ORIGINAL ARTICLE

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

Prerna Juneja [1], Chandni Gupta[2], Vikram Palimar [1], Deepak Nayak M[3]*

[1]Department of Forensic Medicine, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India, 576104, [2]Department of Anatomy, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India, 576104, [3]Department of Pathology, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India, 576104

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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Author for Correspondence:
Dr. Vikram Palimar, Department of Forensic Medicine, KMC Manipal, India-576104
Email: vpalimar@gmail.com Cell: 9886340969

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