as chemo-attractants and cause the build-up of monocytes forming foam cells. The activity of this catalyst conjointly results in alterations in HDL making it less potent to suppress inflammation. These oxidized lipoprotein molecules promote vascular inflammation and result in atherosclerotic plaque formation. The Lp-PLA2 may play an important role in the assessment in the development and progression of atherosclerosis. It may also help as a risk predictor for coronary and carotid events and is thought to be a promising approach to treating vascular diseases [15]. In the current study, we found significantly high Lp-PLA2 levels in RA individuals who were exposed to COVID-19 than RA individuals who were not exposed to COVID-19. Santos *et al*. (2019) have found in their study that patients exposed to COVID-19 had higher levels of Lp-PLA2 compared to the control, which suggested increased vascular inflammation [16]. The present finding suggested a relationship between inflammation caused by autoimmune disease and vascular risk, even though there was a quantitatively larger concentration of Lp-PLA2 in the patients with RA at all time points. Increased levels of Lp-PLA2 in the COVID-19-exposed RA individuals indicated that COVID-19 is also an inflammatory disease that could have aided the inflammatory process, leading to plaque build-up and atherosclerosis and vascular risk [16]. Studies suggest that COVID-19 infection facilitates endothelial dysfunction as a direct consequence of viral involvement and the host inflammatory response. Moreover, it has been suggested that the induction of apoptosis and pyroptosis might be essential in endothelial cell damage in patients with COVID-19 [17, 18].

It has been demonstrated from previously published literature that individuals with RA develop atherosclerosis more frequently than people in the general population, which might be due to the expression of Lp-PLA2 by inflammatory cells in atherosclerotic plaques. Lp-PLA2 hydrolyses phospholipids in LDL to yield proinflammatory products such as oxidized free fatty acids which stimulate the burden of ROS leading to vascular inflammation indirectly or directly and increased the chance of atherosclerosis [19, 20]. To the best of our comprehension, relatively few prospective studies have been conducted regarding Lp-PLA2 and the emergence of subclinical atherosclerosis, and even fewer have focused on individuals with inflammatory diseases. Measuring Lp-PLA2 has been linked to the development of atherosclerosis over time in studies on diabetes mellitus patients, and these findings were also confirmed in the general population [21].

Additionally, a meta-analysis of Lp-PLA2 in cardiovascular disease found that Lp-PLA2 concentrations (activity and mass) were significantly correlated with one another, proatherogenic lipid markers, and CVD risk continuously and graded [14]. Previous studies also report that Lp-PLA2 strongly correlates with apolipoprotein B-containing lipoproteins [23], highlighting the need to consider this. Plasma lipoproteins were assessed to check the Lp-PLA2's effects on occult CVD and subclinical atherosclerosis.

In previously published research, Lp-PLA2 had a positive correlation with total cholesterol, LDL cholesterol, total cholesterol to HDL cholesterol ratio, and non-HDL cholesterol [16,17]. Another study on Lp-PLA2 also stated a significant association between lipid levels and Lp-PLA2 activity. However, in the current investigation, there was no correlation between the lipid profile parameters and the levels of Lp-PLA2 in either the exposed or unexposed RA groups [24, 25]. The therapeutic intervention of the patients over their lipid parameters could have been a reason for the non-significant association between lipid profile parameters and sd LDL. This study looked into the relationship between serum Lp-PLA2 and CRP for predicting cardiovascular comorbidities in inflammatory disease. CRP levels were not statistically significant in RA individuals exposed and non-exposed to COVID-19. In a previous study, Lp-PLA2 was assessed in individuals with acute chest discomfort suggestive of acute Coronary Syndrome (ACS), which is a variety of critical cardiac diseases caused by restricted blood supply to the heart muscle owing to plaque rupture and clot development in the coronary arteries. ST-elevation MI, NSTEMI, and unstable angina were included. The diagnosis relied on clinical symptoms, ECG abnormalities, and increased cardiac biomarkers such as troponins, CK-MB, CRP, and Lp-PLA_2. Blood circulation, heart damage prevention, and life depend on early medical intervention.

Depending on the later confirmation of ACS and the usage of statins. They discovered that Lp-PLA2 was higher in the ACS patient group, regardless of statin medication, than in the non-ACS patient group. It's interesting to note that CRP levels were similar across all patient groups in the study [26]. This indicates that measuring Lp-PLA2 rather than CRP may be more beneficial in predicting cardiovascular comorbidities in inflammatory disease. Recent studies indicate that compared to the general population with low Lp-PLA2 levels, the subset of metabolic syndrome patients with greater Lp-PLA2 levels had an enhanced risk for CVD [27]. Lp-PLA2 activity was also demonstrated to have a significant correlation with atherogenic lipoprotein

sLDL in plasma, and the chance of having CVD was three times higher for people with higher CRP, LDL, and Lp-PLA2 levels [28,29]. Based on the current findings, Lp-PLA2 can assess risk even when the traditional lipid profile parameters are within the reference intervals. It can help evaluate and adjust the patients across risk groups and assist in care [30].

Conclusion

The key finding of our study was that RA individuals exposed to COVID-19 have significantly higher Lp-PLA2 levels than those not exposed, suggesting that COVID-19 exacerbates vascular inflammation and increases the risk of atherosclerosis in these patients. Most other lipid profile parameters do not show significant differences between the two groups. This indicates that Lp-PLA2 may be a more sensitive marker for assessing vascular risk in RA patients exposed to COVID-19 and acts as an independent marker in relation to lipid profile.

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ORIGINAL ARTICLE

Age-related morphometric and histological changes in liver and gallbladder: A postmortem study

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Abstract

Background: Morphometric data helps us determine the age of an individual as a definite organ has a specific morphology during a certain age group. Aging results in variation of histopathology of organs. *Aim and Objectives:* To establish a relationship between age and various morphometric parameters of liver and gallbladder with histological changes amongst the post-mortem samples subjected to autopsy. *Material and Methods:* The study was conducted on 50 liver and gallbladder from postmortem cases. Length, breadth, and thickness of gallbladder and liver were noted. Weight of liver was also noted. Histological examination was performed on representative tissue sections. Statistical analysis of the measured parameters was conducted using SPSS software. *Results:* Length of gallbladder was found to be ranged between 3.5 cm to 11 cm. Breadth of gallbladder was found to be ranged between 1 cm to 5 cm. Thickness of gallbladder was found to be ranged between 0.3 cm to 4 cm. Length of liver was found to be ranged between 21.1 cm to 41 cm. Breadth of liver was found to be ranged between 11.4 cm to 30 cm. Thickness of liver was found to be ranged between 2.5 cm to 11.5 cm. Weight of liver was found to be ranged between 886 g to 2295 g. Mean percentage of liver weight was found to be 2.31% which indicates that liver occupies approximately 2.31% of the total body weight of an individual. Correlation coefficient (r) for length and breadth of gallbladder with respect to age showed a weak positive correlation. Lipofuscin pigment, periportal fibrosis and steatosis were the histopathological changes that were present in liver. These changes reflect progressive age-related decline in liver morphology and gallbladder function. *Conclusion:* The results of the above study can be used to determine the age of a person in forensic science. It can be used in identification of people during forensic investigations. As the age advances gallbladder muscular layers hypertrophied which leads to less contraction and increase stasis of bile leading to gall stones formation. In liver as the age advances peri portal fibrosis increases leading to decrease functioning of liver.

Keywords: Autopsy, Age-related Changes, Morphometric Analysis, Histopathology, Forensic Identification

Introduction

Morphometric anatomical studies help us to know whether the morphometric parameters are of normal value or not [1]. With the increase in age of an individual, morphological parameters of an organ changes and thus help us to determine the problem as well as diseases related to that organ. In most cases morphometric measurements help us to identify the age group of an individual as specific morphometric measurements are seen during a certain age group. Aging also results in change in histopathology of an organ and therefore helps us to identify changes in organ during a particular age group. Length of gallbladder is around 7 to 10 cm, width is around 3cm and have a capacity of 30 to 50 ml [2-7]. Weight of the liver in males is about 1600 grams and in females it is about 1300 grams [8, 9]. It

accounts for around 2-3% of average body weight [10]. Size of a liver depends on age, sex, and body weight of an individual [11].

Aging is a situation where a person's capacity to maintain homeostasis starts to decrease because of structural variation or dysfunction and therefore becomes susceptible to more stress and harm [12, 13]. Aging is marked by natural, progressive reduction in function that decreases the organisms, organs, or cell's ability to counter the internal or external factors [14]. Factors that are associated with aging eventually lead to death after maturing, alteration in biochemical and physical characteristics of tissue, a gradual reduction in physiological capabilities, a decreased ability to respond environmental factors, higher susceptibility and vulnerability towards diseases [13].

Among abdominal organs, the liver and gallbladder show distinct age-related changes that are clinically and forensically significant. Gallbladder's size, length, diameter, and volume increase with age and helps in determination of contraction and function of gallbladder [3,15,16]. It has been reported that ageing affects the pathophysiology of gallstone production. Gallstone disease is more common as people get older. Men have a lower prevalence than women [17]. As the age of an individual increases, gallbladder's contractility decreases which results in biliary stasis as well as formation of sludge [15].

Blood flow and liver volume reduces with the age of an individual [12-14,17-19]. A rise in average cell size happens because of numerical growth of cells having high ploidy, with decrease in overall number of hepatocyte and their mitochondria [12,17,18]. In liver age-related changes include thickening of endothelial lining, reduction in total number of endothelial cells, rising level of lipofuscin, reduction in surface area of smooth endoplasmic reticulum, reduced number and dysfunctioning of mitochondria, decrease in phase 1 metabolism of few drugs, change in expression of certain proteins, reduced hepatobiliary function, muted oxidative stress response, reduction in rate of DNA repairing, shortening of telomere and diminished expression of growth regulatory genes [12-14, 18,19]. Albumin production reduces with the age [12,13,17,18]. The aging process also has influence over the morphological characteristics of macrophages [18]. The liver of older people shows a gross appearance which is known as 'brown atrophy', and this brown color is because of collection of lipofuscin which is highly oxidized insoluble protein [13]. Few studies have simultaneously correlated morphometric and histological changes of both liver and gallbladder across age groups in postmortem samples. Therefore, the objective of this study was to correlate age with morphometric parameters and histological changes of the liver and gallbladder in autopsy cases, with the dual aim of enhancing understanding of age-related organ variation and providing data of potential forensic and clinical relevance.

Material and Methods

This was an observational study utilizing convenient sampling method to collect 50 liver and gallbladder, each, from the bodies belonging to South Indian population. Samples were collected consecutively from autopsy cases brought to the Kasturba Hospital Mortuary, ensuring feasibility and direct access to organs for morphometric and histological analysis. Clear inclusion and exclusion criteria were applied to minimize confounding factors, with cases involving abdominal surgery, crush injuries, putrefaction, drowning, or severe burns being excluded.

Institutional ethical clearance (495/2021) was taken before starting the study. Before postmortem analysis, weight of the body was taken using cadaver weighing machine. Liver and gallbladder were taken during the postmortem examination. Length, breadth, and thickness of both the organs were measured using the Vernier calipers.

The following measurements were considered for the study:

Length of gallbladder: The length of the gallbladder was measured from the neck of gallbladder till the fundus.

Breadth of gallbladder: The breadth was taken in the fundus region where it was maximum.

Thickness of gallbladder: The thickness was taken in the fundus region at the lowermost point.

Length of liver: The length of the liver was measured from the left lobe till the right lobe.

Breadth of liver: The breadth was taken from the mid-point of inferior border till the mid-point on posterior surface.

Thickness of liver: The thickness was measured in the mid-point of right lobe from superior to inferior surface.

After measurements, sections of liver and gallbladder were stored in formalin and then sent for histological examination. For histological examination, fixation was done for 8 hours in 10% neutral buffered formalin. Then the water was removed completely by the process of dehydration. This was done for 1 hour and 30 minutes each by the series of alcohol from 70% to 80% and then 95%. Then clearing was done with the help of toluene that acts as a clearing agent. Tissue was placed in xylene 1 and xylene 2. Tissue was then infiltrated with embedding agents such as molten paraffin wax, agar or gelatin which was placed in a hot air oven for about 6 to 8 hours. After this, tissue was hardened which was done by placing the tissue in a metallic angle or leuckharts moulds. The blocks were then kept in an ice tray and then paraffin blocks were cut by using a rotatory microtome having a thickness of 4 to 5 micrometer. After sectioning, the sectioned tissue was put in warm water bath which helps to eliminate the creases. Sectioned tissue were kept on a glass slide and then put in hot air oven for 15 minutes so that sections were adhered to the slides properly. Tissues were rinsed in xylol for 10 dips to remove the wax and then rinsed in graded alcohol from 70% to 80% to 95% for 5 dips each to remove the xylol. Then tissues were stained using hematoxylin and eosin and covered by a thin piece of plastic or glass with DPX. Slides were dried in hot air oven for 5 minutes and then stained slides were assessed for age related changes in histology of liver and gallbladder.

All morphometric data were entered into the Statistical Package for the Social Sciences (SPSS) software (version 27) for analysis. Descriptive statistics were used to calculate mean values and ranges for each parameter. Normality of data distribution was calculated using the Shapiro–Wilk test. Pearson's correlation coefficient was applied for normally distributed variables, while Spearman's correlation coefficient was applied for nonparametric data to evaluate the relationship between age and morphometric parameters of the liver and gallbladder. Correlation coefficients (r) were reported along with corresponding p-values and confidence intervals to indicate the strength and significance of associations. Histological findings were analyzed qualitatively across age groups; categorical comparisons were noted, and where appropriate, chi-square testing was considered.

Results

Morphometric parameters of gallbladder (Table 1)

Length of gallbladder was found to be ranged between 3.5 cm to 11 cm. Mean length of gallbladder was found to be 7.302 cm. In 86% of samples length ranged between 5 - 10 cm. Breadth of gallbladder was found to be ranged between 1 cm to 5 cm. Mean breadth of gallbladder was found to be 2.962 cm. In 70% of samples breadth ranged between 2 - 4 cm. Thickness of gallbladder was found to be ranged between 0.3 cm to 4 cm. Mean thickness of gallbladder was found to be 1.33 cm. In 76% of samples, the thickness ranged between 0 - 2 cm.

Table 1: Correlation of age with length, breadth and thickness of gallbladder

Length	N (%)	Correlation with age (r)
0-5 cm	3 (6)	0.069
5-10 cm	43 (86)	
10-15 cm	4 (8)	
Breadth		
0-2 cm	3 (6)	0.05
2-4 cm	35 (70)	
4-6 cm	12 (24)	
Thickness		
0-2 cm	38 (76)	-0.033
2-4 cm	11 (22)	
4-6 cm	1 (2)	

Morphometric parameters of liver (Table 2)

Length of liver was found to be ranged between 21.1 cm to 41 cm. Mean length of liver was found to be 28.32 cm. In 58% of samples, length ranged between 20 - 30 cm. Breadth of liver was found to be ranged between 11.4 cm to 30 cm. Mean breadth of liver was found to be 20.37 cm. In 46% of samples breadth ranged between 20 - 25 cm. Thickness of liver was found to be ranged between 2.5 cm to 11.5 cm. Mean thickness of liver was found to be 7.774 cm. In 72% of samples the thickness ranged between 5 - 10 cm. Weight of liver was found to be ranged between 886 g to 2295 g. Mean weight of liver was found to be 1394.52 g. In 40% of samples the weight ranged between 1200 - 1600 g. In the above study, a relation between liver weight and body weight was calculated which was found to range between 1.09% to 5.46%. Mean percentage of liver weight was found to be 2.31% which indicates that liver occupies approximately 2.31% of the total body weight of an individual. After documenting the morphometric variations of the liver and gallbladder across age groups, correlation analysis was performed to assess the statistical relationship between these parameters and chronological age, directly addressing the study objective of age-related morphometric assessment.

Relation between age and length, breadth, thickness of gallbladder (Table 1)

Relation between age and length, breadth, thickness of gallbladder was found using Pearson correlation coefficient. A weak positive correlation was observed between age and length of gallbladder as well as between age and breadth of gallbladder. With every unit year increase in age, the length and breadth of gallbladder is expected to increase by 0.069 cm and 0.05 cm respectively. A weak negative correlation was observed between age and thickness of gallbladder. With every unit year increase in age, the thickness of gallbladder is expected to decrease by 0.033 cm.

Relation between age and length, breadth, thickness, weight of liver (Table 2)

A relation between age and length, breadth, thickness, weight of liver was found using Pearson Correlation Coefficient. A weak negative correlation was observed between age and length, age and breadth, age, and thickness as well as between age and weight of liver. With every unit year increase in age, the length, breadth, thickness, and weight of liver is expected to decrease by 0.019 cm, 0.213 cm, 0.013 cm and 0.164 g respectively.

In addition to morphometric correlations, histological examination was undertaken to identify microscopic changes in liver and gallbladder tissue across age groups, thereby fulfilling the objective of correlating age with histological alterations. Tables 3 and 4 show the histological changes in gallbladder and liver, respectively, with respect to age group. Hypertrophied muscularis with more lymphoplasmacytic infiltrate and lipofuscin pigment were the predominant changes observed as the age advanced in gallbladder. In liver lipofuscin pigment, fatty changes and intrahepatic cholestasis were the predominant changes observed as the age advanced. Periportal fibrosis was noted in 21-30 years and 41-50 years. Steatosis was seen in all age groups starting from 11-20 years to 71-80 years.

Histological changes in gallbladder and liver in relation to age are shown in Tables 3 and 4, respectively. Taken together, morphometric correlations and histological progression demonstrate age-related changes in liver and gallbladder, with potential forensic application in age estimation.

Table 2: Correlation of age with length, breadth and thickness of liver

Length	N (%)	Correlation with age (r)
20-30 cm	29 (58)	-0.019
30-40 cm	20 (40)	
40-50 cm	1 (2)	
Breadth		
10-15 cm	6 (12)	-0.213
15-20 cm	14 (28)	
20-25 cm	23 (46)	
25-30 cm	7 (14)	

Continued...

Thickness		
0-5 cm	4 (8)	-0.013
5-10 cm	36 (72)	
10-15 cm	10 (20)	
Weight		
800-1200 g	18 (36)	-0.164
1200-1600 g	20 (40)	
1600-2000 g	9 (18)	
2000-2400 g	3 (6)	

Table 3: Distribution of age-related histological changes in gallbladder

Age Group (years)	Lipofuscin pigment	Muscularis hypertrophy	Lymphoplasmacytic infiltrate	Fibrosis / lipoid infiltrates	Other changes
21–30	Present	Focal	Sparse	Variable	Denuded mucosa, papillary infoldings, biliary sludge
31–40	Present	Focal	Minimal	Not prominent	Papillary infoldings
41–50	Present	Moderate	Moderate	Present	Biliary concretions
51–60	Present	Moderate	Moderate	Present	Papillary infoldings, concretions
61–70	Present	Marked	Dense	Present	Papillary infoldings
71–80	Present	Marked	Dense	Present	Concretions, advanced fibrosis

Table 4: Distribution of age-related histological changes in liver

Age Group (years)	Lipofuscin pigment	Steatotic (Micro/Macrovesicular) changes	Lymphoplasmacytic infiltrate	Fibrosis / lipoid infiltrates	Other changes
21–30	Present	Present	Present	Present	-
31–40	Present	Present	-	-	Necrosis

Continued...

41–50	-	Present	Present	Present	Hepatic parenchyma with sinusoidal and centrivenular congestion, lobular inflammation, periportal mild chronic inflammation, mixed inflammatory infiltrate and bile ductular proliferation
51–60	Present	Present	-	Present	
61–70	Present	Present	Present	Present	Intracanalicular cholestasis
71–80	Present	Present	-	-	Mild atrophy of hepatocytes and intrahepatic cholestasis

Discussion

The present study demonstrates that morphometric variations of the liver and gallbladder show only weak statistical correlations with age, while histological examination reveals progressive microscopic changes such as lipofuscin deposition, fibrosis, and steatosis. These findings highlight the limited predictive value of morphometry alone and emphasize the greater sensitivity of histological markers in reflecting age-related alterations. The observed trends align with previous reports of gradual degenerative changes in hepatobiliary tissues and suggest potential forensic utility in age estimation, particularly when morphometric data are interpreted alongside histological evidence.

Morphometric measurements of gallbladder

The length of gallbladder was found to be ranged between 3.5 cm to 11 cm. Other studies by Nadeem (2016) (100 gallbladders in south Indian population), Shivanal *et al*. (2021), Desai and Bhojak (2015) (50 gallbladders in South Indian population), Umarani *et al*. (2018) (50 gallbladders in south Indian population), Pirraci *et al.* (2013) (9481 gallbladders in Albania) and Nayak *et al*. (2021) (40 gall bladder in Eastern Indian Population) show the length of gallbladder ranged between 3 cm to 14 cm which was like the present study [4, 6-7, 20-22]. The breadth of gallbladder was found between 1 cm to 5 cm. Other studies documented the breadth of gallbladder ranged from 1.5 cm to 5.5 cm which was similar to the present study [4, 7, 21].

Morphometric measurements of liver

The mean length of liver was found to be 28.32 cm. The findings of the present study were found same as that of Emue *et al*. (2013) (25.9cm) (62 livers in Nigerian population) but was different from Mohammadi *et al*. (2017) who documented the mean length of liver as 23.56 cm. This might be because he conducted the study in 600 auto-

psies in Iranian population [23, 24]. The mean thickness of liver was found to be 7.77 cm. The results of this study were matching with the findings of Emue *et al*. (2013) (5.75cm) 22 The weight of liver was noted between 886 g to 2295 g. Mean weight of liver was reported as 1394.52 g. The present study showed similar results to that of Vinnakota and Jayasree (2013) (900 g-2 kg) (58 liver in South Indian population), Emue *et al*. (2013) (1424 g), Mohammadi et al. (2017) (1357g) [23-25].

Relation between age and morphometric parameters of gallbladder

The study showed weak positive correlation between age and length of gallbladder (r = 0.069), breadth of gallbladder (r = 0.046). These findings were consistent with that of Kariuki *et al*. (2017) where they found a positive correlation between gallbladder length and diameter 0.282, and 0.485 among 92 gallbladders of African population [15]. Yoo *et al*. (2003) conducted a study in 610 gallbladders of Korean population and found that the length of the gallbladder displayed substantial positive correlations with age (r = 0.65), while gallbladder width displayed modest but substantial correlations with age (r = 0.48) [16].

Relation between age and morphometric parameters of liver

The present showed weak negative correlation between age and weight of liver (r=-0.164). The is consistent with that of Wynne *et al*. (1989) who conducted a study in 65 livers of United Kingdom population and found a significant negative correlation between age and liver volume ($p < 0.001$), expressed in per unit body weight [26].

Relation between liver weight and body weight

In above study, mean percentage of liver weight to body weight was found to be 2.31%. These results are consistent with Abdel-Misih & Bloomston (2010) who found that the liver accounted for roughly 2% to 3% of average body weight [10].

Histopathological examination of liver and gallbladder

Lipofuscin pigment was the change which was predominately seen in liver histology as the age advanced. Similar findings were observed in other reported studies. Schmucker (2005) reported an increase in the hepatic dense body compartment (lipofuscin) with advancing age. Kim *et al.* (2015) described aging-associated alterations in liver cells, including the accumulation of dense bodies (lipofuscin) within hepatocytes.

Stahl *et al*. (2018) indicated that age-related morphological changes in hepatocytes involved the buildup of lipofuscin in the cytoplasm, which ultimately had a detrimental effect on hepatocyte function. Additionally, Grizzi *et al*. (2013) stated that the classic gross appearance of the liver in elderly individuals was referred to as "brown atrophy," with the brown discoloration resulting from the accumulation of highly oxidized, insoluble proteins known as lipofuscin stored within hepatocytes [12–14,18]. Periportal fibrosis was noted in 21-30 years and 41-50 years age groups. Similar findings were reported by other authors. Kim *et al*. (2015) stated that aging increased the susceptibility to liver fibrosis. Stahl *et al*. (2018) reported that aging was a major risk factor for the development of liver fibrosis. Likewise, Gan *et al*. (2011) indicated that advanced age was associated with the progression of liver fibrosis [12, 18, 19]. Steatosis was observed in all age groups, ranging from 11–20 years to 71–80 years, which was consistent with the available literature. Wynne (2002) and Kim *et al*. (2015) reported that the

incidence of non-alcoholic fatty liver disease showed an increasing trend with advancing age. Stahl *et al*. (2018) stated that the mechanism underlying age-related steatosis was not fully understood; however, it was attributed to hepatocyte aging, which led to decreased mitochondrial metabolism, reduced insulin transport across the sinusoidal endothelium, diminished autophagic flux, and chronic low-grade inflammation. These changes ultimately resulted in the accumulation of toxic free fatty acids in the liver [12, 17, 18].

Lipofuscin pigment was the change which was predominately seen in histology of gallbladder as the age advanced. Similar findings were noted by Zaki and Al-Refeidi (2009) who conducted the study in 6 gallbladders from Saudi Arabian population [27].

Conclusion

This study demonstrates that morphometric parameters of the liver and gallbladder show only weak correlations with age, whereas histological changes such as lipofuscin deposition, fibrosis, and steatosis display more consistent age-related progression. These findings suggest that microscopic evaluation provides more reliable markers of age than morphometry alone, with potential forensic relevance in age estimation. Given the limited sample size and descriptive nature of histological analysis, further studies with larger cohorts are warranted to validate and extend these observations.

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ORIGINAL ARTICLE

Comparative analysis of risk factors and severity of retinopathy of prematurity in appropriate for gestational age vs small for gestational age preterm infants

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Abstract

Background: Retinopathy of Prematurity (ROP) is a leading cause of childhood blindness worldwide, with a growing burden in developing countries. *Aim and Objectives*: To examine risk factors associated with ROP in preterm infants, assess disease severity, and compare outcomes between appropriate for Gestational Age (AGA) and Small for Gestational Age (SGA) categories. *Material and Methods*: A prospective cohort of 350 preterm infants (≤ 36 weeks GA, ≤ 2000 g birth weight) was studied from 2019–2021. Infants were categorized as AGA or SGA and screened for ROP. Risk factors were analysed using logistic and multivariate regression. Babies were followed until complete regression of disease. *Results*: Overall incidence of ROP was 40.2% (36.3% in AGA, 45.3% in SGA). Type 1 ROP requiring treatment occurred in 9.8% overall (3% AGA, 13% SGA). Significant risk factors included gestational age and hypoglycaemia in AGA, and gestational age and low APGAR score in SGA. All treated infants (laser photocoagulation or anti-VEGF) showed complete regression. *Conclusion*: SGA infants had higher incidence and severity of ROP compared to AGA. Early identification of risk factors enables timely intervention and improved outcomes. Study limitations included reduced SGA sample size and follow-up challenges during the COVID-19 pandemic.

Keywords: Retinopathy of prematurity, small for gestational age, appropriate for gestational age, risk factors, preterm infants

Introduction

Retinopathy of Prematurity (ROP), as the name suggests, is a potentially devastating retinal vascular disease that causes irreversible loss of vision in preterm infants all over the world. Due to the advances in modern neonatal medicine and improved access to healthcare, extremely preterm infants are now surviving. With the advent of the same, the disease burden and incidence of ROP have increased significantly. The term Retrolental Fibroplasia (RLF) was coined by Terry, in 1942. The appearance of a fibrovascular membrane behind the crystalline lens gave rise to the term RLF. The disease process was little understood and hardly any treatment options existed [1].

All around the world, about 9.9% of births are preterm that is before gestational age 37 full weeks [2]. ROP affects about 50,000 babies worldwide, as estimated by Blencowe *et al*. (2013) in a study [3]. The maximum burden is seen in developing countries, because access to neonatal care has improved without an improvement in the quality of neonatal care. Babies who are born before term or have related neonatal morbidity, like respiratory distress syndrome, poor weight gain, and hyperglycemia, are at the highest risk of ROP. Therefore, rates of ROP depend on the quality of care received, which includes oxygen delivery. Most cases of ROP are mild and resolve naturally with no treatment. A

minor percentage proceed to more severe ROP, which, if left untreated, can lead to retinal detachment or scarring of the retina, which can cause permanent blindness [3]. In 2010, India bore the burden of approximately 10% of all projected global visual loss secondary to ROP, with at least 5,000 acquiring severe disease and 2,900 children surviving with visual loss [4].

Various studies have been conducted to compare the incidence ROP in AGA (appropriate for gestational age) vs SGA (small for gestational age) babies, with contrasting results. Some found that the incidence of ROP was similar in both the groups, but some found that SGA babies were at more risk for ROP as compared to AGA babies [5-7]. Knowledge about the risk factors can help in an improved knowledge of the disease, preventing it and modifying the exposure to the risk factors. Given the high burden of ROP in developing countries and the conflicting evidence regarding the influence of gestational growth categories, this study was designed to systematically analyze risk factors and disease severity in AGA versus SGA preterm infants, with the aim of informing targeted screening and management strategies.

Material and Methods

A prospective cohort design was employed to systematically identify antenatal, natal, and postnatal risk factors, assess disease severity, and compare outcomes between AGA and SGA infants. Although this design was appropriate for achieving the study objectives, its single-center setting and instances of loss to follow-up may limit the generalizability of the findings. The study was conducted from September 2019 to December 2021 after obtaining approval from the Institutional Ethics Committee (IEC number 777/2019), and it was registered with the Clinical Trials Registry of India (CTRI/2020/04/0246842). A total of 350 babies were included in the study.

Sample size calculation

The number of patients necessary to carry out the study was computed utilizing the following formula: $N = 2\sigma 2 (Z1-\alpha/2 + Z1-\beta) 2$

d2 for effect size of $d/\sigma = 0.3$; $Z1-\alpha/2 = 1.96$ at 5% level of significance; $Z1-\beta = 0.84$ at 80% power

The minimum sample size required for each group was 175 infants (N), so in total 350 babies (700 eyes) were studied. Informed consent was taken from a parent before enrolment of the baby in the study.

Inclusion criteria: Babies with Gestational Age (GA) at birth of ≤ 36 weeks and a Birth Weight (BW) of ≤ 2000 g, neonates born after 36 weeks gestational age or birth weight above 2000 grams who were at risk of acquiring ROP were included, at the neonatologist's discretion

Exclusion criteria: Babies with inadequate documents on BW or GA were eliminated when born outside our hospital; babies with incomplete records of antenatal clinic details and maternal comorbidities were omitted; babies who died before complete resolution of ROP or did not attend the outpatient clinic for follow-up assessments were eliminated from the study.

Babies were divided as SGA and AGA and then grouped as ROP present (group 1) and not present (group 2). Group 1 was subdivided as Type 1 and type 2 (based on treatment requirement). Babies were screened by a single experienced ophthalmologist under aseptic precautions with an indirect ophthalmoscope and a scleral depressor after pupillary dilation with eye drops containing tropicamide and phenylephrine.

Eyedrops were prepared by taking commercially available tropicamide 0.8 % and phenylephrine 5%

and diluting them to tropicamide 0.4% and phenylephrine 2.5% with a tear supplement in a sterile aseptic environment to achieve a concentration of 1:1, which causes less systemic side effects for these preterm. The clinical condition and vitals of the baby were monitored throughout the screening procedure. The anterior segment of each eye was observed for tunica vasculosa lentis, amount of pupillary dilation, and media transparency. Then the posterior segment was examined to identify retinal alterations. ROP was graded based on the International Classification of ROP. The retinal maturity, zone of involvement, clock hours of involvement, plus disease if present, and stage was entered into the proforma. Treatment was initiated for infants with type 1 ROP. Babies were followed up till complete vascularization and till complete regression of the disease after treatment. On each follow up visit, the above procedure was repeated, and examination findings after instilling dilating eyedrops were recorded. Data from records of babies screened for ROP in the Neonatal Intensive Care Unit (NICU), inpatient neonates in the department of pediatrics and babies attending the outpatient department of ophthalmology was collected.

Risk factors studied

Antenatal

Use of antenatal steroids, multiple gestation such as twins and triplets (monochorionic vs dichorionic), antenatal neuroprotection with magnesium sulphate, chorioamnionitis leading to preterm birth, use of assisted reproductive technology for conception.

Natal

Respiratory Distress Syndrome (RDS) at birth, poor APGAR score, Intraventricular Haemorrhage (IVH), Low Birth Weight (LBW), location of birth (at our hospital or delivered outside)

Postnatal

Use of pulmonary surfactant for RDS, exposure to oxygen therapy, packed red blood cell transfusion, Patent Ductus Arteriosus (PDA), Necrotizing Enterocolitis (NE), recurrent apnea, tachypnoea, development of sepsis, early postnatal hypoglycemia, early enteral nutrition, and Kangaroo care. Data analysis for incidence, risk factors and severity of the disease was done using the Statistical Package for the Social Sciences (SPSS) statistical software. Multivariate regression was done to determine substantial risk factors of ROP in both AGA and SGA.

Results

Identification of risk factors for all preterm babies

Logistic regression was carried out for all the 350 infants to identify the risk factors. The variables that were identified to have significant effect on occurrence of ROP included: GA, LBW, indications for Lower Segment Cesarian Section (LSCS), eclampsia, preterm Premature Rupture of the Membranes (PPROM), preterm labour and twin labour, Intraventricular Hemorrhage (IVH), early neonatal hypoglycemia.

Identification of risk factors in AGA babies

Separate regression analyses were carried out for AGA cases to see if there was any variation in the risk factors. A total of 198 AGA cases were subjected to regression analysis.

The variables that were identified to have significant effect on occurrence of ROP in AGA babies were: GA, head circumference, gender, indications for LSCS, eclampsia, PPROM, preterm labor, oligohydramnios, Absent End Diastolic Flow (AEDF) on Ultrasound (USG), tachypnea, IVH, early neonatal hypoglycemia.

Identification of risk factors in SGA babies

A total of 152 SGA cases were subjected to regression analysis. The variables that were identified to have significant effect on occurrence of ROP in SGA babies were: GA, low APGAR score at 5 minutes of life, being born outside our institute (out born), neonatal hyperbilirubinemia, IVH, hypoglycemia and NE. Binomial logistic regression was done to determine the risk factors and to compare the two categories and it was found to be significant as the values of p was < 0.05. Out of the 350 babies, 40.3% were males and 59.7 % were females who had ROP. The incidence of ROP in AGA/SGA is shown in Table 1. Overall incidence was 40.2%. Distribution of ROP in both the groups is shown in Table 2 and Figure 1. ROP was seen in 72 and 69 cases in AGA and SGA group, respectively. The stage of ROP present in AGA/SGA is shown in Table 3 and Figure 2. Most common stage was zone II and stage II in SGA and AGA group i.e. in 25 babies in both. Of the 350 infants, 125 were < 30 weeks, 104 were born at 31-32 weeks, and 121 were born after 32 weeks of gestation. Average GA at birth was 31 weeks as shown in table 4.

Maternal and antenatal risk factors vs stage of ROP in both groups

The stage of ROP in relation to maternal and antenatal risk factors which were indications for LSCS is shown in Table 5.

Table 1: Incidence of ROP in AGA/SGA babies and their treatment methods

	ROP		Total n (%)
	No n (%)	Yes n (%)	
SGA	83 (54.6)	69 (45.3)	152 (100)
AGA	126 (63.6)	72 (36.3)	198 (100)
Total	209 (59.7)	141 (40.2)	350 (100)

ROP – retinopathy of prematurity; AGA – appropriate for gestational age; SGA – small for gestational age

Table 2: Distribution of ROP in AGA/SGA babies

	Incidence of ROP n (%)	Incidence of treatment requiring ROP n (%)	Treatment with laser photocoagulation n (%)	Treatment with intravitreal anti-VEGF n (%)
Overall incidence	40.2 (141)	9.8 (14)	13 (3.7)	1 (0.002)
SGA	45.3 (69)	11.5 (8)	7 (53.8)	1 (100)
AGA	36.3 (72)	8.3 (6)	6 (46.1)	0 (0)

ROP – retinopathy of prematurity; AGA – appropriate for gestational age; SGA – small for gestational age

Table 3: Stage of ROP present in AGA/SGA babies

	Stage of ROP							Total
	No ROP	Zone II stage I	Zone II Stage II	Zone II Stage III	Zone III Stage I	Zone III Stage II	Zone III Stage III	
SGA	83	13	25	6	9	13	3	152
AGA	126	8	25	4	13	21	1	198
Total	209	21	50	10	22	34	4	350

ROP – retinopathy of prematurity; AGA – appropriate for gestational age; SGA – small for gestational age

Table 4: Average GA (gestational age) at birth of babies

GA in weeks	ROP		Total
	No	Yes	
24	0	1	1
25	1	1	2
26	2	9	11
27	2	11	13
28	8	18	26
29	12	27	39
30	19	14	33
31	20	21	41
32	43	20	63
33	33	12	45
34	29	4	33
35	25	1	26
36	14	2	16
37	1	0	1
Total	209	141	350

Table 5: Stage of ROP in relation to maternal and antenatal risk factors which were indications for LSCS

Risk factors	Stage of ROP							Total
	No ROP	Zone II stage I	Zone II Stage II	Zone II Stage III	Zone III Stage I	Zone III Stage II	Zone III Stage III	
Preeclampsia	26	6	9	1	6	6	0	54
Eclampsia	8	0	3	1	1	0	0	13
PPROM	29	5	7	2	3	3	0	49
Preterm labour	14	0	1	2	0	2	0	19
Fetal distress	16	2	10	0	3	4	0	35
Maternal COVID-19	10	1	0	1	0	1	1	14
Complicated pregnancy	4	1	0	0	0	0	0	5
Placenta previa	7	0	5	0	0	0	0	12
Oligohydramnios	17	2	0	1	4	4	0	28
Decreased fetal movements	5	0	0	0	0	0	0	5
Twin labour	16	0	0	0	0	1	0	17
AEDF	7	1	0	0	0	2	0	10
Cord prolapses	0	1	1	0	0	0	0	2
Fetal malposition	3	0	5	0	0	0	0	8
Miscellaneous (Rh negative pregnancy, Non-progession of labour, Elective LSCS)	47	2	9	2	5	11	3	79
Total	209	21	50	10	22	34	4	350

ROP – retinopathy of prematurity; PPROM - preterm premature rupture of the membranes; AEDF - absent end diastolic flow; LSCS – lower segment caeseran section

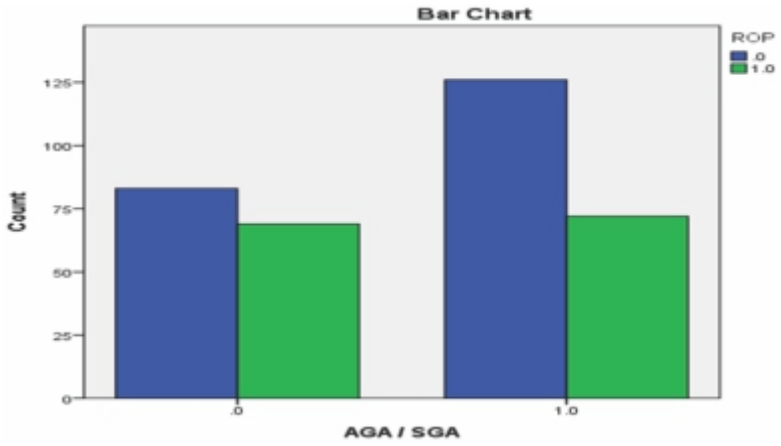

Figure 1: Distribution of ROP in AGA/SGA babies, 0- No ROP, 1- ROP Present

ROP – retinopathy of prematurity; AGA – appropriate for gestational age; SGA – small for gestational age

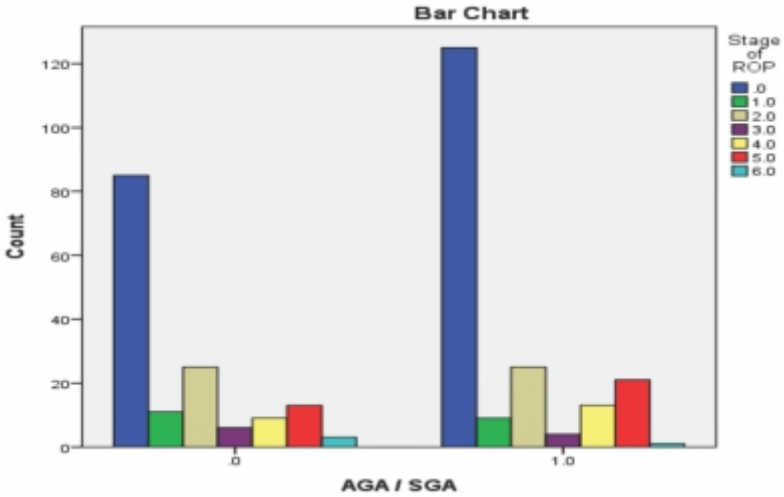

Figure 2: Stage of ROP present in AGA/SGA babies, 0- No ROP, 1.0- Zone II Stage I, 2.0- Zone II Stage II, 3.0- Zone II Stage III, 4.0- Zone III Stage I, 5.0- Zone III Stage II, 6.0- Zone III Stage III.

ROP – retinopathy of prematurity; AGA – appropriate for gestational age; SGA – small for gestational age

Risk factors for development of ROP identified in both the categories

GA (weeks), birth weight (g), maternal and antenatal risk factors like preterm labour, twin labour, hypoglycemia and IVH were significant factors as the values of *p* were < 0.001, 0.013, 0.034, < 0.001, 0.040 and 0.043. Maternal and antenatal risk factors like eclampsia and PPROM were found to be not significant as the values of *p* were 0.078 and 0.061.

Risk factor for development of ROP identified in AGA categories

GA (weeks), head circumference (cm), maternal and antenatal risk factors like eclampsia, AEDF, tachypnoea, IVH and early hypoglycemia were

significant factors identified as the values of $p <$ 0.01, 0.027, 0.012, 0.042, 0.038, 0.044 and 0.002. Gender, maternal and antenatal risk factors like PPROM, preterm labour and oligohydramnios were found to be not significant as the values of *p* were 0.051, 0.073, 0.083, and 0.083.

Identified risk factors in SGA category

GA (weeks), APGAR at 5 min of life, outborn status, IVH and neonatal hyperbilirubinemia were significant factors as the values of p < 0.01, 0.016, 0.038, 0.039 and 0.045 respectively. Hypoglycemia and NE were found to be not to be significant as the values of *p* were 0.064 and 0.054.

Multivariate analysis

Multivariate regression performed to determine significant risk factors of ROP in both AGA and SGA revealed that GA in weeks ($p < 0.001$) and hypoglycemia (p = 0.002) were noteworthy independent risk factors of ROP after correcting for confounding factors. With the increase in GA, risk of ROP significantly decreased. Neonates with early hypoglycemia had significantly higher risk of ROP (Table 6). Multivariate regression performed to determine significant risk factors of ROP in AGA showed that GA ($p = 0.002$) and early neonatal hypoglycemia (p = 0.023) were noteworthy independent risk factors of ROP after correcting for confounding factors. With the increase in GA (weeks), risk of ROP significantly decreased (Table 7). Multivariate regression performed to determine significant risk factors of ROP in SGA showed that gestational age ($p < 0.001$) and low APGAR score at 5 minutes of life ($p = 0.032$) were found to be noteworthy independent risk factors of ROP. With the increase in GA (weeks), risk of ROP substantially decreased. Patients with low APGAR at 5 minutes had significantly high risk of ROP (Table 8).

Table 6: Multivariate logistic regression to determine significant risk factors of ROP in both AGA and SGA

Risk Factors	Beta coefficient	Standard error	*p*	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Gestational age (weeks)	-0.461	0.060	<0.0001	0.631	0.560	0.709
Low birth weight	1.466	1.676	0.382	4.331	0.162	115.647
IVH	-0.716	0.629	0.255	0.489	0.142	1.678
Hypoglycemia	1.099	0.351	0.002	3.002	1.510	5.968

IVH – intraventricular haemorrhage

Table 7: Multivariate logistic regression in AGA cases

Risk Factors	Beta coefficient	Standard error	*p*	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Gestational age (weeks)	-0.384	0.122	0.002	0.681	0.537	0.865
Head circumference	-0.148	0.108	0.169	0.862	0.698	1.065
Gender						
Female				1.000		
Male	-0.206	0.348	0.554	0.814	0.412	1.609
Tachypnea	-0.615	0.370	0.097	0.541	0.262	1.118
IVH	-1.543	0.948	0.103	0.214	0.033	1.370
Hypoglycemia	1.410	0.618	0.023	4.097	1.220	13.764

IVH – intraventricular haemorrhage

Table 8: Multivariate logistic regression in SGA cases

Risk Factors	Beta coefficient	Standard error	*p*	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Gestational age (weeks)	-0.435	0.086	<0.0001	0.647	0.547	0.765
Low APGAR at 5 min	1.557	0.724	0.032	4.745	1.147	19.623
Outborn	0.479	0.510	0.348	1.614	0.594	4.385
Neonatal hyperbilirubinemia	-0.262	0.427	0.540	0.770	0.333	1.778
Early Hypoglycemia	0.656	0.482	0.173	1.928	0.750	4.955
IVH	-0.033	1.014	0.974	0.968	0.133	7.064
NE	-1.529	0.966	0.114	0.217	0.033	1.441

IVH – intraventricular haemorrhage; NE – necrotizing enterocolitis

Treatment group

14 out of 350 babies over the study period underwent treatment for ROP as shown in Table 1. Treatment was done by bilateral laser photocoagulation in most cases, and in one case by intravitreal injection of anti-VEGF. Intravitreal injections were given under aseptic precautions. ROP regressed in all eyes after treatment.

Discussion

ROP is an emerging cause of preventable childhood visual impairment in developing countries, especially in India, which has a high birth rate as well as increasing access to advanced neonatal care. Knowledge about the risk factors can aid in improved knowledge of the disease, preventing it and modifying the exposure to the risk factors. This study aimed to estimate the occurrence of ROP in our hospital, to add to the existing information about the risk factors and to explore other new associations. Various studies have been conducted to compare incidence ROP in AGA vs SGA babies, with contrasting results [5-8]. Our study found that being born with SGA predisposed the infant to a higher risk of developing ROP as well as requiring treatment for ROP than its AGA counterparts. The incidence of ROP in our study population was 40.2% overall, 36.3% in the AGA category and 45.3% in the SGA category. Similarly western studies showed the incidence of ROP to be in a range of 40-65%. The reported incidence in India varies between 38%-51.9% [9-11].

The occurrence of type 1 ROP or treatment requiring ROP in our study population overall was 9%, incidence was 3% in the AGA category and 13% in the SGA category. Similar studies report the incidence of type 1 ROP to be between 6 -27% [12, 13]. The incidence of ROP among SGA babies is reported to be between 23-60%, and in AGA between 20-58 % [14]. SGA infants had a higher incidence of ROP in our study than the AGA category. Other researchers have also reported similar findings that SGA preterms are at a greater risk of ROP development [7, 15]. A study conducted at Israel in a large population of infants with GA in weeks between 24-31 found that SGA infants were at 2 times more risk than that of AGA of developing stage 3–4 ROP [16]. Darlow *et al*. (2005) had reported that the greater the restriction in growth of the neonate before birth, the more severe was the disease [15].

In another similar study, Singh *et al*. described a greater occurrence of retinopathy in the SGA group in extremely premature babies [7]. Dhaliwal *et al*. (2009) described SGA babies with gestational ages between 26–32 weeks to have a higher risk of having retinopathy than the AGA babies [17]. SGA and IUGR have been described as risk factors for developing type 1 disease by Allegaert *et al.* (2003) [18]. In their research, Shah *et al*. (2005) and Arora *et al.* detailed no difference in the occurrence of ROP between SGA and AGA [6, 19]. Shah *et al*. (2005) and Procianoy *et al*. (1980) stated a higher occurrence of ROP in RDS [19, 20]. Administration of pulmonary surfactant and repeated blood transfusions for the infant was identified as risk factors by Shohat *et al*. (1983), Seiberth and Linderkamp (2000), and Maheshwari *et al*. (1996) also reported analogous results [21-23]. Studies like Maheshwari *et al*. (1996) and Hakeem *et al*. (2012) noted that post-natal sepsis was an independent risk factor for developing ROP [23, 24]. Unfortunately, we did not have this correlation in any of our study groups. Studies by Seiberth and Linderkamp (2000) and Kim *et al*. (2004) found that mechanical ventilation for any reason or the use of continuous positive airway pressure were risk factors for the

development of ROP [22, 25]. We could not get this significance in our study. Congenital heart disease has been found to be a risk factor for ROP by Shah *et al.* [19]. Apnea of prematurity has been reported as a risk factor for developing ROP, in studies done by Shah *et al*. (2005), Shohat *et al*. (1983), and Kim *et al*. (2004) [19, 21, 25]. With regards to the risk factors, in our study, a low GA was noted to have the strongest association with the development of ROP ($p < 0.01$) in all 3 groups, in consensus with previous published research [6]. Newer studies like the G-ROP have studied the effect of poor postnatal weight gain and IGF – 1 as an indirect measure of poor weight gain and identified them as risk factors in the development of retinopathy. Further research is warranted into this topic [26].

Current guidelines in neonatal care advocate the use of titrated oxygen therapy in treatment of mechanically ventilated preterm infants. In our NICU setup as well, titrated oxygen therapy is used with strict monitoring of the SpO2 levels. This may be the reason our study did not find any association with exposure to oxygen therapy and the development of ROP, in contrast to previously published literature which implicates oxygen exposure as the main cause of disease development [27]. Neonatal sepsis in our study population, if clinically suspected, was treated early with empirical antibiotics. This early treatment and detection are probably the reason we did not find any association with sepsis and ROP, which is contradictory to previous studies like the ELGAN study [28]. Introduction of early enteral nutrition has been found to be highly beneficial for preterm neonates to improve early postnatal weight gain and decrease morbidity such as NE, BPD and ROP. In our NICU, early enteral nutrition with the mother's breastmilk is practiced routinely and is protective against the development of ROP [5, 17, 29].

Kangaroo care is also in practice at our NICU. It has been shown to significantly improve neonatal outcomes in all aspects, including good postnatal weight gain, decreasing the risk of BPD, NE, neonatal sepsis, requirement of oxygen and may thus have an indirect effect on decreasing the incidence and severity of ROP as well. Further research is warranted into this matter [30].

The study was limited due to the following factors:

Due to the COVID 19 pandemic, once discharged from the NICU, parents were reluctant to bring the preterm babies to the hospital for follow up. The number of SGA births occurring was very less as compared to AGA births. By virtue of a smaller number of births and also loss to follow up due to COVID 19, the sample size in the SGA group could not be achieved. We were able to recruit 152 out of the 175 babies.

Conclusion

Management of ROP in the coming years is going to be an uphill battle due to the ever-increasing preterm population that is now surviving. A collaborative and multidisciplinary approach by the neonatologist and ophthalmologist is necessary to prevent visual disability for future generations. Future multicenter studies are warranted to validate the results of this research.

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ORIGINAL ARTICLE

Genetic characterization of CTX-M-15, AmpC, and metallo-β-lactamase genes among clinical *Klebsiella pneumoniae* isolates in a tertiary-care hospital

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Abstract

Background: The co-existence of Extended-Spectrum β-Lactamases (ESBL), AmpC β-lactamases and Metallo-β-Lactamases (MBL) in *Klebsiella pneumoniae* limits therapeutic options and complicates infection control in tertiary-care settings. *Aim and Objectives:* To determine the phenotypic and molecular profile of β-lactamase-mediated resistance and associated co-resistance to aminoglycosides and fluoroquinolones among clinical *K. pneumoniae* isolates. *Material and Methods:* In this laboratory-based cross-sectional study, 254 non-duplicate clinical isolates were subjected to antimicrobial susceptibility testing and phenotypic detection of ESBL, AmpC and MBL. Multiplex polymerase chain reaction was performed for blaTEM, blaSHV, blaCTX-M-15, blaDHA and blaNDM genes in multidrug-resistant isolates. *Results:* Resistance to third-generation cephalosporins and fluoroquinolones exceeded 70%, while carbapenem resistance ranged from 20.9% to 25.6%. ESBL, AmpC and MBL production was detected in 68.1%, 22.0% and 9.8% of isolates, respectively. Among the genotyped isolates, blaTEM and blaSHV were present in 98.0% each and blaCTX-M-15 in 88.0%, with blaNDM co-detected in 64.0%. Frequent co-resistance to aminoglycosides and fluoroquinolones was observed. *Conclusion:* The convergence of ESBL, AmpC and blaNDM-mediated resistance in K. pneumoniae highlights the need for routine molecular surveillance and targeted antimicrobial-stewardship interventions in tertiary-care hospitals.

Keywords: *Klebsiella pneumoniae*, AmpC beta-Lactamases, Metallo-beta-Lactamases, Carbapenem Resistance

Introduction

Klebsiella pneumoniae (K. pneumoniae) is a major cause of healthcare-associated infections worldwide and is recognised for its remarkable capacity to acquire, accumulate, and disseminate Antimicrobial Resistance (AMR) determinants. The World Health Organization (WHO) classifies carbapenem -resistant *K. pneumoniae* as a Priority 1: Critical pathogen, underscoring its global clinical significance and the urgent need for enhanced diagnostic and surveillance strategies [1]. Infections caused by Multidrug-Resistant (MDR) *K. pneumoniae* are associated with prolonged hospitalisation, increased morbidity and mortality, and severely restricted therapeutic options, particularly in resourcecons-trained settings [2, 3].

Among β-lactam resistance mechanisms, Extended -Spectrum β-Lactamases (ESBLs), AmpC β-lactamases, and Metallo-β-Lactamases (MBLs) represent some of the most clinically consequential determinants. CTX-M-15 has emerged as the predominant ESBL type globally and is strongly linked to resistance to third-generation cephalo-sporins [4, 5]. Plasmid-mediated AmpC enzymes further compromise the efficacy of broad-spectrum

cephalosporins and are often challenging to detect reliably using routine phenotypic assays [6]. MBLs such as New Delhi Metallo-β-lactamase (NDM), Verona Integron-encoded Metallo-β-lactamase (VIM), and Imipenemase (IMP) hydrolyse nearly all β-lactams, including carbapenems, leaving extremely limited treatment options [7, 8].

The co-harbouring of CTX-M-15, AmpC, and MBL genes in *K. pneumoniae* is frequently associated with co-resistance to aminoglycosides and fluoroquinolones, driven largely by plasmid-mediated resistance determinants, efflux mechanisms, and selective pressure from empirical broad-spectrum antibiotic use [9–11]. This MDR phenotype considerably narrows viable therapeutic options, complicates empirical treatment decision-making, and increases the likelihood of clinical failure. The simultaneous presence of ESBL, AmpC, and MBL genes combined with resistance to non-β-lactam agents poses substantial challenges for formulating evidence-based Antimicrobial Stewardship (AMS) strategies, strengthening Infection Prevention and Control (IPC) programmes, and developing rational, hospital-specific antibiotic policies in tertiary-care settings.

The present study aimed to characterise the phenotypic and genotypic profiles of clinical K. pneumoniae isolates co-harbouring CTX-M-15, AmpC, and MBL genes in a tertiary-care hospital. By correlating antimicrobial susceptibility profiles with Polymerase Chain Reaction (PCR) based gene detection and integrating relevant clinical data including treatment compliance we seek to identify co-resistance trends, particularly resistance to aminoglycosides and fluoroquinolones, which commonly accompany β-lactamase-mediated resistance. Additionally, the study aimed to determine associated clinical risk factors and to evaluate the implications of these MDR profiles for therapeutic decision-making, AMS planning, and hospital antibiotic policy development.

Although ESBL, AmpC and MBL genes have been reported individually from India, data on their simultaneous coexistence with non-β-lactam co-resistance and associated clinical risk factors from this region remain limited. Furthermore, genotype –phenotype correlation for CTX-M-15-dominant ESBL backgrounds co-harbouring blaNDM and blaDHA in a single institutional setting has not been adequately explored. The present study therefore provides integrated phenotypic, molecular and clinical correlation to support AMS decision-making.

Material and Methods

Study design and bacterial isolates

This prospective laboratory-based cross-sectional study conducted in the Department of Microbiology, Symbiosis Medical College for Women and Symbiosis University Hospital and Research Centre, Symbiosis International (Deemed University), Pune, India, between May 2022 and December 2022. Consecutive non-duplicate clinical isolates were included using a consecutive sampling technique.

The minimum sample size was calculated using the formula

$n = Z^2P(1-P)/d^2$ taking ESBL prevalence 55%, 95% confidence level and 6% precision → required sample ≈ 250. Two hundred and fifty-four non-duplicate *K. pneumoniae* isolates were recovered from diverse clinical specimens, including urine, blood, respiratory secretions, pus, and indwelling-catheter tips, obtained from hospitalised patients.

Patient demographic and clinical data including age, sex, immune status, underlying comorbidities, hospitalisation history, antimicrobial exposure, mechanical ventilation, indwelling catheterisation, focal site of infection, and indicators of disease severity were collected from hospital records.

This study was approved by the Institutional Ethics Committee of Symbiosis International (Deemed University), Pune (Ref: SMCW/IEC/2022/034). All data were anonymised; no patient identifiers were recorded.

Isolation and identification

Clinical specimens were inoculated onto Mac Conkey agar and blood agar (HiMedia Laboratories, Mumbai, India), whereas urine samples were cultured on Cystine Lactose Electrolyte Deficient (CLED) agar. All plates were incubated aerobically at 37 °C for 24 hours. The isolation and preliminary identification of *K. pneumoniae* were performed using standard conventional microbiological procedures [12]. Confirmatory Identification and antimicrobial susceptibility testing, including Minimum Inhibitory Concentration (MIC) determination, were conducted using the VITEK-2 Compact automated system (bioMérieux, France) in accordance with the manufacturer's instructions. Phenotypic confirmation of ESBL, AmpC and MBL production was undertaken using established phenotypic methods [7, 11, 13].

Phenotypic detection of β-lactamase production

ESBL detection

Screening for ESBL production was undertaken using cefotaxime (30 µg) and ceftazidime (30 µg) discs alone, and in combination with clavulanic acid (10 µg). An increase of ≥ 5 mm in the inhibition-zone diameter for either cephalosporin–clavulanate disc compared with the corresponding cephalosporin disc alone was interpreted as indicative of ESBL production, in accordance with the Clinical & Laboratory Standards Insitute (CLSI) recommendations [7, 11, 14, 15].

AmpC β-lactamase detection

Isolates exhibiting cefoxitin resistance (zone diameter < 18 mm) were screened for AmpC β-lactamase production using an inhibitor-based method employing Phenyl-Boronic Acid (PBA). PBA (95% benzeneboronic acid; Sigma-Aldrich, India) was dissolved at 120 mg in 3 mL dimethyl sulfoxide and 3 mL sterile distilled water; 20 µL of this solution was dispensed onto a cefoxitin (30 µg) disc. An increase of ≥ 4 mm in the inhibition-zone diameter for the cefoxitin–PBA disc relative to cefoxitin alone was interpreted as AmpC positive [12]. Additionally, MICs for cefoxitin alone and in combination with cloxacillin were evaluated, with a cefoxitin/cefotetan–cloxacillin MIC ratio ≥ 8 confirming AmpC β-lactamase production [2, 3, 14, 15].

MBL detection

MBL production was assessed using the imipenem –EDTA combined disc test as described by Yong et al. [14]. Two imipenem (10 µg) discs (HiMedia, India) were placed onto Mueller–Hinton agar plates inoculated with a 0.5 McFarland suspension of the test isolate. Ten microlitres of EDTA solution (750 µg/disc) was applied to one disc. Plates were incubated at 35 °C for 16–18 hours, and an increase of ≥ 7 mm in the inhibition-zone diameter around the imipenem–EDTA disc compared with the imipenem disc alone indicated MBL production [2, 3, 14, 15]

Carbapenemase screening: Modified Hodge test (MHT)

Carbapenemase activity was further evaluated using the MHT following CLSI recommendations [11]. A lawn culture of *Escherichia coli* ATCC 25922 was prepared on Mueller–Hinton agar. After drying, the test strain was streaked from the central imipenem (10 µg) disc toward the periphery. Following overnight incubation at 35°C, the presence of a characteristic clover-leaf-shaped indentation in the *E. coli* lawn along the test-strain streak was interpreted as indicative of carbapenemase production [14, 15].

Quality control

All phenotypic β-lactamase detection assays were Quality-Controlled (QC) using CLSI recommended reference strains. *Escherichia coli* ATCC 25922 served as the negative control for ESBL, AmpC, MBL, and carbapenemase testing; *Klebsiella pneumoniae* ATCC 700603 as the ESBL-positive control; *Enterobacter cloacae* ATCC 13047 as the AmpC-positive control; *Klebsiella pneumoniae* ATCC BAA-1705 and ATCC BAA-1706 as carbapenemase-positive and -negative controls, respectively. All control strains produced expected results, and only QC validated runs were included in the analysis.

Genotyping of antimicrobial resistance genes

Molecular detection of β-lactamase genes

Plasmid-encoded β-lactamase genes conferring resistance to β-lactams, including extended-spectrum β-lactamases (ESBLs; *SHV, TEM, CTX-M-15*), AmpC β-lactamases (*DHA*), and carbapenemases (*KPC-2, NDM-1*), were detected in 50 MDR *K. pneumoniae* isolates using conventional multiplex PCR. Previously sequenced gene templates were used as positive controls (*SHV* KY115-613, *TEM* KY115614, *CTX-M-15* KY115615, *DHA* KY115612, *KPC-2* KY364013, *NDM-1*) while nuclease-free water functioned as the negative control.

Primer sequences and expected amplicon sizes were used as previously described. Each primer set was designed to amplify clinically relevant β-lactamase determinants associated with ESBL, AmpC and carbapenemase-mediated resistance as described by Jacob *et al.,* Nordman *et al.*, Ranjan *et al.* [2,3,8].

PCR amplification conditions

PCR amplification was performed in 25 µL reaction mixtures containing 100–200 ng genomic DNA, 1× PCR buffer, 2.5 mM $MgCl_2$, 50 µM dNTPs, 0.2 µM of each primer, 1 U Taq DNA polymerase, 5 % (v/v) dimethyl sulfoxide and nuclease-free water. Amplification was undertaken in a 2720 Thermal Cycler (Thermo Fisher Scientific, USA), using an annealing temperature of 55 °C for all primer sets, consistent with published multiplex PCR protocols.

Agarose gel electrophoresis

Amplified PCR products were resolved on 2 % (w/v) agarose gels prepared in 0.5× TBE buffer containing LabSafe nucleic-acid stain (G-Biosciences, USA). Each reaction (5 µL) was mixed with 1 µL of 6× loading dye and electrophoresed alongside a molecular weight ladder at 5 V cm^{-1} until adequate band separation was achieved. Bands were visualised under UV illumination using a BIO-RAD GelDoc XR system, and amplicon sizes were verified against expected sizes. All target genes produced amplicons of the anticipated lengths.

Results

Demographic distribution and clinical profile

A total of 254 non-duplicate clinical isolates of K. pneumoniae were obtained from various specimens collected between May 2022 and December 2022.

Table 1 summarises the demographic profile, clinical characteristics, hospital distribution, specimen sources, and predisposing factors of patients from whom *Klebsiella pneumoniae* was isolated, and analyses their association with carbapenem resistance. Of these, 149 (58.7 %) originated from male patients and 105 (41.3 %) from female patients. The mean patient age was 49.6 ± 18.3 years, ranging from 1 month to 87 years. Most isolates were derived from patients admitted to Intensive Care Units (ICUs) (87 isolates; 34.2 %), followed by the

medical wards (67 isolates; 26.3 %), surgical wards (55 isolates; 21.6 %), and outpatient clinics (45 isolates; 17.9 %). The predominant specimen type was urine (96 isolates; 37.8 %), followed by respiratory secretions (62 isolates; 24.4 %), pus/wound swabs (48 isolates; 18.9 %), blood (36 isolates; 14.1 %), and catheter-related samples (12 isolates; 4.7 %) (Table 1, Figure 1). Most isolates were recovered from patients with underlying conditions such as diabetes mellitus (70 patients; 27.5 %), chronic kidney disease (46 patients; 18.1 %), or prolonged hospitalisation (> 10 days) (91 patients; 35.8 %) (Table 1). Of the 254 isolates included in the study, 118 (46.5%) were Carbapenem-Resistant (CRKP) and 136 (53.5%) were Carbapenem-Susceptible (CSKP). There was no statistically significant difference in sex distribution between the two groups (p = 0.21). However, patients with CRKP infection were significantly older than those with CSKP (mean age 52.4 ± 17.6 vs 47.2 ± 18.7 years; p = 0.02). A significantly higher proportion of CRKP isolates originated from ICUs compared with CSKP isolates (47.5% vs 22.8%), and ICU stay emerged as a strong risk factor for carbapenem resistance (OR = 3.06, 95% CI 1.78–5.26; p < 0.001). With regard to specimen type, respiratory samples and blood cultures were significantly associated with carbapenem resistance. Respiratory isolates showed more than three-fold higher odds of being carbapenem-resistant (OR = 3.17, p = 0.001), while bloodstream isolates demonstrated a 2.5-fold increased risk (p = 0.03).

Among the healthcare-associated risk factors, prolonged hospitalisation, prior exposure to broad-spectrum antibiotics, presence of indwelling medical devices, and mechanical ventilation were all strongly associated with CRKP (p < 0.001 for each). Mechanical ventilation showed the highest risk (OR = 6.68, 95% CI = 3.14–14.2). Regarding comorbid conditions, diabetes mellitus (OR = 1.80, p = 0.04) and chronic kidney disease (OR = 2.54, p = 0.006) were significantly associated with carbapenem resistance, whereas hypertension, chronic lung disease, malignancy, chronic liver disease, and immunosuppression did not show statistically significant associations.

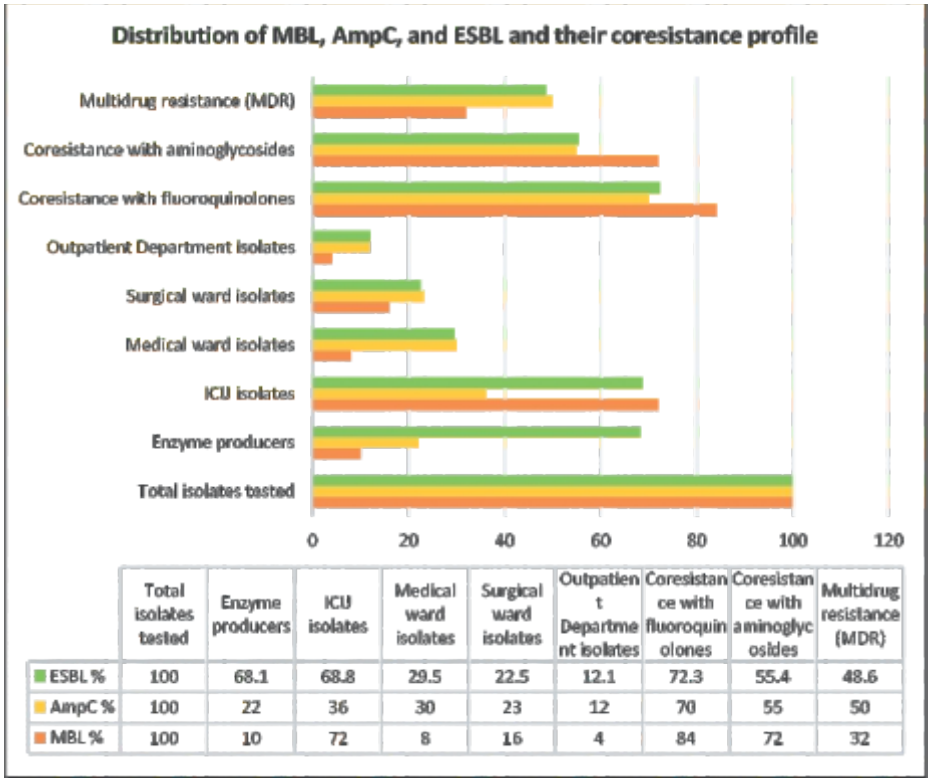
Table 1: Association of clinical and demographic factors with carbapenem resistance among *K. pneumoniae* isolates (n = 254)

Variable	Category	CRKP (n = 118) n (%)	CSKP (n = 136) n (%)	Total n (%)	Odds Ratio (95% CI)	*p*
Sex	**Male**	74 (62.7)	75 (55.1)	149 (58.7)	1.36 (0.83–2.21)	0.21
	Female	44 (37.3)	61 (44.9)	105 (41.3)		
Age (years)	**Mean ± SD**	52.4 ± 17.6	47.2 ± 18.7	49.6 ± 18.3		0.02†
Hospital location	**ICU**	56 (47.5)	31 (22.8)	87 (34.2)	3.06 (1.78–5.26)	<0.001
	Wards/OPD	62 (52.5)	105 (77.2)	167 (65.8)		

Continued...

Specimen type	**Urine**	32 (27.1)	64 (47.1)	96 (37.8)		
	Respiratory	38 (32.2)	24 (17.6)	62 (24.4)	3.17 (1.63–6.17)	0.001
	Pus / wound	22 (18.6)	26 (19.1)	48 (18.9)	1.69 (0.79–3.59)	0.17
	Blood	20 (16.9)	16 (11.8)	36 (14.1)	2.50 (1.08–5.77)	0.03
	Catheter	6 (5.1)	6 (4.4)	12 (4.7)	2.00 (0.55–7.21)	0.29
Prolonged hospitalisation	**>10 days**	62 (52.5)	29 (21.3)	91 (35.8)	4.10 (2.34–7.17)	<0.001
Prior antibiotic exposure	**Yes**	78 (66.1)	36 (26.5)	114 (45.0)	5.40 (3.09–9.44)	<0.001
Indwelling medical device	**Yes**	55 (46.6)	21 (15.4)	76 (30.0)	4.76 (2.63–8.61)	<0.001
Mechanical ventilation	**Yes**	41 (34.7)	10 (7.4)	51 (20.0)	6.68 (3.14–14.2)	<0.001
Diabetes mellitus	**Yes**	40 (33.9)	30 (22.1)	70 (27.5)	1.80 (1.01–3.21)	0.04
Hypertension	**Yes**	31 (26.3)	29 (21.3)	60 (23.7)	1.32 (0.72–2.43)	0.36
COPD / CLD	**Yes**	33 (28.0)	29 (21.3)	62 (24.4)	1.43 (0.79–2.59)	0.23
CKD	**Yes**	30 (25.4)	16 (11.8)	46 (18.1)	2.54 (1.29–5.01)	0.006
Malignancy	**Yes**	18 (15.3)	13 (9.6)	31 (12.2)	1.71 (0.78–3.76)	0.17
Chronic liver disease	**Yes**	11 (9.3)	9 (6.6)	20 (7.9)	1.44 (0.57–3.66)	0.43
Immunosuppression	**Yes**	10 (8.5)	5 (3.7)	15 (5.9)	2.42 (0.79–7.41)	0.11

CRKP: carbapenem-resistant Klebsiella pneumoniae; CSKP:carbapenem-susceptible Klebsiella pneumoniae; ICU: intensive-care unit; COPD: chronic obstructive pulmonary disease; CKD: chronic kidney disease; OR: odds ratio; CI: confidence interval.



	Total isolates tested	Enzyme producers	ICU isolates	Medical ward isolates	Surgical ward isolates	Outpatient Department isolates	Coresistance with fluoroquinolones	Coresistance with aminoglycosides	Multidrug resistance (MDR)
ESBL %	100	68.1	68.8	29.5	22.5	12.1	72.3	55.4	48.6
AmpC %	100	22	36	30	23	12	70	55	50
MBL %	100	10	72	8	16	4	84	72	32

Figure 1: Distribution of MBL, AmpC, and ESBL Producers and Their Coresistance Profiles

Detection of CTX-M-15

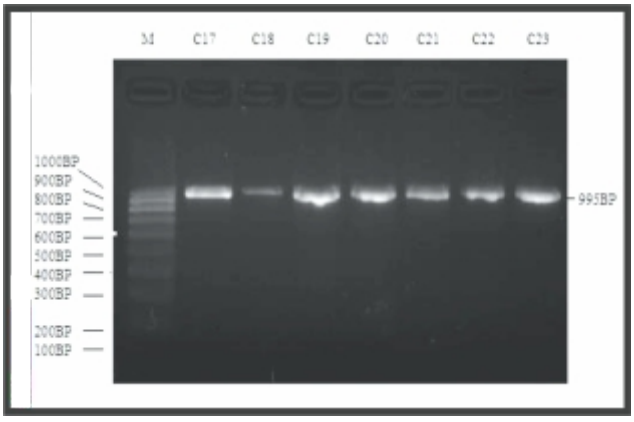


Figure 2: Agarose (2% w/v) gel electrophoresis of amplification products from CTXM gene

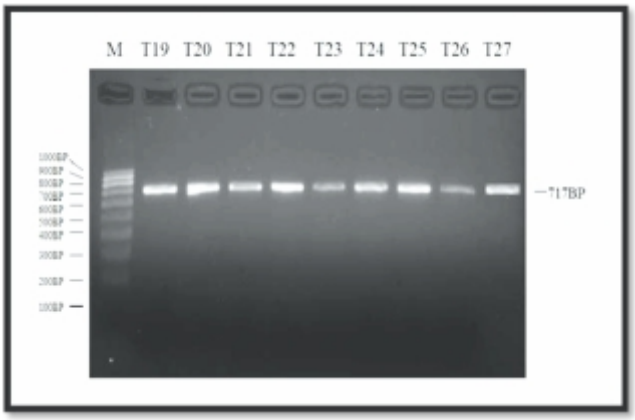


Figure 3: Agarose (2% w/v) gel electrophoresis of amplification products from TEM gene

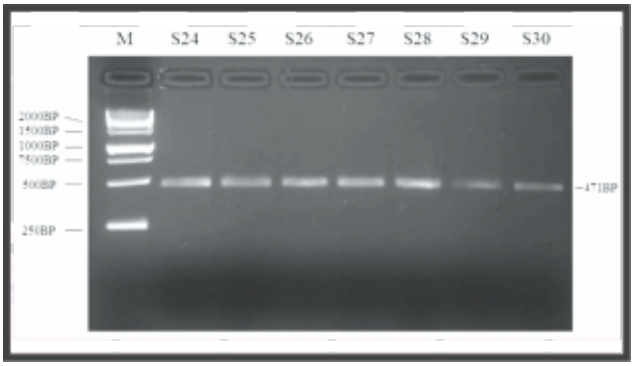


Figure 4: Agarose (2% w/v) gel electrophoresis of amplification products from SHV gene

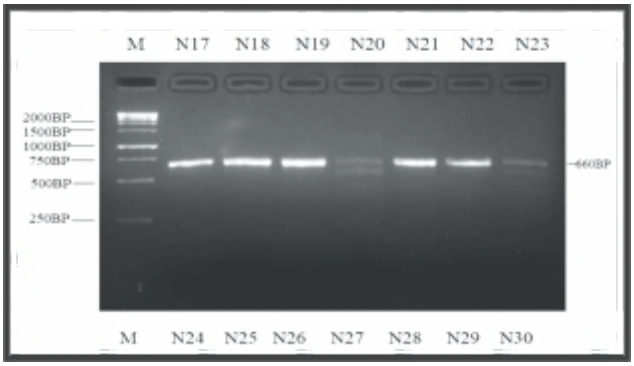


Figure 5: Agarose (2% w/v) gel electrophoresis of amplification products from NDM gene

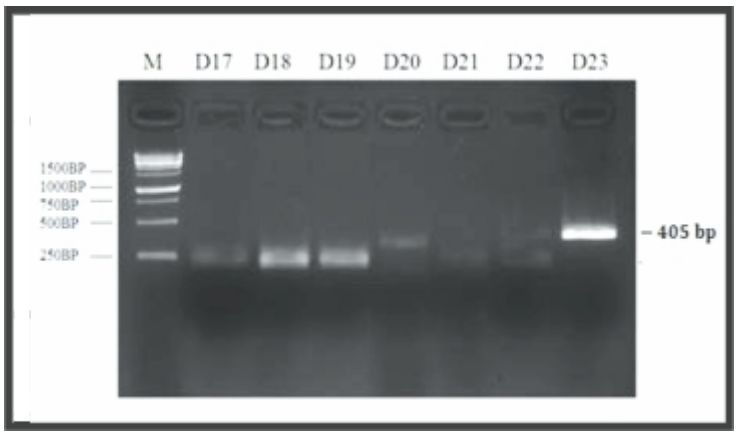


Figure 6: Agarose (2% w/v) gel electrophoresis of amplification products from BLA-DHA gene

Antimicrobial susceptibility profiles

Of the total 254 samples tested; high level of resistance was observed against third-generation cephalosporins, with 182 isolates (71.7%) resistant to cefotaxime, 176 (69.3%), ceftazidime, and 172 (67.8%) to ceftriaxone. Resistance to cefoxitin was seen in 142 isolates (55.9%), indicating possible AmpC β-lactamase activity. Among β-lactam/β-lactamase inhibitor combinations, 148 isolates (58.3%) were resistant to piperacillin–tazobactam.

Carbapenem resistance was detected in 53 isolates (20.9%) for imipenem and 65 (25.6%) for meropenem, suggestive of MBL production. For aminoglycosides, 107 isolates (42.1%) were resistant to gentamicin and 88 (34.6%) to amikacin, reflecting moderate sensitivity retention. Resistance to ciprofloxacin was widespread, affecting 179 isolates (70.5%), while cotrimoxazole resistance was noted in 162 isolates (63.8%). In contrast, last-resort agents colistin and tigecycline showed low resistance rates 10 isolates (3.9%) and 14 isolates (5.5%), respectively indicating retained efficacy. Phenotypic β-lactamase detection confirmed 173 isolates (68.1%) as Extended-Spectrum β-Lactamase (ESBL) producers, 56 isolates (22.0%) as AmpC β-

lactamase producers, and 25 isolates (9.8%) as MBL, positive.

Overall, 115 isolates (45.3%) demonstrated MDR, defined as non-susceptibility to ≥ 3 antimicrobial classes. These findings collectively indicate a high prevalence of ESBL-mediated resistance with emerging carbapenem resistance, underscoring the urgent need for reinforced infection control and AMS interventions in tertiary-care hospital settings (Table 2).

Table 2: Antimicrobial susceptibility pattern and phenotypic β-lactamase resistance among *K. pneumoniae* isolates (n = 254)

Antimicrobial class	Antimicrobial agent	Resistant isolates (n)	Resistance (%)
β-lactams / Cephalosporins	**Cefotaxime**	182	71.7
	Ceftazidime	176	69.3
	Ceftriaxone	172	67.8
	Cefoxitin	142	55.9
β-lactam / β-lactamase inhibitor	**Piperacillin–tazobactam**	148	58.3
Carbapenems	**Imipenem**	53	20.9
	Meropenem	65	25.6
Aminoglycosides	**Gentamicin**	107	42.1
	Amikacin	88	34.6
Fluoroquinolones	**Ciprofloxacin**	179	70.5
Sulphonamides	**Cotrimoxazole**	162	63.8
Polymyxins / Others	**Colistin**	10	3.9
	Tigecycline	14	5.5
Phenotypic β-lactamase detection	**Extended-spectrum β-lactamase positive**	173	68.1
	AmpC β-lactamase positive	56	22.0
	Metallo-β-lactamase positive	25	9.8

Phenotypic detection of β-lactamase production

ESBL

Of the 254 isolates, 173 (68.1%) demonstrated a ≥ 5 mm increase in inhibition-zone diameter with clavulanic acid, confirming ESBL production. The majority of ESBL-producing isolates were recovered from ICUs (62/173, 35.8%), followed by medical wards (51/173, 29.5%), surgical wards (39/173, 22.5%), and outpatient departments (21/173, 12.1%). Co-resistance to fluoroquinolones and aminoglycosides was observed in 125 (72.3%) and 96 (55.4%) isolates, respectively. MDR was detected in 84 (48.6%) ESBL producers (Table 2)

AmpC β-lactamase

AmpC production was confirmed in 56/254 isolates (22.0%). Ward-wise distribution showed a predominance in ICUs (20/56, 35.8%), followed by medical wards (17/56, 29.5%), surgical wards (13/56, 22.5%), and outpatient settings (7/56, 12.1%). Wound specimens constituted the most common source (22/56, 39.3%), followed by respiratory samples (14/56, 25.0%), urine (13/56, 23.2%), and blood cultures (7/56, 12.5%). Prior β-lactam exposure was documented in 38 cases (67.8%), and indwelling devices were present in 18 (32.1%). Co-resistance to fluoroquinolones and aminoglycosides occurred in 39 (70.0%) and 31 (55.0%) isolates, respectively, while MDR was observed in 28 (50.0%) (Table 2).

MBL

MBL production was detected in 25/254 isolates (9.8%) using the imipenem–EDTA combined disc test; of these, 22 (88.0%) were positive by the MHT. Most MBL producers were from ICUs (18/25, 72.0%), followed by surgical wards (4/25, 16.0%), medical wards (2/25, 8.0%), and outpatient settings (1/25, 4.0%). A prolonged hospital stay (> 10 days) and the presence of indwelling devices were noted in 19 (76.0%) and 16 (64.0%) cases, respectively. High rates of co-resistance were observed for fluoroquinolones (21/25, 84.0%) and aminoglycosides (18/25, 72.0%), whereas MDR was present in 8 isolates (32.0%) (Table 2).

Molecular genotyping of K. pneumoniae for the production of blaCTX-M, blaTEM. blaSHV, blaKPC, blaNDM and blaDHA genes to identify the resistance mechanism

Among the 50 K. pneumoniae isolates analysed, ESBL genes were highly prevalent. blaTEM and blaSHV were detected in 98% of isolates each (95% CI 89.4–99.9), while blaCTX-M was identified in 88% (95% CI 75.7–95.5). The prevalence of blaTEM and blaSHV was significantly higher than that expected by chance ($p < 0.001$) (Table 3). Among carbapenemase genes, blaNDM was detected in 64% of isolates (95% CI 49.2–77.1), whereas blaKPC was not detected in any isolate (0%; 95% CI 0.0–7.1), and this difference was statistically significant ($p < 0.001$). The AmpC gene blaDHA was present in 14% of isolates (95% CI 5.8–26.7), which was significantly lower than the ESBL gene prevalence ($p < 0.001$).

The present analysis demonstrates that carbapenem resistance in K. pneumoniae is predominantly driven by healthcare-associated factors rather than patient sex or most underlying comorbidities. ICU stay emerged as a major determinant, reflecting the selective antimicrobial pressure, high device utilisation, and increased severity of illness in critically ill patients. The strong association with prior broad-spectrum antibiotic exposure underscores the pivotal role of AMS in limiting the emergence and dissemination of carbapenem-resistant strains.

Table 3: Distribution of β-lactamase genes among *Klebsiella pneumoniae* isolates (n = 50)

Gene category	Gene	Amplicon size (bp)	Positive isolates n (%)	95% CI for proportion	*p*
ESBL	**blaCTX-M**	995	44 (88.0)	75.7–95.5	0.07
	blaTEM	717	49 (98.0)	89.4–99.9	<0.001
	blaSHV	471	49 (98.0)	89.4–99.9	<0.001
Carbapenemase	**blaKPC**	863	0 (0.0)	0.0–7.1	<0.001
	blaNDM	660	32 (64.0)	49.2–77.1	—
AmpC	**blaDHA**	406	7 (14.0)	5.8–26.7	<0.001

95% Confidence Intervals (CI) for proportions were calculated using the Wilson method. The one-sample proportion test was used to assess the significance of gene prevalence. A two-tailed p value < 0.05 was considered statistically significant

Mechanical ventilation and indwelling devices showed the highest effect sizes, highlighting the importance of biofilm-mediated persistence and cross-transmission in hospital settings. The significant association with respiratory and bloodstream infections suggests that invasive infections are more likely to be caused by resistant strains, possibly due to prolonged hospital stay and repeated antibiotic exposure. Among comorbidities, diabetes mellitus and chronic kidney disease were independent clinical correlates, which may be explained by frequent healthcare contact, immune dysregulation, and repeated antimicrobial therapy in these patient groups. Overall, these findings reinforce that carbapenem resistance in K. pneumoniae is a marker of healthcare exposure and invasive supportive care, and they provide clinically actionable targets for infection-control interventions and AMS programmes.

Discussion

This study reveals a substantial burden of antimicrobial-resistant K. pneumoniae across diverse clinical specimens, with infections disproportionately affecting critically ill patients, those with prolonged hospitalisation, and individuals exposed to broad-spectrum antimicrobials. The predominance of isolates from ICUs reflects established risk factors for healthcare-associated K. pneumoniae infection, including invasive procedures, indwelling devices, and sustained antimicrobial pressure. Similar demographic clustering has been reported by Diekema et al. (2019) who documented increased incidence among hospitalised adults with multiple comorbidities [18]. The high proportion of urinary isolates further supports earlier observations identifying K. pneumoniae as a key uropathogen, particularly among patients with diabetes mellitus or long-term catheterisation [19].

The antimicrobial resistance profile observed is concerning, with resistance exceeding 70% to third-generation cephalosporins and fluoroquinolones. These patterns are consistent with the widespread dissemination of ESBL-producing K. pneumoniae, particularly across South and South-East Asia, where

antimicrobial consumption remains high [20]. Carbapenem resistance rates ranging from 20.9% to 25.6% are comparable to those reported in East and South Asian settings, indicating sustained regional transmission of carbapenem-resistant lineages [21]. In contrast, relatively preserved susceptibility to colistin and tigecycline mirrors global reports suggesting that last-resort agents retain activity, although their continued effectiveness depends on strict antimicrobial stewardship [1]. At the mechanistic level, ESBL production was the dominant resistance determinant, identified in 68.1% of isolates. This prevalence aligns with regional and global data documenting the expansion of CTX-M-type enzymes within healthcare environments [5, 20, 22]. The concentration of ESBL producers in ICUs highlights the role of ecological pressures, including prolonged antibiotic exposure and frequent patient-to-patient transmission, in amplifying resistance. The frequent co-resistance to fluoroquinolones and aminoglycosides further supports plasmid-mediated co-selection of resistance determinants associated with ESBL genes, with nearly half of the isolates exhibiting multidrug-resistant phenotypes [1, 14, 18, 23].

AmpC β-lactamases were detected in 22% of isolates, predominantly from high-risk hospital settings. Their distribution across wound and respiratory specimens is consistent with nosocomial acquisition pathways and reflects the selective impact of repeated β-lactam exposure and device-associated interventions [24]. The observed co-resistance patterns parallel global trends in which AmpC-encoding plasmids frequently harbour additional resistance loci, contributing to complex multidrug-resistant profiles. The detection of MBL production in 9.8% of isolates represents the most clinically significant component of the resistance landscape. MBLs, predominantly encoded by blaNDM variants, are among the most critical antimicrobial resistance threats due to their ability to hydrolyse nearly all β-lactams and their efficient plasmid-mediated dissemination [24]. Their predominance in ICU patients with prolonged hospitalisation and indwelling devices aligns with recognised risk factors for colonisation by carbapenemase-producing organisms. The high levels of fluoroquinolone and aminoglycoside co-resistance reflect the dense genetic linkage of MBL genes with multiple resistance modules. Concordance between EDTA-based synergy testing and the MHT supports the reliability of accessible phenotypic detection strategies in high-burden settings [12, 14, 24, 25].

Genotypic analysis demonstrated extensive co-harbouring of β-lactamase genes, underscoring the remarkable genomic plasticity of K. pneumoniae in tertiary-care hospitals. The near-universal presence of blaTEM and blaSHV (98.0% each), together with a high prevalence of blaCTX-M-15 (88.0%), indicates that ESBL-mediated resistance is firmly entrenched within dominant hospital-adapted clones. Frequent intra-isolate coexistence of these ESBL determinants suggests dissemination through multi-replicon plasmids and composite mobile genetic elements, facilitating both horizontal transfer and long-term stability under sustained antimicrobial pressure [26]. Such clustering may synergistically enhance hydrolytic activity against third-generation cephalosporins, further narrowing therapeutic options [27].

Of particular concern is the high rate of blaNDM co-detection (64.0%) among ESBL-producing isolates. This convergence of ESBL and MBL reflects the successful mobilisation of carbapenemase-encoding platforms into pre-existing ESBL backgrounds, likely mediated by conjugative

plasmids, insertion sequences, and integron-associated gene cassettes [3, 20, 26-28]. ESBL–NDM co-harbouring strains exhibit resistance to nearly all β-lactam agents, including carbapenems, thereby severely constraining treatment options and complicating infection-control efforts [5, 7, 28].

Although blaDHA was detected at a lower frequency (14.0%), its coexistence with ESBL and blaNDM genes is clinically relevant. AmpC β-lactamases can compromise the activity of β-lactam/β-lactamase inhibitor combinations and may remain undetected by routine phenotypic methods, facilitating silent dissemination within healthcare environments. The absence of blaKPC in this cohort highlights region-specific carbapenemase epidemiology and reaffirms NDM as the dominant mechanism of carbapenem resistance in the Indian subcontinent.

Importantly, the β-lactamase genes identified in this study are highly mobile and readily disseminate across genera within the Enterobacteriaceae and to other clinically important Gram-negative pathogens, including Escherichia coli and Acinetobacter species. This interspecies transfer has driven the widespread emergence of multidrug-resistant and extensively drug-resistant phenotypes across health-care and community settings. The largely silent dissemination of these resistance determinants underscores antimicrobial resistance as an evolving global "silent pandemic", necessitating coordinated surveillance and robust AMS to curb further spread.

Conclusion

Clinical K. pneumoniae isolates in this study exhibited extensive multidrug resistance driven by the co-occurrence of ESBL, AmpC, and metallo-β-lactamase genes, frequently accompanied by resistance to aminoglycosides and fluoroquinolones. The convergence of plasmid-mediated resistance mechanisms within hospital-adapted clones severely constrains therapeutic options and facilitates long-term persistence in high-risk healthcare environments. These findings underscore the urgent need for routine molecular surveillance, targeted infection-control interventions, and robust AMS strategies to limit the dissemination of high-risk K. pneumoniae lineages in tertiary-care settings.

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ORIGINAL ARTICLE

Study of ergonomic risks to lower limb muscles in occupations involving prolonged standing and sitting

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Abstract

Background: Musculoskeletal alterations can lead to changes in posture and movement. Due to functional adaptations following postural realignments, lower extremity muscles can be overloaded, causing discomfort and pain. Biomechanical adaptations can lead to functional limitations, resulting in more stress on muscles during everyday activities. *Aim and Objectives:* This study seeked to promote ergonomic workplace strategies for healthier postures and interventions. *Material and Methods:* In this study, we recruited 554 healthy volunteers from urban settings via stratified random sampling, with one group composed of workers engaged in extended sitting time, whereas the other group consisted of individuals standing for extended periods of time. We compared lower limb muscle power values between workers engaged in prolonged standing and those in prolonged sitting using an unpaired t-test, with statistical significance set at $p < 0.05$. *Result:* Long-term standing and sitting workers exhibited statistically significant differences in lower limb muscle power. These findings indicate that stretching and strengthening exercises help to prevent overuse syndrome, musculoskeletal injuries, and alterations in muscle power associated with prolonged standing or sitting occupations. *Conclusion:* Exploratory analyses further revealed a strong association between muscle power changes and these occupational postures, highlighting the potential value of ergonomic interventions.

Keywords: Ergonomics, lower limb muscles, muscle power, manual muscle test

Introduction

Physical fitness has become a major focus in India and globally. People are making greater efforts to enhance their physical fitness, and the sports industry is also growing. Static positions for prolonged periods of time puts excessive strain on the legs, especially on the joint [1, 2]. Maintaining fixed standing or sitting positions over time often results in stressed and taut lower limb tendons and ligaments. As a result, soreness, joint pain, and chronic musculoskeletal injuries can occur. Also, the legs' muscles can become weak and fatigued, making it difficult to walk or exercise. It is important to take breaks throughout the day to reduce the amount of time spent in the same position and to engage in light stretching or movement exercises to help maintain healthy and strong leg muscles [3, 4]. Awkward postures can lead to tendinitis, bursitis, and nerve compressions. Preventing Musculoskeletal Disorders (MSDs) requires incorporating regular work breaks, conducting ergonomic evaluations, implementing job rotation and task modifications, and ensuring the use of appropriate tools and equipment. Employees should also receive training on correct body mechanics and

early recognition of MSD symptoms. These disorders arise from multifaceted causes, encompassing individual, biomechanical, and psychosocial elements. While initial job assessments often target workplace design or administrative practices that contribute to MSDs, broader evaluations are essential, recognizing workers as individuals with distinct beliefs and attitudes toward their roles. Such personal perspectives on work demands have been linked to on-the-job musculoskeletal discomfort and can affect workers' willingness to adopt workplace improvement recommendations [5]. Understanding static versus dynamic postures is crucial for workers to adopt job-specific ergonomics. Static postures (standing, sitting, crouching) remain fixed, appearing comfortable for prolonged periods but risking fatigue. Dynamic postures (bending, reaching) involve movement, reducing muscular strain and enhancing circulation. Balancing both prevents health issues, as excessive static loading impairs blood flow while dynamic activity promotes recovery. Workers maintaining this equilibrium ensure ergonomic health and productivity [6-8].

Posture is an important indicator of overall health and wellbeing, as it can give insight into physical, mental, and emotional states. Muscle activity causes increase in intramuscular tension and length changes in muscles. During muscle activity, muscle fibers are contracted and relaxed. According to the difference in the length and tone of the muscles, these muscle works can be categorized into isotonic contractions and isometric contractions. In deter-mining muscle power, work-related activities exert considerable influence. Muscle and soft tissue undergo changes due to continuous maintenance of one posture, which may modify walking patterns. Due to their occupation, the person may adopt specific postures or develop different gait patterns, which may affect their normal gait patterns. To increase stability on a ship, sailors require a wider base, which leads to adjusting their posture, the abductor of hip may tighten, and the adductor of hip may be stretched and wasted. According to Lakshmi Narayanan (2005), changes in muscular attributes require adopting a new walking pattern for regular tasks [9]. Gait awareness aids occupational health by integrating body structures for postural stability, countering internal/external forces in standing/sitting. This study assesses gait variability's link to lower limb muscle power amid control deficits. Posture alters lower limb loading during work, necessitating interventions against MSDs. Prior literature overlooks lower limb ergonomics in mixed standing /sitting jobs, focusing broadly on MSDs. Our work fills this gap, providing data to guide ergonomic strategies and healthier practices [10-13]. The existing literature lacks comprehensive understanding of the ergonomic effects on the lower limbs among employees who are involved in both standing and sitting activities. Previous studies have mainly focused on general aspects of MSDs rather than specific regional impacts, such as those related to the lower limbs. Recognizing these lacunae, the present study was conducted to provide valuable data and insights pertaining to lower limb health in the realm of occupational health. Furthermore, our research aimed to raise awareness about improved workplace practices, to guide workplace interventions, and to facilitate the formation of ergonomic strategies that should promote healthier work habits.

Material and Methods

A cross-sectional study was designed to evaluate the extent of ergonomic effects in lower extremity muscle strength. In addition, the study assessed the impact of standing and sitting for long duration on muscle power variability. This study received

Institutional Ethics Committee approval (Ref. No. 002/ SBMC/IHEC/2019/1221) and was conducted at Sree Balaji Medical College, Chennai, India. Total of 554 subjects were chosen from the Chennai population through the random sampling method via direct interaction with participants at their workplace and who volunteered to take part in the research. Sample populations were drawn from the participants of workers in two groups from different sectors in Chennai. One group of workers was allotted from prolonged sitting positions, including drivers, IT professionals, and bank staff of both genders. A second group comprised male and female prolonged-standing workers, including traffic officers, builders, and shop/textile sales people.

Eligible study participants were healthy individuals aged 20–60 years who had provided informed consent, possessed at least one year of work experience in their respective occupations, worked in jobs requiring extended standing and sitting for at least eight hours per day, and had no pre-existing lower limb musculoskeletal conditions or injuries. Individuals with known chronic musculoskeletal disorders, a history of lower limb surgeries, fractures, or injuries within the past year and pregnant women were excluded from the study. Additionally, employees with prior cardiovascular or neurological conditions or substance abuse or any clinical condition that impact their participation or data reliability and participants unwilling to comply with study procedures or provide accurate information were excluded.

Manual muscle testing

Muscle testing forms an essential component of the physical examination. It provides information not obtained by other procedures, useful in the differential diagnosis, prognosis and treatment of neuromuscular and musculoskeletal disorders. Musculoskeletal conditions often exhibit characteristic patterns of muscle imbalance. Few patterns are related with handedness, some with habitually poor posture. Muscle imbalance occurs due to occupational or recreational activities in which there is the persistent use of certain muscles without the adequate exercise of opposing muscles. The imbalance that affects body alignment is an essential factor in many painful postural conditions. Muscle imbalance distorts alignment and sets the stage for undue stress and strain on joints, ligaments, and muscles. Manual muscle testing is the tool of choice to determine the extent of imbalance. The muscle strength is graded on a scale. We used the Medical Research Council (MRC) proposed standard manual muscle testing scale in the study [14]. Key lower limb muscles were tested manually against resistance, and strength was rated using a standard 0-5 grading system.

0 - No activity of muscle

1 – Trace muscle activation, such as flickering, without achieving a full range of motion.

2 – Muscle movement with elimination of gravity, achieving complete joint range of motion

3 – Movement of muscle against gravity, complete joint range of motion

4 - Movement of muscle against moderate resistance, complete joint range of motion

5 - Movement of muscle against examiners maximum resistance, complete joint range of motion

Statistical analyses were performed using unpaired t-test, Chi-square test, and Pearson's correlation test. A value of $p < 0.05$ was considered statistically significant. Data were entered into Microsoft Excel (version 2021) and analyzed accordingly.

Results

The prevalence of muscle power alterations in the study population was computed as percentages. In this study, prolonged standing and sitting workers demonstrated statistically significant differences in lower limb muscle power.

Measurements of muscle power

In rehabilitation step-ups, muscle strength testing has long been an important assessment procedure. Manual Muscle Testing (MMT) is a simple method, yet an effective way to evaluate the strength of muscles in a person's body. It is a non-invasive technique that involves applying resistance to a muscle while the person contracts that muscle. The aim of manual muscle testing is to identify weaknesses or deficiencies in a person's muscular system and to monitor their progress during rehabilitation. A MMT graded from 0 to 5 according to the MRC scale was performed on muscles acting on the joints of lower extremity (hip, knee, ankle) of healthy subjects as shown in Tables1 and 2 and Figures 1 and 2.

Table 1: Association of muscle power of hip joint in long-term standing and sitting workers

Hip-joint Muscle power			Grade			Chi-square	*p*
			Grade 3	Grade 4	Grade 5		
Hip flexors	**Standing**	**Observed Count**	19	166	92	112.144	<0.001
		Expected Count	17.0	107.5	152.5		
		Percentage	6.9%	59.9%	33.2%		
	Sitting	**Observed Count**	15	49	213		
		Expected Count	17.0	107.5	152.5		
		Percentage	5.4%	17.7%	76.9%		
Hip extensors	**Standing**	**Observed Count**	15	91	171	25.569	<0.001
		Expected Count	12.5	120.5	144.0		
		Percentage	5.4%	32.9%	61.7%		
	Sitting	**Observed Count**	10	150	117		
		Expected Count	12.5	120.5	144.0		
		Percentage	3.6%	54.2%	42.2%		

Continued...

Hip abductors	**Standing**	**Observed Count**	44	166	67	173.524	<0.001
		Expected Count	25.0	108.0	144.0		
		Percentage	15.9%	59.9%	24.2%		
	Sitting	**Observed Count**	6	50	221		
		Expected Count	25.0	108.0	144.0		
		Percentage	2.2%	18.1%	79.8%		
Hip Adductors	**Standing**	**Observed Count**	8	83	186	38.608	<0.001
		Expected Count	21.5	102.5	153.0		
		Percentage	2.9%	30.0%	67.1%		
	Sitting	**Observed Count**	35	122	120		
		Expected Count	21.5	102.5	153.0		
		Percentage	12.6%	44.0%	43.3%		
Hip medial rotators	**Standing**	**Observed Count**	9	80	188	54.637	<0.001
		Expected Count	27.5	101.0	148.5		
		Percentage	3.2%	28.9%	67.9%		
	Sitting	**Observed Count**	46	122	109		
		Expected Count	27.5	101.0	148.5		
		Percentage	16.6%	44.0%	39.4%		
Hip lateral rotators	**Standing**	**Observed Count**	84	130	63	164.689	<0.001
		Expected Count	47.5	93.0	136.5		
		Percentage	30.3%	46.9%	22.7%		
	Sitting	**Observed Count**	11	56	210		
		Expected Count	47.5	93.0	136.5		
		Percentage	4.0%	20.2%	75.8%		

Table 2: Association of muscle power of knee and ankle joints in long-term standing and sitting workers

Knee/Ankle-Joint Muscle power			Grade			Chi-square	*p*
			Grade 3	Grade 4	Grade 5		
Knee-joint Flexors	**Standing**	**Observed Count**	34	133	110	67.589	<0.001
		Expected Count	21.0	99.0	157.0		
		Percentage	12.3%	48.0%	39.7%		
	Sitting	**Observed Count**	8	65	204		
		Expected Count	21.0	99.0	157.0		
		Percentage	2.9%	23.5%	73.6%		
Knee-joint Extensors	**Standing**	**Observed Count**	6	71	200	46.316	<0.001
		Expected Count	13.0	103.0	161.0		
		Percentage	2.2%	25.6%	72.2%		
	Sitting	**Observed Count**	20	135	122		
		Expected Count	13.0	103.0	161.0		
		Percentage	7.2%	48.7%	44.0%		
Ankle-joint Plantarflexors	**Standing**	**Observed Count**	5	128	144	64.602	<0.001
		Expected Count	4.0	85.0	188.0		
		Percentage	1.8%	46.2%	52.0%		
	Sitting	**Observed Count**	3	42	232		
		Expected Count	4.0	85.0	188.0		
		Percentage	1.1%	15.2%	83.8%		
Ankle-joint Dorsiflexors	**Standing**	**Observed Count**	24	175	78	34.892	<0.001
		Expected Count	12.0	165.0	100.0		
		Percentage	8.7%	63.2%	28.2%		
	Sitting	**Observed Count**	0	155	122		
		Expected Count	12.0	165.0	100.0		
		Percentage	0.0%	56.0%	44.0%		

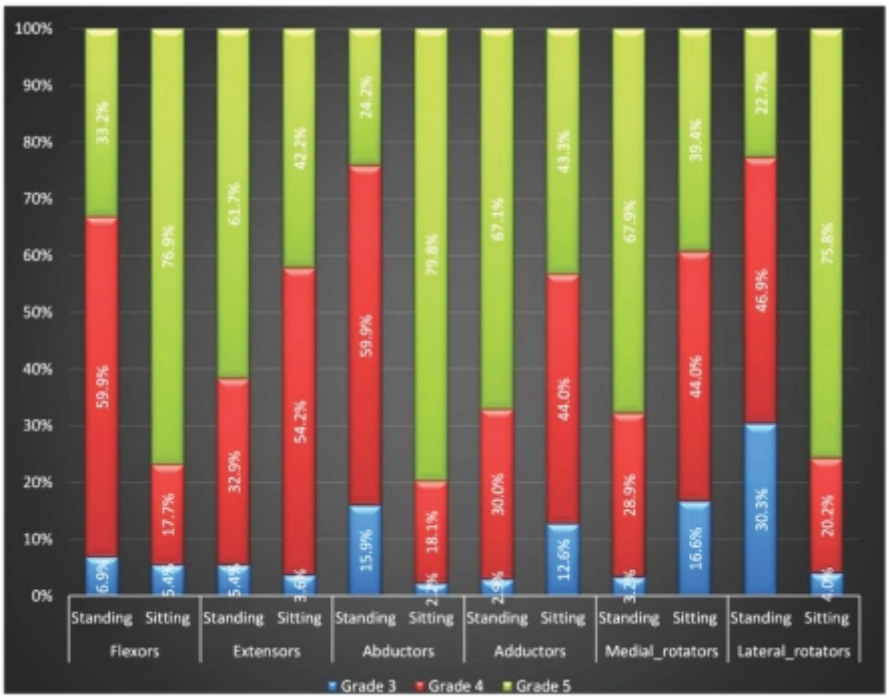

Figure 1: Comparison of hip-joint muscle power between prolonged standing and sitting workers

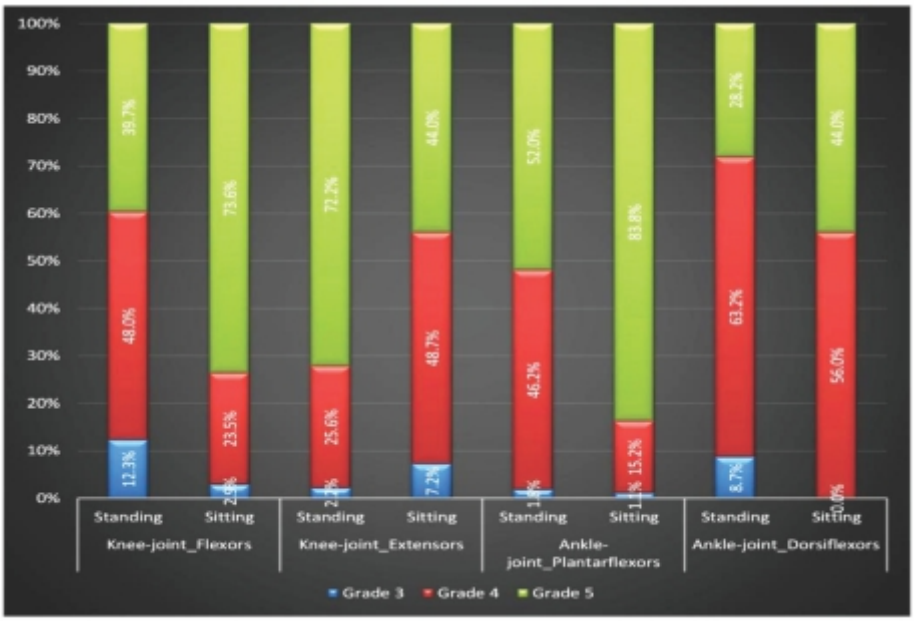

Figure 2: Comparison of knee and ankle joint muscle power between long-term standing and sitting workers

Association of muscle power in long-term standing and sitting workers

In the standing position, we found a significant association between hip flexor muscle power and work position (chi-square = 112.144, $p < 0.001$) (Table 1). Specifically, Grade 4 and Grade 5 muscle power were more prevalent in standing workers compared to Grade 3. In the sitting position, there was also a significant association (chi-square = 76.9, $p < 0.001$), with Grade 5 muscle power being highly prevalent among sitting workers. Similar to hip flexors, there was a notable correlation between hip extensor muscle power and work position in both standing (chi-square = 25.569, $p < 0.001$) and sitting (chi-square = 42.2, $p < 0.001$) positions. Grade 5 muscle power was more prevalent in both positions. The data revealed a notable correlation between hip abductor muscle power and work position in both standing (chi-square = 173.524, $p < 0.001$) and sitting (chi-square = 79.8, $p < 0.001$) positions. Grade 5 muscle power was highly prevalent among sitting workers, while in the standing position, Grade 4 muscle power was also notable. There was a notable correlation between hip adductor muscle power and work position in both standing (chi-square = 38.608, $p < 0.001$) and sitting (chi-square = 43.3, $p < 0.001$) positions. Grade 5 muscle power was more prevalent in both positions. Similarly, the data revealed a notable correlation between hip medial rotator muscle power and work position in both standing (chi-square = 54.637, $p < 0.001$) and sitting (chi-square = 39.4, $p < 0.001$) positions. Grade 5 muscle power was highly prevalent among sitting workers, while in the standing position, Grade 4 muscle power was also notable. There was a notable correlation between power of muscles responsible for hip lateral rotation and work position in both standing (chi-square = 164.689, $p < 0.001$) and sitting (chi-square = 75.8, $p < 0.001$) positions as shown in Figure 1. Grade 4 muscle power was more prevalent in both positions. These findings indicate the importance of considering hip muscle power in understanding the physical demands and adaptations associated with different work positions that could influence occupational health and ergonomics

MMT of lower limb muscles in workers with long-term standing and sitting: correlation with age

In both standing and sitting positions, there was a negative correlation between age and the strength of hip flexor muscles. This indicates that as individual's age, their hip flexor muscle strength tends to decrease. These associations exhibit statistical significance. Similar to hip flexors, there was a marked negative correlation between age and hip extensor muscle strength in both standing and sitting positions. Older individuals tend to have weaker hip extensor muscles. These associations exhibit statistical significance ($p < 0.001$). There was a marked inverse relation between age and hip abductor muscle strength in both standing and sitting positions. This suggests that with advancing age, their capacity to abduct the hip joint (move the leg away from the body) decreased significantly. These associations were highly statistically significant ($p < 0.001$). In both standing and sitting, there was a significant negative relationship between age and hip adductor muscle strength. As individuals age, their hip adductor muscles tend to become weaker. These associations were statistically significant ($p < 0.001$). Both the medial and lateral rotator muscles of the hip showed a marked negative

correlation with age in both standing and sitting positions. Older individuals tend to have weaker hip rotator muscles. These associations exhibited statistical significance ($p < 0.001$).

In both standing and sitting, there was a marked negative correlation between age and the strength of knee-joint flexor and extensor muscles. Older individuals tend to have weaker knee muscles. These associations exhibit statistical significance ($p < 0.001$). In both standing and sitting, there was a significant negative correlation between age and the strength of ankle-joint plantar flexor and dorsiflexor muscles. As individuals age, their ankle muscle strength tends to decrease. These associations exhibit statistical significance ($p < 0.001$) as shown in Table 3. These findings highlight the importance of considering age-related changes in muscle strength when assessing the health and functional capacity of workers in jobs that require prolonged standing and sitting.

A correlation relation between weight and muscle power in prolonged standing and sitting workers

In the standing position, there was a marked inverse relationship between weight and hip flexor muscle power, meaning that individuals with a higher weight tend to have weaker hip flexor muscles. In the sitting position, there was no significant correlation between weight and hip flexor muscle power. In both standing and sitting positions, there was a marked inverse relationship between weight and hip extensor muscle power. This indicated that individuals with elevated body weight tend to have weaker hip extensor muscles in both occupational positions. In the standing position, there was a marked inverse relationship between weight and hip abductor muscle power, suggesting that obese individuals tend to have weaker hip abductor muscles. In the sitting position, there was no correlation between weight and hip abductor muscle power. In both standing and sitting positions, there

Table 3: Correlation between age and MMT of lower limb muscles in workers prolonged standing and sitting

Muscles	Standing		Sitting	
	Correlation	*p*	Correlation	*p*
Hip Flexors	0.541	<0.001	0.365	<0.001
Hip Extensors	0.381	<0.001	0.399	<0.001
Hip Abductors	0.670	<0.001	0.246	<0.001
Hip Adductors	0.304	<0.001	0.501	<0.001
Hip Medial rotators	0.383	<0.001	0.400	<0.001
Hip Lateral rotators	0.620	<0.001	0.399	<0.001
Knee Flexors	0.338	<0.001	0.393	<0.001
Knee Extensors	0.341	<0.001	0.454	<0.001
Ankle Plantarflexors	0.477	<0.001	0.431	<0.001
Ankle Dorsiflexors	0.557	<0.001	0.425	<0.001

was a significant negative correlation between weight and hip adductor muscle power. This indicated that individuals with elevated body weight tend to have weaker hip adductor muscles in both occupational positions. Similar to hip adductors, there was a significant negative correlation between weight and hip medial rotator muscle power in both standing and sitting positions. In the standing position, there was a significant negative correlation between weight and hip lateral rotator muscle power, suggesting that obese individuals tend to have weaker hip lateral rotator muscles.

In the sitting position, there was a significant positive correlation, indicating that individuals with elevated body weight tend to have stronger hip lateral rotator muscles when sitting. In both standing and sitting positions, there was no significant correlation between weight and knee-joint flexor and extensor muscle power. This suggested that weight does not strongly influence the strength of these muscle groups in either position. In the standing position, there was a marked negative correlation between weight and ankle-joint plantar flexor muscle power, suggesting that obese individuals tend to have weaker ankle plantar flexor muscles. In the sitting position, there was a positive correlation, indicating that individuals with elevated body weight tend to have stronger ankle plantar flexor muscles when sitting. Weight was also negatively correlated with ankle-joint dorsiflexor muscle power in the standing position (Table 4). These findings highlight the significance of considering weight as a variable when assessing lower limb muscle power in workers who engage in prolonged standing and sitting, as it may have implications for their physical capabilities and occupational health.

Table 4: Relationship between weight and MMT among long-term standing and sitting workers

Muscles	Standing		Sitting	
	Correlation	*p*	Correlation	*p*
Hip Flexors	0.194	0.001	0.069	0.255
Hip Extensors	0.317	<0.001	0.090	0.133
Hip Abductors	0.251	<0.001	0.041	0.500
Hip Adductors	0.344	<0.001	0.022	0.721
Hip Medial rotators	0.319	<0.001	0.096	0.113
Hip Lateral rotators	0.265	<0.001	0.203	0.001
Knee Flexors	0.093	0.121	0.005	0.940
Knee Extensors	0.024	0.689	0.205	0.001
Ankle Plantar flexors	0.514	<0.001	0.218	<0.001
Ankle Dorsiflexors	0.402	<0.001	0.103	0.089

In prolonged standing and sitting workers, lower limb muscle power correlated with time spent at work

In the standing position, there was a strong direct association between the amount of time spent working and hip flexor muscle power. This indicated that individuals who spend more time at work standing tend to have stronger hip flexor muscles. In the sitting position, a similar positive correlation was observed, indicating that more work hours spent sitting were associated with stronger hip flexors. In the standing position, there was a strong direct association between work duration and hip extensor muscle power, meaning that individuals who spend more time standing at work tend to have stronger hip extensors. However, in the sitting position, there was no significant correlation between work duration and hip extensor muscle power. Both in standing and sitting positions, there was a strong direct association between work duration and hip abductor muscle power. This suggested that spending more time at work was associated with a stronger hip abductor. In both standing and sitting positions, there was a strong direct association between work duration and hip adductor muscle power. This indicated that individuals who spend more time at work tend to have stronger hip adductors. There was a strong direct association between work duration and hip medial rotator muscle power in both standing and sitting positions. This suggested that more work hours were associated with stronger hip medial rotators. In both standing and sitting positions, there was a strong direct association between work duration and power of muscles responsible for hip lateral rotation. This indicated that individuals who spend more time at work tend to have stronger hip lateral rotators.

In both standing and sitting positions, there was a strong direct association between work duration and knee-joint flexor muscle power. This indicated that increased work hours were associated with stronger knee-joint flexors. In the sitting position, there was a strong direct association between work duration and knee-joint extensor muscle power, indicating that more work hours spent sitting were associated with stronger knee-joint extensors. In both standing and sitting positions, there was a strong direct association between work duration and ankle-joint plantar flexor muscle power. This suggested that more work hours were associated with stronger ankle-joint plantar flexors. In the standing position, there was a significant positive correlation between work duration and ankle-joint dorsiflexor muscle power, suggesting that more duration spent standing at work was associated with stronger ankle dorsiflexors. However, in the sitting position, there was no correlation between work duration and ankle dorsiflexor muscle power (Table 5).

Table 5: Correlation of working hours and MMT in long-term standing and sitting workers

Muscles	Standing		Sitting	
	Correlation	*p*	Correlation	*p*
Hip Flexors	0.121	0.045	0.174	0.004
Hip Extensors	0.187	0.002	0.018	0.770

Continued...

Hip Abductors	0.190	0.002	0.328	<0.001
Hip Adductors	0.204	0.001	0.128	0.033
Hip Medial rotators	0.322	<0.001	0.268	<0.001
Hip Lateral rotators	0.219	<0.001	0.152	0.011
Knee Flexors	0.111	0.066	0.206	0.001
Knee Extensors	0.133	0.027	0.215	<0.001
Ankle Plantar flexors	0.107	0.076	0.303	<0.001
Ankle Dorsiflexors	0.129	0.031	0.034	0.568

In summary, the study results indicate that generally positive correlation between duration of occupational activity and muscle power of lower extremity muscle groups in workers who engage in prolonged standing and sitting. This suggests that long work durations were correlated with enhanced lower limb muscles strength, which was due to the physical demands of these occupations.

Long-term standing and sitting employees' MMT of lower limb muscles was correlated with their years of work experience

In both standing and sitting positions, there was a marked inverse relationship between the number of years of experience in specific work and the muscle power of hip the flexor muscles. This indicated that as employees accumulated more years of work experience, their hip flexor muscle power tend to decrease. These correlations exhibited high statistical significance ($p < 0.001$). Similarly, there was a marked inverse relationship between work experience and hip extensor muscle power in both standing and sitting positions. This suggests that as employees gained more years of work experience, their hip extensor muscles tend to become weaker. These correlations exhibited high statistical significance ($p < 0.001$). In both standing and sitting positions, there was a marked inverse relationship between work experience and hip abductor muscle power. As employees accumulated more work experience, their hip abductor muscles tend to weaken. These correlations exhibited high statistical significance ($p < 0.001$).

There was an inverse relationship between work experience and hip adductor muscle power in both standing and sitting positions. This indicated that as employees gained more years of work experience, their hip adductor muscles tend to become weaker. These correlations exhibited high statistical significance ($p < 0.001$). In both standing and sitting positions, there was a marked inverse relationship between work experience and the muscle power of hip medial rotators and lateral rotators. As employees accumulated more years of work experience, their hip rotator muscles tend to weaken. These correlations exhibited high statistical significance ($p < 0.001$). In both standing and sitting positions, there was a marked inverse relationship between work experience and the muscle power of knee joint flexors and extensors. This suggested that as employees gained more years of work experience, their knee muscles tend to become weaker. These correlations exhibited high statistical significance ($p < 0.001$). In both standing and sitting positions, there was a marked inverse relationship between

Table 6: The relation between work experience and MMT for long-term standing and sitting workers.

Muscles	Standing		Sitting	
	Correlation	*p*	Correlation	*p*
Hip Flexors	0.537	<0.001	0.444	<0.001
Hip Extensors	0.361	<0.001	0.638	<0.001
Hip Abductors	0.605	<0.001	0.406	<0.001
Hip Adductors	0.295	<0.001	0.743	<0.001
Hip Medial rotators	0.327	<0.001	0.694	<0.001
Hip Lateral rotators	0.574	<0.001	0.517	<0.001
Knee Flexors	0.391	<0.001	0.617	<0.001
Knee Extensors	0.286	<0.001	0.716	<0.001
Ankle Plantar flexors	0.397	<0.001	0.556	<0.001
Ankle Dorsiflexors	0.503	<0.001	0.707	<0.001

work experience and the muscle power of ankle joint plantar flexors and dorsiflexors. As employees accumulated more years of work experience, their ankle muscles tend to weaken. These correlations exhibited high statistical significance ($p < 0.001$) as shown in Table 6. These results emphasize the necessity of considering the effect of long-term work experience on lower limb muscle strength in individuals with extended exposure to standing and sitting positions.

Discussion

The proposed method enables physicians to evaluate medical conditions based on muscle strength using MMT. For strength conditioning programs and rehabilitative procedures, MMT is sufficient for quantifying muscle strength. Lower socio-economic status people could benefit from MMT. By delving into this topic, we can enhance workplace conditions and mitigate the negative consequences of prolonged sitting and standing.

Our findings on elevated hip muscle power (Grade 4-5) in both standing and sitting workers align with Reid and Fielding (2012) [15], who established skeletal muscle power as a critical determinant of physical functioning, declining faster than strength with age and strongly predicting mobility. Higher power supports postural demands in occupational settings, mirroring their evidence that leg extensor power better explains functional tasks like stair climbing and gait than strength alone.

These results also resonate with demonstration of Messier *et al*. (2005) [16] that weight loss proportionally reduces knee-joint loads, implying sustained muscle power in our cohorts mitigates hip/knee strain from prolonged postures. Unlike

their OA-focused older adults, our working population showed preserved power, potentially buffering ergonomic risks; however, contradictions may arise in aging workers, as power deficits precede overt disability. Future studies should explore interventions enhancing hip power for occupational health.

Our data also showed consistent negative correlations between age and strength across all lower limb movements in standing/sitting workers. These age-related declines mirror Neumann's (2016) [17] kinesiology framework, linking sarcopenia to reduced lower limb torque production and impaired gait/posture stability. Supporting evidence from this study showed quadriceps weakness emerging by middle age, marked hip abductor/adductor losses (34-56%), and ankle plantar flexor deficits. No major contradictions noted, emphasizing targeted training for occupational health. We also found strong negative correlations between years of work experience and muscle power across all lower limb movements in both standing and sitting workers ($p < 0.001$). These findings indicated cumulative occupational strain eroded lower limb strength over time. Consistent with Robbins and Waked (1997) [18], who linked prolonged vertical loading to balance deficits and muscle fatigue, our results extend this to hip/knee/ ankle power declines in static postures. Supporting Buckwalter and Martin (2006) [19] on osteoarthritis progression from repetitive stress, no contradictions noted; interventions targeting veteran workers are urged.

According to the results of the study, the mean age and weight of long-term standing workers were statistically significant. These results align with other studies demonstrating the positive impact of standing workstations on reducing sitting time and maintaining a healthy weight [20, 21]. The study also revealed that work experiences were significantly different for prolonged standing and sitting workers. Another research on the relationship between running and mental health found that exercise had a positive impact on mental health and well-being [22].

Using ergonomic principles by adjusting workstations, promoting proper posture, and providing ergonomic tools reduces these risks. Investing in ergonomic interventions not only safeguards employee health but also enhances productivity, demonstrating the imperative role of ergonomics in preventing occupational-related injuries. Prolonged standing and sitting both have negative impacts on musculoskeletal health. While prolonged standing can cause strain over lower back region and pain in the leg, prolonged sitting can lead to hip joint stiffness and reduced range of motion [23-24]. According to Serbest *et al*. (2015) [25], prolonged standing and sitting were related with negative effect on health. The study's findings indicated that promoting physical activity and reducing sedentary behavior in the workplace was vital for maintaining musculoskeletal health.

The study found that prolonged standing workers had significantly reduced lower limb joint range of motion in the knee and ankle, while prolonged sitting workers had higher muscle power in the knee and ankle. This aligns with prior research documenting adverse musculoskeletal effects of prolonged standing, including heightened risks of varicose veins, low back pain, and foot disorders [26, 27]. However, the research also found that prolonged sitting had negative health outcomes, such as obesity and cardiovascular condition [25]. In conclusion, our findings on posture-related hip muscle power, age/work experience declines, and occupational strain emphasizing lower limb adaptations. Prasetiowati *et*

al. (2017) [28] and Guzmán-Muñoz *et al*. (2023) [29] respectively linked body mass index and physical activities to impaired balance and relative hip/knee extensor weakness in upright postures, while the study by Mohan *et al*. (2017) [30] confirmed obesity-driven peripheral strength alterations relevant to prolonged standing/ sitting. These corroborate ergonomic needs, advocating targeted training for workers. Future interventions can mitigate risks, enhancing occupational health. The strengths of this study are that it provides insights into the impact of prolonged standing and sitting on lower-limb health. Thus, occupational health initiatives become essential interventions to promote physical activity and reduce prolonged sedentary behavior at work, and that the ergonomic needs of standing and sitting workers are also addressed. The study used MMT grades, which could be a reliable instrument for objectively evaluating muscle strength in rehabilitation settings.

However, the study has limitations too. First, the study was cross-sectional, which means limiting the generalizability of these findings to other populations or contexts. Moreover, the study did not account for potential confounders like age, gender, and physical activity levels, which could have affected the outcomes. Finally, the study relied on self-reported data, which may be subject to bias and inaccuracies. Although the study has some limitations, the results suggest that occupational health interventions should focus on promoting physical activity, reducing long lasting sedentary behavior, and addressing workers' ergonomic needs. People who maintain the same posture for a long time, like workers in the fields of construction, carrying heavy loads, automotive, agriculture and the army are highly exposed to fatigue and work-related injuries. This study reconsiders the occupational hazard as a threat to the alteration of lower limb muscle power which can be approached for prevention.

The study's future scope includes conducting prospective studies accounting for confounding variables, using objective measures of physical activity, and exploring the effectiveness of workplace interventions. These interventions could include ergonomic adjustments to workstations, promoting physical activity during work breaks, and encouraging employees to engage in regular exercise. Moreover, the study's findings may inform policy decisions intended to reduce sedentary behavior in the workplace and promote musculoskeletal health. Furthermore students, researchers, and investigators in different disciplines can integrate the understanding of occupational-related movement changes. Therefore, we should sensitize workers across multiple worksites about the effect of alterations in lower limb muscle power in the workplace and the risks associated with various occupations.

Conclusion

This study reveals significant decline in lower limb muscle power among workers maintaining prolonged standing or sitting postures, with notable associations between work position and hip muscle strength grades. Age and occupational tenure further exacerbate these deficits across hip flexors, extensors, abductors, adductors, rotators, knee flexors/ extensors, and ankle plantar/dorsiflexors. These findings underscore ergonomic hazards in static occupational settings, linking muscle imbalances to heightened musculoskeletal disorder risk. Targeted interventions —such as workstation modifications, periodic movement breaks, and strength training— are essential to preserve muscle function, improve postural stability, and safeguard worker health and productivity.

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ORIGINAL ARTICLE

Enhancement of regeneration and early vascular function as measured by SIRT1 activity, endothelial progenitor cell population, and nitric oxide expression following exercise training in adults

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Abstract

Background: Early endothelial dysfunction can begin in young adulthood and is characterized by reduced Nitric Oxide (NO) bioavailability and diminished endothelial regenerative capacity mediated by Endothelial Progenitor Cells (EPCs). Exercise may enhance vascular repair through Sirtuin-1 (SIRT1)–related pathways, but the optimal training intensity to maximize SIRT1 activity, EPC populations, and NO levels remains unclear. *Aim and Objectives:* To evaluate the effects of moderate- and high-intensity exercise on SIRT1 levels, EPC populations, and NO levels in young adults, compared with a control group, and to determine the optimal exercise intensity for vascular regeneration. *Material and Methods:* This interventional study included 45 young adults. The exercise intervention consisted of 24 sessions over eight weeks. Participants were equally assigned to three groups: moderate-intensity exercise (n = 15), high-intensity exercise (n = 15), and a control group that maintained usual daily activities (n = 15). Peripheral Blood Mononuclear Cells (PBMCs) were measured for SIRT1 activity using flow cytometry. EPCs were identified by the double expression of CD41 and CD62E, while NO expression was identified using fluorescence intensity. *Results*: This study showed a significant increase in SIRT1 activity ($p = 0.017$), followed by an increase in EPC percentage ($p < 0.001$) and NO expression ($p < 0.001$) after exercise training prescription. The study also demonstrated that moderate-intensity exercise increased SIRT1 activity, EPC populations, and NO levels compared with other intensity exercises. *Conclusion*: Exercise training improved endothelial regeneration and vascular function by increasing SIRT1 activity, EPC populations, and NO levels, with moderate-intensity being the best at increasing the three parameters.

Keywords: Aerobic exercise, endothelial progenitor cell, nitric oxide, Sirtuin1, vascular function

Introduction

Recent reports from the World Health Organization indicate that cardiovascular disease is the foremost cause of mortality globally, accounting for an estimated 17.9 million deaths each year [1]. This condition is driven by multiple risk factors, including diabetes, dyslipidemia, obesity, smoking, and physical inactivity [2, 3]. These exposures contribute to endothelial dysfunction, a key process in the

development, progression, and prognosis of cardiovascular disease. Modifiable risk factors are frequently present in adults, particularly when physical activity is lacking. Exercise is a practical, low-cost strategy for cardiovascular prevention; accordingly, structured exercise training is recommended to mitigate the harmful effects of these risk factors [4]. Although various clinical explanations indicate the role of exercise in improving vascular health, few studies have explained the molecular mechanisms of blood vessel regeneration and function. In addition, it is not known which exercise intensity best modulates these three parameters in increasing regeneration and vascular function.

Exercise intensity is a key factor in determining the physiological benefits of physical activity and can be categorized into low-, moderate-, and high-intensity, each corresponding to different heart rate zones. Low-intensity exercise, typically 50-60% of maximum heart rate, or ~ 90-110 beats per minute (bpm) for a 30-year-old, includes activities such as walking or gentle yoga, which slightly elevate heart rate and are ideal for beginners or recovery sessions [5]. Moderate-intensity exercise (60-70% of maximum heart rate, ~114-133 bpm for a 30-year-old), such as brisk walking, cycling, or swimming, noticeably increases heart rate and breathing and improves cardiovascular endurance and endothelial function [6]. High-intensity exercise, 70-85%+ of maximum heart rate, ~ 133-162+ bpm for a 30-year-old, including sprinting or High-Intensity Interval Training (HIIT), pushes the heart rate near its maximum, enhancing vascular adaptation, metabolic efficiency, and cardiorespiratory fitness [7]. Although all intensities provide health benefits, the optimal level for improving vascular regeneration and function remains under investigation, as molecular responses may vary. Tailoring exercise intensity based on the heart rate zones can help maximize cardiovascular protection and overall health [8].

This study postulated that Sirtuin-1 (SIRT1) is an early upstream mediator of exercise-induced vascular regeneration and improved vascular function. SIRT1 belongs to the sirtuin family and functions as a Nicotinamide Adenine Dinucleotide (NAD)-dependent histone deacetylase. It regulates metabolic processes in key vascular cell types, including endothelial cells, perivascular adipose tissue, and vascular smooth muscle cells. This mechanism increases glucose metabolism, mitochondrial activity, and the life span of cells. In contrast, low levels of SIRT1 cause failure of the endothelial cell survival mechanism, leading to cell damage and apoptosis, which can induce atherosclerosis [9]. Studies indicate that acute exercise transiently increases SIRT1 levels, particularly in skeletal muscle and endothelial cells, likely due to elevated NAD^+ availability and metabolic stress. Chronic exercise training, especially moderate to high-intensity aerobic and resistance exercise, has been shown to sustain higher SIRT1 concentrations, enhancing mitochondrial biogenesis, endothelial Nitric Oxide Synthase (eNOS) activation, and antioxidant defense [10]. The maintenance of normal endothelial cells also relies on their capacity for regeneration. Endothelial Progenitor Cells (EPCs) are stem/progenitor cells that can differentiate into endothelial cells and contribute to vascular repair by restoring blood flow and facilitating reperfusion in ischemic regions. EPC populations in the blood can indicate an individuals' ability to repair endothelial cells. [11].

Notably, SIRT1 appears to influence Nitric Oxide (NO) metabolism and EPC activity, which are crucial for vascular homeostasis. Endothelial dysfunction occurs when there is an imbalance

between vasodilator and vasoconstrictive factors. NO, a key vasodilator produced by endothelial cells via eNOS, regulates vascular tone, leukocyte migration, and blood coagulation. SIRT1 enhances eNOS activity by deacetylating and stabilizing the enzyme, thereby promoting NO bioavailability. Additionally, SIRT1 may support EPC function, which is vital for endothelial repair and angiogenesis [12]. These exercise-related alterations in SIRT-1 contribute to improved metabolic health, reduced inflammation, and delayed vascular aging, highlighting its potential role in preventing cardiovascular and metabolic diseases [13].

We hypothesized that moderate-intensity exercise would yield the most pronounced improvements in SIRT1 activity, EPC mobilization, and NO bioavailability because this intensity optimally balances metabolic stimulation with oxidative stress modulation. To investigate this, we examined how different exercise intensities affect SIRT1 expression, circulating EPC populations, and NO levels in young adults. By comparing these physiological markers across intensity groups, we aimed to identify the optimal exercise regimen to enhance vascular regeneration and endothelial function. The focus on young adults allowed us to minimize age-related confounders while elucidating the direct effects of exercise intensity on early vascular adaptation mechanisms.

Material and Methods

Ethical Clearance

The Ethics Committee of the Universitas Brawijaya, Faculty of Medicine, issued letter number No. 7168/UN10.F17.10.4/TU/2023 granting ethical approval. The participants signed an informed consent form to indicate their agreement to participate in the study, following the Helsinki Declaration.

Research design

This study employed an experimental pretest –posttest design with a control group to assess differences in SIRT1 activity, EPC percentage, and NO expression at baseline (pretest) and after the intervention (posttest). Changes in SIRT1, EPC, and NO were evaluated following completion of all training sessions over eight weeks in the moderate-intensity and high-intensity exercise groups, as well as in the control group (daily activities). Participants were allocated into three groups (n = 15 per group). This allocation followed the minimum sample size estimation using G*Power (effect size 0.8, $\alpha = 0.05$, three groups), which indicated a minimum of 10 participants per group.

Participants

The population of this research was students aged 19-30 years who live in the student dormitory of the Universitas Brawijaya. Participants were recruited through voluntary participation after they read the announcement of this research project in the dormitory. Eligibility criteria included nonsmoking status, being clinically healthy without medication use, and having normal body mass index (BMI), blood glucose, blood pressure, and cholesterol levels. A total of 45 eligible individuals were recruited, and participants were randomly allocated using a computer-generated randomization procedure to one of three groups: moderate-intensity exercise (n = 15), high-intensity exercise (n = 15), or control (usual daily activities; n = 15). Written informed consent was obtained from all participants, who indicated their willingness to adhere to the study procedures.

Participant age was verified using official student identification.

Non-smoking status was confirmed by salivary cotinine testing using an immuno-chromatographic assay, with concentrations of 0–10 ng/mL classified as non-smokers, and overall health status was checked via university clinic medical records. BMI was calculated as weight/height² (normal 18.5–24.9 kg/m²), glycemic status was assessed using fasting blood glucose (hexokinase method; normal < 5.55 mmol/L) and HbA1c (HPLC; normal 4.0%–5.6%), blood pressure was measured supine using a digital sphygmomanometer (three readings averaged; normal 110–120/70–80 mmHg), and total cholesterol was determined enzymatically (normal < 200 mg/dL).

Procedure

In this study, there were three groups of participants. Control group participants were students who were not given any exercise training. They carried out routine daily activities while studying on campus and living in dormitories without physical exercise or sports. The second and third groups included participants who underwent moderate- and high-intensity exercise, respectively. Exercises were performed for 30 min daily, three times a week for eight weeks. They received aerobic exercise training from a certified trainer and were supervised by sports medicine specialists. The moderate-intensity group performed 100 steps/min. Meanwhile, the high-intensity exercise group did more than 100 steps per minute. The trainer tracked the participant's steps using motion sensors. The participant's heart rates determined the intensity of the exercise. The formula 220 minus age was used to calculate the maximum heart rate. Moderate-intensity exercise was estimated at 65-75% of the maximum heart rate, and high-intensity was 76-96% of the maximum heart rate [14]. The participants used a smartwatch to calculate their maximum heart rate in beats per minute (bpm).

SIRT1 activity, EPC populations, and NO levels measurement: Flow cytometry

A 5 mL blood sample was drawn from the median cubital vein, and Peripheral Blood Mononuclear Cells (PBMCs) were isolated for SIRT1 activity assessment by flow cytometry after incubation with NAD and specific substrates at room temperature. EPC percentage was quantified by flow cytometry (Biology Laboratory, Universitas Brawijaya) using PerCP-conjugated anti-human vWF/CD41 (Bio-Legend; 303720) and PerCP-conjugated anti-human CD62E (BioLegend; 322606) after Ficoll-based PBMCs separation, washing, fixation/staining, and a 20-minute dark incubation before acquisition.

NO expression was quantified using an NO Assay Kit (Abcam Flow Cytometry – Red, ab219934). Whole blood samples were stained with a NO-sensitive red fluorescent dye and incubated at 37°C for 30 min. For experimental samples, 1 mM DA/NONOate in assay buffer was added prior to incubation, whereas control samples were processed without buffer addition. Flow cytometry analysis was performed using a flow cytometer with Ex/Em 610/620 nm excitation. Fluorescence intensity was measured in the PE/red channel emission, with positive events gated based on fluorescence intensity compared to the unstained controls. Data were collected for a minimum of 10,000 cellular events per sample, and the percentage of NO-positive cells was determined from the gated population. The 104 events threshold was established to ensure statistical reliability while maintaining analysis efficiency, as preliminary studies showed that this provided a <5% coefficient of variation between technical replicates.

Data analysis

The Statistical Package for the Social Sciences (SPSS) version 23 was used to analyze the data normality distribution of SIRT1 activity, EPC populations, and NO levels, which were tested using Shapiro-Wilk, with p-values of 0.607, 0.745, and 0.770, respectively. These results indicated that the data were normally distributed. All participants' characteristics were examined by One-way Analysis of Variance (ANOVA), except for sex, which was analyzed using Chi-Square. ANOVA was also used to determine the mean differences in SIRT1 activity, EPC populations, and NO levels during pre-exercise and post-exercise training. Finally, post-hoc analysis was performed using Least Significant Difference (LSD) to determine differences in mean SIRT1 activity, EPC population, and NO levels between groups.

Results

Baseline characteristics of participants

Study participants were assessed for age, sex, cotinine levels, weight, height, BMI, fasting blood glucose, blood pressure, and total cholesterol. All participants resided in the same dormitory facility to ensure similar daily activity patterns. Prior to the exercise intervention, participants commuted primarily by walking. During the study period, participants performed exercises according to their assigned intensity groups. As shown in Table 1, the baseline characteristics were homogeneous across all study groups. While minor age variations existed between the intensity groups (range: 19-30 years), all participants fell within the predetermined age range selected to account for optimal vascular regeneration capacity. This age range was specifically chosen because young adults typically demonstrate robust vascular repair mechanisms, which minimizes age-related variations in endothelial function and allows for a more precise assessment of exercise effects without significant confounding from age-related vascular changes. Table 1 confirms no significant differences in body weight (p = 0.165), BMI (p = 0.075), or other metabolic parameters between the groups, strengthening the validity of our exercise intervention results by reducing the likelihood of observed effects stemming from pre-existing group differences rather than the exercise protocols themselves.

Most participants were females (73.3%), and the number of male and female participants was distributed equally within the intensity groups (p = 0.376). Participants were also screened for a history

Table 1: Baseline characteristics of study participants in each group

Variable	Daily Activities (n=15)		Moderate Intensity (n=15)		High Intensity (n=15)		*p*
	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test	
Age (years)	22.8 ± 1.7	22.8 ± 1.7	24.2 ± 1.5	24.2 ± 1.5	25.0 ± 1.2	25.0 ± 1.2	<0.001* S
Gender (Male/Female)	3/12	3/12	3/12	3/12	6/9	6/9	0.376** NS

Continued...

Weight (kg)	56.8 ± 8.3	56.6 ± 10.5	54.3 ± 11.6	52.8 ± 12.2	58.6 ± 11.2	58.9 ± 12.1	0.165* NS
Height (m)	1.56 ± 0.05	1.56 ± 0.05	1.58 ± 0.05	1.58 ± 0.05	1.61 ± 0.06	1.61 ± 0.06	0.165* NS
Body Mass Index (kg/m²)	24.8 ± 4.2	24.8 ± 4.6	21.3 ± 3.4	21.3 ± 3.6	22.8 ± 3.8	22.8 ± 4.0	0.075* NS
Fasting Blood Glucose (mmol/L)	4.57 ± 0.45	4.57 ± 0.46	4.84 ± 0.51	4.79 ± 0.57	4.76 ± 0.49	4.65 ± 0.53	0.468* NS
Haemoglobin A1c (%)	5.0 ± 0.6	5.0 ± 0.6	4.9 ± 0.5	4.9 ± 0.5	4.8 ± 0.6	4.8 ± 0.6	0.711* NS
Blood Pressure (mmHg) Systolic	113.7 ± 1.8	113.7 ± 2.0	125 ± 2.9	115.6 ± 3.1	126 ± 2.3	114.6 ± 2.7	0.168* NS
Diastolic	80.7 ± 2.2	80.7 ± 2.4	80.2 ± 2.5	80.4 ± 2.7	80.3 ± 2.6	80.4 ± 2.6	0.689* NS
Total Cholesterol (mmol/L)	4.69 ± 0.11	4.74 ± 0.10	4.80 ± 0.10	4.72 ± 0.11	4.71 ± 0.12	4.76 ± 0.12	0.645* NS

S = Significant ($p < 0.05$)
NS = Not significant
**One-Way ANOVA test with the level of significance 5%*

of illnesses such as cardiovascular disease, metabolic disease or chronic disease. There were no significant differences in body weight ($p = 0.165$) and BMI ($p = 0.075$) across the intensity groups. To rule out diseases that could affect the results of this study, fasting blood glucose testing, haemoglobin A1c measurement, blood pressure measurement, and total cholesterol testing were conducted. These parameters were within the normal range before and after exercise training. There were no significant differences between the intensity groups, indicating that the division of the participant groups was homogenous. Statistically significant baseline characteristics between groups were needed to ensure that these factors do not contribute to differences in SIRT1 activity, EPC population, and NO levels in the study.

The mean SIRT1, EPC, and NO levels pre- and post-exercise training are shown in Table 2 and Figure 1-3. The data analysis showed a significant increase in SIRT1 activity in moderate and high exercise intensity groups but a slight decrease (0.5 ± 13.1) in the daily activity group. EPC populations significantly increased after exercise training ($p < 0.001$), showing that moderate-intensity exercise training had the highest average increase in EPC populations. Similar results were also observed for NO levels after the exercise training prescription. This study showed a significant increase in NO levels ($p < 0.001$), with the highest increase

Table 2: Difference in the number of Sirtuin 1 activity, EPC Populations, and NO Levels Measurement pre and post exercise training

Variable	Daily Activities (n=15)		Moderate (n=15)		High (n=15)		*p**	
	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test		
SIRT1 Activity	23.9 ± 5.6	23.4 ± 17.1	10.8 ± 5.8	17.7 ± 7.5	15.5 ± 5.3	21.3 ± 16.8	0.017	S
Δ (Post–Pre)	0.5 ± 13.1[a]		7.9 ± 7.3[b]		5.8 ± 20.3[c]			
EPC Populations	2.7 ± 0.7	3.7 ± 1.9	2.6 ± 1.6	6.4 ± 4.7	1.8 ± 0.9	3.0 ± 1.1	<0.001	S
Δ (Post–Pre)	0.9 ± 2.7[a]		3.9 ± 3.4[b]		1.3 ± 0.9[c]			
NO Levels	2.8 ± 0.5	5.2 ± 2.4	3.3 ± 0.9	8.7 ± 4.8	3.4 ± 2.2	6.3 ± 1.7	<0.001	S
Δ (Post–Pre)	2.4 ± 2.7[a]		5.4 ± 4.1[b]		2.9 ± 2.5[c]			

S = Significant (p < 0.05)
NS = Not significant
**One-Way ANOVA test with the level of significance 5%*

observed in moderate-intensity exercise. Table 2 shows moderate-intensity exercise consistently had more significant positive effects than daily or high-intensity exercise. In addition, the highest average increase in SIRT1 activity (7.9 ± 7.3), EPC populations (3.9 ± 3.4), and NO levels (5.4 ± 4.1) occurred in the moderate-intensity exercise group, indicating an optimal regeneration process and vascular function in the moderate-intensity exercise group.

Discussion

Roles of SIRT1 in improving regeneration and vascular function measured by EPCs and NO after exercise training in adults. Our research indicates that moderate-intensity exercise training significantly enhances SIRT1 activity (7.9 ± 7.3), EPC counts (3.9 ± 3.4), and increases NO levels (5.4 ± 4.1) ($p < 0.001$), outperforming both high-intensity and regular activity regimens. This corroborates previous findings in animal studies which have shown that exercise-induced activation of SIRT1 boosts defense against oxidative stress and extends these observations to human subjects by underscoring the importance of exercise intensity [14, 15]. The superior results in the moderate-intensity group indicate an optimal balance between metabolic activation and the mitigation of oxidative stress, suggesting that overly intense activities, such as high-intensity exercise, could hinder EPCs mobilization (2.1 ± 2.8 vs. 3.9 ± 3.4; p = 0.011) via ROS-mediated pathways [16]. The mechanistic role of SIRT1, particularly through the deacetylation of eNOS, reflected in elevated NO levels (Table 2), is likely central to its vascular benefits, which enhance vasodilation and promote endothelial cell survival. These findings resonate with prior research connecting SIRT1 to the upregulation of antioxidants driven by FOXO3 [17]. However, our study links these pathways to exercise intensity, providing a foundation for

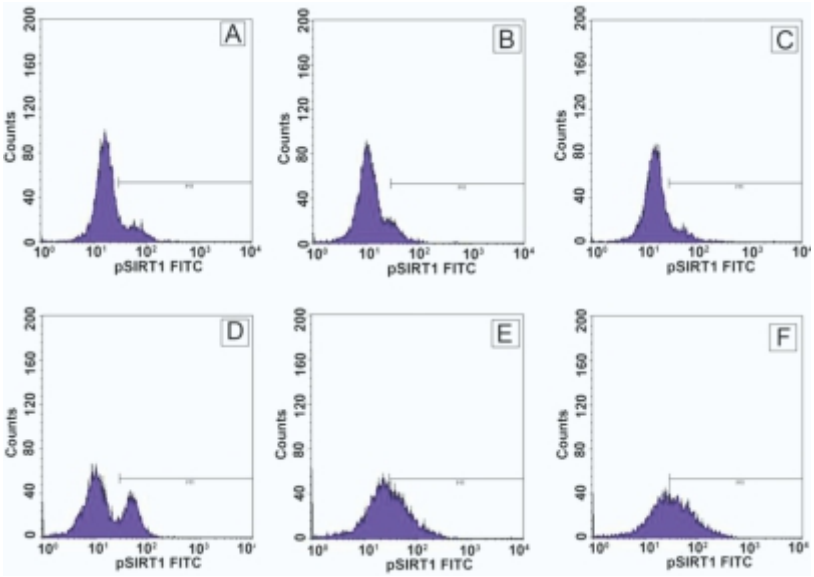

Figure 1: Gate view of Sirtuin 1 activity pre and post exercise training analysed using flow cytometry.

(A) Daily activity group pre-test, (B) moderate-intensity pre-test, (C) high-intensity pre-test, (D) daily activity post-test, (E) moderate-intensity post-test, and (F) high-intensity post-test.

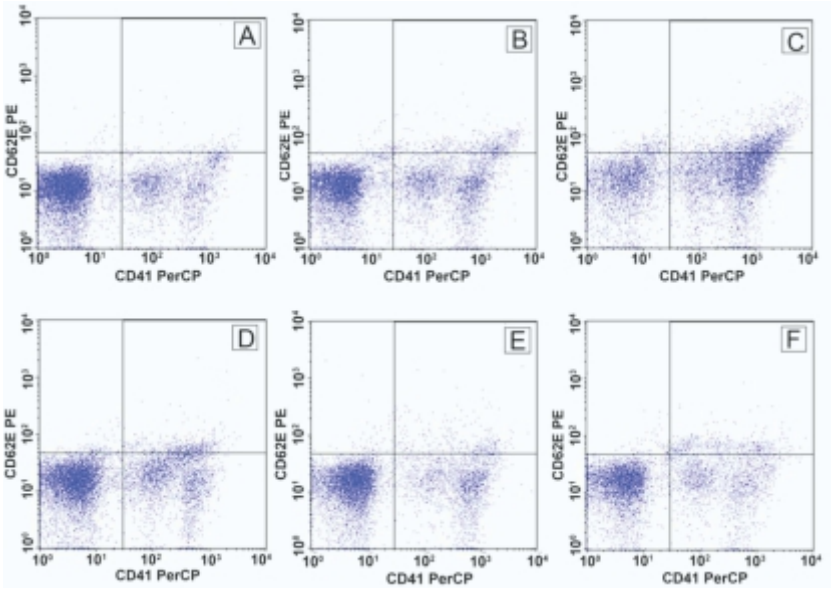

Figure 2: Gate view of endothelial precursor cell population pre and post exercise training analysed using flow cytometry.

(A) Daily activity group pre-test, (B) moderate-intensity pre-test, (C) high-intensity pre-test, (D) daily activity post-test, (E) moderate-intensity post-test, and (F) high-intensity post-test.

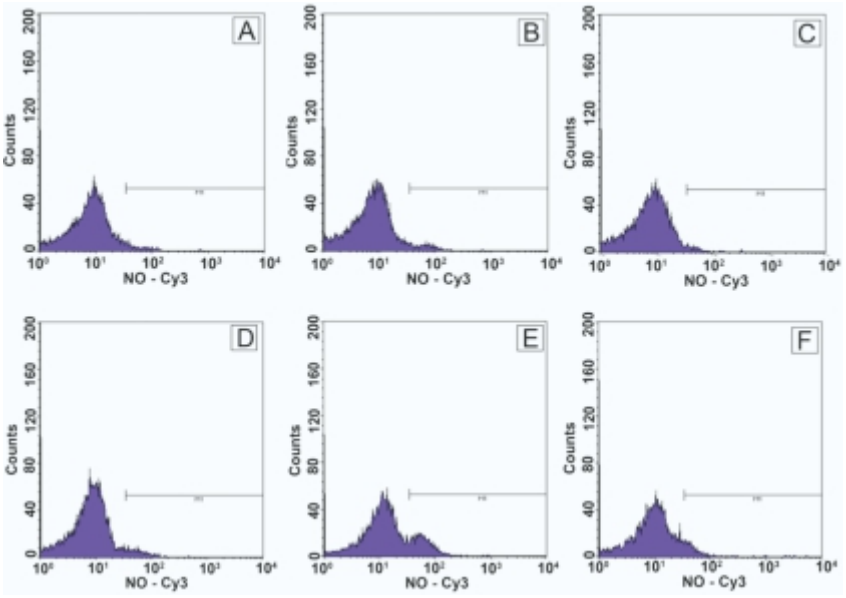

Figure 3: Gate view of nitric oxide levels pre and post exercise training analysed using flow cytometry.

(A) Daily activity group pre-test, (B) moderate-intensity pre-test, (C) high-intensity pre-test, (D) daily activity post-test, (E) moderate-intensity post-test, and (F) high-intensity post-test.

tailoring clinical exercise recommendations. Additionally, the detected SIRT1 activity in PBMCs underscores its systemic importance in vascular adaptation. The association of increased SIRT1 with reduced oxidative stress and lower inflammatory gene expression aligns with its function in deacetylating p53, which inhibits apoptosis and boosting FOXO3-mediated antioxidant responses. Although similar mechanisms have been observed in models of coronary artery disease [18], our study uniquely associated these beneficial effects with exercise-induced improvements in vascular health among healthy young adults. The interaction between SIRT1 and eNOS [19], which is crucial for NO production, may explain the maintained endothelial function in the moderate-intensity group. However, unlike previous studies focusing on aging or disease [35, 39], our results highlight SIRT1's sensitivity to regulated exercise stimuli, emphasizing its viability as a target for primary cardiovascular prevention.

EPC percentage improvement by exercise training

EPCs, originating from the bone marrow, play a pivotal role in vascular repair and prevention of atherosclerosis in response to conditions such as hypoxia, shear stress, and growth factors such as VEGF and SDF-1α [20, 21]. Heiss *et al*. demonstrated that EPC-mediated vascular homeostasis is age-dependent, with significantly reduced EPC survival, migration, and proliferation in older adults (61 ± 2 years) compared with younger individuals (25 ± 1 years) [21]. Vascular regeneration capacity varies based on multiple factors, particularly the extent and site of vascular damage [23]. In adult populations, this regenerative process primarily involves bone marrow-derived progenitor cells, specifically EPCs, which play a crucial role in endothelial repair and maintenance [23].

EPCs progress through distinct developmental stages: early EPCs (E-EPCs) are characterized by the expression of CD34, CD45, and CD31, whereas later stages (L-EPCs) exhibit increased levels of CD41, CD62E, and NO production, which facilitates their direct incorporation into the developing vasculature [24]. This transition from a signalling role to a structural function in endothelial repair is facilitated by L-EPCs promoting angiogenesis via HIF-1-driven pathways [21, 24]. Our research supports these processes, revealing that exercise prompts EPC mobilization ($p < 0.001$), with moderate-intensity exercise showing the most significant rise (3.9 ± 3.4 versus high-intensity: 2.1 ± 2.8; $p = 0.011$), likely due to optimal activation of HIF-1/VEGF without excessive oxidative stress [25].

Exercise is beneficial for neovascularization because it stimulates the synthesis of EPCs and their release from the bone marrow [21, 25]. Moderate-intensity exercise optimally recruits EPCs by balancing metabolic demands and oxidative stability. In contrast, high-intensity routines, such as marathon running, can compromise EPC survival through excessive production of free radicals, thereby reducing their migratory effectiveness [16]. In contrast, routine daily activities did not provide sufficient stimuli for significant EPC responses. These findings on the impact of exercise intensity underscore the need for well-adjusted exercise prescriptions to optimize EPC-mediated vascular repair, especially in individuals prone to endothelial dysfunction.

NO expression improvement by exercise training

Exercise training enhances endothelial function by augmenting shear stress, which stimulates NO production via eNOS activation. Regular exercise also upregulates antioxidant enzymes (e.g., superoxide dismutase), reducing free radicals that degrade NO into peroxynitrite ($ONOO^-$) [26, 27]. This dual mechanism, eNOS stabilization and oxidative stress mitigation, preserves NO bioavailability, as evidenced by our findings: a significant post-intervention NO increase ($p < 0.001$), particularly in the moderate-intensity group (5.4 ± 4.1 vs. other groups, $p = 0.003$). SIRT1 further amplifies this process by deacetylating eNOS [26], linking its activity to NO elevation. However, the excessive intensity may offset benefits through oxidative eNOS uncoupling [26], underscoring the need for balanced exercise regimens that optimize metabolic adaptation without overwhelming redox defense.

Endothelial dysfunction, marked by NO deficiency and proinflammatory factor dominance, drives atherosclerosis and cardiovascular disease [28, 29]. Our study demonstrated that exercise counteracts this by synergistically elevating EPCs and NO, key mediators of vascular repair. EPCs, mobilized by VEGF and SDF-1α during hypoxia [20, 21], integrate into the damaged endothelium, while NO promotes vasodilation and stem cell regulation [30]. Notably, moderate-intensity exercise maximized both EPCs (3.9 ± 3.4) and NO, highlighting its superiority in sustaining vascular health. These adaptations occur independently of traditional risk factors (e.g., lipids and BMI), emphasizing the direct role of exercise in preserving endothelial integrity and preventing cardiovascular pathology.

Limitations of the study

This study had several limitations that should be acknowledged. As a pilot investigation with a modest sample size, the findings may lack sufficient statistical power to detect subtle physiological effects. They may not be fully representative of broader population responses. The exclusive focus on young adults (19–30 years) further limits

generalizability, as age-related changes in vascular biology and the potential presence of comorbidities in older populations could substantially alter the observed relationships between exercise intensity and biomarkers of vascular regeneration (SIRT1, EPCs, and NO).

Methodologically, the absence of a non-exercise control group prevented definitive attribution of observed effects solely to the intervention, as natural biological fluctuations and unmeasured lifestyle factors could contribute to the outcomes. Although baseline anthropometric and metabolic parameters (e.g., BMI and blood glucose) were well balanced, the lack of standardized cardiorespiratory fitness assessments (e.g., VO_2 max) both before and after the intervention represents an important oversight, as baseline fitness levels may modulate individual responses to different exercise intensities.

Furthermore, potential confounding variables, including dietary patterns, sleep quality, and psychosocial stress, were not systematically controlled or measured, any of which could independently influence the biomarkers under investigation. Future research should benefit from larger-scale, longitudinal designs incorporating diverse age cohorts, comprehensive fitness profiling, and stricter control of lifestyle variables to enhance the validity and clinical applicability of the findings.

Conclusion

Moderate-intensity exercise significantly enhanced SIRT1 activity, EPC population, and NO levels, confirming its superiority in promoting vascular regeneration. High-intensity exercise showed lesser gains, likely due to oxidative stress, whereas daily activity had negligible effects. These findings suggest that moderate-intensity exercise is a non-pharmacological strategy for optimizing endothelial health in young adults.

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ORIGINAL ARTICLE

Evaluation of glycated haemoglobin and hepatitis B antigenic markers in people living with HIV in Ilorin, north-central Nigeria

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Abstract

Background: Metabolic and viral co-morbidities, particularly Hepatitis B Virus (HBV) infection and dysglycemia, are increasingly recognized among People Living with HIV (PLHIV) in sub-Saharan Africa. Antiretroviral Therapy (ART) improves survival but may alter glucose metabolism, while HBV co-infection exacerbates hepatic and metabolic complications. *Aim and Objectives:* To determine the prevalence of Hepatitis B surface Antigen (HBsAg) positivity and assess glycated haemoglobin (HbA1c) levels among HIV-positive patients on ART in Ilorin, North-Central Nigeria. *Material and Methods:* A case-control study was conducted among 150 participants (80 HIV-positive on ART, 70 HIV-negative controls). HIV status was confirmed serologically. HBV antigenic markers were identified using commercial immunoassay kits (Rapid Labs, UK). HbA1c was determined by cation-exchange chromatography. Diabetes and prediabetes were defined using ADA criteria: HbA1c $\geq$ 6.5% and 5.7-6.4%, respectively. Data were collected by trained laboratory scientists under faculty supervision and analyzed with SPSS v25 using t-tests and ANOVA (significance set at $p < 0.05$). *Results:* The mean HbA1c was similar between HIV/HBV co-infected, HIV-only, and control groups ($p = 0.162$). HIV patients on ART for < 1 year had significantly higher HbA1c (5.01 ± 0.84%) than those on ART > 1 year (4.56 ± 0.99%) ($p = 0.031$). The prevalence of HIV/HBV co-infection was 35%. No HIV patient met the HbA1c threshold for diabetes. *Conclusion:* HIV/HBV co-infection was common, while HbA1c values suggested that HbA1c may underestimate glycemia in PLHIV. Strengthening HBV screening and using glucose-based tests for glycemic monitoring are essential for improved patient management.

Keywords: HIV, Hepatitis B, glycated Haemoglobin (HbA1c), antiretroviral therapy, Nigeria, co-infection

Introduction

Antiretroviral Therapy (ART) has markedly improved survival and quality of life among people living with HIV [1, 2]. However, as HIV infection transitions into a chronic condition, the long-term complications of treatment and co-infections have emerged as major health challenges. Among these, metabolic disorders such as dysglycemia and chronic viral hepatitis are particularly important

because they significantly contribute to morbidity and mortality [3, 4]. HIV-infected individuals are at increased risk of developing Diabetes Mellitus (DM), partly due to the metabolic effects of ART. Large cohort studies have shown that cumulative ART exposure increases the incidence of DM, with HIV-infected populations having up to a two-fold higher risk compared to the general population [5-7]. Poor glycemic control further raises the risk of cardiovascular disease, renal impairment, and dyslipidemia, making routine monitoring essential [4,8]. Glycated Hemoglobin (HbA1c) is widely used to assess glycemic control and diagnose DM [9], though concerns have been raised about its accuracy in HIV-infected patients due to factors such as altered red blood cell turnover and haemolysis [10,11]. At the same time, Hepatitis B Virus (HBV) co-infection is common among people living with HIV because of shared transmission routes [12]. Globally, about 10% of HIV-infected individuals are estimated to be co-infected with HBV, with prevalence rates in sub-Saharan Africa reaching 15–60% in some populations [13-14].

HIV/HBV co-infection accelerates liver disease progression, increases HBV replication, and raises the risk of cirrhosis and hepatocellular carcinoma compared to HBV infection alone [15-17]. Nigeria, where both HIV and HBV are highly prevalent, faces a particular burden of these overlapping infections [18, 19]. Despite the recognized risks, there are limited data from Nigeria on the combined burden of dysglycemia and HBV co-infection among HIV patients receiving ART. Understanding these risks is essential for designing effective screening, preventive, and treatment strategies in resource-limited settings.

HIV and HBV co-infection is common in sub-Saharan Africa due to shared transmission routes, leading to increased liver-related morbidity and mortality [12, 15-17]. Concurrently, ART has been linked to metabolic complications such as dysglycemia [5-7]. Despite these dual risks, few Nigerian studies have examined both metabolic and viral co-morbidities in HIV patients on ART. This study therefore aimed to determine the prevalence of HBV co-infection and evaluate HbA1c levels among HIV-positive patients on ART in Ilorin, Nigeria, to assess potential interactions between ART duration, HBV co-infection, and glycemic status.

Material and Methods

Study area

The study was conducted at the HIV clinic of Sobi Specialist Hospital, Ilorin, Kwara State, Nigeria. Ilorin is a major city in north-central Nigeria with a population engaged mainly in commerce and small-scale industry [20].

Ethical considerations

Ethical approval was obtained from the Ethics and Research Committee of the Kwara State Ministry of Health, Ilorin, Nigeria, and the management of Sobi Specialist Hospital. Written informed consent was obtained from all participants, and confidentiality was maintained throughout the study.

Study design and participants

This was a comparative case-control study conducted over three months. A total of 150 participants were recruited by random sampling: 80 HIV-positive patients on ART and 70 apparently healthy controls. The HIV-positive group was further divided into patients on ART for less than one year (n = 40) and those on ART for more than one year (n = 40).

Operational definitions

Diabetes was defined as HbA1c $\geq$ 6.5%, prediabetes as 5.7-6.4%, and normal glycemia as < 5.7% according to the American Diabetes Association

criteria [11]. HBV positivity was defined as serologic detection of HBsAg. HIV-positive status was confirmed by parallel rapid testing using Determine™ HIV-1/2 and Uni-Gold™ kits per national guidelines.

Inclusion criteria: HIV-seropositive patients aged 18–50 years receiving ART for at least one year (experienced) or less than one year (naïve).

Exclusion criteria: Pregnancy, extremes of age, HIV/TB co-infection, use of anticoagulants, cytotoxic chemotherapy, or radiotherapy, as well as self-reported conditions that might alter immune status.

Sample size determination

The minimum sample size was calculated using the Cochran Sample Size Formula:

$$N = \frac{Z^2 \times P\,(1-P)}{d^2}$$

where P = prevalence of HIV/HBV co-infection in Nigeria (10%) [21], d = desired precision (0.05), and Z = 1.96 at 95% confidence interval.

$$N = \frac{1.96^2 \times 0.10\,(1-0.10)}{0.05^2}$$

$$N = \frac{3.8416 \times 0.09}{0.0025}$$

The calculation yielded 138, which was rounded up to 150 to improve statistical power.

Sample collection and laboratory analysis

Venous blood (2 mL) was collected into EDTA tubes. HbA1c was measured using the A1c cation exchange method on a Beckman Coulter Chemistry Analyzer (Beckman Coulter, USA). HIV screening was performed only for the control group to confirm HIV-negative status using Determine™ HIV-1/2 (Abbott Laboratories, USA), with reactive samples confirmed using Uni-Gold™ HIV (Trinity Biotech, Ireland), following national testing algorithms [22, 23]. HBV infection was screened using a rapid HBsAg Bioline™ Hepatitis test kit, and positive samples were further evaluated with a multi-marker HBV rapid test (detecting HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc) according to manufacturer's instructions.

Statistical analysis

All samples and data were collected by certified medical laboratory scientists under supervision of senior faculty from the Department of Medical Laboratory Science, Kwara State University, Malete. Data integrity and test reliability were ensured through double-entry verification and daily calibration of analytical instruments. Socio-demographic and clinical data were obtained through interviewer-administered questionnaires. Data were analysed using the Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM Corp., USA). Descriptive statistics were used to summarize participant characteristics. Differences between groups were tested using Student's t-test and one-way ANOVA where appropriate. Pearson's correlation coefficient was applied for relationships between continuous variables. Statistical significance was set at $p < 0.05$.

Results

Participant characteristics

A total of 150 participants were included in the study: 80 HIV-positive patients on ART and 70 HIV-negative controls. The mean age of HIV patients was 39.7 ± 13.1 years, significantly higher than that of controls (29.2 ± 12.6 years; $p < 0.001$). Females predominated among HIV patients (67.5%), while males predominated among controls (48.6%), a difference that approached statistical significance ($p = 0.05$).

Coexistence of HIV, HBV and glycaemic status

Table 1 presents a summary of demographic and biochemical findings. The mean HbA1c did not

differ significantly among HIV-only patients (4.58 ± 0.59 %), HIV/HBV co-infected patients (4.63 ± 0.81 %), and HIV-negative controls (4.86 ± 1.00 %) (p = 0.162). The proportion of participants in the pre-diabetic HbA1c range (≥ 5.7 %) was higher among HIV-infected individuals (21.3 %) than in controls (11.4 %), but the difference was not statistically significant (p > 0.05). When ART duration was considered, patients on therapy for < 1 year had significantly higher mean HbA1c (5.01 ± 0.84 %) than those on ART > 1 year (4.56 ± 0.99 %) (p = 0.031), suggesting an early treatment-related rise in glycemia that stabilised over time.

Table 1: Coexistence of HIV, HBV, and HbA1c status

Parameter	HIV Only (n=52)	HIV + HBV (n=28)	Controls (n=70)	*p*
Age (years, mean ± SD)	39.4 ± 13.2	40.1 ± 12.9	29.2 ± 12.6	<0.001
Sex (Male %)	32.5	30.2	48.6	0.05
HbA1c (%) ± SD	4.58 ± 0.59	4.63 ± 0.81	4.86 ± 1.00	0.162
HbA1c ≥ 5.7 % (Prediabetic)	17 (21.3 %)	0 (0 %)	8 (11.4 %)	-
ART < 1 year	26 (50 %)	14 (50 %)	-	0.031

HIV – Human immunodeficiency virus; HBV – hepatitis B virus; HbA1c – glycated haemoglobin; ART – antiretroviral therapy

Table 2: Distribution of Hepatitis B markers by ART duration

ART Duration	HBsAg+	HBsAb+	HBeAg+	HBeAb+	HBcAb+	Total HBV+ (%)
< 1 year	19	0	0	14	19	67.9
> 1 year	9	0	0	3	9	32.1
Total (n=28)	-	-	-	-	-	100

HBsAg = hepatitis B surface antigen; HBsAb = hepatitis B surface antibody; HBeAg = hepatitis B envelope antigen; HBeAb = hepatitis B envelope antibody; HBcAb = hepatitis B core antibody; ART – antiretroviral therapy

Discussion

This study examined the coexistence of HBV co-infection and HbA1c levels among people living with HIV receiving ART in Ilorin, North-Central Nigeria. The analysis showed a high prevalence of HIV/HBV co-infection and a modest rise in HbA1c during early ART exposure, with normalization over time. These findings have important implications for metabolic monitoring and integrated management of viral co-infection in HIV patients.

Glycemic status and ART duration

No participant met the diagnostic threshold for diabetes (HbA1c ≥ 6.5%), and only about one-fifth of HIV patients were in the prediabetic range. The mean HbA1c levels did not differ significantly

between HIV-only, HIV/HBV co-infected, and HIV-negative participants, suggesting that HBV co-infection did not significantly affect glycemia. However, patients on ART for less than one year had significantly higher HbA1c than those on therapy for more than one year (p = 0.031). This pattern implied an early metabolic effect of ART initiation, which appeared to attenuate with prolonged therapy. Similar transient dysglycemic responses have been reported in other cohorts, particularly with Nucleoside Reverse Transcriptase Inhibitor (NRTI)-based regimens [7–9,]. The reduction of HbA1c values after extended ART use could reflect adaptation of glucose metabolism or regimen changes minimizing mitochondrial toxicity [10, 11]. The absence of overt diabetes in this cohort supported earlier observations that HbA1c tends to underestimate glycemia in people living with HIV due to shortened red blood cell lifespan, haemolysis, or macrocytosis. This finding reaffirmed the need for periodic fasting glucose or oral glucose tolerance testing in this population, as recommended by clinical guidelines.

HIV/HBV Co-infection and ART

The prevalence of HIV/HBV co-infection in this study (35%) was higher than previous Nigerian reports ranging between 10% and 20% [16-18]. Such variation may result from differences in diagnostic sensitivity, sampling frame, or regional endemicity. All co-infected patients were HBsAg positive and HBsAb negative, reflecting incomplete immune clearance and chronic infection. The absence of HBeAg with persistent HBcAb positivity suggested a low-replicative state, consistent with earlier observations of immune-tolerant or inactive carrier phases in HIV co-infection [13, 15].

Interestingly, HBV co-infection was twice as frequent among participants on ART for less than one year compared with those on ART for longer durations. This pattern might indicate immune reconstitution unmasking occult HBV in the first months of ART or gradual suppression of viral replication with sustained treatment [12,13]. Reduced HBeAb positivity in patients with longer ART exposure further supported the hypothesis of partial viral suppression.

Clinical and public health implications

The concurrent burden of metabolic and viral co-morbidities among people living with HIV underscores the importance of comprehensive monitoring. The data suggested that early ART may transiently disturb glucose regulation, while prolonged ART appeared to confer hepatic and metabolic stability. Routine HBV screening, vaccination for susceptible individuals, and periodic assessment of glycemia are therefore critical components of HIV care in Nigeria and similar settings [21, 22, 24-26].

Study limitations

This study was cross-sectional and could not establish causal relationships between ART duration, metabolic changes, and HBV infection patterns. The reliance on HbA1c alone to assess glycemia might have underestimated true glucose intolerance, and the absence of molecular HBV testing limited full characterization of viral replication. Nonetheless, rigorous laboratory procedures, adequate sample size, and internal validity strengthen the credibility of the findings.

Strengths of the study

This study was among the few to simultaneously examine HBV co-infection and glycemic status in Nigerian HIV patients using standardized assays and verified data collection protocols. The inclusion of ART duration provided new insights into the temporal interaction between therapy and metabolic

response, while regional focus contributed valuable epidemiological data for Ilorin and the wider North-Central zone.

Implications

Despite the limitations, our findings provide important local data. Routine use of HbA1c in HIV patients should be interpreted cautiously, and confirmatory glucose-based testing is advisable. The high prevalence of HIV/HBV co-infection highlights the need for expanded HBV vaccination, routine screening, and coordinated management strategies. Together, these interventions could reduce long-term cardiovascular and liver-related complications in HIV populations in Nigeria.

Conclusion

HBV co-infection was prevalent among HIV-positive patients on ART in Ilorin, affecting over one-third of participants. HbA1c levels indicated a low prevalence of diabetes and no significant difference between HIV-only and HIV/HBV co-infected patients. However, patients within the first year of ART had significantly higher HbA1c levels compared to those on longer treatment. These findings suggest that HBV co-infection did not influence glycemic status, while early ART exposure was associated with transient elevations in HbA1c.

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ORIGINAL ARTICLE

Iron status and total iron-binding capacity in patients with essential hypertension: A case-control study

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Abstract

Background: Essential hypertension is a multifactorial disease with an unknown cause. Despite extensive research, the underlying mechanisms contributing to essential hypertension are not fully understood. Recent studies have explored various physiological factors that may influence blood pressure regulation, including disturbances in mineral metabolism. Iron, a crucial element in various physiological processes, has been implicated in blood pressure regulation. Abnormal iron metabolism, measured by Total Iron-Binding Capacity (TIBC), has also been explored. Emerging evidence suggests that altered iron metabolism could play a role in the pathogenesis of hypertension. *Aim and Objectives:* This case-control investigates the association between iron and TIBC in essential hypertension patients. *Material and Methods:* The study involved 100 participants, 50 individuals with hypertension and 50 healthy controls, selected based on specific inclusion and exclusion criteria. Clinical histories were obtained from each participant using a data collection form. Blood pressure measurement was done as per the Eighth Joint National Committee guidelines followed by anthropometric measurements as per the World Health Organization STEPwise approach to non-communicable risk factor surveillance guidelines. Biochemical parameters such as iron and TIBC were estimated by a semi-auto analyser using a commercially available kit. *Results:* Mean iron levels were significantly increased and TIBC levels were significantly reduced (179.26 ± 53.47 vs 136.90 ± 38.89; $p < 0.001$, 167.09 ± 80.03 vs 261.33 ± 34.41; $p < 0.001$ respectively) in cases compared to controls. A positive association was found between iron and systolic blood pressure in essential hypertension cases and an inverse association was found between iron and TIBC significantly predicting essential hypertension ($p < 0.001$). Conclusion: The study revealed significant differences in iron metabolism between patients with essential hypertension and healthy controls, suggesting a disruption in iron homeostasis. Elevated iron levels may contribute to oxidative stress and endothelial dysfunction, while reduced TIBC may indicate an imbalance in iron homeostasis or an adaptive response to changes in iron availability. Understanding these associations could pave the way for novel approaches to managing and treating essential hypertension.

Keywords: Essential hypertension, total iron binding capacity, systolic blood pressure, diastolic blood pressure

Introduction

Hypertension is a prevalent medical condition characterized by a persistent increase in arterial pressure [1]. It possesses a significant risk for myocardial infarction, heart failure, stroke, and renal failure [2]. Up to 45% of individuals are affected, with over one billion adults affected globally [3]. The prevalence is constant across all socioeconomic and income levels, making up as much as 60% of the population who are 60 or older [3]. According to the latest estimates, there may be 15–20% more individuals with hypertension by 2025, bringing the total number of patients to approximately 1.5 billion [4].

Hypertension, also known as essential hypertension, is primarily idiopathic and increases with increased salt intake [5]. The genetic ability to

respond to salt is a significant factor in developing essential hypertension [6,7]. Around 50-60% of patients are salt-sensitive, leading to hypertension [8]. Obesity and insulin resistance are major risk factors that contributes to cardiovascular complications. Obesity leads to the accumulation of dietary fatty acids in adipose tissue, causing a saturated storage capacity. This results in lipid spillover to lean tissues like the liver and muscles, leading to inflammation and insulin resistance. This combined state of inflammation and ectopic fat deposition is a significant risk factor for cardiovascular complications [9]. Additionally, recent studies have highlighted disruptions in iron homeostasis as a potential factor in the heightened risk of hypertension, though the role of iron in the development of hypertension remains uncertain [10].

Iron is essential for maintaining physiological balance; nonetheless, excessive iron can cause free radical damage, resulting in tissue damage [11]. Iron storage and blood pressure are not completely understood [12, 13]. It was recently found that an increase in iron levels upregulates oxidative stress, which contributes to the development of hypertension. Experimental studies have further demonstrated that iron-catalysed free radicals cause endothelial damage and eventually lead to an increase in blood pressure [14]. Dietary iron restriction can reduce oxidative stress in the aorta, potentially reducing high salt-induced hypertension and cardiovascular remodeling [11]. Iron deficiency and overload can increase reactive oxygen species, oxidative stress, inflammation, and endothelial function, increasing blood pressure [12]. Thus, one of the major independent factors leading to the development of hypertension is the dysregulation of iron metabolism [13]. Despite a considerable amount of research that has focussed on linking iron and the Total Iron Binding Capacity (TIBC) with essential hypertension, the results were inconsistent.

Material and Methods

Study design

The case-control study was conducted in the Department of Biochemistry at Integral Institute of Medical Sciences and Research (IIMS&R), Lucknow, India. The Institutional Ethics Committee of IIMS&R, Lucknow, Uttar Pradesh, approved human participant enrolment and blood sample collection (IEC No.: IEC/IIMS&R; R/2023 /66) following the ethical standards of the 1964 Helsinki Declaration and its amendments. Clinical histories were obtained from each participant using a data collection form. A written informed consent was obtained from each participant in the study.

Sample size estimation and sampling method

The sample size was calculated using the formula

$$\frac{r+1}{r}\frac{(SD2)Z\beta+2\alpha/2)2}{\alpha 2}$$

where r = ratio of control to cases, 1 for an equal number of cases and control, Z_{β} = standard normal variate for power = for 80% power it is 0.84. $Z_{\alpha/2}$ = standard normal variate for a level of significance as mentioned in the previous section, d^2 = expected mean difference between case and control may be based on previously published studies. Non-probability purposive sampling method was used to identify the study population. A total of 100 participants, 50 hypertension cases, and 50 healthy controls were selected based on specific inclusion and exclusion criteria.

Inclusion and exclusion criteria for study subjects

The study included individuals aged 30-60 years with essential hypertension as per the Eighth Joint

National Committee (JNC 8) guidelines [15], and healthy individuals aged 30-60 as controls. Individuals having a history of iron-deficient anemia, pregnant or lactating women, and those suffering from acute or chronic diseases were excluded from both cases and controls.

Blood pressure measurement

Blood pressure measurement was done as per JNC 8 guidelines. Patients were asked to sit quietly for a few moments ensuring that the patient was relaxed before taking the measurements. Three readings were recorded and an average of the two readings was considered for the final blood pressure readings [16].

Anthropometric measurements

Body Mass Index (BMI) was determined using the formula: body weight (kg) / height (m^2). Measurements of weight and height for BMI estimation followed the World Health Organization STEPwise approach to noncommunicable disease risk factor surveillance (STEPS) guidelines [17].

Laboratory investigations

Sample collection and serum separation

Each participant in the study had 2 mL of venous blood drawn, which was then centrifuged at 3000 rpm for 5 minutes to separate the serum, which was then used to measure iron and TIBC levels.

Estimation of iron and TIBC

Iron, as well as TIBC levels, were estimated using a commercial kit and ERBA CHEM 7 semiauto-analyzer machine, using the ferrozine/magnesium carbonate method.

Statistical analysis

The statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software version 20.0 (IBM, USA) and GraphPad Prism 2023 (GraphPad Software, Boston, MA, USA). The findings were reported as mean ± Standard Deviation (SD). An unpaired student's t-test was performed to compare research parameters in the case and control groups. Pearson's correlation coefficient and regression analysis were used to determine the relationship between variables. A value of $p < 0.05$ indicated statistical significance.

Results

Table 1 shows a significant increase in mean levels of Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), and iron in essential hypertension cases compared to healthy controls. TIBC levels were significantly reduced in these cases compared to controls (179.26 ± 53.47 vs 136.90 ± 38.89; $p < 0.001$, 167.09 ± 80.03 vs 261.33 ± 34.41; $p < 0.001$ respectively). Age and BMI were also increased in cases, although not statistically significant. A significant positive association between iron and SBP and an inverse association between iron and TIBC in essential hypertension cases is shown in Table 2; Figures 1 and 2. Regression analysis indicated that iron and TIBC were associated with essential hypertension. Model 1 shows the regression analysis of TIBC ($F(5,44) = 6.426$, $p < 0.001$). $r^2 = 0.422$ depicts that model 1 explains 42.2% of the variation in hypertension whereas model 2 shows the regression analysis of iron ($F(5,44) = 11.132$, $p < 0.001$). $r^2 = 0.558$ depicts that model 2 explains 55.8 % of the variation in hypertension illustrated in Table 3.

Table 1: Characteristics of subjects

Anthropometric and biochemical parameters	Cases (N=50) Mean ± S. D	Controls (N=50) Mean ± S. D	*p*
Age (Years)	46.88 ± 9.21	43.88 ± 9.46	0.111
BMI (Kg/m^2)	24.68 ± 3.53	23.70 ± 3.17	0.147
SBP (mmHg)	157.64 ± 17.21	120.3 ± 4.77	<0.001
DBP (mmHg)	93.08 ± 8.02	80.84 ± 4.73	<0.001
Iron (µg/dl)	179.26 ± 53.47	136.90 ± 38.89	0.001
TIBC (µg/dl)	167.09 ± 80.03	261.33 ± 34.41	<0.001

***Statistically significant at 0.01 level (2-tailed), p<0.01, ∗Statistically significant at 0.05 level (2-tailed), p<0.05, BMI is body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure: TIBC: total iron binding capacity*

Table 2: Correlation between variables in cases

Parameters	BMI	Age	SBP	DBP	Iron	TIBC
BMI (Kg/m^2)	1	0.156	**0.535****	271	276	0.030
Age (Years)	-	1	0.124	0.052	0.251	-0.021
SBP (mmHg)	-	-	1	0.301	**0.500****	-0.204
DBP (mmHg)	-	-	-	1	0.203	-0.022
Iron (µg/dl)	-	-	-	-	1	**-0.603****
TIBC (µg/dl)	-	-	-	-	-	1

***Statistically significant at 0.01 level (2-tailed), p<0.01, ∗Statistically significant at 0.05 level (2-tailed), p<0.05, BMI is body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure: TIBC: total iron binding capacity*

Table 3: Regression analysis model

Model	r	r^2	Adjusted r^2	Std. Error of the Estimate	Change Statistics				
					r^2 Change	F Change	df1	df2	Sig. F Change
1	0.650[a]	0.422	0.356	64.20847	0.422	6.426	5	44	0.001
2	0.747[a]	0.558	0.508	37.49153	0.558	11.132	5	44	0.001

1. Predictors: (Constant), iron, diastolic blood pressure, body mass index, age, systolic blood pressure; Dependent variable- total iron binding capacity

2. Predictors: (Constant), total iron binding capacity, body mass index, age, systolic blood pressure, diastolic blood pressure; Dependent Variable- Iron

**p<0.05 is considered significant.*

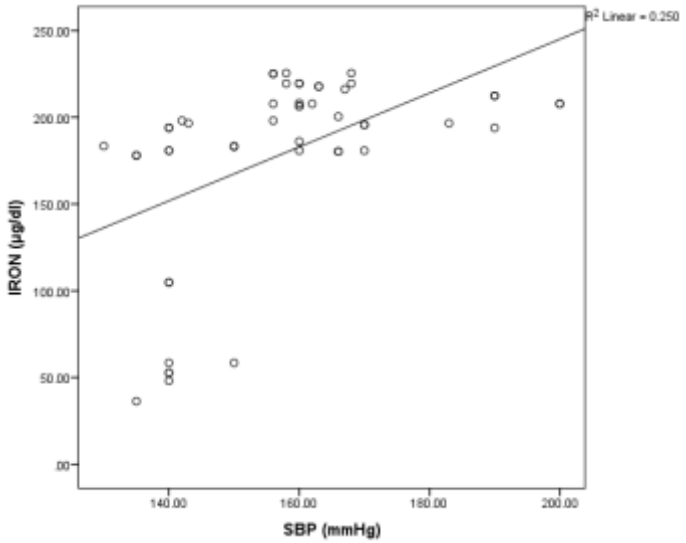

Figure 1: Relationship between systolic blood pressure and iron in cases

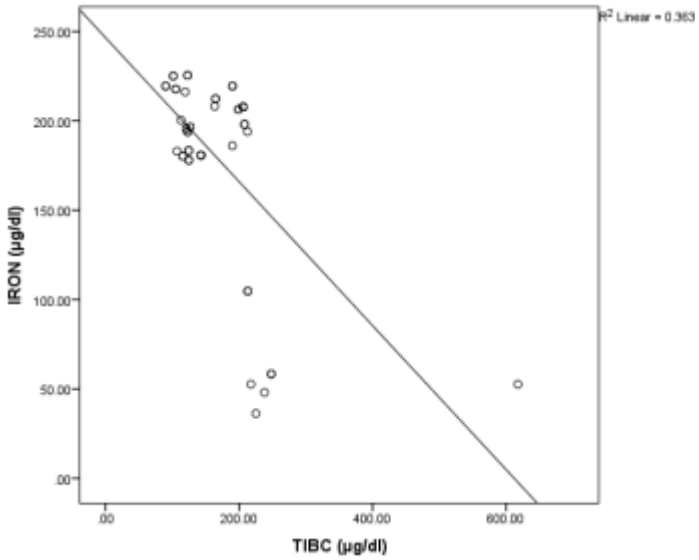

Figure 2: Relationship between iron and TIBC in cases

Discussion

Hypertension remains a significant global public health issue. Increased serum iron levels and reduced TIBC can lead to the production of free radicals and the process of lipid peroxidation, both of which are linked to a higher risk of cardiovascular disease, a leading cause of illness and death worldwide [18]. The study observed that patients with essential hypertension had higher serum iron levels and lower TIBC than controls. Iron was found to have a positive relationship with SBP and an inverse relationship with TIBC in cases with essential hypertension. According to regression analysis, the iron profile, including iron and TIBC, may predict essential hypertension and its compli-

cations. Numerous studies have shown that changes in levels of iron and TIBC in the blood have negative effects [18]. The generation of reactive oxygen compounds, which leads to inflammation as well as oxidative stress has negative effects on the functioning of mitochondria and results in hypertension, which may be influenced by iron overload [19].

Elevated body iron levels can lead to stress from oxidation that may transform less reactive free radicals into more reactive forms such as hydroxyl radicals (OH), hydroxide radicals (OH), & (O_2^-) superoxide anions, which may contribute to the development of hypertension and cardiovascular diseases [20]. The formation of Reactive Oxygen Species (ROS), which increases inflammation and oxidative stress and may lead to an increase in arterial blood pressure, may be influenced by excess iron and inadequate iron levels [21]. The combination of Low-Density Lipoprotein (LDL) and isoprostane oxidation, which act as indicators for oxidative stress, can cause endothelial damage from excessive amounts of iron, which can lead to the development and progression of atherosclerosis. Following is the atherosclerosis process, which could raise the possibility of hypertension [22]. Early work by Kiechl *et al*. 2007) found that cardiovascular disease and body iron levels were positively associated [23]. Another study on the topic by Sempos *et al*. (2004) asserts no association between stored iron and high blood pressure [24]. Similarly, Rauramaa et al. (1994) also reported an insignificant correlation between iron reserves and heart disease [25]. A recent work conducted by Ruiter *et al*. (2011) found that TIBC levels were increased and iron levels were decreased in patients with hypertension and may also show a positive correlation [26]. Likewise, Kim *et al*. (2012) in their study found that the emergence of hypertension was positively correlated with increased iron and decreased TIBC [27].

Previous studies have certain limitations, such as the fact that the inclusion and exclusion criteria differed in these studies, with varying age and ethnicity groups. This study does not yield consistent findings regarding the essential hypertension risk and the link between an iron profile and essential hypertension. To confirm the findings, more longitudinal and clinical studies with larger sample sizes controlled for confounding factors are recommended, including removing smoking and alcohol.

Conclusion

This study found that patients with essential hypertension had significantly higher iron levels and lower TIBC compared to healthy controls. This suggests that iron metabolism may be disrupted in essential hypertension. The positive association between iron levels and SBP in hypertensive patients, along with the inverse relationship between iron and TIBC, suggests iron dysregulation may play a role in the pathophysiology of essential hypertension. Iron and TIBC are valuable biomarkers or therapeutic targets in hypertension management, but further research is needed to understand the mechanisms and therapeutic implications.

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ORIGINAL ARTICLE

"They still think that I don't know enough about myself"- lived experiences of mental health distress among sexual minority women in Maharashtra, India

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Abstract

Background: Sexual Minority Women (SMW) in India experience significant mental health disparities, yet their specific needs remain understudied. *Aim and Objectives:* To explore the lived experiences of mental health distress and mental health needs among SMW. *Material and Methods:* Between 23 May 2022 and 27 February 2024, a total of 29 interviews (19 in-depth and 10 key informant interviews) were conducted, face-to-face and telephonically in Maharashtra, India. Participants were recruited purposively through LGBTQ+ NGOs and using the snowball sampling method. Data were audio-recorded, verbatim transcribed, and translated in English, and analysed using a grounded theory approach. *Results:* Participants reported persistent family non-acceptance, moral judgment, and dismissal of their self-knowledge, which contributed to emotional distress and led to corrective treatments by health care providers. These experiences were compounded by a lack of queer-affirmative knowledge among healthcare professionals. Additionally, judgmental attitudes and high costs of counselling emerged as barriers to accessing mental healthcare services. SMW expressed their mental health needs, highlighting the need for safe, affordable, and queer-affirmative services delivered by trained health care providers. *Conclusion:* SMW's mental health challenges arise from social and institutional exclusion, underscoring the need for queer-affirmative training, strict legal laws for conversion practices, and expanding accessible public mental health services.

Keywords: India, lesbian, mental health, healthcare access, qualitative research, sexual minority women

Introduction

Lesbian, Gay, Bisexual, Transgender, and Queer (LGBTQ) individuals are at increased risk of mental, physical, and sexual health risks such as emotional distress, anxiety disorders, self-harm, suicidal ideation, substance use, sexually transmitted diseases, and cervical and breast cancers due to marginalisation and minority status [1]. Further negative encounters during healthcare access, such as discrimination, invalidation of identity, and lack of queer affirmative care, contributes to delayed or forgoing of healthcare among LGBTQ individuals [1-3]. In scientific research, the focus has been predominantly on human immunodeficiency virus (HIV) and other Sexually Transmitted Infections (STIs) in homosexual men, with limited focus on Sexual Minority Women (SMW), viz. lesbian, bisexual, and queer women. More often, the health needs of SMW are either ignored or are assumed to be the same as those of women in the general community or the same as those of LGBTQ people. Most health studies among SMW are conducted in high-income countries

In India, despite legal advancements recognizing same-sex relationships, LGBT individuals continue to face significant disparities, particularly in health care services [4]. In the Indian LGBTQ context, studies have predominantly focused on Men who have Sex with Men (MSM) and Transgender

Women (TGW), especially about HIV and STIs. Studies among SMW have a limited focus on psychiatric care, marriage, and substance use [5,6]. Women's perceptions of distress and their health-care-seeking behaviour are often influenced by gender norms and sociocultural expectations [7], which may further shape how SMW access healthcare. Furthermore, owing to existing prejudices, discrimination, and heteronormative attitudes among health care providers in health care settings; SMW hesitate, cannot communicate, and delay in accessing health care, and suffer mental trauma [1,5]. Cultural norms, patriarchal structures, and a lack of space to deviate around sexuality further shape the lived experiences and health-seeking behaviours of SMW. There is a paucity of data on how identity invalidation and family affect their mental health and healthcare access. We conducted a qualitative study aimed at exploring the lived experiences of SMW in the context of their mental health distress and needs.

Material and Methods

Saturation is considered the cornerstone of rigor in determining sample sizes in qualitative research, which can be achieved in a narrow range of interviews (9–17) or focus group discussions (4–8), particularly in studies with relatively homogenous study populations [8]. Accordingly, between May 2022 and February 2024, a total of 29 interviews, including 19 in-depth interviews (IDIs) among SMW, and 10 key informant interviews (KIIs) among LGBTQ community representatives, LGBTQ NGO leaders, college teachers, and LGBTQ healthcare providers, were conducted using a grounded theory approach. Participants were recruited using purposive and snowball sampling through LGBTQ NGOs and the community network. IDIs included women who were assigned female at birth who self-identified as lesbian, bisexual, pansexual, or queer.

Interview/topic guides were developed with the focus of enquiry on SMW's social status and issues, health needs, networks, and relationships. The KII guide focused on the KIs' experiences with the SMW community, their social issues, networking, health problems, access to healthcare, and solutions to the SMW's problems. The socio-demographic data were captured on age, gender, sexual identity, education, occupation, and income.

As directed by the institutional ethics committee, IDIs were conducted by master's-level, educated women interviewers with more than four years of experience in qualitative research. These interviewers were trained and supervised by the PhD scholar (DU) and the guide (SS) to work with the sensitive population. IDIs and KIIs were conducted by master's-level women interviewers and DU, respectively, in-person (face-to-face) or virtually, i.e., telephonically or through zoom meetings, as per the participants' request, in a confidential setting convenient to the participants, in their preferred language. Interviews were conducted until saturation was achieved. No pictures were taken during the interview. The IDI/KII lasted between 30 and 170 minutes. FGD was not conducted to prevent breach of confidentiality.

Using GoldWave software, all audio-recorded interviews were transcribed and translated verbatim into English by DU. All the audio-recorded files and identifying forms were stored separately, with access only to DU and SS. The translated transcripts were thoroughly reviewed by DU and SS. Using a grounded theory approach [9], initially, open coding was used to break the data into smaller units, allowing the emergence of new concepts and categories directly from the data. A total of 534

codes were developed during the open coding process. Data were constantly compared across interviews, and related codes were grouped. Selective coding was used to integrate categories into themes representing SMW experiences.

The data were managed and analyzed using NVivo 14 software. Four coders were involved in the coding process for generating the code list. Each coder independently coded different transcripts, and the resulting code lists were compared and discussed to resolve discrepancies and reduce the influence of individual researchers' assumptions on the findings.

Data triangulation was ensured by incorporating both IDI and KII. During interviews, participants' responses were clarified, and key points were briefly summarised and checked with the participants for correct understanding of the researcher about the issue being discussed and to ensure accurate interpretation. Verbatim quotations are presented in the findings to ensure the interpretation remains grounded in participants' narratives. Due to the sensitive nature of the study population, transcripts were not returned to participants for comments to protect confidentiality, respecting their privacy and sensitivity. The study was approved by the Institutional Ethics Committee of ICMR-National Institute of Translational Virology & AIDS Research. Written informed consent was obtained from all the participants before conducting and audio recording the interview.

Definitions/ terms used

'Sexual Minority Women' refers to women who are assigned female at birth and self-identify themselves as lesbian, bisexual, queer, or any identity or label other than cis-woman, straight, or heterosexual women.

'Queer-affirmative' care refers to healthcare practices that recognize, validate, and support sexual identities without any attempts to change or 'psychiatrization' of identity.

Results

The IDI respondents' ages ranged from 23 to 72 years; all were literate, and more than three-fourths of the participants reported completing a bachelor's degree (Table 1). The majority of the participants were unmarried (15/19). Of the 19 participants, 7 reported living with a romantic partner. There were seven students, and the rest were working as IT personnel (05); social workers (03); a therapist (01); a pub hostess (01), while 02 were unemployed. Out of the 10 key informants, 2 identified as male, 5 as female, and 3 as transgender (Table 2). Key informants reported diverse occupations. Their experience working in the LGBTQ field ranged from 3 to 31 years.

Participants narrated their experiences of identity expression, family responses, and encounters with mental health systems. Through the iterative coding process, following grounded theory principles, a code list of 534 codes emerged. Using the constant comparison method, codes were merged. Finally, 58 related codes were grouped during axial coding into broader categories, including identity disclosure, acceptance of SMW identity, cultural norms, internalized stigma, mental health issues, conversion treatment, barriers to accessing mental health care, and needs for inclusive and accessible mental health services. These categories were further compared and four core emerging themes captured the layered mental health challenges faced by SMW: (i) Limiting the autonomy with conditional threat – psychosocial distress, (ii) non-affirmative health system, (iii) mental health needs, and (iv) addressing the gap: mental healthcare.

Table 1: Socio demographic information of SMW participants

Characteristics	Response	N= 19 (%)
Median age (range)	25 years; range (22-72)	
Gender	Woman	18 (95)
	Non-binary	1 (5)
Highest education completed	High school/ Higher secondary	4 (21)
	Graduate	9 (47)
	Postgraduate and above	6 (32)
Sexual orientation	Lesbian	13 (68)
	Bisexual	3 (16)
	Pansexual	3 (16)
Marital status	Unmarried	15 (79)
	Married	2 (11)
	Separated	1 (5)
	Divorced	1 (5)
Currently living with	Family	6 (32)
	Living alone	3 (16)
	With friends	1 (5)
	With a male romantic partner	2 (11)
	With a female romantic partner	5 (26)
	Hostel	2 (11)
Occupation	Unemployed	2 (11)
	IT sector	5 (26)
	Student	7 (37)
	Social work	3 (16)
	Therapist	1 (5)
	Hostess (pub)	1 (5)

Table 2: Socio demographic information of KII participants

Characteristics	Response	N= 10 (%)
Gender	**Male**	02
	Female	05
	Transgender person	03
Highest education completed	**Graduate**	02
	Masters and above	08
Occupation	**Programme/ researcher**	02
	CBO/ NGO leader	02
	Community leader	02
	Teacher	01
	Health care providers	03
	Therapist	01
	Psychiatrist	01
	Medical officer	01
Years of experience	**Up to 5 years**	01
	6-10 years	05
	11 years and above	04

KII – Key informant interview; CBO – community based organization; NGO – nongovernmental organization

Limiting the autonomy with conditional threat – psychosocial distress

"They still think that I don't know enough about myself." SMW voiced this in the study. When family members discovered participants' sexual identity, either because the participant disclosed it or by accident, the family often responded with denial, disbelief, or rejection.

"So, she [/mother/] was full on against it [/lesbian identity/] that there is nothing like this [/lesbian/], this [/lesbian/] is all nonsense." [IDI-02, lesbian woman]

The non-acceptance by family is due to the heteronormative norms and concerns regarding social reputation in society. A participant narrated her conversation with her mother regarding her sexual identity, where she outrightly rejected her identity, framing it as morally unacceptable and socially risky.

"You know, like, she said [/mother/], what are you

saying, this is not right, what will those people say?" [IDI-16, lesbian woman]

Another participant narrated how rejection is not a single event but a continuous struggle she is facing with her family. She further highlights how parents think that she is confused or lacks understanding of her own identity. This repetitive invalidation resulted in psychological distress.

"With my family, my parents, I did face lot of trouble. I am still facing a lot of trouble, because they don't agree or accept the idea that I am, [/or may/] may not [/be/] straight, they still think that I don't know enough about myself, they are in continuous denial… That struggle is still ongoing. I don't think that reduces any time soon." [IDI-01, pansexual woman]

Non-affirmative health system

This theme captures the frustration of participants with healthcare providers who lacked the knowledge, sensitivity, or willingness to treat SMW with dignity. It illustrates how structural ignorance and heteronormativity constrained women's ability to access meaningful care, even when they urgently needed support.

Mental healthcare providers lack basic scientific understanding of SMW identities, as cited by a key informant.

"99% of the doctors do not have scientific knowledge about this [/LGBTQ/]." [KII-01, community representative]

The non-acceptance of identity by family is a major setback for SMW, affecting their mental health. This distress further becomes intolerable when parents take SMW to the doctors to fix them.

As elicited by a key informant, doctors prescribe conversion therapy:

"I would like to stress about the conversion therapy that people from this community undergo, they are forced by their parents, sometimes even by the doctors, who recommend their parents to take them to conversion therapy, and they will be fine." [KII-05, psychiatrist]

This shift in sexual orientation is often pursued through conversion therapies. Participants emphasized that such treatments, aimed at "fixing" or "converting" one's identity, can be highly traumatic.

A bisexual woman stressed the pain she had to endure during the conversion treatment. Unable to bear, out of compulsion, she chose marriage as an escape.

"For almost one and half month to two months, he was giving me shock [/shock treatment/]… what he [/doctor/] used to do through syringe [/injection/] he [/doctor/] was giving me some kind of medicine and due to that my body used to get 'Jhatke' [/get seizures/], then sometime in every 15 days, he used to keep cloth in my teeth and used to give me electric shock… Then I thought, rather than enduring all this, it is better to get married, and so I got married." [IDI-14, bisexual woman]

Similarly, a key informant reported that a psychologist suggested her friend to go through the conversion therapy, where the doctor would attempt to gauge her reaction to pornographic images. She was subjected to aversion therapy, which she found to be extremely humiliating.

"To my friend… he [/psychiatrist/] was like just go through this therapy, whatever I do at the end of it if it doesn't work out, you can tell your parents you did manage, I'll tell your parents I was not successful… but they made her go through that process. They made her go through 'aversion therapy' where the doctor did try to gauge her reaction to different pornographic images, and it's extremely humiliating." [KII-04, psychologist]

This *treatment to 'fix' or to 'convert identity'* is not only because of family pressure and non-acceptance

but is also reflected in the healthcare provider's own lack of queer affirmative understanding, combined with heteronormative assumptions embedded in clinical practice as described in the theme 'Mental health needs'.

Mental health needs

SMW kept talking about the lack of affirmative care and how they experienced stigma and non-affirmative treatment when they needed mental health care. It is a matter of grave concern that mental health professionals who are needed the most are actually putting SMW through the dreaded conversion therapies. Experience of stigma was shared.

"Oh yes, the doctors they talk to, usually are very homophobic and transphobic. So it's a challenge for them while they are talking about their sexual health or when they are talking about their mental health also. So when I am talking about mental health, the mental practitioner will be the psychiatrist or a therapist who is homophobic and also puts into conversion therapies which like leads to lot of like mental trauma and even suicide for lot of people. So that is an issue a community as a whole is facing right now." [KII-02, community representative]

This lack of affirmative mental health care, along with persistent heteronormative ideology among mental health care practitioners to cure a person's identity, can be seen in the narration below, where a participant emphasized the importance of choosing Healthcare Professional (HCP) very wisely.

"I have chosen a therapist who is queer-affirming. That also, I had to choose very wisely. My friend has been to a therapist, and they told her that you know, like… let's cure you and make you 'a straight', you know, and this is in 2022, and this still happens and in places like =Mumbai= [/big metro city in India/], and I don't really have a lot of hope, but I had to sit and choose very wisely." [IDI-01, pansexual woman]

Another SMW expressed that HCPs should be well-oriented towards the SMW, so that they can feel comfortable; a lack of this leads to stopping her therapy.

"Yeah, there was one [/time/] when I stopped going for therapy because the therapist was not well-oriented with the queer community. I don't feel comfortable, because even if I, even I know that they're going to be very understanding, but if that person is not well oriented, then they will never really understand us." [IDI-11, lesbian woman]

Thus, the presence of judgmental, heteronormative behaviours, and coercive conversion practices were highlighted in the themes above. However, participants also voiced a clear vision of what supportive mental health care could look like, which is described in the theme 'addressing the gap: mental health care'.

Addressing the gap: mental health care

SMW expressed that the healthcare system should be more inclusive and understanding towards them. Training of general practitioners in mental health care emerged as an expectation.

"Like I said, I think even a generic doctor needs to be taught some basics of mental health and how to behave with a patient [/SMW/]. That should be very important… I think there should be a little awareness." [IDI-18, lesbian woman]

The curriculum needs to be inclusive of diversity so that doctors are non-judgmental and can provide due care, including mental health care, at the initial stages of diagnosis.

"So, even I don't think there's a lack of knowledge. I'm sure this is somewhere covered in their education about the LGBTQ community, but there is a lack of acceptance among these doctors. They don't want to accept that these people exist… I think even a generic doctor needs to be trained on some

aspects of mental health." [KII-09, NGO representative]

The need for online mental health counseling was cited.

"I think nowadays everyone's comfortable with online, like, counseling and therapy. I think that would be a good thing, online counseling." [IDI-09, pansexual woman]

There is a need for affordable mental health counselling, as there is a lack of public therapy. Online therapy can close the gap. Virtual sessions may allow SMW to access therapists, which is an affordable and accessible option available anywhere.

"There is no public therapy available. There are no public therapists which are affordable. Like, there are no government therapy spaces. So, therapy becomes expensive. So, they don't go for counseling. You know, they live with that burden. They live with that. Things have, of course, changed now over the years." [IDI-06, bisexual woman]

Further participants expressed the need for a separate healthcare facility with queer-affirmative counselling due to the presence of stigma towards SMW.

Discussion

This study brings out an urgent need to build inclusivity among HCPs, which requires strong structural curriculum improvements to include SMW care [10]. The findings from this study highlight a multilayered mental health landscape for SMW in India, shaped by family rejection, coercive psychiatric interventions, structural barriers in healthcare, and the absence of accessible queer-affirmative mental health services. Participants repeatedly described non-acceptance of identity within families, often expressed through moral condemnation, and questions of 'what people will say.' These align with global evidence, where family rejection significantly affects the mental health outcomes of LGBTQ+ individuals [11]. In the Indian context, the family plays an especially central role in regulating sexuality and conformity. The belief that SMW do not know themselves enough, voiced by participants' families, resonates with findings that Indian families often frame same-sex desire as confusion or immaturity [12], thereby legitimizing subsequent corrective actions.

Following this rejection, participants reported being taken by families to doctors or healers in attempts to "fix" them. These interventions ranged from moral advice and visualization exercises to unnecessary medication and coercive practices. Participants described these experiences as highly traumatic, echoing with evidence that conversion therapy increases the risk of depression, self-harm, and suicide [13]. Despite condemnation of conversion therapies, such practices continue in India due to weak regulatory frameworks [14], where HCPs reinforce societal expectations of heteronormativity.

Among participants, healthcare setting emerged as a site of fear and exclusion. Participants cited a lack of queer-affirmative HCPs, judgmental attitudes, and heteronormative assumptions as barriers for identity disclosure and care-seeking [1,3]. Mental health needs emerged as a foremost need of the SMW population who are vulnerable and in need of support and care. Additionally, India's large privatization of mental health hinders access to care. Collectively, these barriers reproduce minority stress [15], reinforcing psychological burden on SMW and avoid healthcare. The National Tele Mental Health Programme of India aims to exponentially scale up the reach of mental health services to anybody who reaches out, across India, any time, by setting up a 24x7 tele-mental health

facility in each State and UT in India, and to extend services to vulnerable and difficult to reach populations [16]. However, visibility of this program is limited among LGBTQ+ populations, as none of the participants spoke about it. We recommend enhancing the visibility of these services through banners displayed in all primary health care facilities, private hospitals, and through television and radio broadcasts.

SMW expressed a need for queer-affirmative therapists, inclusive counselling spaces, and mental health professionals to be trained in LGBTQ specific concerns. The need for affordable and accessible services underscores the integration of queer affirmative training into medical and psychological curricula. Other studies have also highlighted the need to incorporate LGBTQ health issues into medical education to improve healthcare providers' knowledge and cultural competence [10, 17]. Addressing these gaps is essential to reducing mental health disparities among SMW and to ensuring equitable and affirming care.

Conclusion

This study highlights that mental health challenges among SMW in India arise not only from sexual identity but from social and structural exclusion. Family non-acceptance often leads to corrective or conversion-based treatment through healthcare, leading to emotional distress. The lack of queer affirmative HCPs, judgmental attitudes, high costs of therapy, and confidentiality concerns compound these experiences. Participants emphasized the need for safe, affordable, respectful, and affirming mental health services delivered by trained professionals. Strengthening queer-affirmative competencies and expanding accessible public mental health services are essential steps to support the well-being of SMW in India.

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SHORT COMMUNICATION

Hospital-based trends in adverse drug reactions: A descriptive analysis

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Abstract

Background: Adverse Drug Reactions (ADRs) remain a significant contributor to morbidity and hospital admissions, especially in settings with high rates of polypharmacy and irrational drug use. In India, hospital-based pharmacovigilance systems provide vital insights into community prescribing patterns and medication safety. *Aim and Objectives:* To assess the frequency, severity, and characteristics of spontaneously reported ADRs at a tertiary care ADR Monitoring Centre (AMC) and to explore their implications for public and community health. *Material and Methods:* A retrospective observational study was conducted at the AMC under the Pharmacovigilance Programme of India (PvPI) at KMCH Institute of Health Sciences & Research, Coimbatore, from January 2021 to December 2024. All spontaneously reported ADRs were analysed using the WHO-UMC causality assessment scale and the Hartwig and Siegel severity scale. Data on demographics, drug class, route of administration, affected organ systems, and ADR characteristics were evaluated using SPSS version 27. Results: A total of 131 ADRs were reported. The majority were observed in adults aged 19–65 years (85%) and in females (66%). Intravenous administration accounted for the highest number of ADRs (55%). Antimicrobials were the leading drug class (60%), particularly cephalosporins, penicillins and quinolones. Cutaneous reactions were most frequent (64%), including itching (34%) and rashes (22%). Most ADRs were classified in severity as mild (53%) moderate (38%) and severe (9%) *Conclusion:* This study underscores the burden of antimicrobial-related ADRs and highlights the utility of hospital-based pharmacovigilance in promoting safe prescribing practices. Regular feedback, prescriber education, and an improved ADR reporting culture are critical for reducing drug-related morbidity and informing community-level health interventions.

Keywords: Adverse Drug Reactions, Pharmacovigilance, Hospital-Based Study, Causality Assessment, ADR Reporting

Introduction

An Adverse Drug Reaction (ADR) is defined by the World Health Organization (WHO) as an unpleasant and unexpected reaction that happens in patients after they take pharmaceuticals for the diagnosis, treatment, or prevention of a disease at dosages that are normally used. Since 2012, the phrase has broadened to include not only the authorized use of a medication in prescribed quantities but also reactions resulting from errors, misuse, or abuse, as well as suspected reactions to off-label or unlicensed medications. Although this shift may alter the reporting and surveillance practices of pharmaceutical regulators and manufacturers, it should not affect clinical practice in terms of managing ADRs [1]. ADRs are commonly observed in clinical settings, often resulting in unplanned hospital admissions, developing during hospital stays, and presenting symptoms after

discharge, according to significant studies carried out in the United States and the United Kingdom in the late 20th and early 21st centuries. Despite several preventative efforts, research shows that 5-10% of patients may have an ADR upon admission, during hospitalization, or after discharge. Over time, the frequency of ADRs has stayed quite constant [2].

A large variety of prescription and over-the-counter medications are readily available in the Indian pharmaceutical market, which frequently encourages polypharmacy and self-medication [3]. Communities are especially susceptible to drug-related issues because of factors including illiteracy, lack of information, and the impact of aggressive pharmaceutical marketing, which all contribute to irrational drug usage [4]. A strategic method for identifying, assessing, and reducing such medication-related hazards is hospital-based ADR monitoring. These establishments frequently act as sentinel surveillance facilities, providing vital information about medication safety profiles in practical settings [5].

Adverse drug reaction Monitoring Centers (AMCs) are a countrywide network. India's pharmacovigilance initiative, which was started in 2010 under the Pharmacovigilance Programme of India (PvPI), seeks to improve ADR reporting procedures [6]. Healthcare workers frequently lack the time, motivation, or training necessary to report suspected ADRs. Hence underreporting is still a major problem [7].

Notwithstanding these difficulties, ADR data from hospitals could be used as a stand-in for patient behavior and community-level prescribing practices, especially in rural and resource-constrained areas. Furthermore, identifying high-risk medication classes, vulnerable patient groups, and frequently impacted organ systems is made possible by tracking ADR trends over time [8]. In addition to helping hospitals make the best clinical decisions possible, this data also informs public health initiatives, laws, and educational initiatives meant to raise community knowledge of drug safety [9]. Single-center studies provide valuable insights into institution-specific prescribing patterns and drug safety issues influenced by local patient demographics and clinical practices. Accordingly, this study evaluates the trends, occurrence, and severity of spontaneous ADRs reported to the AMC at KMCH Institute of Health Sciences & Research, Coimbatore, with the aim of identifying locally relevant safety patterns and potential community health implications.

Material and Methods

Study design and type: This was a retrospective observational study conducted to evaluate the occurrence and severity of spontaneously reported ADRs.

Study setting: The study was carried out in the Department of Pharmacology, which functions as an AMC under the PvPI, at KMCH Institute of Health Sciences & Research, Coimbatore.

Inclusion and exclusion criteria: All spontaneously reported ADRs received at the AMC from January 2021 to December 2024 were included in the study. Reports with incomplete data or those lacking essential information for assessment were excluded.

Ethical approval: The study was approved by the Institutional Human Ethics Committee (IHEC Approval No. 03/IHEC/2024). Patient confidentiality was strictly maintained, and data was anonymized prior to analysis. As the study involved retrospective review of existing data, informed consent was not applicable.

Strategy for data collection: ADRs reported during the study period were recorded using a standardized data collection form. Information on patient demographics, drug details (name, class, route of administration), ADR characteristics (clinical presentation, affected organ system), and temporal relationship with drug intake was collected.

Causality assessment was done using the World Health Organization-Uppsala Monitoring Centre (WHO-UMC) Causality Assessment Scale. Severity of ADRs was graded using the Hartwig and Siegel Severity Assessment Scale [10, 11].

Statistical analysis: Data was entered in Microsoft Excel and analyzed using Statistical Package for the Social Sciences (SPSS) version 27. Descriptive statistics were applied, and results were presented as frequencies and percentages.

Results

A total of 131 spontaneous ADRs were reported at the AMC from January 2021 to December 2024. ADRs were reported in individuals ranging from 3.5 months to 79 years of age, and was predominant in the age group of 19-65 years of age (85%). ADRs were more frequently observed in female patients (66%) in comparison to male patients (34%). Drugs given via Intravenous route (IV) (55%) showed most ADRs, followed by oral route (27%) (Table 1).

Antimicrobials were responsible for 60% of the ADRs, with cephalosporins accounting for the majority (30%), followed by penicillins (23%) and

Table 1: Demographic parameter and route of drug administration among the patients developing ADRs

Parameter	Patients with ADR n (%)
Adverse drug reactions	131
Age group (years)	
0-18	8 (6)
19- 65	111 (85)
>65	12 (9)
Sex	
Female	87 (66)
Male	44 (34)
Route of drug administration	
Intravenous	72 (55)
Oral	36 (27)
Intradermal	12 (9)
Intramuscular	5 (4)
Topical	6 (5)

quinolones (19%). The most commonly reported drugs in these antimicrobial categories were ceftriaxone (third-generation cephalosporins), piperacillin (penicillins), and ciprofloxacin (quinolones). Followed by antimicrobials, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) (5%) and anticonvulsants (4%) caused substantial ADRs shown in figure 1. Among all organ system classes, skin and subcutaneous tissue disorders were the most prevalent ADRs (64%), followed by immune system related disorders (13%) and gastrointestinal tract effects (6%). Most frequently reported ADRs are itching (34%), rashes (22%) and allergic reactions (5%). In accordance with the WHO causality assessment scale, 93% of the ADRs were probable and 5% were certain. With reference to the Hartwig scale for severity assessment, most of the reported ADRs were mild (53%), (38%) were moderate and (9%) were severe. No serious adverse events had been reported.

Discussion

The four-year hospital-based examination of ADRs offers important information about pres-cribing patterns, drug safety, and the implications of community-level pharmacovigilance. This study highlights the wider community health issues brought on by drug-related morbidity in addition to identifying frequent patterns of ADRs in a tertiary care context. One important finding of this study was that adults aged 19–65 years accounted for 85% of all ADRs. This is in line with research by Patel *et al*., (2016) which found that 80% of ADRs occurred in this age group[12].This is likely due to their higher exposure to pharmaceutical agents as a result of the burden of chronic diseases and their higher healthcare utilization. Previous studies have found lower ADR incidence in geriatric (9%) and pediatric (6%) populations, which may be related to underreporting or more cautious prescribing in these populations [13]. According to the gender-based distribution, ADRs were more common in

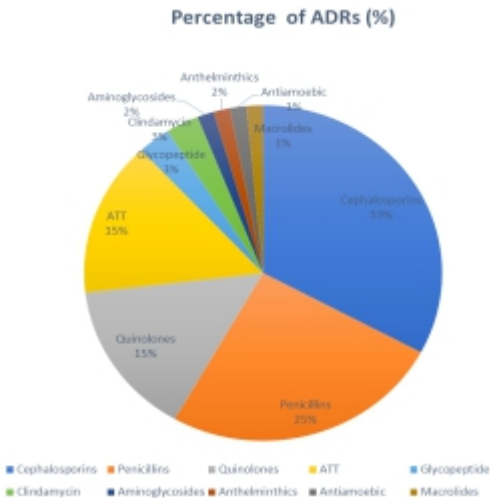

Figure 1: ADRs related to antimicrobials

ADR - Adverse drug reaction; ATT - Anti-tubercular drugs

women (66%) than in men (34%). This conclusion supports earlier findings by Ramesh *et al*., and who ascribe this trend to changes in drug metabolism, physiological and hormonal differences, and immunological responses in females [14].

IV route was linked to a considerable percentage of ADRs (55%), which is consistent with results from other institutional studies. Although IV delivery guarantees quick medication action, it also puts patients at risk for severe systemic reactions, such as anaphylaxis and hypersensitivity. Despite being safer, 27% of ADRs occurred via the oral route, highlighting the fact that even widely used formulations carry some risk. Healthcare providers must also be vigilant due to the incidence of ADRs through less often utilized routes, such as intradermal (9%) and intramuscular (4%) notably during allergy testing or injectable therapy. The predominance of antimicrobials (60%) as causative agents, particularly cephalosporins (30%), penicillins (23%), and quinolones (19%), underscores the prevailing antibiotic prescribing trends in both hospital and community settings [15, 16]. This pattern mirrors the high burden of infectious diseases and the potential for overprescription or empirical antibiotic use, which can lead to both ADRs and Antimicrobial Resistance (AMR). Ceftriaxone, piperacillin, and ciprofloxacin were frequently implicated-drugs commonly used for moderate to severe infections, reflecting their widespread utility and risk potential.

NSAIDs (5%) and anticonvulsants (4%) were also notable contributors, consistent with global pharmacovigilance data that highlight these drug classes as common culprits due to their narrow therapeutic indices and immunologic reaction potential [17]. These findings suggest the need for more robust prescribing oversight and patient education in both inpatient and outpatient settings. The most often impacted organ systems were the skin and subcutaneous tissue (64%), followed by the gastrointestinal (6%) and immunological (13%) systems. This pattern highlights the frequency of cutaneous ADRs and is consistent with worldwide pharmacovigilance data from the Uppsala Monitoring Centre and Indian investigations [18, 19]. Mohan *et al*., demonstrated that fixed-dose antituberculous combinations can cause significant cutaneous ADRs. This highlights the need for vigilant pharmacovigilance monitoring and prompt reporting to improve drug safety in tuberculosis therapy [20]. Ghodge *et al*., (2017) identified antimicrobials and antiepileptics as common causes of adverse cutaneous drug reactions, pre-dominantly presenting as maculopapular rashes. This underscores the need for prompt pharmacovigilance reporting to enable early signal detection and improve patient safety [21]. In line with research by Sumith Kumar *et al*., the most common symptoms, itching (34%) and rashes (22%), were mainly associated with beta-lactams and NSAIDs [22]. According to a study by Ahmed NJ, metronidazole and macrolides were commonly related with gastrointestinal Adverse Drug Reactions (ADRs), such as vomiting and diarrhea, and antibiotic-associated diarrhea was identified as a common hospital-based ADR [23]. According to the study by Paulmann *et al*., (2019) global research of severe cutaneous adverse reactions, anticonvulsants were the main cause of severe ADRs such as Stevens - Johnson syndrome (SJS) and drug reaction with eosinophilia and systemic symptoms (DRESS) [24]. Using the WHO-UMC criteria, the majority of

ADRs were categorized as probable (93%), with a lesser percentage being certain (5%). This is consistent with research by Naranjo *et al*., (1981) which found that "probable" ratings were more common because of limited follow-up data and ethical restrictions in the rechallenge [25, 26]. According to the Hartwig severity scale, the majority of ADRs were classified as mild (53%), moderate (38%), and severe (9%). These results were consistent with those of Shinde *et al*., who also observed a similar distribution [27]. Findings of our study on the prevalence of cutaneous manifestations and ADRs due to antibiotics are consistent with pharmacovigilance data from Thailand and other Southeast Asian nations. According to one study, skin reactions including urticaria and rashes are the most common clinical presentations, and antibiotics, especially beta-lactams, are the main cause of ADRs [28]. Similar ADR patterns to those observed in India are observed in Southeast Asia due to the region's extensive empirical use of antibiotics, easy availability to over-the-counter medications, and low public knowledge of responsible drug use.

These similarities highlight the need for community-level drug safety education, standardized ADR monitoring systems, and improved antimicrobial stewardship in the region. The broader public health relevance of these findings lies in their utility for strengthening pharmaco-vigilance mechanisms and antibiotic stewardship. Systematic ADR documentation can generate early warning signals and guide drug safety communications. Reports linked to anti-microbial use, in particular, are vital in curbing the irrational use of antibiotics and combating antimicrobial resistance through informed prescribing policies.

This study emphasizes the critical role of hospital-based pharmacovigilance in understanding and responding to community health challenges. By identifying trends in age, gender, drug class, and organ system involvement, healthcare institutions can design targeted interventions to reduce the incidence and severity of ADRs.

In the community context, such data are invaluable in advocating for better regulation of over-the-counter drug use, enhanced public awareness, and improved integration of ADR reporting systems across primary care settings. By identifying high-risk medications and influencing evidence-based clinical guidelines, labeling and reporting novel ADRs might help change prescribing patterns. It has been demonstrated that providing prescribers with feedback on ADR trends increases reporting rates and decreases needless prescriptions.

In addition, effective communication and ongoing training for Healthcare Professionals (HCPs) regarding medication safety and the significance of reporting ADRs can improve patient care and guarantee safer therapeutic results in the community. When reporting ADRs, it is crucial to make sure that HCPs receive objective, fact-based information and are shielded from potential legal action.

Enhancing healthcare organizations' open and non-punitive reporting cultures can promote a more proactive pharmacovigilance environment, which will ultimately improve public health outcomes.

Conclusion

This study highlights the burden and patterns of ADRs in a hospital setting, with antimicrobials, adult patients, and females being most affected. Cutaneous reactions were predominant, and most ADRs were mild to moderate in severity. The findings underscore the importance of hospital-based pharmacovigilance

in guiding safer prescribing practices, enhancing ADR reporting, and informing community-level drug safety policies. Strengthening surveillance and promoting a non-punitive reporting culture are key to reduc-ing drug-related morbidity and improving public health outcomes.

Limitations of the study

Despite its strengths, the study is limited by its reliance on spontaneous reporting, which is inherently subject to underreporting and selection bias. Additionally, lack of follow-up data may have led to an underestimation of long-term ADR outcomes. Nevertheless, the insights provided form a foundation for strengthening pharmacovigilance training, particularly among undergraduate health professionals, and support the need for active surveillance systems at the community level.

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CASE SERIES

Extra-nodal Kimura disease of head and neck region: A clinical perspective

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Abstract

Kimura's Disease (KD) is a rare, chronic inflammatory disorder of unknown etiology, potentially linked to dysfunctional immune responses or aberrant allergic reactions. It typically involves the head and neck, manifesting as subcutaneous nodules, often accompanied by regional lymphadenopathy, peripheral blood eosinophilia, and elevated serum IgE levels. While most commonly reported in young Asian males, cases in older individuals and females also occur. The definitive diagnosis is primarily based on histopathological examination of a biopsy specimen. Case series: This is cross-sectional observational study which reviewed 4 cases of extra-nodal KD, emphasizing its rarity and highlighting its presentation across different demographics. The patients presented with painless, slow-growing pre and post auricular swellings. Consistent findings included marked peripheral eosinophilia and imaging showed well-defined subcutaneous masses and reactive lymph nodes. Fine-needle aspiration cytology was non-specific. Histopathological evaluation of excised tissue confirmed KD, demonstrating characteristic hyperplastic lymphoid follicles with germinal centers, diffuse eosinophilic infiltration (including microabscesses), vascular proliferation, and fibrosis, correlating with the observed eosinophilia. These features aid in differentiating KD from similar conditions like angiolymphoid hyperplasia with eosinophilia. Treatment primarily involved surgical excision and systemic steroids were used for a recurrent case. Extra nodal KD is benign tumor yet can clinically and histologically mimic malignancy emphasizing the need for high clinical and pathological suspicion for accurate diagnosis and treatment.

Keywords: Kimura disease, head & neck, eosinophilia

Introduction

Kimura's Disease (KD) is a rare, chronic inflammatory disorder of unknown etiology. It was first described by Kimm and Szeto in 1937 as "eosinophilic hyperplastic lymphogranuloma" [1]. It gained prominence as KD following a report by Kimura and coworkers in 1948, which elaborated on an "unusual granulation combined with hyperplastic changes in lymphoid tissue" [2].

KD primarily involves the head and neck region specially in preauricular region, forehead or scalp and typically manifests as subcutaneous tumor-like nodules, often accompanied by regional lymphadenopathy, peripheral blood eosinophilia, and elevated serum Immunoglobulin E (IgE) levels [3].

Exact etiology remains elusive with possible theories considered are dysfunctional immune response, aberrant allergic response to viral, arthropod and tumor antigens. Presence of eosinophils, mast cells, interleukins and IgE suggest an abnormal T cell response to hypersensitivity type of reactions [4-5]. Association with autoimmunity and endocrine disorders have also been reported [3]. The exact prevalence of KD is not known. Most cases of this rare disease are reported in East and Southeast Asia, with a small number of cases reported in Europe. Male to female ratio ranges

from 3.5:1 to 9:1 in most series so far reported, with some exceptions. KD is usually seen in young adults during the third decade of life, with the median age being 28–32 years [6].

Morphologically KD is characterized by lymphoid follicles with germinal centers, marked eosinophilic infiltration, vascular proliferation, and fibrosis involving lymph nodes or extra nodal subcutaneous tissue. This research paper aimed to discuss the frequency and pathology of this rare disease.

Methods

This was a ten-year cross sectional observational study (2015 to 2025). Institutional ethical clearance was obtained from the Institutional Ethics Committee (SDMIEC/2023/517). Biopsy of confirmed cases (nodal/extra-nodal) of KD received in SDM College of Medical Sciences and hospital were noted from clinical records. Patient data were documented as demographic data (including age, gender etc), clinical presentation, haematological examination findings including haemoglobin, erythrocyte sedimentation rate, peripheral smear examination findings (and blood eosinophils levels), serum IgE levels, and Ultrasonography (USG) findings at the time of biopsy. The biopsy specimens obtained were processed as per standard operating procedure. The tissue sections were 4-6 microns thick and stained routinely with H&E. The H&E-stained slides were evaluated and correlated with serum IgE levels and blood eosinophil count.

Results

Considering KD is a rare entity, out of 11 KD encountered in present study, 4 were extra-nodal which are discussed here and remaining 7 cases of KD were in axillary and cervical lymphnodes.

Case 1: A 22-year-old male patient presented with a 10-year history of a gradually increasing swelling on the right side post auricular area. The swelling was painless with no other associated functional symptoms.

On examination: On local examination, an ill-defined swelling was palpated measuring about 6 cm × 5 cm, soft in consistency. Right level V discrete lymph nodes were enlarged. The skin over the swelling appeared normal with no signs of infection or inflammation. Fixation to the underlying structures was not present. Intraoral findings were unremarkable with no foci of infection. No other distant lymphadenopathy was elicited clinically.

Investigations: Haematological investigations revealed leucocyte count with normal limit with marked eosinophilia (46%) with all other peripheral blood findings within normal limits. High Resolution USG (HRUSG) of post auricular region showed a well-defined, lobulated, heterogenous solid, cystic, subcutaneous mass measuring 6 × 1.3 × 3.4 cm with minimal oedema. Numerous enlarged hypoechoic, level V discrete lymph nodes were seen with maintained USG morphology and raised vascularity suggestive of reactive change. Aspiration cytology revealed chronic inflammatory lesion.

Clinical diagnosis: The clinical differential diagnoses comprised a wide spectrum of conditions such as infected dermoid cyst or benign soft tissue lesion.

Treatment: Excision biopsy was performed and the specimen received on histopathological examination revealed KD.

Case 2: A 71 year female presented with a painless right preauricular swelling progressive in nature for last 6 years. The swelling was painless with no other associated functional symptoms.

On examination: On local examination a swelling was palpated measuring about 4 × 5cm, soft in consistency. The skin over the swelling appeared

normal with no signs of infection or inflammation. Fixation to the underlying structures was not present. Intraoral findings were unremarkable. No cervical lymphadenopathy was seen.

Investigations: Haematological investigations revealed leucocyte count within normal limits with marked eosinophilia (35%) with all other peripheral blood findings within normal limits. HRUSG showed a well-defined, lobulated, heterogenous solid subcutaneous mass measuring 4 × 4.2 × 4.8cm suggesting benign salivary neoplasm. Fine needle aspiration cytology was done and reported as chronic sialadenitis.

Clinical diagnosis: The clinical differential diagnoses were salivary benign neoplasm.

Treatment: Excision biopsy was performed and tumor diagnosed as KD.

Case 3: A 73 year female presented with a painless left postauricular swelling progressive in nature for last 1 year. The swelling was painless with no other associated functional symptoms.

On examination: On local examination a swelling was observed measuring about 3 × 3.5 cm, soft in consistency. The skin over the swelling and underlying tissue appeared normal with no signs of infection or inflammation.

Investigations: Haematological investigations revealed mild rise in leucocyte count with marked eosinophilia (38%) with all other peripheral blood findings within normal limits. HRUSG showed a well-defined, lobulated, heterogenous solid subcutaneous mass is seen measuring 3.2 × 2.8 × 2.9 cm. Fine needle aspiration cytology was done and reported as inflammatory lesion.

Clinical diagnosis: The clinical differential diagnoses was neurofibroma or schwannoma.

Treatment: Excision biopsy was performed and the specimen diagnosed as KD.

Case 4: A 56-year-old male patient presented with the complaint of right parotid swelling for the past 9 years which was insidious in onset and gradually progressive. He had history of left parotidectomy done 3 years back.

On examination: On examination 1 × 1 cm swelling was seen in the right preauricular area. It was firm in consistency. Scar noted on left preauricular area measuring 1.5cm in length.

Investigations: Haematological examination revealed eosinophilia with 58.7% eosinophils. HRUSG of right parotid showed a hypoechoic homogenous lesion measuring 2 × 1.8 × 1.5cm. CT done outside showed features suggestive of lymphoma sialadenitis. Fine needle aspiration of parotid swelling showed focal areas of micro abscesses with lympho-eosinophilic infiltrates. Diagnosis of chronic inflammatory lesion favouring KD was considered. On interrogation patient revealed left parotidectomy was diagnosed as KD.

Clinical diagnosis: A clinical diagnosis of recurrent KD was established.

Treatment: Excision of right parotid lesion was done and sent for histopathologic evaluation. As it was recurrent KD, the consulting hematologist treated the patient with systemic steroid therapy with a loading dose of 20 mg of prednisolone in divided doses with cetirizine for 4 months. There was good response to steroid therapy, and the lump started to regress >70% within 3 months which was confirmed by CT scan. Steroid dose was tapered. Short course of dexamethasone (60 mg) also started for a month.

Histopathological evaluation of all four excised tissue revealed similar morphology. (Figures 1, 2, 3 & 4)

Microscopy showed hyperplastic lymphoid follicles with prominent germinal centers with variable amount of fibrosis. Diffuse infiltration of eosinophils, histiocytes, plasma cells and mast cells were seen along with eosinophilic micro abscess formation destroying the follicles. Occasional multinucleated giant cells were also noted. Polykaryocyte were evident. Lymphoid folliculysis and follicular hyperplasia were evident, correlating with the hematological finding of eosinophilia. All the cases were diagnosed as KD.

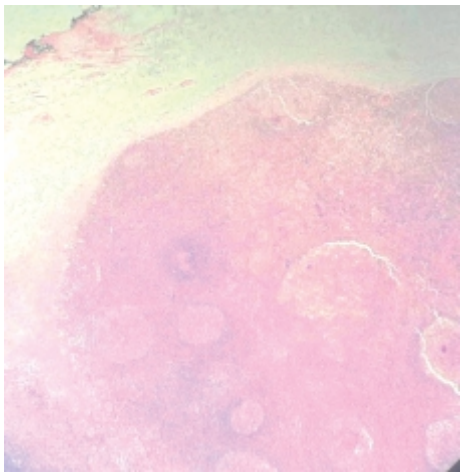

Figure 1: Scanner view H&E stain: Tissue displaying hyperplastic lymphoid follicles with prominent germinal centers with variable amount of fibrosis

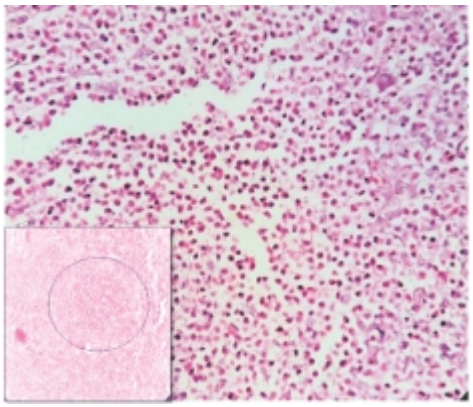

Figure 2: H&E stain High power displaying diffuse infiltration of eosinophils, histiocytes, plasma cells and mast cells seen along with eosinophilic micro abscess formation destroying the follicles (Inset). Occasional multinucleated giant cells were also noted

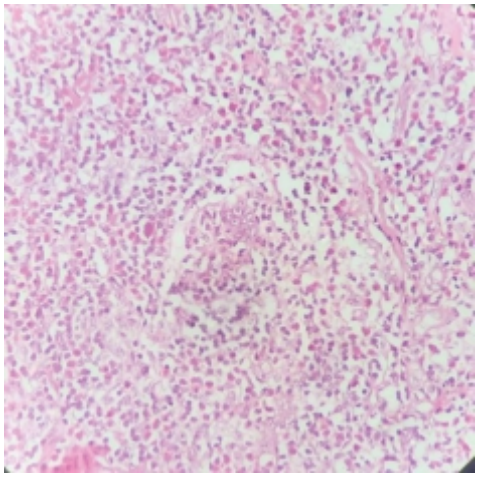

Figure 3: H&E stain High power displaying polykaryocyte in the follicles.

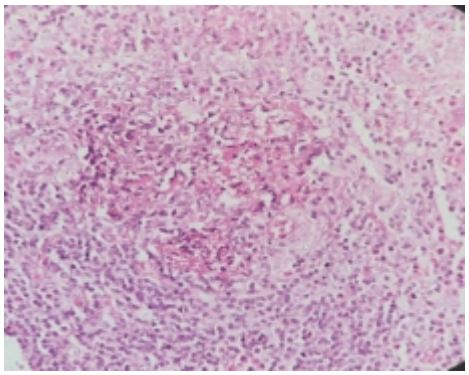

Figure 4: H&E stain High power displaying lymphoid folliculolysis

Discussion

KD is a chronic inflammatory disease of uncertain etiology and is proposed to be an immune response to unknown antigen. However, various theories in literature suggest dysfunctional immune response, aberrant allergic response to viral, arthropod and tumor antigens. Presence of eosinophils, mast cells, interleukins and IgE suggest an abnormal T cell response to hypersensitivity type of reactions. Association with autoimmunity and endocrine disorders have also been reported [3-5]. The cause of KD is still not well understood. Research has indicated that immunological imbalance related to IgE-mediated type 1 hypersensitivity and T Helper (Th)-2 cytokines significantly contribute to its pathogenesis, where tissue damage, allergies, infections, hormonal disorders, and autoimmunity may act as potential triggers. A crucial function of Th 2 cytokines in the development of KD is discussed in literature [6, 7]. Increased expression of Interleukin (IL)-4, IL-5, and IL-13 mRNA has been observed in the peripheral blood mononuclear cells of individuals with KD.

Immunohistochemical analysis of KD tissues showed heightened infiltration of IL-4C and IL-5C mast cells and T cells, along with increased levels of eotaxinC and C–C motif ligand 5 and mast cells, T cells, and activated eosinophils, reinforcing the prevalence of Th2 response. The disease's reactive characteristics are evident in the unique histopathologic patterns, including conspicuous follicular hyperplasia, dense eosinophilic and lymphoplasmacytic infiltration both intra and interfollicular, eosinophilic micro-abscesses (with or without Charcot-Leyden crystals), an abundance of small blood vessels, and marked stromal fibrosis. Lymphoproliferation is linked to the growth of postcapillary venules. The substitution of regular germinal centers with deposits of IgE or eosinophils, necrosis, and polykaryocytes (giant cells of the Warthin–Finkeldey type) may occur. In certain patients, the infiltration of nerve fibers by inflammation leads to skin irritation and itching [7]. The clinical presentations of KD described in the literature exhibit several consistent features, along with some variability.

Age and sex prediction

The literature suggests a predilection for young males of Asian descent. Study of 21 cases reported by Kung *et al.* (1984) included 18 male and 3 female patients with a mean age of onset of 28 years [1]. Similarly, study by Lee *et al.* (2022) with 23 Taiwanese patients showed a male predominance (16 males, 7 females) with a median age at diagnosis of 39 years [3]. A review of orofacial cases also noted a 94% male predominance by Iguchi et al. (1986) [8]. A case report by AlGhamdi *et al.* (2016) also emphasized that KD has no age or ethnicity limit, presenting a case of an 11-year-old Saudi boy [9]. Glibbery *et al.* (2018) reported a rare case in a 41-year-old Caucasian female [10]. Present study had 2 middle aged men affected with extranodal KD and 2 elderly females being affected by extranodal KD, which highlights that KD can occur in older individuals and females, although less commonly reported. These instances underscore the importance of considering KD even in atypical demographics.

Location of lesions

The head and neck region are the most frequently involved anatomical site in KD. Kung *et al*. (1984) found head and neck involvement in 23 out of 33 instances [1]. Lee *et al*. (2022) reported that 21 out of 23 patients presented with unilateral or bilateral head and neck masses [3]. In present study, 2 cases

had pre auricular and 2 cases post auricular swelling. These locations are consistent with the common presentation of KD. However, case series with an inguinal lymph node involvement by Punia *et al*. (2013) and Lee *et al*. (2022) reported cases with right flank and right arm lesions, indicating that KD can occur outside the head and neck, although less frequently [3, 11].

Nature of swelling

The lesions of KD are typically described as firm, painless, circumscribed subcutaneous masses. The painless nature and slow progression reported are characteristic of KD as was evident in present study. However, pruritus of the overlying skin has also been reported, which was not explicitly seen in present study. The size of the masses in the literature varies, ranging from 3-10 cm in the study by Kung *et al*. (1984) and 0.4 to 4.9 cm in maximal diameter in series by Lee *et al*. (2022) [1, 3]. Present cases had size variation from 1.8 to 5 cm.

Associated symptoms and findings

Regional lymphadenopathy is a common finding in KD. Kung *et al*. (1984) reported superficial regional lymph nodes affecting 14 of 21 patients. In present study the radiology revealed few discrete lymph nodes with reactive change. Peripheral blood eosinophilia and elevated serum IgE levels are considered typical laboratory findings. All cases in present study had significant eosinophilia of 46%, 35%, 38% and 58.7% respectively. These findings strongly support the diagnosis of KD and align with the literature, where eosinophil counts often range from mild to marked elevation. AlGhamdi *et al*. (2016) and Guimaraes *et al*. (2009) reported significantly elevated IgE level [7, 12]. While specific IgE levels were not provided in present cases but the presence of eosinophilia, a common association with elevated IgE in KD, further strengthens the clinical picture. Notably, the study by Lee et al. (2022) found increased eosinophils in 56.25% of patients and elevated IgE in 100% of the tested patients [3].

Key histological findings include:

Lymphoid follicles

These are hyperplastic with prominent germinal centers. The lymph node architecture is generally preserved. Extra nodal KD also displays hyperplastic lymphoid tissue.

Eosinophilic infiltrate

There is an intense infiltration of eosinophils, which can be focal or diffuse and may be massive in some lesions. Discrete foci of necrosis with eosinophils ("eosinophil abscesses" or eosinophilic microabscesses) are often present. Eosinophils may sometimes impinge on lymphoid follicles.

Vascular proliferation

There is proliferation of capillaries and small blood vessels, often with swollen endothelial cells. These are typically canalized vessels with flat endothelial cells, unlike the uncanalized masses with plump endothelial cells seen in Angiolymphoid Hyperplasia with Eosinophilia (ALHE). An increased amount of postcapillary venules is a constant feature. Hyalinization of sinusoidal vessels may be observed. Punia *et al*. (2013) illustrated vascular proliferation in their cases [11].

Fibrosis

Fibrosis surrounds the lesions, and fibrous bands may extend into the lesions as septa or outwards into adjacent structures, including lymph nodes. The fibrous tissue is variably cellular, with hyalinization being prominent in older lesions. Punia *et al*. (2013)

also reported areas of fibrosis in their cases [11]. Sparse plasma cells and histiocytes may be present, but giant cells, epithelioid cells, or granulomas are typically absent, which helps to distinguish KD from other inflammatory conditions. Immunochemical staining may reveal an IgE reticular network in germinal centers and IgE-coated non degranulated mast cells. Increased recruitment of IgG4+ plasma cells may be seen in some cases.

Other histological features

Occasional multinucleated giant cells were noted in present study, which can be seen in KD. Hyalinization of sinusoidal vessels with thick collagen bundles has also been described. The study by Lee *et al*. (2022) reported tissue eosinophilia (100%), florid follicular hyperplasia (78.26%), interstitial fibrosis (52.17%), lymphoplasmacytic infiltrates (48.82%), and increased small blood vessels (48.82%) as the most frequent histological features, which were concordant with present cases [4, 13,14].

Differential diagnosis

Besides ALHE, KD needs to be differentiated from other conditions presenting with head and neck masses and lymphadenopathy, including lymphoma, reactive lymphadenopathies, salivary gland tumors (benign and malignant), sarcoidosis, Kikuchi-Fujimoto disease, and infections (e.g., tuberculosis, fungal infections). The characteristic histological triad of lymphoid follicles with germinal centers, marked eosinophilic infiltration, and vascular proliferation is crucial for accurate diagnosis. The absence of granulomas, significant atypia, or malignant features helps to exclude other entities. In cases with atypical presentations, such as unusual location or association with panniculitis, careful pathological assessment is essential [13, 14].

Distinction from ALHE is critical

Clinically KD typically affects young Asian males with deeper subcutaneous nodules in the head and neck, often accompanied by lymphadenopathy, peripheral eosinophilia, and elevated IgE. ALHE, conversely, is more commonly found as superficial dermal nodules in middle-aged women, with less frequent lymphadenopathy and relatively lower eosinophilia and IgE levels [4].

Pathological differences

Histologically, KD shows well-developed lymphoid follicles with germinal centers, marked eosinophilic infiltration often forming microabscesses, vascular proliferation with canalized vessels and flat endothelial cells, and prominent fibrosis. ALHE is characterized by vascular proliferation with plump, atypical endothelial cells that may form solid masses without lumina, fewer lymphoid follicles often lacking germinal centers, variable eosinophilic infiltration without microabscesses, and less evident fibrosis [4].

Treatment and prognosis

There is no consensus on the optimal treatment for KD. Given its benign nature and the lack of malignant potential, the primary goals of treatment are to alleviate symptoms, reduce the size of lesions for cosmetic or functional reasons, and prevent recurrences [5, 7, 13, 15].

Surgical excision is often considered the first-line treatment for localized lesions. However, recurrences are common after surgical removal, occurring in a significant percentage of patients. Present cases were majorly managed surgically [5, 7]. Corticosteroids (topical, intralesional, or systemic) can effectively reduce the size of the nodules and alleviate symptoms. However, tumors may become refractory, and

long-term use is associated with side effects, with relapses often occurring upon discontinuation. AlGhamdi *et al*. (2016) showed temporary improvement with intravenous steroids and oral prednisone [9]. In present study one case was managed by steroids as there was history of recurrence.

Local radiation therapy has been used for recurrent or persistent lesions and has shown some success in achieving local control [10, 15]. Various other drugs like cyclosporine, interferon-alpha, thalidomide, leflunomide, intravenous immunoglobulins, antihistamines (e.g., cetirizine, loratidine), leukotriene receptor antagonists (e.g., pranlukast), imatinib, and omalizumab (anti-IgE antibody) have shown good response [15].

Conclusion

KD is a chronic inflammatory condition of unknown etiology; however, it is immune response to various antigenic stimuli. It does not increase the risk of malignancy but can clinically and histologically mimic malignancies. Histopathological examination of excision biopsy specimen with associated peripheral blood eosinophilia is the gold standard for diagnosis. High index of suspicion is warranted by the clinicians and pathologists to avoid misdiagnosis and unnecessary diagnostic and therapeutic modalities.

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CASE SERIES

Airway challenges in maxillofacial injuries and anterior neck

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Abstract

Maxillofacial and penetrating neck traumas pose significant airway challenges due to anatomical distortion and increased risk of obstruction. This case series highlights the importance of prompt assessment, interdisciplinary coordination, and use of advanced airway techniques such as video laryngoscopy to ensure safe airway control in complex trauma cases, to ensure safety of the patients.

Keywords: Maxillofacial Trauma, Difficult Airway, Video Laryngoscope

Introduction

Trauma to the maxillofacial region and neck, complicates airway management due to disrupted anatomy, bleeding, and tissue damage. Rapid evaluation and stabilization, often requiring a multidisciplinary approach, are essential; any delays can result in rapid deterioration. Standard assessment methods like Mallampati grading are often not possible due to pain, structural damage, or altered mental status. A step wise team approach using strong communication and global mental model facilitated by definitive airway management in maxillofacial injuries and neck trauma is required [1]. Maintaining airway being top priority, along with immobilization of cervical spine [2]. Securing the airway is complex and crucial requiring co-operation between anaesthesiologist, surgeon, and trauma physician [3]. Anesthesiologists must rely on clinical judgment, adaptable techniques, and advanced tools such as video laryngoscopes and awake intubation strategies.

Case 1: Severe facial trauma following animal attack

A case of 50-year-old male with severe midfacial injury and mandibular disruption from an animal attack with a classical presentation of "cannot ventilate and cannot intubate" scenario. Patient presented with extensive loss of facial tissue and exhibiting signs of hypovolemia. Our primary concern was high risk of aspiration and the impossibility of effective mask ventilation.

Airway strategy

Awake video laryngoscopy was selected as the first-line approach to visualize the glottis before induction, patient was counselled and oxygen was insufflated. Lignocaine (10%) was sprayed and mild sedation was administered, C-MAC video laryngoscope with blade size 3 was chosen; mandibular instability made it even more difficult. Multiple attempts were made, bloody field made the visualization poor. Upon visualization of the epiglottis, sedation was cautiously administered using intravenous propofol (30 mg), ketamine (30mg) and fentanyl (50 mcg). Anaesthetic plane was deepened, then a bougie was carefully introduced under direct vision, and 7 mm flexo metallic endotracheal tube was successfully railroaded over it into the trachea. Tube placement was confirmed with capnography; anaesthesia was maintained with oxygen: air: sevoflurane: atracu-

rium. The patient was hemodynamical maintained with adequate fluid and blood. The tube was kept in situ and extubated the following day.

Case 2: Paediatric mid facial injuries from road traffic accident (RTA)

Managing a traumatized child, especially with midfacial injuries, adds layers of complexity. This 8-year-old's extensive nasal and maxillary disruption made conventional mask ventilation impossible.

Airway strategy

Conventional mask ventilation and intubation was deemed impractical, hence using video laryngoscopy (C-MAC blade size 2) was again our chosen path. Providing counselling to the child was challenging, and administering sedation required careful judgment, as there was a genuine risk of compromising the airway. The surgeon remained prepared to establish a surgical airway if necessary. Minimal sedation using ketamine (1 mg/kg) along with glycopyrrolate 5 mcg, combined with 10% lignocaine spray, helped suppress airway reflexes while maintaining airway patency. Insertion was challenging though, due to anatomical instability and bleeding. The critical takeaway here is the importance of having the surgical team ready for an immediate surgical airway as a backup. This highlights the vital "plan B". As soon the epiglottis was visualized, injection propofol 1 mg/kg and fentanyl 1 mcg/kg was given to deepen the plane and cuffed 5.0 mm endotracheal tube was passed. Tube placement was confirmed with end-tidal CO_2, maintained with oxygen: air: sevoflurane: atracurium. This child was ventilated until she was hemodynamically stable and extubated.

Case 3: Penetrating neck injury with retained foreign body

This 26-year-old man, with a motorcycle brake handle embedded in the anterior neck, presented a different but equally complex and demanding situation. The foreign body's interaction with phonation immediately flagged potential laryngeal or tracheal involvement

Airway strategy

Our strategy here revolved around meticulous assessment and multidisciplinary collaboration. Given his stable vitals, we had the luxury of a controlled approach. A multidisciplinary airway plan was developed use of a Blade 3 C-MAC video laryngoscope along with lignocaine spray and mild sedation with injection fentanyl 70 mcg and propofol 50mg. After confirmation of no internal injuries, anaesthetic plane was deepened and orotracheal intubation was performed with a 7.5 mm tube, confirmed via capnography maintained with oxygen: air: sevoflurane: atracurium. The surgical team then safely removed the foreign object, repaired tissues, and the patient was extubated without complication. This case underscores the importance of a calm, coordinated effort when a foreign body is involved, prioritizing imaging and surgical readiness.

Discussion

The key lessons and best practices drawn from these cases of maxillofacial and neck trauma management lie in the importance of adaptability. Standard airway assessment is often unreliable; we cannot depend on them. Anatomical distortion makes mask ventilation incredibly difficult, increasing the risk of obstruction [4]. Endotracheal intubation remains the gold standard for ventilation but supraglottic airway devices have been replacing endotracheal tubes in many scenarios but it's difficult to replace them in traumatic airway [5]. The availability of video laryngoscopy during surgical mission would prove as a game changer [6]. Its indirect visualization capability, even with limited mouth opening or active bleeding, significantly improves our chances of

successful intubation. They enhance intubation success rate and reduces complication, particularly in difficult airway [7] .They also have higher first pass success rate in difficult airway [8]. Fibreoptic bronchoscopy, while excellent, can be severely hampered by blood, as noted in this series. Surgical airway readiness is non-negotiable. We must always have the option of a surgical airway (cricothyroidotomy) as a rapid rescue. Interdisciplinary collaboration with surgeons, emergency physicians, and radiologists is essential for optimal patient outcomes. Above all, awake intubation remains a fundamental technique in airway management, alongside the use of various other devices [9]. Delaying here is simply not an option; a patient's condition can deteriorate alarmingly fast [10]. Careful sedation management is paramount and must avoid over-sedation that could lead to airway collapse. Smaller endotracheal tubes often offer better manoeuvrability in distorted anatomy and minimize further trauma. Overall interdisciplinary collaboration and rapid decision-making are vital.

Conclusion

Successful airway management in maxillofacial and neck trauma hinges on a multidisciplinary, proactive, and highly adaptable approach.

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CASE SERIES

Reinforcing infection prevention practices: *Providencia stuartii* outbreak in ICU, an observational study

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Abstract

Gaps in infection control practices have long been significant contributors to healthcare-associated infections (HAIs). Both multidrug-resistant isolates and outbreaks caused by rarely reported bacterial species are increasingly being recognized adding to the concern. This study reports an outbreak of *Providencia stuartii* in a single Intensive Care Unit (ICU) of a tertiary care center. The reported observational study was initiated with the identification the index case of *Providencia stuartii* during routine HAI surveillance in an adult ICU. Subsequently, four additional cases with the same pathogen and susceptibility profile were detected in the same unit. Infection control practices were immediately reinforced, and environmental sampling taken from multiple sites of the concerned ICU was carried out to trace the source. Multiple interventions, including additional teaching sessions and audits, were implemented. *Providencia stuartii* was first isolated from the index case during routine surveillance and later from samples of four additional patients in the same ICU. The pathogen was identified in a total of eleven samples over three months, with all isolates showing identical susceptibility profiles. Although the source could not be determined despite extensive sampling, the implemented interventions, including enhanced teaching sessions and hand hygiene audits helped control the outbreak and strengthen infection control practices. The pathogen was transferred possibly due to the lapses in the infection control practices. Reinforcement of infection control measures, in addition to regular teaching sessions and audits for hand hygiene, played a major role in controlling the outbreak and further highlighted the importance for their periodic implementation to prevent such lapses.

Keywords: Hospital acquired infections, *Providencia stuartii*, intensive care unit, HAI surveillance, multi drug resistant organisms, hand hygiene, infection control practices, healthcare workers

Introduction

Infections caused by emerging and re-emerging infections have increasingly been recognized as significant contributors to the Healthcare Associated Infections (HAIs) [1]. These pathogens have the ability to colonize and survive in harsh environments, facilitating their spread part-icularly among immunocompromised and critically ill patients. *Providencia* species are increasingly being reported and recognized as important micro-organisms causing HAIs [2] Number of published studies have reported and identified *Providencia* species as potential nos-ocomial pathogens responsible for outbreaks [3] These are gram-negative bacteria capable of forming biofilms and causing device-associated infections, urinary tract infections (UTIs) being most common. Frequent contact among patients, residents, and healthcare workers, along with poor adherence to hand hygiene (HH) and infection prevention and control (IPC) practices, facilitates the transmission of such microbes and leads to outbreaks. Intensive Care Unit (ICU) settings are particularly vulnerable as

mostly immunocompromised patients are admitted in intensive care. The best way to prevent such infections is through strict adherence to appropriate infection control practices, which requires continuous reinforcement through regular teaching sessions and audits. We report an outbreak of Multidrug-Resistant (MDR) *Providencia stuartii* in an adult ICU at our tertiary care institute. Despite extensive efforts, the source of the outbreak could not be identified. However, rigorous IPC measures successfully contained the outbreak within three months. The key factors that helped halt further transmission were repeated teaching sessions emphasizing proper IPC practices, regular audits to monitor healthcare worker compliance, and strict supervision of disinfection policy adherence.

Outbreak report

This cross-sectional observational study for over a period of three months was done in an adult ICU of a 1200-bedded tertiary care health facility of North India.

ICU setting

The adult ICU at our institute comprises 10 beds. The patient profile usually includes immuno-compromised individuals or those in critical conditions requiring ventilator support or invasive devices, making them highly susceptible to HAIs. As part of routine protocol, HAI surveillance is carried out for Ventilator-Associated Events (VAE), catheter-associated UTI (CAUTI), and Central Line-Associated Bloodstream Infections (CLAB SI) in reference with the National Healthcare Safety Network (NHSN) guidelines [4].

Index case and case series

During routine HAI surveillance, *Providencia* species was isolated from the urine sample of a catheterized patient (index case) admitted on bed no. 9 in the adult ICU. Two days later, blood culture from the central line of the same patient also grew *Providencia* spp. Antimicrobial Susceptibility Testing (AST) of both isolates showed susceptibility to imipenem, piperacillin tazobactam, and amikacin, and resistance to cotrimoxazole, tetracycline, ceftazidime, cefepime, and ciprofloxacin. Within 10 days, the same organism i.e. *Providencia* spp. was isolated from a pus sample of another patient (case 2) on bed no. 1, who had previously occupied bed no. 10. Subsequently, it was detected in urine and tracheal aspirate samples of a third patient (case 3) on the adjacent bed (bed no. 2). One month later, *Providencia* spp. was isolated from another patient also, (case 4) admitted on bed no. 9 (the previous bed of the index case) and from the urine sample of another patient on bed no. 10 (case 5). The susceptibility profiles of all isolates were identical to that of the index case. The identified index case was a 48-year-old male diagnosed with aspiration pneumonia and Guillain –Barré Syndrome (GBS). The patient had a urinary catheter, central line, and nasogastric tube in situ. He was admitted for three months and was receiving injectable amikacin (750 mg once daily), cefepime (1.5 g twice daily), colistin (2 IU twice daily), and linezolid (600 mg twice daily). After isolation of *Providencia* spp. from the urine sample, the treatment was modified to intravenous piperacillin-tazobactam (4.5 g twice daily) and colistin was discontinued. However, the patient succumbed to his illness. All the other patients with *Providencia* infection were treated with amikacin and piperacillin-tazobactam and they responded favourably. Species-level identification of every isolate was done using Matrix-assisted Laser

Desorption /Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS). A log score > 2 was obtained for *Providencia stuartii* in all 11 isolates. All isolates were MDR organisms and exhibited identical susceptibility patterns.

Actions taken and root cause analysis

Providencia stuartii was first isolated from the index case and subsequently from various clinical samples of four other patients in the same ICU. The organism was reported a total of 11 times over a period of three months. All isolates exhibited identical antimicrobial susceptibility profiles. Table 1 presents the details of *Providencia stuartii* isolates obtained from ICU patients. Isolation of this rare organism from two initial patients raised concern for the Hospital Infection Control (HIC) team. Hand Hygiene (HH) practices and cleaning and disinfection protocols were immediately reinforced in the ICU. This incident occurred during the COVID-19 pandemic, a period when the fear of viral spread was high and misuse of Personal Protective Equipment (PPE), gloves, and gowns was common, likely compromising infection control practices. Analysis of the root cause for the outbreak revealed that case 2 (bed no. 1) had been shifted from bed no. 10, which was adjacent to bed no. 9 (index case). Furthermore, the same attendant was responsible for patients on beds 1 and 2. The concerned staff received on-site education regarding hand hygiene and IPC measures. Disinfection protocols were meticulously followed, further reinforcement of infection control practices among all the Healthcare Workers (HCWs) was ensured. Repeated education sessions were conducted, and the frequency of Hand Hygiene (HH) audits was increased to ensure compliance. Before admitting new patients to beds 1, 2, 9, and 10, all bedding materials—including mattresses, sheets, pillows, and covers—were replaced, and the beds were disinfected. All potential routes of transmission were addressed through targeted corrective and preventive measures.

Environmental assessment

Swab sampling from the ICU environment was undertaken for the possible identification of the source of infection and eliminate potential reservoirs of transmission. Multiple rounds of sampling were performed by the HIC team. Swabs were collected from sterilized patient-care equipment, equipment screens and monitors, high-touch surfaces, and various disinfectant and solution containers, including distilled water, chlorhexidine, alcohol-based solutions, povidone-iodine, and chlorhexidine mouthwash. Samples were also collected from heparin vials, drinking and tap water, respiratory equipment surfaces and filters, cleaning devices and carts, medical equipment (e.g., thoracic drainage aspirators, ECG machines, portable X-ray units, cassettes, ECMO water heaters), haemodialysis units, patient surroundings, and also the bare hands of ICU staff. A total of 273 environmental swab samples were analysed over a three-month period. The first round of sampling was done within one week of isolating *Providencia* spp. from two ICU patients. None of the environmental samples yielded *Providencia* spp. Seven additional rounds of environmental sampling were subsequently conducted, but the source of contamination could not be identified. Though the environmental cultures were negative, still multiple infection control measures were initiated. Following these interventions, *Providencia stuartii* was no

longer isolated from any patient samples in the concerned ICU after the treatment of case 4.

HH auditing

HH auditing is a routine component of our infection control program. The HH audit is done using a direct observation method adapted and modified, based on the World Health Organization (WHO) hand hygiene audit tool via the Ibhar HH audit application. HH complete adherence rate, partial adherence rate, and total adherence rate (complete + partial) was determined. Profession-specific (e.g., doctors, nurses, others) and moment-specific, both HH adherence rates were calculated for all WHO-defined HH moment. The initial HH audit showed complete adherence, partial adherence, and total adherence rates of 13.1%, 41.2%, and 54.3%, respectively. Moment-specific observations revealed lower compliance during WHO Moments 1 and 5 (i.e., before touching a patient and after touching a patient's surroundings), at 38.9% and 35.5%, respectively. After reinforcement of HH practices and repeated training sessions, the adherence rates improved significantly over a three-month period. Complete Adherence (HHCAR) increased from 13.1% to 38.2%, Partial Adherence (HHPAR) from 41.2% to 47.4%, and Total Adherence (HHTAR) from 54.3% to 85.6%. Profession-specific HH adherence was initially below 25% among attendants and allied staff, which subsequently improved to approximately 50%. Moment-specific HH adherence for Moments 1 and 5 also increased to around 60% after interventions.

Hypothesis

The following possibilities were hypothesized as contributing factors to the outbreak:

Providencia spp. is known to colonize the intestinal

Table 1: Timeline of the isolation of Providencia stuartii from the ICU patients

Sequence of reports	Sample type	Bed No. (as per figure 1)	Isolate
Day 1	Urine	9 (Index case)	*Providencia stuartii*
Day 3	Blood	9 (Index case)	*Providencia stuartii*
Day 8	Urine	10 (Case 2)	*Providencia stuartii*
Day 12	Pus (Bed sore)	1(earlier on bed 10) (Case 2)	*Providencia stuartii*
Day 17	Urine	2 (Case 3)	*Providencia stuartii*
Day 19	Tracheal aspirate	2 (Case 3)	*Providencia stuartii*
Day 20	Tracheal aspirate	1(earlier on bed 10) (Case 2)	*Providencia stuartii*
Day 21	Tracheal aspirate	1(earlier on bed 10) (Case 2)	*Providencia stuartii*
Day 43	Urine	10 (Case 4)	*Providencia stuartii*
Day 48	Urine	9 (next patient) (Case 5)	*Providencia stuartii*
Day 50	Pus	9 (next patient) (Case 5)	*Providencia stuartii*

tract and can intermittently inhabit the periurethral region, leading to catheter contamination, biofilm formation, and infection spread particularly in immunocompromised patients. The index case had GBS and his initial treatment included colistin, to which *Providencia* spp. is intrinsically resistant. This likely facilitated colonization and persistence.

Gaps in practices for infection control, including infrequent change of PPE and gloves, may have a significant role in the spread of infection.

Although environmental cultures were negative, transmission via environmental reservoirs could not be completely ruled out. Ultimately, strengthening IPC measures, reinforcing disinfection protocols, and improving HH compliance appeared to have contained the outbreak.

It was therefore hypothesized that the index patient on bed no. 9, being immunocompromised and receiving colistin, became colonized with *Providencia* spp. in the periurethral region, leading to CAUTI and CLABSI. The organism likely spread from the index case (bed no. 9) to case 2 (bed no. 10), who was later shifted to bed no. 1. Transmission to case 3 (bed no. 2) may have occurred through a healthcare worker who attended both patients. Subsequently, the same organism i.e. *Providencia* spp. was again isolated from a patient occupying bed no. 9, the original bed of the index case. The mattresses and linens of all patients infected with *Providencia* spp. were replaced, and strict practices to control infection were enforced. Follow-up surveillance of ICU samples over the next month showed no isolation of *Providencia* spp. after three months of continued intervention and assessment.

Discussion

A study reported by the WHO across 22 countries reported that although approximately 60–70% of countries have IPC guidelines and training programs but auditing and monitoring are conducted annually in fewer than 30% of these countries only [5]. Outbreaks caused by rare and resistant organisms in hospital settings have become an increasing concern. Recently, various MDR organisms and their spread within hospital environments have been reported. The transmission of Methicillin-Resistant *Staphylococcus Aureus* (MRSA) between patients and their surroundings has been documented in teaching hospitals in the United Kingdom and hospitals including nursing homes in the United States [6, 7]. The isolate in our study was also multidrug-resistant, as defined by the Centers for Disease Control and Prevention (CDC).

Providencia species are commonly associated with CAUTIs, particularly in elderly patients with long-term indwelling urinary catheters [8]. Biofilm formation on the surface of indwelling catheters facilitates bacterial persistence and infection. Furthermore, the urease-producing property of *Providencia* allows these organisms to thrive, often causing severe infections [9]. Biofilm-forming bacteria such as *Proteus* spp. and *Pseudomonas aeruginosa* are globally recognized as major contributors to HAIs. *Providencia* spp. is intrinsically resistant to colistin, which facilitates colonization and persistence in ICU patients receiving this antibiotic. Colistin use has been linked to the emergence of hospital-acquired, Intrinsically colistin-Resistant Enterobacteriaceae (IRCE) [10]. Several outbreaks caused by *Providencia* species have been documented. Saida *et al*. (2008) reported a *P. stuartii* outbreak in a burn unit over four months, with tracheal aspirators

identified as the source [11]. A Tunisian study recovered *P. stuartii* from eight colonized patients and environmental samples, although only one patient developed disease [12].

P. stuartii outbreak in our hospital ICU was hypothesized to have resulted from inappropriate infection control practices. Outbreaks associated with improper use of personal PPE, gloves, and gowns have been previously reported [13]. During our outbreak, similar lapses were observed likely influenced by the post-COVID-19 period, when staff routinely wore gowns and gloves, making frequent changes between patient interactions impractical. Studies from the United States have documented the transfer of Gram-Negative Bacilli (GNB), including MDR *Acinetobacter baumannii*, MDR *Pseudomonas* spp., and *Klebsiella pneumoniae*, from infected patients to gloves, gowns, and hands of HCWs [14]. The transfer of microorganisms between patients via HCWs' hands and reused medical devices has been repeatedly reported in the literature, dating back to the pioneering work of Ignaz Semmelweis (1818– 1865), the father of infection control [15]. These outbreaks highlight persistent gaps in IPC programs at both national and facility levels, particularly in Low- and Middle-Income Countries (LMICs). However, such outbreaks can be effectively prevented and controlled through appropriate IPC interventions. According to WHO, key IPC components include structured programs, guidelines, strategies, training, auditing, and monitoring, supported by adequate staffing and infrastructure. At our facility, IPC guidelines and strategies exist, and periodic training is provided. The staff are not overworked, and facilities are adequate; however, consistent monitoring and auditing were lacking, and infection control personnel were insufficient. These shortcomings likely contributed to the observed IPC gaps. Integration of all IPC components is essential for effective infection prevention in any healthcare facility. Early, targeted interventions addressing standard precautions are crucial for preventing and controlling outbreaks. Although environmental sampling did not reveal any association, the spread of infection through the environment cannot be completely excluded. Despite not isolating *Providencia* spp. from the environment or HCWs, the implementation of stringent infection control measures including reinforcement of HH, correct PPE use, regular HH audits, and meticulous disinfection procedures—over a two-month period effectively contained the outbreak. Continuous reinforcement of ongoing IPC programs is necessary to prevent future lapses. Species-level identification of *Providencia* strains was performed using MALDI-TOF MS, with all isolates showing identical susceptibility profiles. However, variations in sequence types cannot be excluded. Advanced molecular techniques such as multilocus sequence typing or whole-genome sequencing could not be performed due to financial constraints, which is a limitation of this study.

Conclusion

It was concluded that understanding the dynamics of pathogen transmission among patients and healthcare workers is crucial for designing effective infection control policies and for containing inadvertent outbreaks. This reinforces the principle that, regardless of the type of infection, the golden rule for patient safety is the consistent adherence to appropriate infection control practices. Equally important are their regular reinforcement, continuous monitoring, and systematic auditing to strengthen and sustain these practices in routine healthcare settings.

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CASE REPORT

Concurrent cutaneous and skeletal tuberculosis presenting as multifocal extrapulmonary disease: A case report

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Abstract

Tuberculosis continues to pose a significant health burden in developing nations such as India, contributing to nearly one-third of tuberculosis cases globally. The cutaneous form of extrapulmonary tuberculosis, though not life-threatening, poses a diagnostic challenge due to its varied clinical presentation and low incidence; delayed treatment may result in irreversible scarring. Metastatic tuberculous abscesses are more commonly observed in individuals with compromised immune status. Concurrent involvement of the skin and skeletal system as a manifestation of extrapulmonary tuberculosis is rarely reported. Herein, we present a patient with multiple skin abscesses and skeletal involvement, complicated by chronic hepatitis B infection, to emphasize the need to consider tuberculosis, particularly in patients unresponsive to standard antibiotic therapy and the importance of early recognition for timely diagnosis and effective management.

Keywords: Extrapulmonary tuberculosis, skin abscesses, immunocompromised host

Introduction

Tuberculosis, an airborne communicable disease, continues to be endemic in developing countries such as India. According to the Global Tuberculosis Report 2023, the incidence of tuberculosis was estimated at 10.6 million cases globally, with India contributing to nearly one-third (28%) of the overall burden [1]. Extrapulmonary Tuberculosis (EPTB) accounts for approximately 15–20% of all tuberculosis cases, with a higher proportion reported in South-East Asia and immune-compromised individuals [1, 2]. Due to its multisystem involvement and nonspecific clinical manifestations, under-reporting of EPTB is common, reflecting the iceberg phenomenon [2]. Cutaneous tuberculosis constitutes 1–2% of EPTB cases [3, 4]. Due to its close resemblance to other dermatological conditions, early diagnosis continues to be a challenge. We report a patient with compromised immune status presenting with metastatic skin abscesses, who subsequently had a spinal involvement, highlighting an unusual presentation of a common disease and the potential complications associated with delayed diagnosis.

Case Report

A 51-year-old male presented with one-year history of dry cough of insidious onset, which was non-progressive and unresponsive to symptomatic treatment provided at outside hospitals, had complaints of low-grade intermittent fever associated with weight loss since past one month. Over the past nine months, patient noticed development of multiple soft tissue lesions, initially involving the right thigh and subsequently the left thigh, anterior chest wall, limbs, and neck. Approximately two months after the onset of initial swelling, purulent

discharge was noted from the right thigh lesion; pus culture and sensitivity testing done had showed no microbial growth. Additional soft tissue lesions later appeared over the left side of chest and epigastric regions. Despite receiving multiple short courses of antibiotics, no improvement in symptoms was noted. Incision and drainage had been performed at an outside hospital, and cytological examination revealed features of necrotizing chronic granulomatous inflammation, suggestive of a non-tuberculous granuloma or fat necrosis, following which the patient was referred to us for further evaluation and management. Cytology slides from the initial procedure were not available for review. The patient was diagnosed with chronic hepatitis B infection three years prior to presentation and had received treatment with lamivudine; which was discontinued one year post initiation of treatment. A significant contact history of tuberculosis was present, as the patient's mother had been treated for pulmonary tuberculosis eight years ago.

On examination, the patient was moderately built, pallor was noted. There was no clubbing or generalized lymphadenopathy. Vitals were stable, and systemic examination was unremarkable. Local examination revealed a discharging sinus over the left ankle and a hyperpigmented, jelly-like lesion over the lateral aspect of the right thigh (Figure 1). Multiple swellings were noted, which were soft in consistency, erythematous and non-tender (Figure 2). Differential diagnoses then considered were staphylococcal skin abscesses, tuberculosis and melioidosis [8, 9]. Mantoux test done was strongly positive, with an induration measuring 20 mm. Erythrocyte sedimentation rate was markedly elevated at 119 mm/hr. Chest X - ray was normal. Incision and drainage of the left chest wall abscess (Figure 3) was performed and pus samples were sent for further evaluation. Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) detected *Mycobacterium tuberculosis* with rifampicin sensitivity. Hepatitis B viral DNA level was 13,50,000 IU/mL. The patient was initiated on antitubercular medications – weight base and treatment for chronic hepatitis B infection.

One month later, the patient presented with low

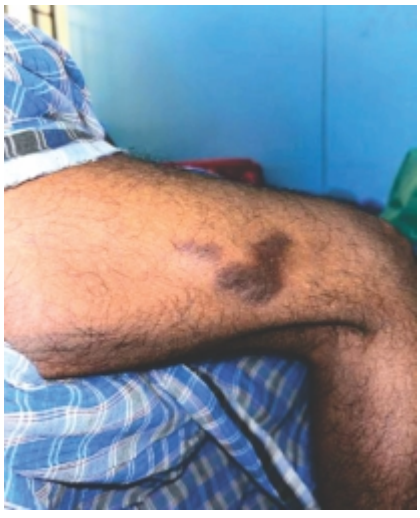

Figure 1: Hyperpigmented jelly like lesion – Lupus vulgaris

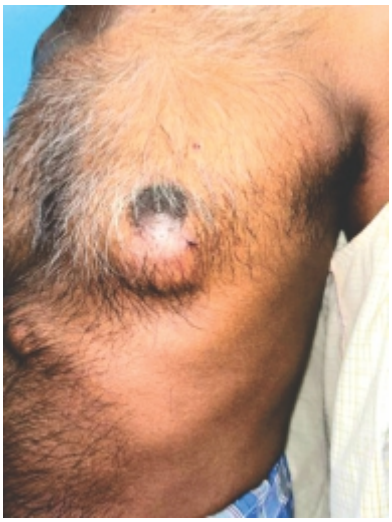

Figure 2: Tuberculous chest wall abscess

back pain with localized tenderness over the dorsolumbar spine. High - resolution computed tomography of the thorax, revealed scattered centrilobular nodules in bilateral lungs, few in a tree-in-bud pattern, patchy consolidation in the left upper lobe, loculated minimal pleural effusion and an enlarged subcarinal lymph node - features suggestive of an infective etiology. Associated skeletal findings included pathological fracture of D10 vertebral body (Figure 3). In view of above mentioned findings, magnetic resonance imaging of the whole spine was performed, which demonstrated altered signal intensities involving the D9 and D10 vertebral bodies with collapse of the D10 vertebra, along with mildly enhancing prevertebral and paravertebral collections. A well-defined, peripherally enhancing round lesion was also noted in the L1 vertebral body, suggestive of a tuberculoma (Figure 4). The patient subsequently underwent posterior spinal instrumentation from D8 to D12 with decompression at D9–D10. Biopsy samples were obtained, and anterior bone grafting was performed. Postoperatively, the patient was mobilized with Thoracolumbosacral Orthosis (TLSO) support. Histopathological examination of the D9–D10 granulation tissue revealed an organising inflammatory process composed of neutrophils, plasma cells and lymphocytes, with areas of fibrosis; no evidence of granuloma or malignancy was identified (Figure 5). These findings were interpreted in conjunction with the clinical, radiological, and microbiological evidence and a tubercular etiology was favored [2, 3]. In view of skeletal involvement, extended antitubercular therapy for a total duration of 18 months was advised. On follow-up after three months, significant resolution of the cutaneous swellings was observed.

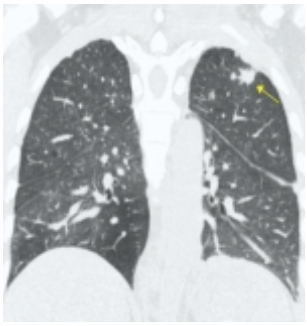

Figure 3: CT Thorax showing patchy consolidation, centrilobular nodules and tree in bud pattern suggestive of an infective etiology

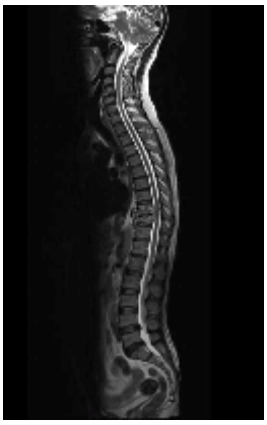

Figure 4: MRI whole spine sequence – suggestive of altered signal intensities in D9, D10 vertebral bodies with collapse of D10 vertebral body and mildy enhancing pre and para vertebral collections

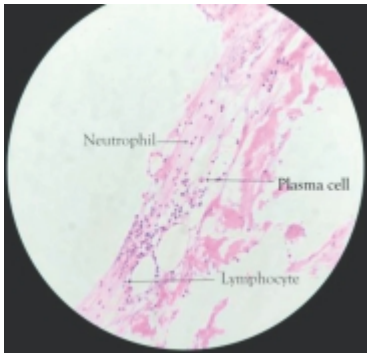

Figure 5: H and E (200×) shows inflammatory cells composed of neutrophils, lymphocytes and plasma cells

Discussion

EPTB, predominantly caused by *Mycobacterium tuberculosis* and occasionally by other mycobacterial species, accounts for approximately 15–20% of all tuberculosis cases worldwide [1]. Due to its diverse clinical manifestations and nonspecific symptomatology, EPTB poses significant diagnostic challenges, often resulting in a delay in diagnosis and uncertainty in management [2, 3]. Cutaneous tuberculosis represents approximately 1–2% of EPTB cases and is reported more frequently in children than in adults [3, 4]. Although it rarely is life-threatening, cutaneous tuberculosis can lead to considerable morbidity, necessitating repeated hospital visits and carries risk of permanent disfigurement and scarring when the diagnosis and treatment get delayed [4]. According to Kumar *et al*., lupus vulgaris is the most common clinical form of cutaneous tuberculosis, followed by scrofuloderma, tuberculosis verrucosa cutis, and tuberculous skin abscesses [6]. Metastatic tuberculous abscesses are uncommon and are more frequently encountered in patients with underlying immunosuppression [7]. Recent epidemiological data suggests that despite sustained global TB control efforts, the burden of EPTB and disseminated TB remains substantial in endemic areas, highlighting the need for recognition of uncommon manifestations such as skin and skeletal involvement.

The differential diagnosis of multiple skin abscesses includes staphylococcal infections, melioidosis, atypical mycobacterial infections, fungal infections, and panniculitis [8, 9]. Establishing a definitive diagnosis relies on histopathological and microbiological investigations. The sensitivity of tuberculin skin test is known to be low in cases of cutaneous tuberculosis [2,6,7] and demonstration of acid-fast bacilli or positive cultures from skin and skeletal lesions is challenging due to the paucibacillary nature of the disease [3,6]. In this patient the coexistence of skeletal tuberculosis may have contributed to the strongly positive tuberculin response. Available literature suggests that the concomitant occurrence of cutaneous and skeletal tuberculosis is rare. Kivanç-Altunay *et al*. (2003) reported no skeletal involvement among patients with cutaneous tuberculosis, whereas Kumar *et al.* (2001) identified associated skeletal tuberculosis in only 4 of 75 pediatric cases [6, 7]. In our patient, markedly elevated erythrocyte sedimentation rate and a strongly positive tuberculin test raised the clinical suspicion of tuberculosis, which was subsequently confirmed by microbiological evidence using CBNAAT [10].

Magnetic resonance imaging of the spine provided radiological confirmation of vertebral involvement. Although histopathological examination of the skeletal lesion demonstrated only an organizing inflammatory process without well-formed granulomas, tuberculosis could not be excluded, as extrapulmonary and skeletal forms of tuberculosis frequently exhibit nonspecific histological features, owing to their paucibacillary nature, particularly in immunocompromised individuals [2, 3]. Immune dysregulation secondary to chronic infection was considered to have contributed to disease dissemination and formation of multiple abscesses.

Conclusion

Tuberculosis should be considered an important differential diagnosis in patients presenting with multiple skin abscesses, especially among immunocompromised individuals. A high index of clinical suspicion, supported by radiological and microbiological evidence, is necessary for early identification and initiation of appropriate treatment to prevent disease progression and related complications.

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CASE REPORT

Spinal muscular atrophy carrier couple with normal child: Demonstrating Mendelian inheritance patterns

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Abstract

Spinal Muscular Atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by mutations in the SMN1 gene, with a carrier frequency of approximately 1 in 40-60 individuals. When both parents are carriers, each pregnancy carries a 25% risk of producing an affected child, 50% chance of a carrier, and 25% probability of a completely unaffected offspring. We report the case of a 27-year-old primigravida and her 33-year-old husband, both confirmed SMA carriers through genetic testing. The wife had heterozygous deletions in SMN1 exons 7 and 8, while the husband showed heterozygous duplication with 3 copies. Despite having a family history of SMA (wife's sister died at 18 months with confirmed SMA), the couple elected to forego prenatal genetic testing after comprehensive genetic counseling. The pregnancy was monitored with serial ultrasounds, revealing bilateral renal pelvis dilatation but no other structural anomalies. The couple delivered a healthy male infant at term via normal vaginal delivery with appropriate growth parameters and normal neonatal examination findings. Postnatal genetic testing using Multiplex Ligation-Dependent Probe Amplification (MLPA) revealed normal copy numbers for both SMN1 and SMN2 genes in exons 7 and 8, with MLPA ratios within normal range (1.05-1.14), confirming the infant was neither affected nor a carrier of SMA. This case demonstrates the classical Mendelian inheritance pattern for autosomal recessive disorders, where two SMA carrier parents achieved the favourable 25% probability of having a completely unaffected child. The outcome emphasizes the importance of com-prehensive genetic counseling in supporting diverse reproductive choices and highlights that positive outcomes remain possible even in high-risk genetic scenarios.

Keywords: Spinal Muscular Atrophy; Genetic Counseling; Heterozygote; Prenatal Diagnosis; Inheritance Patterns; Survival Motor Neuron 1 Protein

Introduction

Spinal Muscular Atrophy (SMA) is a severe autosomal recessive neuromuscular disorder characterized by progressive degeneration of motor neurons in the spinal cord and brainstem, leading to muscle weakness and atrophy [1]. The condition is caused by homozygous deletions or mutations in the Survival Motor Neuron 1 (SMN1) gene located on chromosome 5q13, with an estimated incidence of 1 in 6,000 to 1 in 10,000 live births worldwide [1, 2]. The carrier frequency for SMA mutations varies significantly across different populations, with approximately 1 in 40 to 1 in 60 individuals being carriers in most populations [2]. When both parents are carriers of SMA mutations, each pregnancy carries a 25% risk of producing an affected child, a 50% chance of a carrier child, and a 25% probability of a completely unaffected offspring, following classic Mendelian autosomal recessive inheritance patterns [3]. However, the clinical outcome in any individual pregnancy can vary considerably due to

the complex genetic architecture of the SMN locus [3]. Genetic counseling and carrier screening for SMA have become increasingly important in reproductive medicine, particularly given the availability of preimplantation genetic diagnosis and emerging therapeutic interventions [4, 5]. Understanding the inheritance patterns and genetic counseling implications for SMA carrier couples is crucial for healthcare providers, as it enables informed reproductive decision-making and appropriate medical management [4]. This case report demonstrates the classical Mendelian inheritance pattern in a confirmed SMA carrier couple who had a genetically normal child, illustrating the 25% probability of an unaffected offspring and highlighting the importance of comprehensive genetic counseling in such scenarios.

Case Report

We present a case of a 27-year-old primigravida and her 33-year-old husband, both confirmed carriers of SMA mutations, who delivered a genetically normal male infant. The couple sought genetic counseling at Nityanand Clinic's Genetic Counseling Centre due to their carrier status and family history of SMA. Her family history was significant for her younger sister, who died at 18 months of age with a confirmed diagnosis of spinal muscular atrophy. Her previous genetic testing revealed heterozygous deletions in exons 7 and 8 of the SMN1 gene, confirming her carrier status for SMA. Her genetic analysis demonstrated heterozygous dup-lication in exons 7 and 8 of the SMN1 gene with 3 copies, also indicating SMA carrier status.

During her pregnancy, she underwent routine antenatal care with serial ultrasound monitoring. At 20+3 weeks gestation, fetal ultrasound revealed bilateral renal pelvis dilatation with an antero-posterior diameter of 6mm, though no other gross structural anomalies or soft markers of aneuploidy were detected. The mean uterine artery pulsatility index was greater than the 99th centile, suggesting increased risk for pregnancy complications. Given the 25% risk of having an affected child with both parents being SMA carriers, the couple elected not to undergo prenatal genetic testing, making an informed decision to continue the pregnancy. Comprehensive genetic counseling was provided in their preferred language (Marathi) to ensure complete understanding of inheritance patterns and recurrence risks. She delivered a male infant at term via normal vaginal delivery on March 13, 2025, at our hospital. The newborn had normal birth parameters with a weight of 2.820 kg, length of 49 cm, and head circumference of 34 cm. Apgar scores were 7 at 1 minute and 8 at 5 minutes. The infant cried immediately after birth and demonstrated normal neonatal reflexes and muscle tone.

Postnatal genetic testing was performed on the newborn using Multiplex Ligation-Dependent Probe Amplification (MLPA) analysis at MedGenome Labs. The results showed normal copy numbers for both SMN1 and SMN2 genes in exons 7 and 8, with MLPA ratios of 1.05 and 1.10 for SMN1 exons 7 and 8 respectively, and 1.08 and 1.14 for SMN2 exons 7 and 8 respectively. All values fell within the normal range (0.80-1.20), confirming that the infant was neither a carrier nor affected with SMA. The infant's clinical course was unremarkable with normal feeding patterns, appropriate weight gain, and achievement of expected developmental milestones. Family history documentation revealed that the couple had no consanguinity, and the father's sibling had expired at 2 days of age due to unknown causes, though this was not related to SMA.

This case exemplifies the classical Mendelian inheritance pattern for autosomal recessive disorders, where two carrier parents had a 25% chance

of having an unaffected child, 50% chance of having a carrier child, and 25% chance of having an affected child. The outcome demonstrates the favourable 25% probability of complete genetic normalcy, reinforcing the importance of comprehensive genetic counselling for at-risk couples and the value of postnatal genetic confirmation in such scenarios.

Result

No pathogenic or likely pathogenic variants causative of the suspected phenotype have been identified.

Discussion

This case report demonstrates the classical Mendelian inheritance pattern in a confirmed SMA carrier couple who had a genetically normal child, illustrating the 25% probability of an unaffected offspring. Our findings align with established genetic principles for autosomal recessive disorders and highlight the importance of comprehensive genetic counseling in reproductive decision-making. The carrier frequency observed in our case is consistent with published literature. Studies report SMA carrier frequencies of approximately 1 in 40 to 1 in 60 individuals in most populations [2], with approximately 95% of affected patients showing homozygous absence of SMN1 gene exon 7 [3]. The heterozygous deletion in the mother and heterozygous duplication in the father represent typical genetic variants seen in SMA carriers.

Our case differs from several published studies in the reproductive choices made by the carrier couple. While many studies report high uptake of prenatal diagnosis among at-risk couples, our couple elected to forego prenatal genetic testing. This decision contrasts with findings from various studies where carrier couples opted for comprehensive prenatal testing programs. Recent research on prenatal genetic counseling in India has demonstrated that cultural factors and individual family preferences significantly influence testing decisions.

The favourable outcome in our case represents the 25% probability of having a completely unaffected child, as confirmed by normal SMN1 and SMN2 copy numbers. This outcome demonstrates the

Table 1: Multiplex ligation-dependent probe amplification (MLPA) results showing normal SMN1 and SMN2 gene copy numbers in the newborn

Genes/ Exons	Deletions/ Duplications	MLPA probe ratio (Dosage quotient)	Copy number	Disease (OMIM)	Inheritance	Clasiification
SMN1 (Exon 7)	-	Exon 7 (1.05)	2	-	-	-
SMN1 (Exon 8)	-	Exon 8 (1.10)	2	-	-	-
SMN2 (Exon 7)	-	Exon 7 (1.08)	2	-	-	-
SMN2 (Exon 8)	-	Exon 8 (1.14)	2	-	-	-

SMN - Survival Motor Neuron; OMIM-Online Mendelian Inheritance in Man

importance of understanding that each pregnancy of a couple who are both SMA carriers has approximately 25% chance of producing an affected child, 50% chance of producing an asymptomatic carrier, and 25% chance of producing an unaffected child who is not a carrier [3,4]. Recent advances in SMA treatment have influenced reproductive decision-making patterns. The approval of disease-modifying therapies like nusinersen has transformed the landscape of SMA management [5, 6]. Studies indicate that the availability of effective treatments influences prenatal genetic counseling approaches and parental decision-making regarding testing and pregnancy management [6].

The genetic counseling approach in our case exemplifies best practices recommended in the literature. Formal genetic counseling services must be made available to anyone requesting testing, with counseling including description of the disorder and range of severity [4]. The provision of counseling in the couple's preferred language (Marathi) aligns with recommendations for culturally appropriate genetic services, which is particularly important in the Indian healthcare context. Our case also highlights the importance of postnatal genetic confirmation even when prenatal testing is declined. Carrier screening helps individuals make informed reproductive decisions and take appropriate steps to ensure the health of their future children. The normal genetic results provide valuable information for future reproductive planning and eliminate the need for ongoing SMA-related medical surveillance in this child. The family history of SMA in the maternal lineage, with the death of the mother's sister at 18 months, underscores the significance of cascade testing and family screening. Those who have a family member with SMA or a family member known to be a carrier are at increased risk to be carriers themselves [4], supporting the importance of genetic counseling and testing for at-risk family members. The evolution of genetic counseling approaches in the new treatment era has been documented extensively. With the availability of disease-modifying therapies, the landscape of reproductive decision-making has transformed significantly [6,7]. Studies show that prenatal genetic counselors are now aware of these treatments and feel that this information affects pregnancy management decisions [6].

Our case contributes to the growing body of literature demonstrating that successful outcomes are achievable in high-risk genetic scenarios. Large-scale carrier screening studies have shown the technical feasibility and clinical utility of population-based screening programs [8], emphasizing the need to routinely offer carrier screening in appropriate settings. The prenatal ultrasound findings of bilateral renal pelvis dilatation in our case, while concerning at the time, were ultimately unrelated to SMA and resolved without intervention. This highlights the importance of comprehensive fetal monitoring in high-risk pregnancies, even when genetic testing is declined. The decision-making process illustrated in our case reflects the complex considerations families face when dealing with genetic risk.

Recent research emphasizes that reproductive decisions are intensely personal and that families should have access to comprehensive information about all available options [7]. The support provided by genetic counseling services and the respect for autonomous decision-making demonstrated in this case align with current best practices in reproductive genetics.

Conclusion

This case report exemplifies the fundamental principles of Mendelian inheritance in autosomal recessive disorders, specifically demonstrating the 25% probability of having a genetically normal child when both parents are confirmed SMA carriers. The couple's decision to decline prenatal testing while proceeding with postnatal genetic confirmation illustrates the diverse reproductive choices available to at-risk families and emphasizes the critical role of comprehensive genetic counseling in supporting informed decision-making. The favorable outcome reinforces the importance of understanding inheritance probabilities and highlights that positive outcomes are possible even in high-risk scenarios. This case contributes valuable insights into reproductive decision-making patterns among SMA carrier couples and underscores the necessity of continued genetic counseling support throughout the reproductive journey, regardless of the testing choices made by families.

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About Journal

Aims and Scope:

The Journal of Krishna Institute of Medical Sciences University, (JKIMSU), (ISSN 2231-4261) is peer-reviewed, open-access journal published quarterly (January to March, April to June, July to September and October to December) in two formats; printed and online (epub- ahead of print). The main aims of this journal are to publish novel and interesting observations and advance scientific knowledge in all the branches of medicine and allied sciences. The JKIMSU provides an international and interdisciplinary forum for the dissemination of peer-reviewed review articles, original articles, case reports, short communication, letters to the editor and book reviews in the field of all branches of medical sciences. The advisory board and editorial board of JKIMSU includes well known eminent scientists from India and abroad in all the branches of medicine to produce a high-quality scientific journal an impact on researchers and practitioners.

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Heading	Important Points
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Is the sampling technique appropriate?	
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Tables & figures: Are the data presented according to the objectives?	
Tables & figures: Are all of them necessary to be included?	
Are the findings presented logically?	
Are the findings presented in appropriate sequence	
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