ISSN (O) 3108-2246 https://doi.org/10.66328/ijprmh.2026.0201002

International Journal of Public Research in Medicine and Health (IJPRMH)

Volume 2, Issue 1, Jan-Mar 2026

Original Research Article

Comparative Histomorphometric Analysis of Hepatocyte Nuclear Morphometry and Binucleation in Young and Elderly Adult Cadavers: A Pilot Study

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Received: 31/12/2025
Revised: 29/01/2026
Accepted: 07/02/2026

Abstract

Background: Aging of the liver is accompanied by subtle structural alterations at the cellular level, including changes in hepatocyte nuclear morphology and the frequency of binucleated cells. Quantitative human data describing these changes remain limited, particularly in cadaveric studies employing objective morphometric techniques.

Methods: A comparative cross-sectional histomorphometric study was conducted on liver tissue obtained from 16 adult cadavers. Specimens were categorized into two age groups: young adults (20–40 years, n = 8) and elderly adults (≥60 years, n = 8). Standardized wedge biopsies were obtained from the right hepatic lobe, processed using routine histological techniques, and stained with hematoxylin and eosin. Digital histomorphometry was used to measure hepatocyte nuclear diameter, nuclear area, cytoplasmic area, nuclear-to-cytoplasmic (N:C) ratio, and the percentage of binucleated hepatocytes. Statistical comparisons between groups were performed using appropriate parametric or nonparametric tests, with significance set at $p < 0.05$.

Results: Elderly specimens demonstrated significantly greater nuclear diameter and nuclear area compared with young adults ($p < 0.001$). The N:C ratio was also significantly higher in the elderly group. In addition, the proportion of binucleated hepatocytes showed a marked increase in elderly cadavers, exceeding twofold relative to younger specimens ($p < 0.001$). Age exhibited strong positive correlations with nuclear morphometric parameters and binucleation frequency.

Conclusion: Hepatic aging is associated with measurable enlargement of hepatocyte nuclei and increased binucleation, reflecting structural remodeling of liver parenchyma. Despite the modest sample size, the consistent morphometric trends observed highlight the utility of quantitative histological analysis in characterizing physiological hepatic aging.

Keywords: Hepatic Aging, Hepatocyte Morphometry, Binucleation, Liver Histology, Nuclear Morphometry, Polyploidy, Hepatic Senescence.

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Introduction

The liver is a vital parenchymal organ with central roles in metabolism, detoxification, protein synthesis, and homeostatic regulation throughout life. Although it exhibits remarkable functional resilience, aging induces subtle morphologic and cellular alterations that may compromise hepatic reserve and increase susceptibility to disease in later decades [1]. Age-dependent changes at the cellular level include modifications in hepatocyte size, nuclear morphology, chromatin organization, and polyploidy that can affect mitotic capacity and metabolic function [2–4].

Human hepatocytes possess a unique capacity for polyploidization and binucleation, phenomena that increase with advancing age and reflect alterations in cell cycle dynamics and differentiation status [2,3]. Early studies demonstrated that the proportion of binucleated hepatocytes in human liver tissue increases progressively across the adult lifespan, suggesting that age-related replicative history and genomic dosage compensation contribute to these morphologic changes [2]. Increased polyploidy is considered both a marker of cellular aging and a compensatory response to cumulative oxidative and metabolic stress [4,5]. These features have been corroborated by comparative investigations that identify age-associated enlargement of nuclear and cell size coupled with incremental binucleation in human hepatic tissue.

Despite recognition of these phenomena, quantitative data on hepatocyte nuclear morphometry and binucleation in normal human liver across defined age cohorts remain limited. Most existing evidence derives from small observational series or animal models, with few studies systematically comparing younger and older adult populations using standardized histomorphometric techniques [2,3]. Moreover, the mechanistic implications of such changes for liver functional capacity, regenerative response, and vulnerability to age-related liver diseases are not fully elucidated [1,4].

Accurate histomorphometric characterization of hepatocyte morphology across age groups can therefore serve multiple scientific purposes: establishing normative morphometric baselines, identifying structural hallmarks of hepatic aging, and informing interpretations of age-related hepatic pathology in clinical and forensic practice. Given that the liver's regenerative potential declines with age and that morphologic aging signatures may precede functional deterioration, objective quantification of nuclear and cellular features can yield insights into the biology of hepatic senescence [1,4,6].

In this context, the present study aims to quantitatively compare hepatocyte nuclear size, nuclear-to-cytoplasmic ratio, and the prevalence of binucleation between young and elderly adult cadaveric liver specimens. By employing rigorous digital histomorphometry, we seek to define age-associated microstructural hepatic changes with potential relevance to both basic anatomy and clinical hepatology.

Methodology

Study Design and Setting

A comparative cross-sectional histomorphometric study was conducted in the Department of Anatomy of Lakshmi Narain College of Technology Medical College, Indore. The investigation was designed as an exploratory pilot analysis to evaluate age-associated variations in hepatocyte nuclear morphology and binucleation in adult human cadaveric liver tissue.

Study Sample

A total of 16 adult cadavers were included in the study. Cadavers were allocated into two

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age-defined groups to maximize biological contrast:

- **Group I (Young adults):** 20–40 years (n = 8)
- **Group II (Elderly adults):** ≥60 years (n = 8)

Cadavers in the intermediate age range (41–59 years) were deliberately excluded to enhance intergroup differentiation and improve detection of morphometric effect size.

Inclusion Criteria

- Adult cadavers aged ≥20 years
- Documented age and sex
- Well-preserved bodies with minimal postmortem decomposition
- Absence of grossly visible hepatic pathology

Exclusion Criteria

- Evidence of cirrhosis, nodularity, or advanced fibrosis on gross examination
- Documented history of chronic liver disease or hepatic malignancy (if records available)
- Extensive hepatic trauma
- Severe autolysis affecting tissue architecture

Tissue Sampling Procedure

To ensure uniformity and reduce zonal bias, tissue samples were harvested using a standardized protocol:

- A wedge-shaped specimen measuring approximately 1.5 × 1.5 × 1 cm was obtained from the right lobe of the liver (segment V/VI region).
- The sample was taken approximately 2 cm below the capsular surface to avoid subcapsular architectural distortion.
- All specimens were immediately fixed in 10% neutral buffered formalin for a minimum of 48 hours prior to processing.

Histological Processing

Fixed tissues were processed using routine paraffin embedding techniques. Serial sections of 4–5 µm thickness were obtained using a rotary microtome. Sections were mounted on clean glass slides and stained with Haematoxylin and Eosin (H&E) following standard laboratory protocols.

Slides demonstrating optimal preservation and minimal artifact were selected for morphometric analysis.

Histomorphometric Analysis

Calibration

All measurements were performed using a trinocular research microscope equipped with a digital camera attachment. Calibration was carried out using a stage micrometer prior to analysis. Image analysis was conducted using calibrated digital image processing software.

Field Selection

For each cadaver:

- Ten non-overlapping high-power fields (HPFs) were selected randomly from well-preserved areas avoiding portal tracts and central veins to minimize structural bias.
- Areas showing tissue distortion, folding, or autolytic change were excluded.

Measurement Protocol

Within each high-power field:

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- Fifty hepatocytes with clearly visible cell boundaries and centrally placed nuclei were selected.
- Cells with overlapping nuclei, distortion, or sectioning artifact were excluded.

The following parameters were recorded:

1. **Nuclear Diameter (µm):** Measured along the longest visible axis of the nucleus.
2. **Nuclear Area (µm²):** Calculated using digital tracing tools.
3. **Cytoplasmic Area (µm²):** Determined by subtracting nuclear area from total hepatocyte area.
4. **Nuclear-to-Cytoplasmic (N:C) Ratio:** Calculated as nuclear area divided by cytoplasmic area.
5. **Binucleated Hepatocyte Percentage:** The number of binucleated hepatocytes was counted per 1000 hepatocytes examined and expressed as a percentage.

For each parameter, the mean value per field was calculated, followed by the mean per cadaver. The cadaver mean served as the unit of statistical analysis to avoid pseudo-replication bias.

Observer Reliability

To minimize measurement bias:

- All measurements were performed by the principal investigator.
- A subset of randomly selected slides (20%) was reassessed after a two-week interval to evaluate intra-observer consistency.
- The intraclass correlation coefficient (ICC) was calculated to assess measurement reliability.

Statistical Analysis

Data were entered into a spreadsheet and analysed using statistical software.

1. Normality of distribution was assessed using the Shapiro–Wilk test.
2. For normally distributed variables, intergroup comparison was performed using the independent samples t-test.
3. For non-normally distributed data, the Mann–Whitney U test was applied.
4. Effect size (Cohen's d) was calculated to determine the magnitude of age-related differences.
5. A p-value < 0.05 was considered statistically significant.

Results were expressed as mean ± standard deviation for continuous variables.

Ethical Considerations

The study was conducted after obtaining approval from the Institutional Ethics Committee of Lakshmi Narain College of Technology Medical College, Indore. Cadaver procurement and utilization were carried out in accordance with institutional guidelines and prevailing legal provisions governing the use of human anatomical material for academic and research purposes. No personal identifiers were recorded, and confidentiality was strictly maintained throughout the study.

Results

Baseline Characteristics

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A total of 16 cadaveric liver specimens were analysed, comprising 8 young adults (20–40 years) and 8 elderly adults (≥60 years). The mean age of Group I was 31.4 ± 5.8 years, whereas the mean age of Group II was 68.9 ± 6.7 years. There was no statistically significant difference in sex distribution between the two groups ($p = 0.62$).

Hepatocyte nuclear diameter was significantly greater in the elderly group compared with the young group. The mean nuclear diameter in Group I was 7.82 ± 0.48 µm, whereas in Group II it measured 9.14 ± 0.61 µm ($p < 0.001$). **(Figure 1)** The calculated effect size (Cohen's d = 2.35) indicated a large magnitude of difference. Similarly, mean nuclear area was significantly increased in elderly specimens (65.27 ± 6.82 µm²) compared to young adults (48.96 ± 5.11 µm²) ($p < 0.001$). **(Figure 2)** Cytoplasmic area showed a modest but statistically significant increase in the elderly group ($p = 0.041$). Consequently, the nuclear-to-cytoplasmic (N:C) ratio demonstrated a significant elevation with advancing age**. (Table 1)**

Table 1. Comparison of Hepatocyte Nuclear Morphometric Parameters Between Age Groups

Parameter	Young Adults (n=8) Mean ± SD	Elderly Adults (n=8) Mean ± SD	p-value	Effect Size (Cohen's d)
Nuclear diameter (µm)	7.82 ± 0.48	9.14 ± 0.61	<0.001	2.35
Nuclear area (µm²)	48.96 ± 5.11	65.27 ± 6.82	<0.001	2.71
Cytoplasmic area (µm²)	156.42 ± 14.73	169.88 ± 18.94	0.041	0.79
N:C ratio	0.31 ± 0.03	0.38 ± 0.04	0.002	1.95

The proportion of binucleated hepatocytes was markedly higher in the elderly group. Young adults demonstrated a mean binucleation percentage of 7.6 ± 1.8%, whereas elderly specimens exhibited 15.9 ± 3.4% ($p < 0.001$), representing more than a twofold increase. The effect size was large (Cohen's d = 3.01). **(table 2)**

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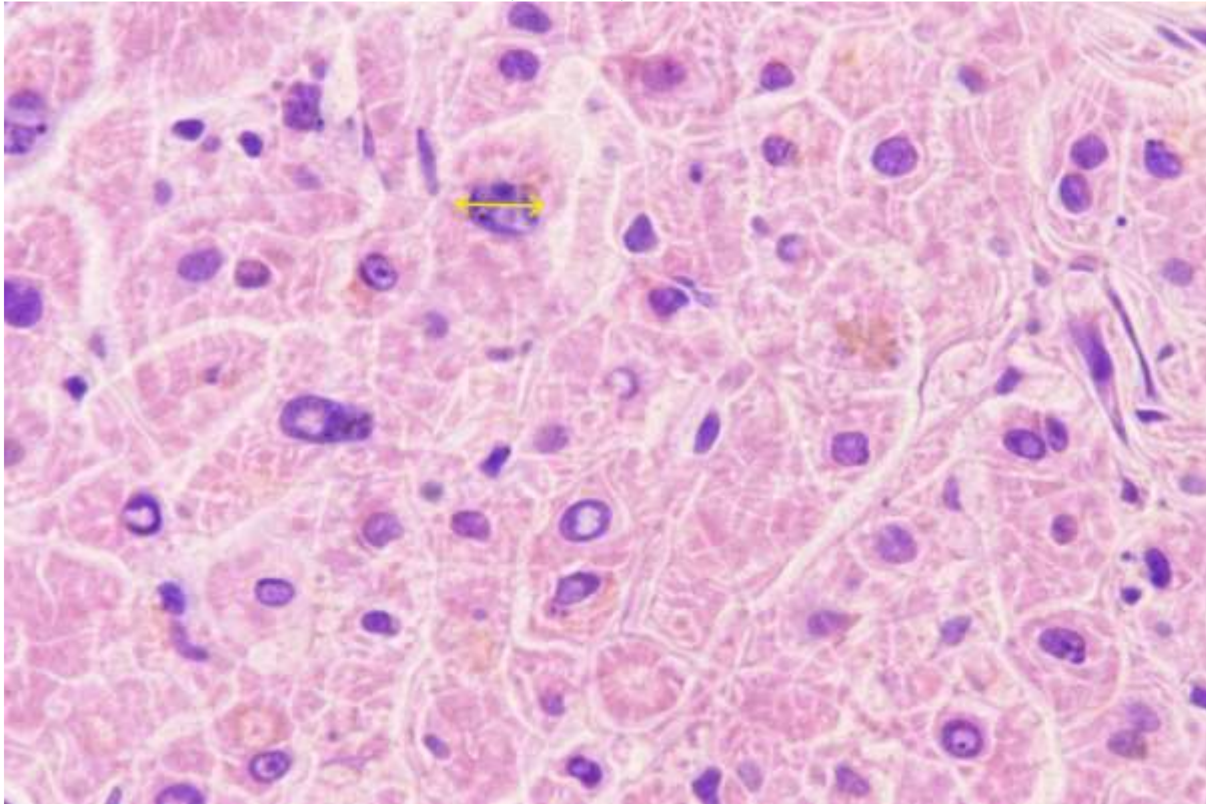

Figure 1: Measurement of hepatocyte nuclear diameter along the longest nuclear axis using calibrated digital image analysis software.

Table 2. Comparison of Binucleation Frequency Between Age Groups

Parameter	Young Adults (n=8) Mean ± SD	Elderly Adults (n=8) Mean ± SD	p-value	Effect Size (Cohen's d)
Binucleated hepatocytes (%)	7.6 ± 1.8	15.9 ± 3.4	<0.001	3.01

Pearson correlation analysis demonstrated a strong positive association between age and nuclear diameter ($r = 0.81$, $p < 0.001$), as well as between age and binucleation percentage ($r = 0.86$, $p < 0.001$). Nuclear area similarly showed a strong correlation with chronological age ($r = 0.84$, $p < 0.001$).

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ISSN (O) 3108-2246 https://doi.org/10.66328/ijprmh.2026.0201002
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Volume 2, Issue 1, Jan-Mar 2026

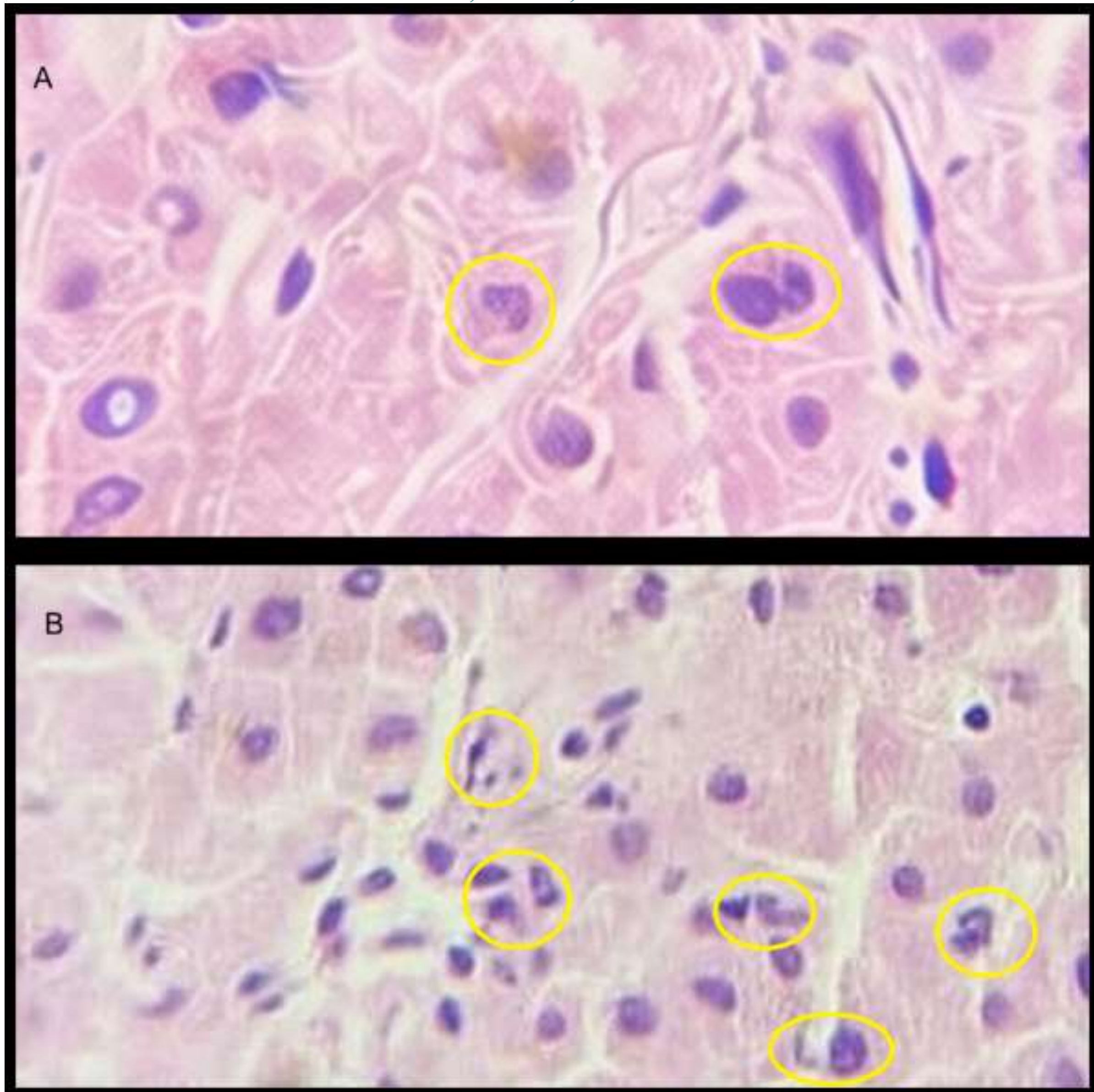

Figure 2: Binucleated hepatocytes (A) Young adult liver (B) Elderly liver showing increased number of binucleated hepatocytes as compared to young adult liver.

Table 3. Correlation of Age with Morphometric Parameters (n=16)

Parameter	Correlation Coefficient (r)	p-value
Nuclear diameter	0.81	<0.001
Nuclear area	0.84	<0.001
N:C ratio	0.69	0.003
Binucleation percentage	0.86	<0.001

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Intra-observer reproducibility analysis demonstrated high measurement consistency. The intraclass correlation coefficient (ICC) was 0.91 for nuclear diameter and 0.88 for nuclear area, indicating excellent reliability. **(Table 3)** The elderly group exhibited statistically significant enlargement of hepatocyte nuclei, increased nuclear area, elevated N:C ratio, and a substantially higher proportion of binucleated hepatocytes compared with young adults. The large effect sizes and strong positive correlations with age suggest that these morphometric alterations represent consistent structural features of hepatic aging rather than random variation.

Discussion

The present study demonstrates significant age-associated alterations in hepatocyte nuclear morphology and binucleation in adult human cadaveric liver. Elderly specimens exhibited increased nuclear diameter, expanded nuclear area, elevated nuclear-to-cytoplasmic ratio, and a markedly higher proportion of binucleated hepatocytes compared with younger adults. The magnitude of these differences, reflected by large effect sizes and strong positive correlations with chronological age, suggests that these changes represent intrinsic structural signatures of hepatic aging rather than incidental variability.

Hepatic aging is characterized by complex cellular remodelling processes that involve genomic, metabolic, and microarchitectural adaptations [7]. Enlargement of hepatocyte nuclei observed in the elderly group aligns with previously described age-related polyploidization. Hepatocytes possess a unique propensity to undergo polyploidy through incomplete cytokinesis or endoreduplication, resulting in either multinucleated or mononuclear polyploid cells [8]. This phenomenon has been interpreted as an adaptive response to chronic metabolic demand and cumulative oxidative stress. Experimental and human studies have demonstrated that polyploidization increases with age and may serve to enhance metabolic capacity or buffer genomic instability [8,9].

The observed increase in nuclear area and N:C ratio in elderly specimens is consistent with reports indicating that nuclear enlargement accompanies replicative aging and altered chromatin organization [2,3]. Nuclear expansion may reflect increased DNA content and transcriptional reprogramming associated with senescence. Age-related chromatin remodelling, including heterochromatin redistribution and epigenetic drift, has been shown to influence nuclear morphology and transcriptional regulation in multiple tissues, including liver [10]. These structural nuclear modifications are often accompanied by diminished proliferative responsiveness and impaired regenerative potential, phenomena well documented in aging hepatic tissue [1,7].

The marked rise in binucleated hepatocyte frequency in elderly cadavers constitutes one of the most striking findings of the present study. Earlier morphologic investigations reported progressive increases in binucleation across the adult lifespan, suggesting that incomplete cytokinesis becomes more frequent with advancing age [2]. Contemporary experimental data further indicate that age-dependent alterations in cyclin-dependent kinase regulation and mitotic checkpoint fidelity may predispose hepatocytes to binucleation [8,11]. While binucleation may enhance cellular metabolic output, it has also been proposed as a morphological correlate of replicative exhaustion and cellular senescence.

From a mechanistic standpoint, several converging pathways may underlie the morphometric changes documented herein. Oxidative stress accumulation with aging

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contributes to DNA damage and activation of senescence pathways, including p53- and p16-mediated responses [12]. Mitochondrial dysfunction, a recognized hallmark of aging, further amplifies reactive oxygen species production and may influence hepatocyte genomic stability [13]. These processes collectively promote structural nuclear remodelling and may explain the positive correlation observed between age and nuclear metrics in this cohort.

Importantly, the enlargement of hepatocyte nuclei and increased polyploidy may not be uniformly deleterious. Some investigators propose that polyploidization confers protective advantages by increasing genomic redundancy and enhancing resistance to mutational stress [8,14]. However, excessive polyploidization has also been implicated in altered metabolic regulation and susceptibility to hepatocarcinogenesis [14]. The balance between adaptive compensation and maladaptive remodelling likely shifts with advancing age, and quantitative morphometric assessment may provide objective insight into this transition.

The strong correlation coefficients identified in the present analysis underscore the consistency of age-related morphologic remodelling across individuals. The use of standardized sampling and digital histomorphometry strengthens the validity of these findings and reduces subjective bias inherent in purely descriptive histology. Notably, prior human studies often relied on qualitative observation or limited measurement parameters [2,3]. By integrating quantitative nuclear metrics with binucleation frequency, the current study expands upon existing knowledge and provides measurable benchmarks for hepatic aging.

From a broader biological perspective, hepatic aging does not occur in isolation but interacts with systemic metabolic alterations. Age-related changes in hepatic sinusoidal endothelial fenestration, reduced perfusion, and altered inflammatory milieu have been documented and may indirectly influence hepatocyte morphology [7,15]. Although vascular and stromal parameters were not the focus of this investigation, such factors may contribute to the structural remodelling observed at the cellular level.

The findings of this pilot study also bear potential relevance for forensic and clinical practice. Establishing normative age-associated morphometric parameters may assist in distinguishing physiological aging from early pathological transformation in autopsy material. Furthermore, objective nuclear morphometry may provide a supplementary tool in research exploring age estimation or age-related susceptibility to hepatic disease.

Certain limitations merit consideration. The relatively small sample size reflects the pilot nature of the study and precludes extrapolation to population-level estimates. Detailed clinical histories, including metabolic and alcohol-related factors, were not uniformly available and may represent unmeasured confounders. Additionally, embalming-related tissue shrinkage, although minimized through standardized processing, cannot be entirely excluded. Nevertheless, consistent sampling protocols and strong effect sizes support the internal validity of the observations.

Conclusion

This study demonstrates that advancing age is associated with quantifiable enlargement of hepatocyte nuclei, increased nuclear-to-cytoplasmic ratio, and a substantially higher frequency of binucleated hepatocytes in human liver, supporting the concept that polyploidization and nuclear remodelling constitute consistent structural hallmarks of hepatic aging. The use of standardized sampling, calibrated digital

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histomorphometry, and cadaver-level statistical analysis strengthens the internal validity and reproducibility of the findings. Despite the limited sample size and incomplete clinical background data inherent to cadaveric research, the large effect sizes and strong correlations observed suggest biological robustness. These results provide objective baseline morphometric parameters that may aid future investigations into hepatic senescence and facilitate differentiation between physiological aging and early pathological change in both anatomical and translational research settings.

Source of Funding: Nil
Conflict of Interest: None
Ethical approval: Institutional Research Committee

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Cite this Article: Sarda SKL, Apacible ADC, Mahajan R, Dandapat S. Comparative Histomorphometric Analysis of Hepatocyte Nuclear Morphometry and Binucleation in Young and Elderly Adult Cadavers: A Pilot Study. International Journal of Public Research in Medicine and Health. Jan-Mar 2026 (2)1: 5-15.

https://doi.org/10.66328/ijprmh.2026.0201002

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