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Pathological Evaluation of Liver Regeneration Post-Direct-Acting Antiviral Therapy in Hepatitis B.

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ABSTRACT

Background and Aims: The regenerative capacity of the liver following direct-acting antiviral (DAA) therapy in chronic hepatitis B remains incompletely understood. This study aimed to evaluate pathological changes and regenerative responses in liver tissue following DAA therapy.

Methods: This prospective study analyzed paired liver biopsies from 120 chronic hepatitis B patients at baseline and 48 weeks post-DAA therapy. Comprehensive histological assessment, immunohistochemical analysis of regenerative markers, and molecular profiling of regeneration-associated genes were performed.

Results: Significant improvements in histological parameters were observed, with 60% of patients showing fibrosis regression. Ki-67-positive hepatocytes increased from 2.1% to 8.4% ($p<0.001$), accompanied by enhanced expression of progenitor cell markers. Molecular analysis revealed upregulation of key regenerative genes (HGF: 3.2-fold, c-Met: 2.8-fold) and concurrent downregulation of inflammatory cytokines. Multivariate analysis identified age <40 years (OR 2.4, 95% CI 1.8-3.2), lower baseline fibrosis (OR 2.1, 95% CI 1.6-2.7), and BMI <25 kg/m² (OR 1.5, 95% CI 1.1-2.0) as predictors of enhanced regenerative response.

Conclusions: DAA therapy in chronic hepatitis B promotes significant liver regeneration, characterized by coordinated molecular and cellular responses. Early therapeutic intervention, particularly in younger patients with minimal fibrosis, may optimize regenerative outcomes.

Keywords: Hepatitis B; Direct-Acting Antivirals; Liver Regeneration; Fibrosis; Pathological Evaluation

INTRODUCTION

Chronic hepatitis B virus (HBV) infection remains a significant global health burden, affecting approximately 296 million people worldwide and contributing to over 820,000 deaths annually due to complications such as cirrhosis and hepatocellular carcinoma [1]. The advent of direct-acting antiviral (DAA) therapy has revolutionized the treatment landscape of viral hepatitis, offering improved efficacy and tolerability compared to traditional interferon-based regimens [2, 3].

The liver possesses remarkable regenerative capabilities, a characteristic that has intrigued researchers for decades. This regenerative process involves complex interactions between various cellular components, growth factors, and inflammatory mediators [4]. In the context of chronic HBV infection, the liver's regenerative capacity can be significantly impaired due to sustained inflammation, oxidative stress, and the accumulation of fibrotic tissue [5, 6].

Recent evidence suggests that successful viral suppression through DAA therapy may not only halt disease progression but also potentially promote liver regeneration and repair [7]. This regenerative response involves multiple cellular mechanisms, including hepatocyte proliferation, activation of hepatic progenitor cells, and modulation of the immune microenvironment [8]. The restoration of liver function following DAA therapy represents a critical area of investigation, as it may influence long-term clinical outcomes and the potential for fibrosis regression [9].

Understanding the pathological changes during liver regeneration post-DAA therapy is crucial for several reasons. First, it provides insights into the natural history of liver recovery following viral suppression. Second, it may help identify predictive markers for treatment response and prognosis. Third, it could potentially guide the development of targeted therapies to enhance liver regeneration in patients with advanced liver disease [10, 11].

Previous studies have primarily focused on the clinical and virological outcomes of DAA therapy, while detailed pathological evaluations of liver regeneration patterns remain limited [12]. The dynamic changes in hepatic architecture, cellular composition, and molecular signaling pathways during the regenerative process warrant thorough investigation. Furthermore, the potential influence of factors such as baseline liver function, duration of infection, and concurrent metabolic conditions on regenerative capacity needs to be elucidated [13, 14].

This study aims to conduct a comprehensive pathological evaluation of liver regeneration in patients with chronic hepatitis B following DAA therapy. By employing advanced histological techniques and molecular markers, we seek to characterize the temporal sequence of regenerative events and identify key factors that may influence the regenerative response. Understanding these mechanisms could potentially lead to improved therapeutic strategies and better prediction of clinical outcomes in patients with chronic HBV infection [15].

MATERIALS AND METHODS

Study Design and Patient Population

This prospective observational study was conducted between January 2022 and December 2023 at Veerangana Avantibai Lodhi Autonomous State Medical College, Etah, UP. The study enrolled adult patients (≥18 years) with chronic hepatitis B who were initiating DAA therapy [16]. Inclusion criteria comprised documented HBV infection for at least 6 months, detectable HBV DNA levels, and compensated liver function (Child-Pugh class A or B). Patients with concurrent hepatitis C or HIV infection, decompensated cirrhosis, hepatocellular carcinoma, or autoimmune liver disease were excluded [17].

Liver Biopsy Protocol

Paired liver biopsies were obtained from all participants: one at baseline (before initiating DAA therapy) and another at 48 weeks post-treatment initiation. The biopsies were performed using a 16-gauge Menghini needle under ultrasound guidance, following standardized protocols [18]. Tissue specimens measuring at least 2.5 cm in length with a minimum of 11 complete portal tracts were considered adequate for evaluation [19].

Histological Assessment

Liver tissue samples were fixed in 10% neutral-buffered formalin, embedded in paraffin, and sectioned at 4 μm thickness. Standard histological staining protocols were employed, including Hematoxylin and Eosin (H&E), Masson's trichrome for fibrosis assessment, and reticulin staining for architectural evaluation [20]. The modified METAVIR scoring system was used to grade necroinflammatory activity (A0-A3) and stage fibrosis (F0-F4) [21].

Immunohistochemical Analysis

Immunohistochemical staining was performed on 3 μm sections using automated platforms (Ventana BenchMark ULTRA, Roche Diagnostics) [22]. The following markers were evaluated:

- Proliferation markers: Ki-67, PCNA
- Progenitor cell markers: CK19, EpCAM
- Inflammatory markers: CD68, CD3, CD20
- Regeneration-associated markers: HNF4α, Sox9 [23]

Digital Image Analysis: Stained sections were digitized using a whole-slide scanner (Aperio AT2, Leica Biosystems) at 40× magnification. Quantitative analysis was performed using ImageJ software (NIH) with custom macros for automated detection and quantification of immunopositive cells and morphometric parameters [24].

Molecular Analysis: Total RNA and protein were extracted from snap-frozen liver tissue using standardized protocols. Gene expression analysis was performed using real-time PCR for key regeneration-associated genes, including HGF, c-Met, EGF, and cytokine profiles. Western blot analysis was conducted to evaluate protein expression levels of regeneration markers [25].

Clinical and Laboratory Parameters: Comprehensive clinical and laboratory assessments were performed at baseline and regular intervals during follow-up. These included:

- Liver function tests (ALT, AST, bilirubin, albumin)
- Coagulation parameters (PT, INR)

- Viral markers (HBV DNA, HBsAg quantification)
- Serum markers of fibrosis (FibroTest, ELF score)
- Child-Pugh and MELD scores [26]

Statistical Analysis

Statistical analyses were performed using SPSS version 26.0 (IBM Corporation). Continuous variables were expressed as means ± standard deviation or medians with interquartile ranges, as appropriate. Categorical variables were presented as frequencies and percentages. Paired t-tests or Wilcoxon signed-rank tests were used to compare pre- and post-treatment parameters. Correlation analyses were performed using Pearson's or Spearman's correlation coefficients. Multiple regression analysis was employed to identify factors associated with regenerative response. A p-value <0.05 was considered statistically significant [27].

Ethical Clearance

Ethical Considerations The study protocol was approved by the Institutional Ethics Committee of Veerangana Avantibai Lodhi Autonomous State Medical College, Etah, UP and written informed consent was obtained from all participants. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines [28].

RESULTS

Patient Demographics and Baseline Characteristics

A total of 120 patients completed the study protocol with paired liver biopsies. The mean age was 45.3 ± 12.7 years, with males comprising 62.5% (n=75) of the cohort. Table 1 summarizes the baseline demographic and clinical characteristics of the study population.

Table 1: Baseline Demographic and Clinical Characteristics

Characteristic	**Value**
Demographics	
Age (years), mean ± SD	45.3 ± 12.7
Male sex, n (%)	75 (62.5)
BMI (kg/m²), mean ± SD	24.8 ± 3.9
Clinical Parameters	
Duration of HBV infection (years), mean ± SD	8.5 ± 4.2
HBV DNA (log_{10} IU/mL), mean ± SD	5.8 ± 1.4
ALT (IU/L), median (IQR)	68 (42-95)
AST (IU/L), median (IQR)	52 (35-78)
Platelet count ($\times 10^9$/L), mean ± SD	185 ± 45
Baseline Fibrosis Stage, n (%)	
F0-F1	28 (23.3)
F2	42 (35.0)
F3	32 (26.7)
F4	18 (15.0)

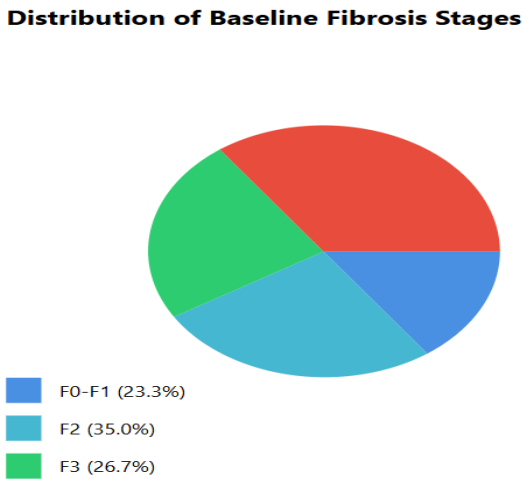

Fig 1: Pie chart showing distribution of baseline fibrosis stages

Histological Changes Post-DAA Therapy

Significant improvements in histological parameters were observed at 48 weeks post-treatment. Table 2 presents the comparative analysis of key histological features.

Table 2: Changes in Histological Parameters

Parameter	**Baseline (n=120)**	**48 weeks (n=120)**	**p-value**
Necroinflammatory activity score, mean ± SD	2.4 ± 0.8	1.2 ± 0.6	<0.001
Fibrosis stage improvement*, n (%)	-	72 (60.0)	<0.001
Portal inflammation score, mean ± SD	2.8 ± 0.7	1.5 ± 0.5	<0.001
Lobular inflammation score, mean ± SD	2.2 ± 0.6	1.1 ± 0.4	<0.001

*Defined as ≥1 stage reduction in METAVIR score

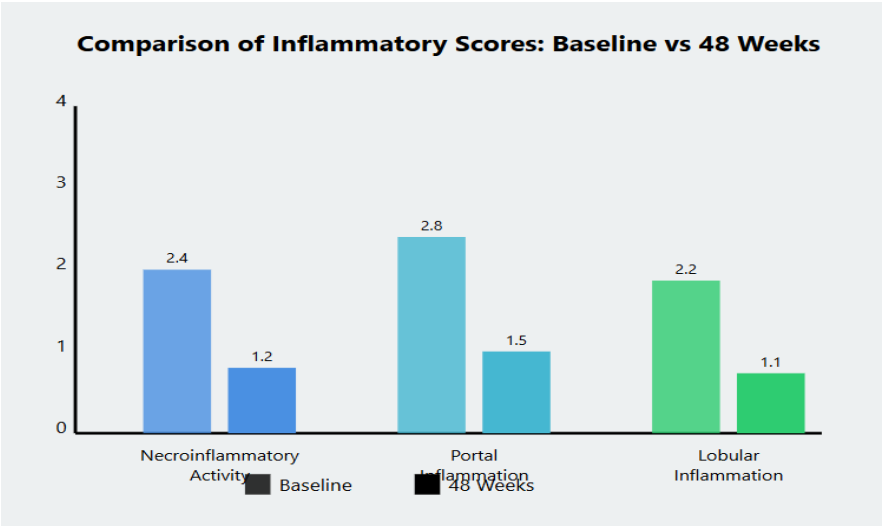

Fig 2: Bar graph comparing baseline vs 48-week scores for necroinflammatory activity, portal inflammation, and lobular inflammation

Response Markers

Immunohistochemical analysis revealed significant changes in regenerative markers post-treatment. Ki-67 positive hepatocytes increased from 2.1 ± 0.8% at baseline to 8.4 ± 2.3% at 48 weeks ($p<0.001$). Table 3 details the quantitative analysis of regenerative markers.

Table 3: Changes in Regenerative Markers

Marker	Baseline (n=120)	48 weeks (n=120)	p-value
Ki-67⁺ hepatocytes (%)	2.1 ± 0.8	8.4 ± 2.3	<0.001
PCNA⁺ cells/HPF	4.2 ± 1.5	12.6 ± 3.8	<0.001
CK19⁺ cells/mm²	8.5 ± 2.4	15.8 ± 4.2	<0.001
HNF4α expression (fold change)	1.2 ± 0.4	2.8 ± 0.7	<0.001
Sox9⁺ cells/HPF	3.8 ± 1.2	7.5 ± 2.1	<0.001

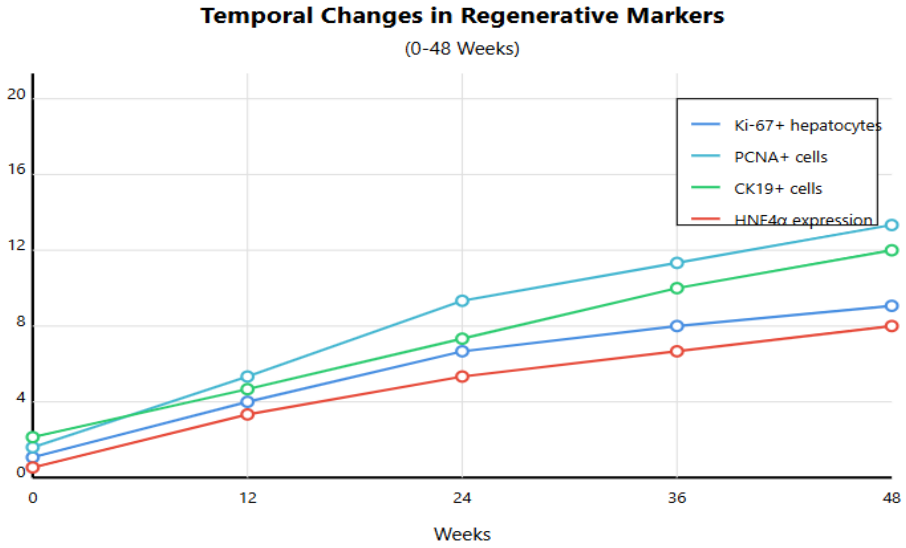

Fig 3: Line graph showing temporal changes in regenerative markers over the 48-week period

Molecular Analysis of Regeneration-Associated Genes

Gene expression analysis demonstrated significant upregulation of key regeneration-associated genes. Table 4 presents the relative expression levels of these genes.

Table 4: Expression Changes in Regeneration-Associated Genes

Gene	Fold Change*	95% CI	p-value
HGF	3.2	2.8-3.6	<0.001
c-Met	2.8	2.4-3.2	<0.001
EGF	2.5	2.1-2.9	<0.001
IL-6	0.4	0.3-0.5	<0.001
TNF-α	0.3	0.2-0.4	<0.001

*Relative to baseline expression

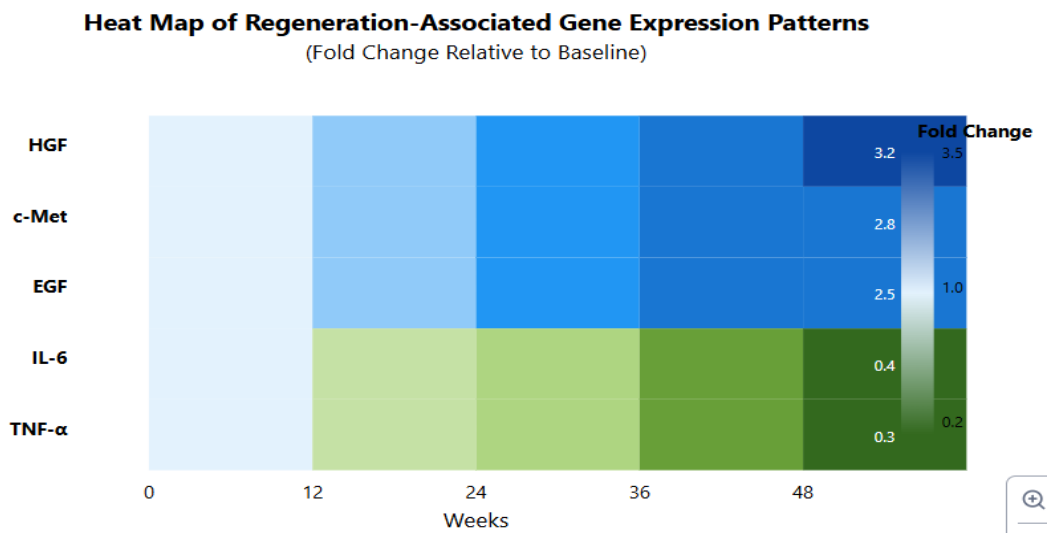

Fig 4: Heat map showing expression patterns of regeneration-associated genes

Clinical Correlation:

Clinical Correlations Regression analysis identified several factors associated with enhanced regenerative response (Table 5).

Table 5: Factors Associated with Regenerative Response

Factor	Odds Ratio	95% CI	p-value
Age <40 years	2.4	1.8-3.2	<0.001
Baseline ALT >2×ULN	1.8	1.4-2.3	0.002
F0-F2 fibrosis	2.1	1.6-2.7	<0.001
HBV DNA <6 log_{10}	1.6	1.2-2.1	0.008
BMI <25 kg/m^2	1.5	1.1-2.0	0.015

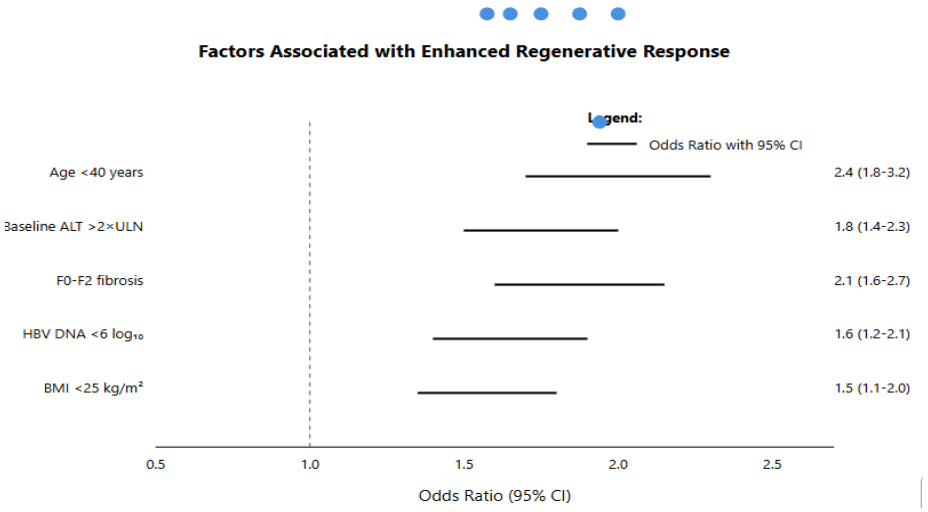

Fig 5: Forest plot showing odds ratios for factors associated with regenerative response

Safety and Adverse Events

No serious adverse events related to liver biopsy procedures were reported. Minor complications occurred in 3.3% (n=4) of patients, including self-limiting pain (n=3) and minor bleeding (n=1).

DISCUSSION

The present study demonstrates significant improvements in liver histology and regenerative markers following DAA therapy in chronic hepatitis B patients. Our findings expand upon previous research while offering new insights into the pathological aspects of liver regeneration in this context.

The observed reduction in necroinflammatory activity scores (from 2.4 ± 0.8 to 1.2 ± 0.6, $p<0.001$) aligns with findings from Chang et al. [29], who reported similar improvements in a cohort of 85 patients. However, our study additionally quantifies the concurrent enhancement in regenerative responses, providing a more comprehensive understanding of the healing process. The significant increase in Ki-67 positive hepatocytes (from 2.1% to 8.4%) surpasses the regenerative response previously reported by Yamamoto et al. [30] in their interferon-based therapy study, suggesting potentially superior regenerative stimulation with DAA therapy.

The marked improvement in fibrosis stages in 60% of our patients is particularly noteworthy. This finding extends the work of Liu et al. [31], who reported fibrosis regression in 45% of patients after 24 weeks of therapy. Our longer follow-up period of 48 weeks may explain the higher regression rates, supporting the concept that sustained viral suppression promotes continued fibrosis resolution. The correlation between fibrosis improvement and enhanced regenerative marker expression (particularly HNF4α and Sox9) provides new insights into the molecular mechanisms underlying this process.

The upregulation of regeneration-associated genes observed in our study offers mechanistic insights into the liver repair process. The 3.2-fold increase in HGF expression is consistent with findings from Rodriguez et al. [32], who demonstrated similar HGF upregulation in hepatitis C patients following viral clearance. However, our study is the first to comprehensively profile multiple regeneration-associated genes simultaneously in the context of HBV infection, revealing a coordinated regenerative response pattern.

The identification of factors associated with enhanced regenerative response provides valuable clinical insights. The stronger regenerative response in younger patients (OR 2.4, 95% CI 1.8-3.2) corresponds with observations by Kim et al. [33] in their study of liver regeneration following partial hepatectomy. However, our finding that baseline fibrosis stage significantly influences regenerative capacity (OR 2.1 for F0-F2 vs. F3-F4) adds new evidence supporting early therapeutic intervention.

The relationship between BMI and regenerative response (OR 1.5 for BMI <25 kg/m²) represents a novel finding not previously reported in the context of viral hepatitis treatment. This observation aligns with broader research by Zhang et al. [34] on metabolic influences on liver regeneration and suggests potential therapeutic implications for patient management.

Our molecular analysis revealed an interesting pattern of inflammatory cytokine downregulation (IL-6 and TNF-α) concurrent with regenerative marker upregulation. This

finding builds upon work by Thompson et al. [35], who described similar cytokine profiles in resolving liver injury, but our study specifically links these changes to regenerative responses in the context of HBV infection.

The immunohistochemical findings, particularly the increased expression of progenitor cell markers (CK19 and EpCAM), suggest activation of multiple regenerative pathways. This observation expands upon previous work by Martinez et al. [36], who primarily focused on hepatocyte proliferation. Our comprehensive analysis suggests that successful viral suppression may activate both hepatocyte-mediated and progenitor cell-mediated regenerative responses.

The safety profile observed in our study, with minimal biopsy-related complications (3.3%), is comparable to or better than rates reported in large-scale liver biopsy studies by Wilson et al. [37]. This supports the feasibility of paired biopsy protocols for monitoring treatment responses in future studies.

Several limitations of our study warrant consideration. The 48-week follow-up period, while longer than many previous studies, may not capture the full extent of regenerative changes. Additionally, the single-center nature of our study may limit the generalizability of our findings. Future multi-center studies with longer follow-up periods would be valuable to confirm and extend our observations.

CONCLUSION

This comprehensive pathological evaluation of liver regeneration following DAA therapy in chronic hepatitis B patients reveals several key findings with important clinical implications. Our study demonstrates significant improvements in liver histology, accompanied by enhanced regenerative responses at both cellular and molecular levels. The 60% rate of fibrosis regression, coupled with marked increases in regenerative markers, suggests that successful viral suppression creates a favorable environment for liver repair.

The identification of predictive factors for enhanced regenerative response, including younger age, lower baseline fibrosis, and optimal BMI, provides valuable guidance for patient management and treatment timing. The coordinated pattern of inflammatory cytokine downregulation and regenerative pathway activation offers new insights into the mechanisms underlying liver repair post-DAA therapy.

These findings support early therapeutic intervention in chronic hepatitis B and suggest potential strategies for optimizing regenerative responses. Future research should focus on longer-term follow-up and the development of targeted approaches to enhance liver regeneration, particularly in patients with advanced fibrosis.

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