ORIGINAL ARTICLE

Assessment of P53 BCL-2 and CD34 in premalignant and malignant cervical lesions

Ekundina Victor Olukayode[1], Ayodele Ore-Oluwa Favour[1], Ejelue Chiamaka Chelsea[1], Oladele Abraham Ayowole[1], Sanya Olurotimi[2], Eze Gerald Ikechi[3]*

[1]Department of Medical Laboratory Science (Histocytopathology Unit), [2]Department of Human Physiology, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Ekiti state, [3]Department of Anatomy, University of Benin, Edo State, Nigeria

Abstract

Background: Cancer cells are characterized by loss of tumor suppressor genes, evasion of apoptosis and an upregulation in angiogenesis. Cervical cancer is the second most prominent cancer amongst young women of reproductive age after breast cancer. With ever increasing knowledge in molecular changes, immunohistochemistry serves as a capable tool for early detection of cervical lesions, the addition of more specific biomarkers to routine diagnosis would improve diagnostic accuracy and unnecessary invasive examination. *Aim and Objectives*: To assess the morphological expression of P53, bcl-2 and CD34 in normal, pre-malignant and malignant cervical lesions. *Material and Methods:* This study was conducted to assess the various expressions of these tumor suppressor oncogenes and angiogenesis in normal, pre-malignant and malignant cervical lesions. A case controlled retrospective study was done with a total of 70 formalin fixed and paraffin wax embedded tissue blocks retrieved from pathology archives; amongst these were 10 normal, 15 Cervical Intraepithelial Neoplasm 1 (CIN1), twenty (20) CIN 2 and CIN3, and twenty five(25) Squamous Cell Carcinoma (SCC) confirmed cases of the cervix. Immunohistochemical analysis was carried out on the samples, alongside Hematoxylin and Eosin (H&E) staining. *Results*: The semi-quantitave analysis results were followed by normal, CIN1, CIN2 and 3 and SCC respectively; p53 (16%, 20%, 57% and 84%), bcl-2 (27%, 40%, 50% and 83%) and CD34 (75%, 81%, 87%and 98%). The data therefore provides information that the P53, bcl-2, CD34 are good diagnostic tool to detect SCC from CIN3. *Conclusion:* The data provides information that IHC markers are useful in differentiating the neoplastic lesions from the invasive carcinoma, but for production of optimum and accurate results, individual markers should not be used alone to avoid bias.

Keywords: Immunohistochemistry, CIN, SCC, P53, BCL-2, CD34

Introduction

Cervical cancer is the second most prominent cancer amongst young women of reproductive age after breast cancer [1]. It causes almost 300 thousand deaths per year globally and about 95% to 98% cervical cancer is caused by human papilloma virus, commonly the high risk E6 and E7 genes with slow disease progression taking up to 5-10 years most time [2]. The premalignant lesions of the cervix are termed as Cervical Intraepithelial Neoplasia (CIN)1, CIN2 and CIN3. The CIN refers to a prospective transformation and atypical growth (dysplasia) of the surface squamous cells of the cervix. It is non-cancerous and can be stabilized, annihilated by the body's immune system mediation, some however become persistent and progressive and led to the

malignancy which is usually the cervical Squamous Cell Carcinoma (SCC) [3]. With ever increasing knowledge in molecular changes, immunohistochemistry serves as a capable tool for early detection of cervical lesions, the addition of more specific biomarkers to routine diagnosis would improve diagnostic accuracy and unnecessary invasive examination [4].

Immunohistochemistry is a technique that detects cellular constituents (antigens) based on the interactions between antigens and antibodies; the antibody sites are identified by labeling and also the use of adjunct [5]. Expression of high risk HPV oncogene E6 and E7 allows mutagenic activities occur in the cell. The specific detection of the antigen responsible for these changes at every stage is important in diagnostic medicine. Oncoprotein E6 induces the destruction of p53 protein, which is critical in HPV cervical cancer cases. In early cervical tumors, most times, p53 is usually intact but is exception in cancer genetics. These variations in expressions of the p53 can help in diagnosis of the CIN1, CIN2, CIN3 and SCC of the cervix [6]. BCL2 is an anti-apoptotic protein, it prolongs cell life and makes it resistant to apoptotic factors. Expressions of the down regulation or over regulation of the BCl2 would indicate the presence of cancerous cells [7]. The overregulation and under regulation of the bcl2 protein can be directly linked to specific prognostic and diagnosis of cervical disease. Immunohistochemistry provides these specific markers [8]. Angiogenesis is the formation of new microcirculation from preconceived vessels, which is essential for tumor growth and progression, various research work has shown that micro vessel thickness in uterine cervix carcinomas is a risk factor linked to a bad prognosis, Wang *et al*. also confirms that the most commonly used method for testing angiogenesis is immuno-histochemistry with monoclonal antibodies [9]. The connective tissue stroma of virtually all human organs contain large amounts of resident CD34+ fibrocytes, which are involved in multiple functions such as wound healing, secretion of cytokines and also participate in stroma remodeling [10]. With improvements to diagnostic medicine, immunohistochemistry offers a high sensitivity of approximately 73.4% against the conventional diagnosis of cervical cancer (the routine pap smear, visual examination with acetic acid or Lugol's iodine and colposcopy examination) which has a low sensitivity of 51.3% which could result to a significant rate of misdiagnosis. Immunohistochemistry provides high specificity and sensitivity to serve as additional test to the routine diagnosis [4]. This study was conducted to assess the expressions of these tumor suppressor oncogenes and angiogenesis in a normal, pre-malignant and malignant cervical lesion.

Material and Methods

Tissue Blocks

In this retrospective review, a total of 70 tissue blocks were selected from the pathology archives of the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC). All tissue blocks retrieved were fixed in formalin and embedded in paraffin wax by conventional techniques. Haematoxylin and eosin stained slides of the samples were reviewed and classified according to the normal, premalignant and malignant cases. Confirmed cervical blocks of normal, CIN and invasive Squamous Cell Carcinoma (SCC) were selected. In total 70 blocks were retrieved; amongst these, 10 cervical tissue blocks were normal, 15 cervical tissue blocks were CIN1 diagnosis, 20

cervical tissue blocks were diagnosed with CIN2 and CIN3, and 25 cervical tissue blocks were diagnosed with SCC of the cervix.

Demonstration of P53, CD34 and BCL-2

P53, bcl-2 and CD34 expression was demonstrated by treating the selected cases with their corresponding antibodies using the immuno-histochemistry method.

Method

All of the specimens were formalin-fixed and paraffin-embedded. 4µ thick sections were cut and the end sections were stained with haematoxylin and eosin to certify that the lesions are still present in the serial section. The sections were processed for histochemical analysis as follows: Deparrafinization was carried out with xylene followed by rehydration through graded alcohol. Epitope retrieval was done by heating the sections for 10 mins in citrate buffer (pH 6.0) at 121 degree Celsius. The sections were incubated in 3% hydrogen peroxide (H_2O_2) in methanol for 5 minutes to block endogenous activity, it was followed by blocking of non-specific binding of primary antibodies to epitopes by a pre-incubation step with 5% normal goat serum for 10mins at 37 degree Celsius. The primary antibodies to be used in this study were p53 antibody (ab131442), bcl-2antibody (ab59348) and CD34 antibody (ab185732). Incubation for these antibodies was done at room temperature for 30 mins. Colour development was done by Diaminobenzidine (DAB). The slides were counterstained in haematoxylin and dehydration in ascending grades of alcohol. Slides was cleared in xylene and mounted in histomount. Staining expressions was evaluated optically using the light microscope at x100 magnification [11].

Photomicrography

The stained sections were examined under a LEICA research microscope (LEICA DM750, SWITZERLAND) interfaced with a digital camera (LEICA ICC50). Digital photomicrographs of the stained sections for the histomorphology, immuno-histochemistry on the tissue blocks studied was taken at various magnifications, and reported for morphological changes.

Immunostaining assessment

Expression of P53, BCL-2 and CD34 was determined through a semi-quantitative method. The immunoreactivity of these markers was determined by assessing the staining intensity and percentage of stained cells per field. The staining intensity was graded as mild, moderate and severe. The staining percentage of cells were graded as follows: <10% cells are negative (-), grade 0, 10% - 39% are positive (+), grade 1, 40% -79% are positive (++), grade 2 and 80% -100% are positive (+++), grade 3 [12].

Results

The staining intensity assessed by semi quantitative method is shown to be increasing from the normal to the SCC of cervical progression. The normal cases had a high negativity rate with 8 of 10 cases unreactive. The pre-malignant lesion CIN1 also showed an almost equal negativity or positivity rate with the all sections staining less than 40% which is mild intensity. The CIN2 and CIN3 also showed moderate staining intensity at the nuclear portions of the cells with a percentage lesser than 80%. The SCC also showed all the staining assessment as 3 were stained mildly, 6 moderately and 15 severely. Table 1 shows the increase in the positivity rate from the normal case to the SCC of the cervix while staining p53 immunohistochemically.

Table 2 depicts the rate of the P53 in the progression of the SCC of the cervix. The negativity reduces from the normal to the SCC while the positivity increases from the normal to the SCC of the cervix.

Table 3 depicts the staining intensity assessed by semi quantitative method. This show to be increasing from the normal to the SCC of cervical progression. The normal cases has high negativity rate with 6 out of 10 cases unreactive. The CIN1 showed negative in 7 cases, 3 cases showed mild reaction and 5 showed moderate staining reactions. The CIN2 and CIN3 also showed high rate of negativity with 7 cases unreactive and 13 cases showing mild and moderate staining intensity. The SCC demonstrates all the staining assessment with 6 unreactive, 1 is mild, 3 cases moderate and 15 cases markedly stained. The table thereby emphasizes the increase in the positivity from the normal cases to the SCC of the cervix when staining section with bcl-2.

Table 4 depicts the rate of the bcl-2 in the progression of the SCC of the cervix. The negativity reduces from the normal to the SCC while the positivity increases from the normal to the SCC of the cervix.

Table 5 shows the staining intensity assessed by semi quantitative method. The above table shows moderate and marked staining intensity across all the progression of cervical cancer.

Table 6 represents the rate of the CD34 in the progression of the SCC of the cervix. The positivity is high in the normal cases to SCC.

Table 1: Qualitative and semi-quantitative staining intensity of P53 in the progression of squamous cell carcinoma of the cervix

Group (P53)	Total cases	-	+	++	+++	Mean Percentage Reactivity
Normal/Control	10	8	2	0	0	16%
CIN1	15	7	8	0	0	20%
CIN2 and CIN3	20	5	7	8	0	57%
SCC	25	1	3	6	15	84%

Table 2: Rate of positive and negative cases of the P53 in the progression of squamous cell carcinoma of the cervix

Groups (p53)	N	Negative (n%)	Positive (n%)
Normal	10	8 (80)	2 (20)
CIN1	15	7 (47)	8 (53)
CIN2 and CIN3	20	5 (25)	15 (75)
SCC	25	1 (4)	22 (96)

Table 3: Qualitative and semi-quantitative staining intensity of bcl-2 in the progression of squamous cell carcinoma

Group (bcl2)	Total cases	-	+	++	+++	Mean Percentage Reactivity
Normal	10	6	1	3	0	27%
CIN1	15	7	3	5	0	40%
CIN2 and CIN3	20	7	4	9	0	50%
SCC	25	6	1	3	15	83%

Table 4: Rate of positive and negative cases of bcl-2 in the progression of squamous cell carcinoma

Group (bcl2)	N	Negative (n%)	Positive (n%)
Normal	10	6 (60%)	4 (40%)
CIN1	15	7 (47%)	8 (53%)
CIN2 and CIN3	20	7 (35%)	13 (65%)
SCC	25	6 (24%)	19 (76%)

Table 5: Qualitative and semi-quantitative staining intensity of CD34 in the progression of squamous cell carcinoma

Group (CD34)	Total cases	-	+	++	+++	Mean Percentage Reactivity
Normal	10	0	0	4	6	75%
CIN1	15	0	0	3	12	81%
CIN2 and CIN3	20	0	0	4	16	87%
SCC	25	0	0	5	20	98%

Table 6: Rate of positive and negative cases of CD34 in the progression of squamous cell carcinoma

Group (CD34)	N	Negative (n%)	Positive (n%)
Normal	10	0 (0%)	10 (100%)
CIN1	15	0 (0%)	15 (100%)
CIN2 and CIN3	20	0 (0%)	20 (100%)
SCC	25	0 (0%)	25 (100%)

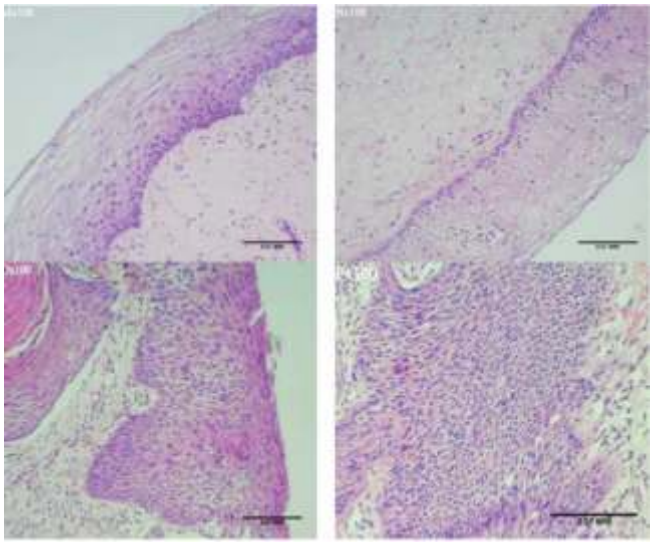

Figure 1: Micrograph of the normal cervix stained with H&E

(M) (NORMAL) the differentiation between the parabasal and basal cell layer. The nucleus stained purple and cytoplasm stained pink. (N) (CIN1)the differentiation between the parabasal and basal cell layer. The vast presence of the koliocytes in the section and mild dysplasia. The nucleus stained purple and cytoplasm stained pink. (O) (CIN 2 and 3) the lack of differentiation between the parabasal and basal cell layer. The presence of severe dysplasia, and anisonucleosis. The nucleus stained purple and cytoplasm stained pink, (P) (SCC) showing the presence of hyperchromasia, severe dysplasia, and anisonucleosis, invasion of cells into the stroma, lack of cell differentiation between parabasal and basal cells.

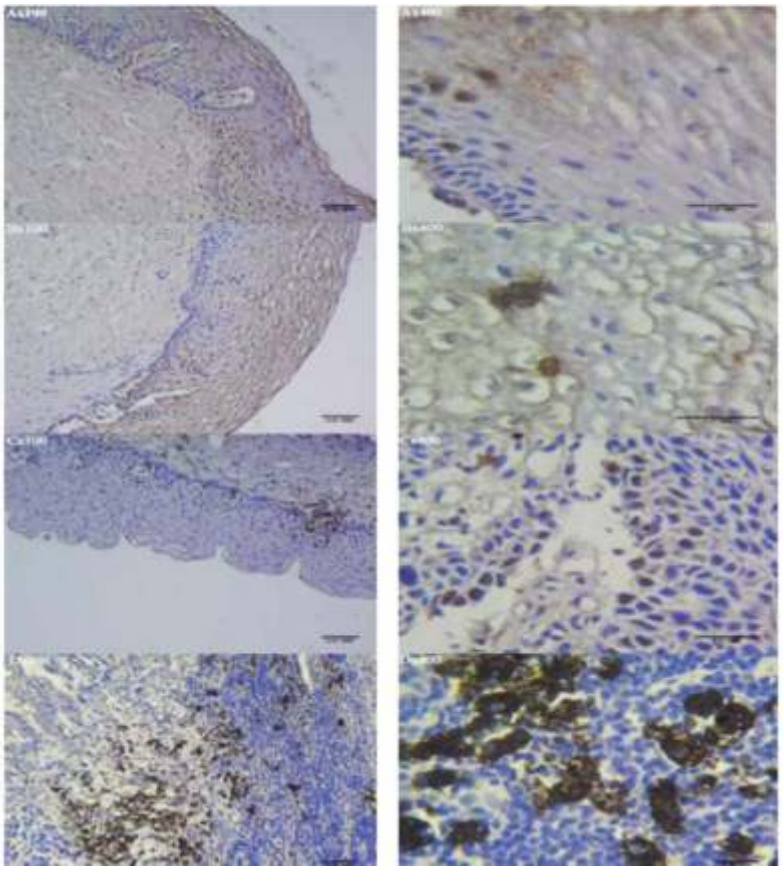

Figure 2: Micrographs of the cervical sections stained with P53

Illustrating (A) (Normal P53×100 and 400 respectively) shows mild staining reaction (B) (CIN1 P53×100 and 400 respectively) also shows a mild staining intensity. (C) (SCC P53×100 and ×400 respectively) shows a moderate staining intensity and (D) (SCC P53×100 and×400 respectively) showing marked expression in the stroma.

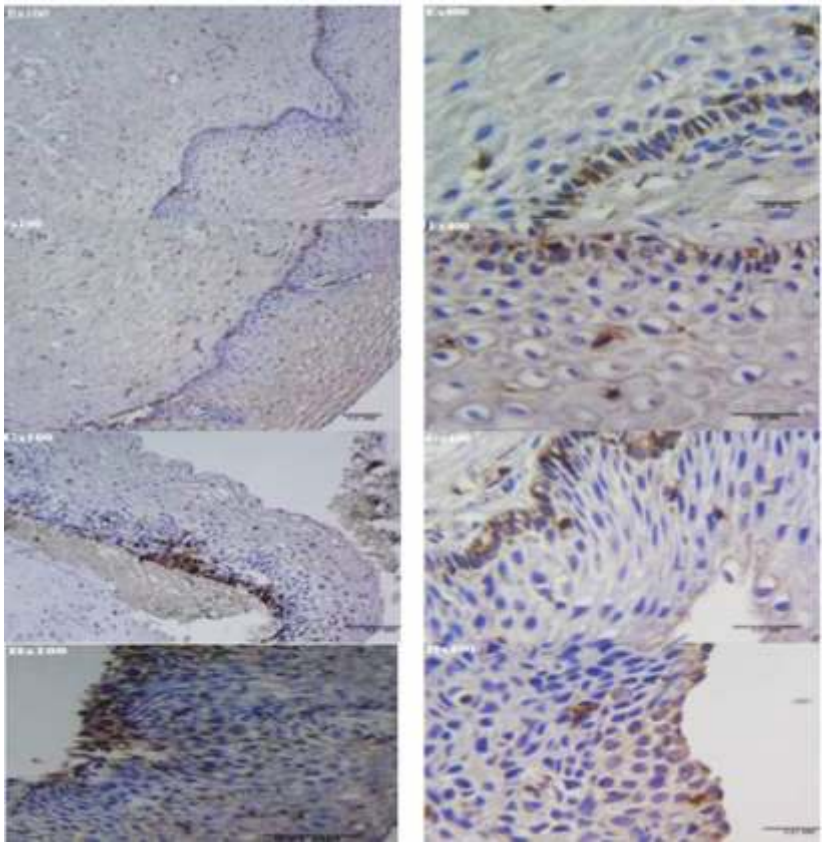

Figure 3: Micrographs of the cervical sections stained with Bcl-2

Illustrating (E) (NORMAL), (F) (CIN1) and (G) (CIN2 and CIN3) shows moderate staining intensity. (H) (SCC) shows marked staining intensity as bcl-2 expression increased with the stages of progression of the SCC of the cervix.

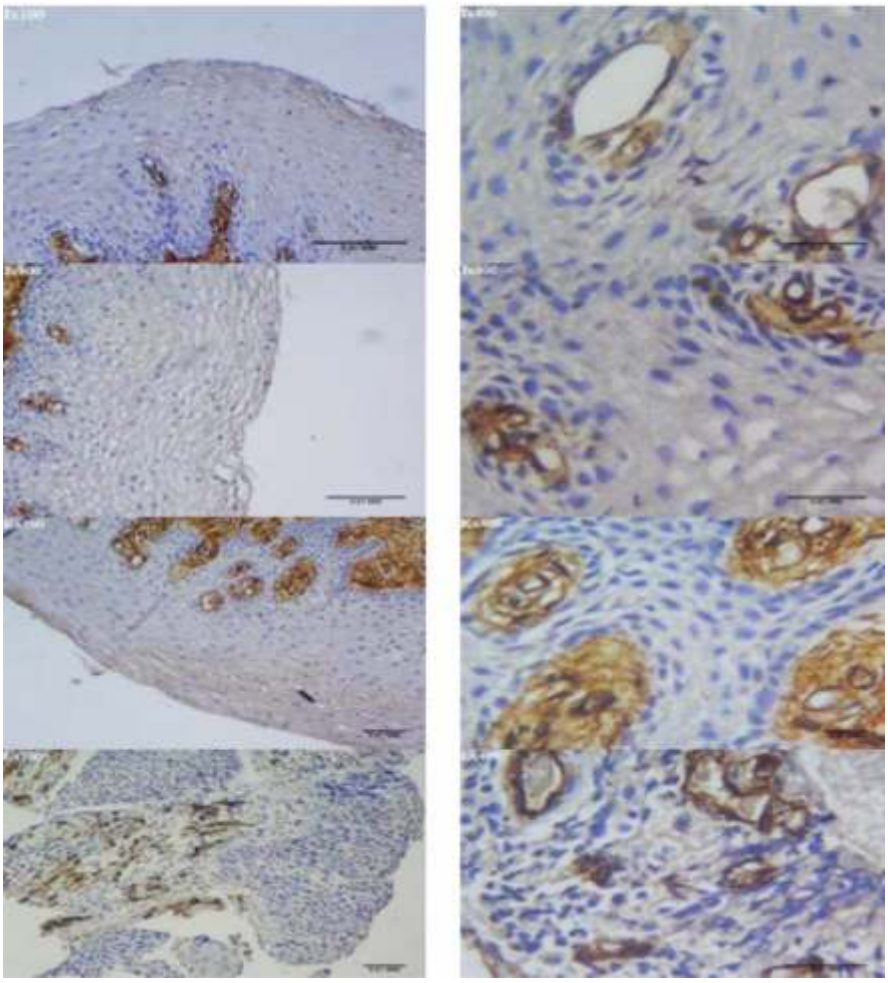

Figure 4: Micrograph of the cervical sections stained with CD-34

Illustrating (I) (NORMAL), (J) (CIN 1), (K) (CIN2 and CIN3) and (H) (SCC CD34x100 and SCC CD34x400) shows marked staining intensity with variation in the quantity of angiogenic cells.

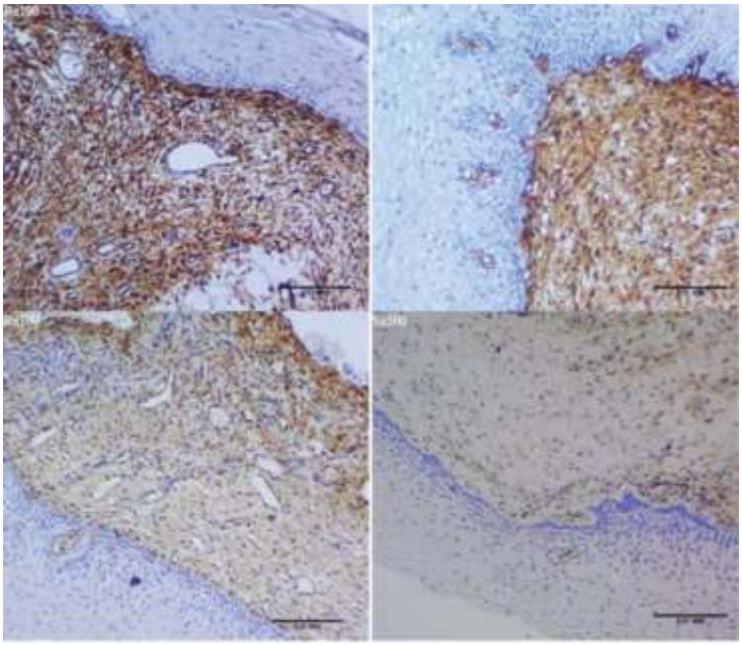

Figure 5: CD34 also stains for fibrocytes

(NORMALx100 (P), CIN1x100 (Q), CIN2 and CIN3 (R) and SCC (S)). The subepithelial and perivascular area are stained with the CD34, the intensity reduces with the increasing grades.

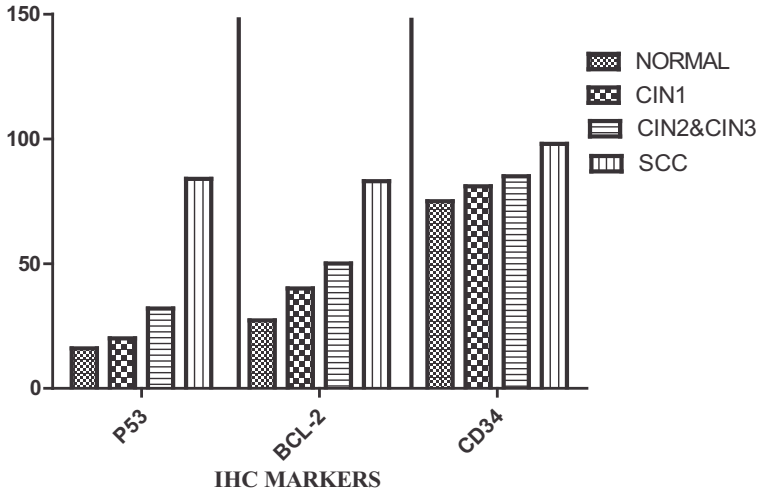

Figure 6: A graphical representation of the expression of the p53, bcl-2 and CD34 in progression of invasive carcinoma

The above figure 6 compares the staining reaction of the IHC markers. The p53 graph reveals its diagnostic tool of the SCC from the CIN3, the bcl-2 also reveals the progression of the staining intensity and cervical progression showing the prognostic value of the bcl-2 marker. The CD34 angiogenic marker shows the same staining intensity across, it reveals that angiogenic properties are only useful when quantified.

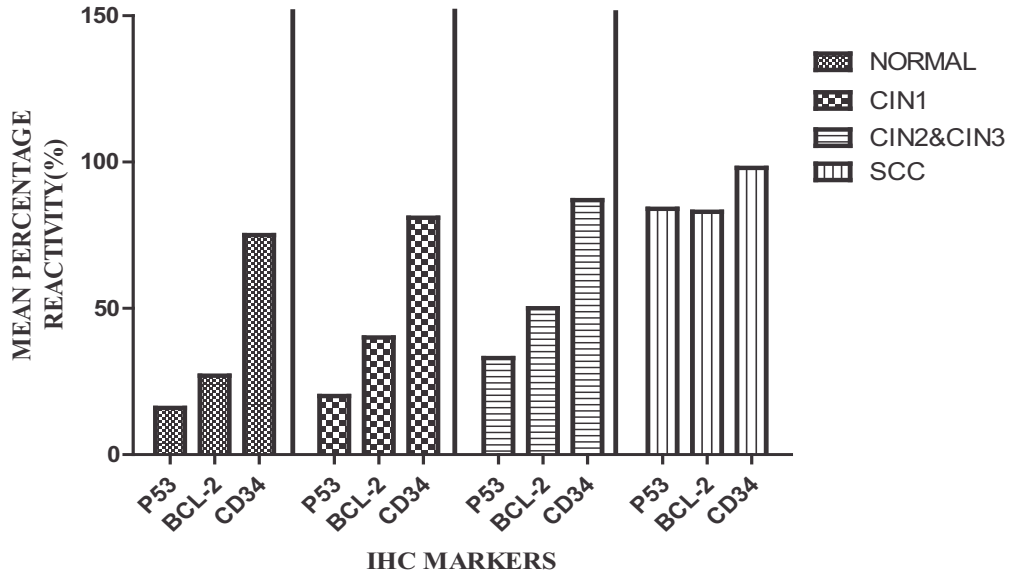

Figure 7: Comparing p53, bcl-2 and CD34 in premalignant and malignant lesions

The above figure 7 compares the staining reaction of the IHC markers with the progression of the cervical carcinoma. The IHC markers are seen to progressively increase except the CD34 which remained static because angiogenetic cells are stained with the same intensity, there is only a difference in the quantity of the angiogenic cells.

Discussion

Cervical cancer is the fourth most common cancer among women in the world [13], it is a common lesion in the developed and developing African countries, it is responsible for a high rate of mortality accounting for about 266,00 deaths in the year 2012. The average period for the premalignant to a malignant lesion is between 10-15 years, this gives a long window for early detection and possible preventive interventions [14].

P53, as an oncosuppressor is a major factor affecting cell proliferation, it serves as a guard in the cell cycle in response to DNA damage by arresting the cell cycle or cell apoptosis [15]. In this study, the p53 expression increased with increasing grades of the progression of cervical cancer. The expression of p53 in the normal cervical section was little or absent, with a little difference observed between the neoplastic grades, however there was significant increase in the intensity in the SCC which is in agreement with Murchala *et al*., and Tan *et al*., [16, 17], whose research shows the p53 well expressed in the SCC, mildly in the CIN grade and absent in the normal cervical section. The present study shows a progressive increase in the expression of the p53 with increasing grades of the cervical carcinoma. Also the p53 in this study was observed in the nucleus of the superficial and intermediate layer of the squamous epithelial, this is however contested in Vasilescu *et al*. as the p53 was expressed at the basal cell layer of the squamous epithelium cell [18], The reason for this disparity is however not

know but it agrees with generalization of Quade *et al*., that p53 can be found in the nucleus of cells attached to DNA anywhere in the body [19]. The expression in this study however suggests that the p53 may be useful in the diagnosis of the invasive cervical cancer, this is demonstrated by Jin *et al*, in the literature demonstrating the prognostic uses of the p53 [20]. P53 is not clearly expressed in all the progression of the cervical cancer, as the mutated form of p53 cannot be found in the normal cervix, the mutated form of the p53 which is deformed of all original functions of the p53 gene is found in the premalignant and malignant cases of the cervix; therefore nearly all 10 of the normal cervical section presented negative in cervical expression. With still no significant change, the CIN grades still didn't express p53 significantly from the normal cervix, the SCC however shows a higher expression with this the p53 maybe experimented more on as a prognostic tool. Alongside Yadav *et al*., and Cha *et al*. [21-22] the p53 is overly expressed in the malignant case, a relationship is not formed between the expression in the premalignant and the malignant cases, there is no direct relationship between the expression of the p53 and the increasing progression of cervical cancer, however the Lindstrom *et al*. from collected data found a significant relationship between the overexpression of the p53 and the increasing grades of the neoplasm [23], In contradiction, there is no connection between the expression of p53 and progression to SCC, this is in accordance with Yadav *et al*. and Cha *et al.* [21-22]. P53 will however be useful in differentiation of premalignant and malignant lesion as difference between the expressions is statistically significant.

Despite its hypothetical uncertainty, bcl-2 has been found to be a useful predictive marker in a handful of review papers [24]. The current study shows an increased expression of the bcl-2 from the normal to the CIN grades to the invasive carcinoma, the data shows the reaction in the basal layer which increased significantly to the SCC. The bcl-2 unlike the p53 was expressed in the normal cervical section with a mild intensity, unlike the p53 whose mutated form is targeted in immunohistochemistry, the bcl-2 protiens remains the same across all the stages, with its important role as a gatekeeper in apoptosis it is found largely in the normal cells. A significant difference between the CIN2 and 3 and the SCC was observed in the study which enables bcl-2 to serve as a prognostic tool to determine how long it takes for invasive carcinoma to occur after neoplasms, this is supported by Ekundina *et al*. which also stated that bcl-2 is observed to be overly expressed as the tumor stages progresses [12], there is similarity in this finding with Singh *et al*. and Kamaraddi *et al*., [25, 8]. However Shukla *et al*. disagrees with distinctive decrease in expression of the bcl-2 in HSIL, but the data was deemed insignificant [26]. This present study however shows that bcl-2 can be detected in all the stages of carcinogenesis in the cervix, with a large distribution in the SCC. The progression pattern of the expression is significant with the increasing grades of the dysplasia. This study shows that the bcl-2 holds capacity for prognostic uses in agreement with Hameetman *et al*. in whose literature Bcl-2 expression was been described as a good prognostic marker, this feature is attributed to the different functions of Bcl-2 in both apoptosis and cell cycle regulation [27]. The significant difference between bcl-2 expression in premalignant and malignant lesions makes it a

useful diagnostic tool.

Amongst many immunohistochemical markers been investigated, the angiogenetic marker CD34 has been largely neglected, Jais-sadu *et al.* suggests anti-CD34 antibody is a highly sensitive biomarker for endothelial cell differentiation that has been extensively studied in tumour angiogenesis. In this study, neovascular cells are also seen to have a moderate and marked staining intensity across all the progression of the cervical progression, the staining intensity shows little or no difference, so quantification is needed to differentiate between the premalignant and malignant lesions, however the smooth progression of the staining intensity and occurrence of the CD34 fibrocytes makes it a valuable diagnostic tool to confirm SCC, also to distinguish between the malignant and premalignant lesions of the cervix. This present study shows a decrease in the staining pattern of the CD34 fibrocytes in the progression of cervical cancer, the normal cervix shows a high percentage of the CD34 as the endocervix stroma contains the CD34 in the subepithelial and perivascular layers, the CIN1 also shows almost the same reaction with normal cervix, however there seems to be a disappearance of the CD34 fibrocytes in the SCC, This level of stroma CD34 staining implies a high degree of peritumoral stroma alteration, which can be seen in a number of squamous cell malignancies, including cervical carcinomas, furthermore, it has been discovered that the decline of CD34 expression in stromal tissue is linked to an increase in -Smooth Muscle Actin (-SMA) positive myofibroblasts [10]. The connective tissue is highly densed in the normal cervical section as the staining intensity is marked, the staining intensity however reduces across the increasing the grades, the CIN1 shows the same reaction with the normal cervix and a slight variation is seen in the CIN2 and CIN3 and a disappearance of the fibrocytes in the CD34, this may be attributed to the loss of connective tissue at invasive carcinoma due to random proliferation of the cells according to Li *et al*. [28], these findings imply that the decrease of CD34-positive stromal cells are related with cervical transition CD34 could be a strong predictive indicator with more than half of the SCC samples CD34 non-reactive and significant difference in staining score between the SCC and the CIN grades.

Conclusion

From the statistical data gathered from this retrospective study, it has been shown that the p53 and bcl-2are expressed in the premalignant and malignant lesions with moderate and severe intensity respectively; their expressions are directly proportionally to the increasing grades of cervical oncogenesis. However, the CD34 showed similar expressions in the premalignant and malignant lesions with the CD34 fibrocytes showing high disappearance in the SCC. The data therefore provides information that IHC markers are useful in differentiating the neoplastic lesions from the invasive carcinoma, but for production of optimum and accurate results, individual markers should not be used alone to avoid bias.

Acknowledgement

The authors acknowledge Olalekan Adegoke Aremu from the department of Morbid Anatomy and Forensic Medicine Obafemi Awolowo Complex (OAUTH) for his contribution to this study in the sample Immunohistochemical process.

References

1. Al-Darwish AA, Al-Naim AF, Al-Mulhim KS, Al-Otaibi NK, Morsi MS, Aleem AM. Knowledge about cervical cancer early warning signs and symptoms, risk factors and vaccination among students at a medical school in Al-Ahsa, Kingdom of Saudi Arabia. *Asian Pac J Cancer Prevent* 2014;15(6):2529-32.
2. Pierangelo A, Nazac A, Benali A, Validire P, Cohen H, Novikova T, Ibrahim BH, Manhas S, Fallet C, Antonelli MR, Martino AD. Polarimetric imaging of uterine cervix: a case study. *Opt Express* 2013;21(12): 14120-14130.
3. Adekunle OO. Cervical intraepithelial neoplasia (CIN) (squamous dysplasia). *Intraepithel Neopl* 2012;1(12): 279-310.
4. Sun H, Shen K, Cao D. Progress in immuno-cytochemical staining for cervical cancer screening. *Cancer Manag Res* 2019; 11:1817.
5. Kabiraj A, Gupta J, Khaitan T, Bhattacharya PT. Principle and techniques of immunohistochemistry-a review. *Int J Biol Med Res* 2015;6(3):5204-5210.
6. Pandey S, Pandey LK, Saxena AK, Patel N. The role of p53 gene in cervical carcinogenesis. *J Obstet Gynecol India* 2016;66(1):383-388.
7. Tuohetimulati G, Zhu M, Chen J, Niyazi M. Expressions and clinical significance of Bcl-2, Bcl-xL and c-IAP1 protein in cervical cancer. *Int J Clin Exp Med* 2018;11(11):12361-12367.
8. Kamaraddi S, Nayak A, Honnappa S, Swarup A. Expression of Bcl-2 marker in premalignant lesions of cervical cancer. *Int J Reproduc Contracep Obstet Gynecol* 2016; 5(4):965-970.
9. Wang B, Xu T, Liu J, Zang S, Gao L, Huang A. Overexpression of activated Cdc42-associated kinase1 (Ack1) predicts tumor recurrence and poor survival in human hepatocellular carcinoma. *Pathol Res Pract* 2014;210(12):787-792.
10. Aijaz M, Alam K, Maheshwari V, Hakim S, Kamal M. Clinicopathological study of role of CD34 expressions in the stroma of premalignant and malignant lesions of uterine cervix. *Ann Diagn Pathol* 2019;38:87-92.
11. Ayowole OA, Hassan YN, Ekundina VO. Prognostic value of Amacr and Ki-67 in progression of prostate cancer. *J Med Dent Sci Res* 2021;8(2):41-51.
12. Ekundina VO, Oladele AA, Wilson HT, Hassan NY, Sanya JO. Diagnostic utility of BCL-2, E-cadherin and PSA in the progression of malignant prostatic lesions. *Int J Med Lab Res* 2021;6(1):1-10.
13. Joshi S, Sankaranarayanan R. Opportunities for cervical cancer prevention in India. *J Krishna Inst Med Sci Univ*2015;4:8-16.
14. Catarino R, Petignat P, Dongui G, Vassilakos P. Cervical cancer screening in developing countries at a crossroad: Emerging technologies and policy choices. *World J Clin Oncol* 2015;6(6):281.
15. Wu J, Li XJ, Zhu W, Liu XP. Detection and pathological value of papillomavirus DNA and p16INK4A and p53 protein expression in cervical intraepithelial neoplasia. *Oncol Letters* 2014;7(3):738-744.
16. Mucharla R, Prathyusha P, Voolapalli G, Ravinder T, Sreenivas V. Study of p53 expression in carcinoma cervix and normal cervical epithelium. *Int Arch Integ Med* 2019;6(7):37-48.
17. Tan GC, Sharifah NA, Salwati S, Shiran MS, Hatta AZ, Ng HO. Immunohistochemical study of p53 expression in premalignant and malignant cervical neoplasms. *Med Health* 2007:125-32.
18. Vasilescu F, Ceauşu M, Tănase CR, Stănculescu RU, Vlădescu TE, Ceauşu Z. p53, p63 and Ki-67 assessment in HPV-induced cervical neoplasia. *Rom J Morphol Embryol* 2009;50(3):357-361.
19. Quade BJ, Yang A, Wang Y, Sun D, Park JJ, Sheets EE, *et al*. Expression of the p53 homologue p63 in early cervical neoplasia. *Gynecol Oncol* 2001;80(1):24-29.
20. Jin Y, Xiao W, Song T, Feng G, Dai Z. Expression and prognostic significance of p53 in glioma patients: a meta-analysis. *Neurochem Res* 2016;41(7):1723-1731.
21. Yadav V, Jaiswal, M, Pandey, S.A comparative study of p53 expression in premalignant and malignant cervical lesions at a tertiary care institute of Rohilkhand region, India. *Int J Med Sci Publ Health* 2018; 7(3): 216-222.
22. Cha JY, Cho TI, Park TY, So JS, Choi SK, Park ES. Analysis of expression of survivin, caspase 3, and p53 protein in cervical neoplasia comparing with Ki-67 index. *Korean J Gynecol Oncol* 2008; 19(1):17-25.
23. Lindström AK, Tot T, Stendahl U, Syrjänen S, Syrjänen K, Hellberg D. Discrepancies in expression and prognostic value of tumor markers in adenocarcinoma and squamous cell carcinoma in cervical cancer. *Anticancer Res* 2009;29(7):2577-2578.
24. Munakata S, Watanabe O, Ohashi K, Morino H. Expression of Fas ligand and bcl-2 in cervical carcinoma and their prognostic significance. *Am J Clin Pathol* 2005;123(6):879-885.

25. Singh M, Srivastava S, Singh U, Mathur N, Shukla Y. Co-expression of p53 and Bcl-2 proteins in human papillomavirus-induced premalignant lesions of the uterine cervix: correlation with progression to malignancy. *Tumor Biol* 2009;30(5-6):276-285.
26. Shukla S, Dass J, Pujani M. p53 and bcl2 expression in malignant and premalignant lesions of uterine cervix and their correlation with human papilloma virus 16 and 18. *South Asian J Cancer* 2014;3(1):48-53.
27. Hameetman L, Kok P, Eilers PH, Cleton-Jansen AM, Hogendoorn PC, Bovée JV. The use of Bcl-2 and PTHLH immunohistochemistry in the diagnosis of peripheral chondrosarcoma in a clinicopathological setting. *Virchows Archiv* 2005; 446(4):430-7.
28. Li Q, Huang W, Zhou X. Expression of CD34, α-smooth muscle actin and transforming growth factor-β1 in squamous intraepithelial lesions and squamous cell carcinoma of the cervix. *J Int Med Res* 2009; 37(2):446-454.

Author for Correspondence:
Ekundina Victor Olukayode, Department of Medical Laboratory Science (Histocytopathology Unit) College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Ekiti state, Nigeria Email: ekundinav@abuad.edu.ng Cell: 08038836691

How to cite this article:
Olukayode EV, Favour AO, Chelsea EC, Ayowole OA, Olurotimi S, Gerald Ikechi E. Assessment of P53 BCL-2 and CD34 in premalignant and malignant cervical lesions. *J Krishna Inst Med Sci Univ* 2022; 11(2):31-45

Submitted: *18-Feb-2022* ***Accepted:*** *15-Mar-2022* ***Published:*** *01-Apr-2022*