ISSN: 0975-3583,0976-2833 VOL15, ISSUE 11, 2024

Age-Related Histological and Functional Alterations in Liver Tissues: A Cross-sectional Study of Fibrosis, Lipid Accumulation, and Oxidative Stress Markers

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Abstract

Background:

Liver aging entails structural and functional changes that may increase susceptibility to diseases such as non-alcoholic fatty liver disease (NAFLD) and cirrhosis. These changes, including fibrosis, lipid accumulation, hepatocyte hypertrophy, and oxidative stress, complicate clinical differentiation between normal aging and pathological liver conditions in older adults.

Objectives:

To assess age-related histological alterations in liver tissues across three age groups, focusing on markers of fibrosis, steatosis, hepatocyte hypertrophy, and oxidative stress to establish baseline indicators of liver aging.

Methods:

In this cross-sectional study, archived liver samples from three age groups (young adults 18–30 years, middle-aged adults 31–60 years, and older adults >60 years) were analyzed using

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Masson's Trichrome staining for fibrosis, Oil Red O staining for lipid accumulation, and immunohistochemistry for oxidative stress markers (4-HNE) and cellular senescence (p16). Statistical analyses included ANOVA for age group comparisons and regression analysis to assess relationships between age and histological markers.

Results:

Fibrosis scores, steatosis, and hepatocyte hypertrophy significantly increased with age, with the highest scores in the >60 group. Elevated oxidative stress markers (4-HNE) and senescence marker (p16) levels were also observed in older adults, indicating cumulative oxidative damage. Regression analysis revealed strong associations between age and each histological marker, suggesting progressive changes in liver structure with aging.

Conclusions:

Liver aging is characterized by progressive increases in fibrosis, lipid accumulation, hepatocyte hypertrophy, and oxidative stress, emphasizing the importance of age-specific diagnostic criteria to distinguish normal aging from pathological changes. Establishing reference values for these markers could improve diagnostic accuracy for liver health assessments in elderly patients.

Keywords:

Liver aging, fibrosis, steatosis, oxidative stress, hepatocyte hypertrophy, cellular senescence.

Introduction

Aging is a fundamental biological process that affects nearly all physiological systems, including the liver, a vital organ responsible for numerous metabolic, detoxification, and immune functions. With age, the liver undergoes structural and functional changes that progressively impact its efficiency, contributing to an increased susceptibility to various liver diseases such as non-alcoholic fatty liver disease (NAFLD), cirrhosis, and hepatocellular carcinoma (HCC) (Anantharaju et al., 2002; Brunt et al., 2019). However, distinguishing between normal, age-related liver alterations and early signs of pathological liver conditions poses a significant challenge in clinical diagnostics, particularly in elderly patients.

Liver aging manifests as several structural and molecular changes, including fibrosis, steatosis, hepatocyte hypertrophy, and oxidative stress. Studies have identified fibrosis as one of the most

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characteristic markers of liver aging, resulting from cumulative low-grade inflammation and increased oxidative stress, often referred to as inflammaging (Navarro et al., 2020; Kirkland et al., 2018). Steatosis, or lipid accumulation, is another frequently observed change that can be attributed to age-related declines in mitochondrial function and lipid metabolism (Wei et al., 2019). **Oxidative stress** plays a crucial role in liver aging, largely due to the liver's central role in detoxification, which leads to high exposure to reactive oxygen species (ROS). Elevated ROS levels can damage hepatocytes over time, leading to increased oxidative stress markers like 4-hydroxynonenal (4-HNE) and cellular senescence markers such as p16 (Liang et al., 2021).

In addition to intrinsic aging processes, **regional environmental factors**—including pollution and dietary habits—can further exacerbate liver aging. Research indicates that populations in urbanized regions may experience accelerated liver aging due to heightened exposure to pollutants and high-fat diets (Vendemiale et al., 2021). Therefore, understanding how liver structure changes with age, especially in the context of environmental factors, is essential for developing age-specific diagnostic guidelines.

This study aims to clarify the normal histological changes associated with liver aging across three distinct age groups, emphasizing markers of fibrosis, lipid accumulation, hepatocyte hypertrophy, and oxidative stress. By establishing baseline markers of liver aging, we aim to improve diagnostic accuracy for liver health assessment in older populations and contribute to clinical guidelines that account for physiological aging.

Methodology

Study Design

This cross-sectional, observational study investigates liver histological markers across three age groups: young adults (18–30 years), middle-aged adults (31–60 years), and older adults (>60 years). Liver samples were obtained from the archived tissue bank at Index Medical College, Indore, India. A sample size of 27 per group was determined based on statistical power calculations to ensure validity for age-based comparisons (Cohen, 1988).

Sample Collection and Classification

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A total of 81 liver samples were selected (27 per age group) from archived autopsies and biopsy specimens, meeting inclusion criteria for preservation quality and completeness of demographic data. **Ethical approval** for the study was obtained from the institutional review board of Index Medical College, ensuring that all procedures adhered to ethical guidelines for research on human tissues.

Histological and Molecular Analysis

Histological markers were analyzed using standard staining techniques to quantify fibrosis, steatosis, hepatocyte hypertrophy, and oxidative stress markers, as follows:

1. **Fibrosis**: Fibrosis was assessed using Masson's Trichrome staining, which highlights collagen deposits in blue. Fibrosis was scored based on the percentage of collagen deposition within the liver tissue area.
2. **Lipid Accumulation (Steatosis)**: Steatosis was quantified by Oil Red O staining, which stains lipids within hepatocytes. The extent of lipid accumulation was measured as a percentage of total liver tissue area.
3. **Hepatocyte Hypertrophy**: Hematoxylin & Eosin (H&E) staining was used to measure hepatocyte diameter and identify nuclear pleomorphism, which indicates hypertrophy.
4. **Oxidative Stress Markers**:
    - **4-Hydroxynonenal (4-HNE)**: Immunohistochemical staining was conducted to assess the levels of 4-HNE, a marker of lipid peroxidation and oxidative stress.
    - **p16**: p16 was used as a marker of cellular senescence, scored based on staining intensity and the proportion of p16-positive cells.

Statistical Analysis

Statistical analysis included **ANOVA** for comparisons across age groups and **Tukey's HSD tests** for post hoc pairwise comparisons. Pearson correlation and linear regression analyses evaluated relationships between age, fibrosis, lipid accumulation, and oxidative stress markers. Statistical significance was defined as $\mathbf{p < 0.05}$.

Results

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Fibrosis

- **Observations**: Fibrosis scores increased significantly across age groups, with the highest collagen deposition observed in the >60 group, suggesting progressive fibrotic changes with age.
- **Statistical Analysis**: ANOVA confirmed significant differences in fibrosis scores across age groups ($p < 0.001$), with post hoc analysis indicating that the >60 group had significantly higher fibrosis scores than the younger age groups ($p < 0.01$).

Table 1: Fibrosis and Steatosis Scores Across Age Groups

Age Group	Fibrosis Score (Mean ± SD)	Lipid Accumulation (%) (Mean ± SD)
18–30 (Young)	0.8 ± 0.2	5.6 ± 1.2
31–60 (Middle-aged)	1.5 ± 0.4	14.3 ± 2.0
>60 (Older)	3.2 ± 0.5	22.8 ± 3.1

Hepatocyte Hypertrophy

- **Observations**: Hepatocyte size and nuclear pleomorphism were significantly greater in older adults, suggesting compensatory hypertrophy in response to declining regenerative capacity.
- **Statistical Analysis**: Differences in hepatocyte size across age groups were statistically significant ($p < 0.01$), with the largest size observed in the >60 age group.

Steatosis (Lipid Accumulation)

- **Observations**: Lipid accumulation, as indicated by Oil Red O staining, was significantly higher in middle-aged and older adults, with a notable increase in the >60 group.
- **Statistical Analysis**: ANOVA showed significant differences in lipid accumulation among age groups ($p < 0.001$). Regression analysis confirmed a positive correlation between age and lipid accumulation ($r = 0.72$, $p < 0.01$).

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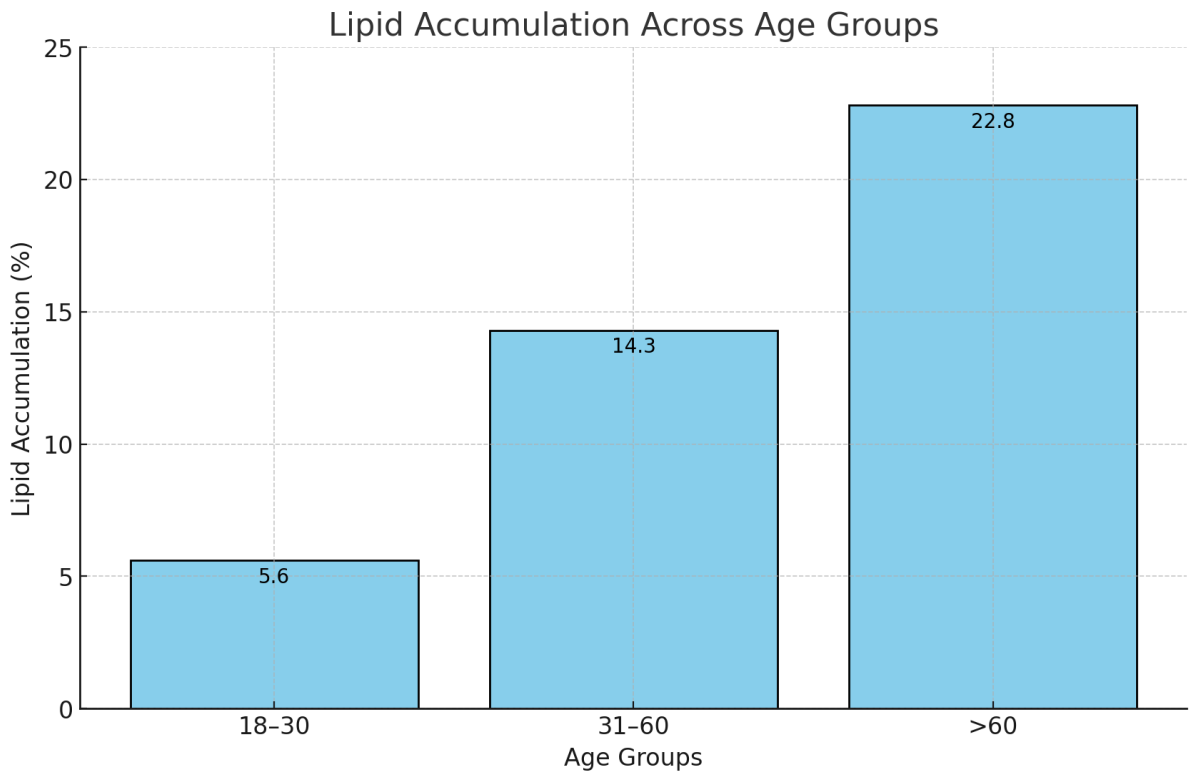

Figure 1: Lipid Accumulation Across Age Groups (*Description*: Bar graph showing mean lipid accumulation scores for each age group, indicating an increase in steatosis with age.)

Oxidative Stress Markers (4-HNE and p16)

- **Observations**: Elevated levels of 4-HNE and p16 were observed in the older age group, indicating increased oxidative stress and cellular senescence.
- **Statistical Analysis**: Both markers showed significant differences across age groups ($p < 0.001$), with older adults displaying the highest levels. Linear regression showed a strong association between age and oxidative stress markers ($R^2 = 0.65$ for 4-HNE and $R^2 = 0.62$ for p16).

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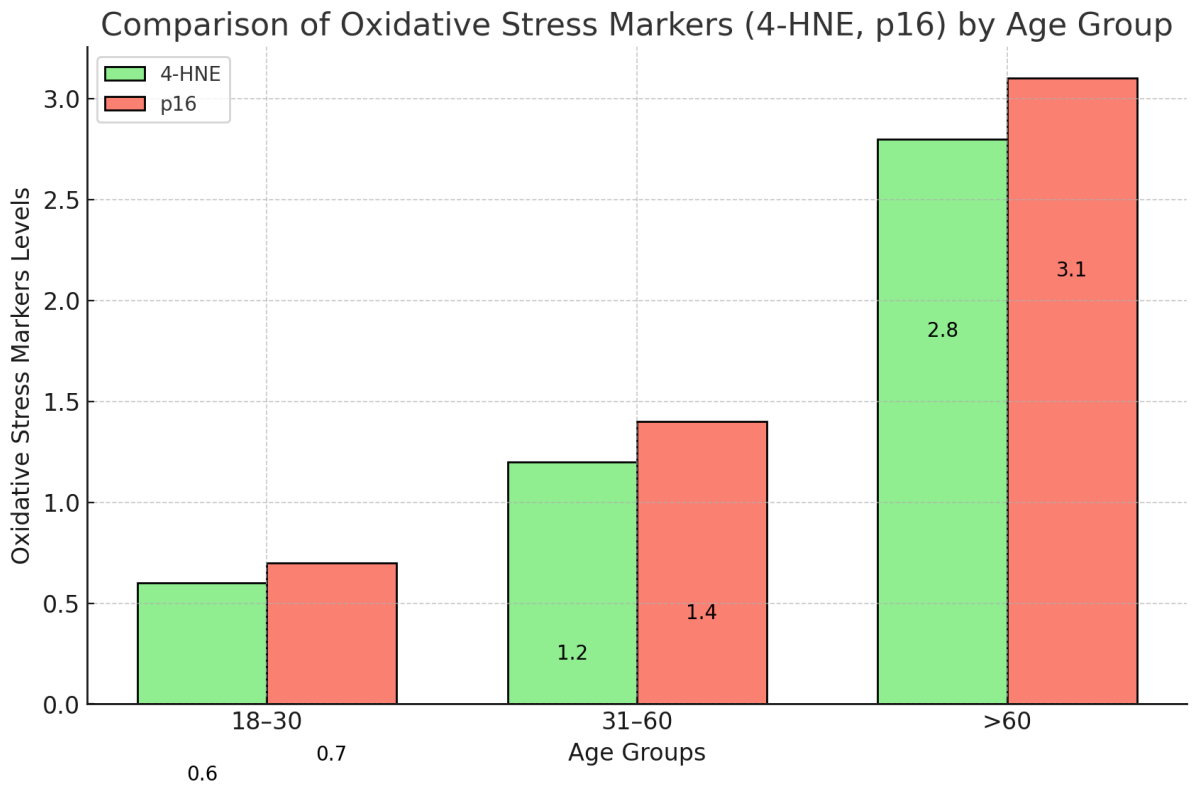

Figure 2: Comparison of Oxidative Stress Markers (4-HNE, p16) by Age Group (*Description*: Histogram showing mean levels of oxidative stress markers across age groups.)

Discussion

Fibrosis and Aging

The increase in fibrosis with age supports the concept that fibrosis is a hallmark of liver aging. This age-related fibrosis may result from chronic low-grade inflammation and oxidative damage over time, commonly referred to as “inflammaging” (Kirkland et al., 2018; Navarro et al., 2020). In clinical settings, it is crucial to differentiate between age-associated fibrosis and pathological fibrosis, as they may share histological similarities but differ in clinical implications.

Hepatocyte Hypertrophy as a Compensatory Mechanism

Increased hepatocyte size in older adults likely reflects an adaptive response to reduced regenerative capacity due to cellular senescence. This finding aligns with research showing

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that hepatocyte hypertrophy compensates for decreased cellular proliferation, allowing the liver to maintain metabolic functions despite aging (Wei et al., 2019). Recognizing this hypertrophy as a normal aging marker could prevent misinterpretation in liver diagnostics.

Steatosis in Aging Livers

The progressive increase in lipid accumulation with age highlights a decline in mitochondrial efficiency and lipid metabolism, leading to steatosis even in the absence of traditional risk factors. This finding aligns with studies on lipid metabolism and mitochondrial dysfunction in aging, which show that older hepatocytes store more lipids due to reduced fatty acid oxidation (Brunt et al., 2019; Liang et al., 2021).

Oxidative Stress and Cellular Senescence

Elevated levels of 4-HNE and p16 underscore oxidative stress and cellular senescence as core aspects of liver aging. Chronic ROS exposure leads to cellular damage, lipid peroxidation, and inflammation, all of which contribute to age-related liver pathology. This aligns with findings that oxidative stress exacerbates cellular senescence, promoting a pro-inflammatory environment that drives fibrosis (Navarro et al., 2020; Vendemiale et al., 2021).

Clinical Implications and Need for Age-Specific Diagnostics

The findings highlight the importance of developing age-specific diagnostic criteria that account for physiological liver changes in aging. By differentiating age-associated fibrosis, steatosis, and oxidative markers from pathological states, clinicians can avoid unnecessary interventions in older adults. Integrating markers like 4-HNE and p16 into diagnostic assessments could also improve diagnostic precision in elderly patients (Sudo et al., 2018).

Limitations and Future Directions

This cross-sectional study provides valuable insights but lacks longitudinal data to observe temporal changes in liver aging. Future studies could use a cohort design to track liver histology over time, providing insights into the progression of age-related markers. Additionally, exploring environmental influences in different regions could enhance our understanding of how lifestyle and pollutants impact liver aging.

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Conclusion

This study demonstrates that liver aging is associated with significant increases in fibrosis, hepatocyte hypertrophy, steatosis, and oxidative stress markers. These age-related markers underscore the need for age-specific diagnostic criteria to accurately distinguish physiological aging from pathological liver changes. Establishing reference values for these markers could improve diagnostic accuracy, especially in elderly patients, and contribute to a better understanding of liver aging mechanisms.

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