Impact of Hepatic Steatosis on Fibrosis and Treatment Response in Chronic Hepatitis B: a Longitudinal Cohort Study

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Background and study aim: Data on the prevalence of NAFLD in chronic hepatitis B (CHB) patients is widely variable. Data about the effect of hepatic steatosis (HS) on CHB patients in the Middle East are scarce. So, we aimed to assess the associations between biopsy-proven HS and HBV viral load, liver biochemistry, and liver fibrosis and inflammation in CHB patients. We also aimed to evaluate the effect of HS on the complete virological response (CVR) within 2 years of follow-up.

Patients and Methods: This hospital-based longitudinal cohort study included all CHB patients who were subjected to liver biopsy between June 2019 to June 2020. Patients were followed up for liver biochemistry and HBV DNA at six months' intervals for two years. CVR was defined as undetectable HBV DNA by PCR testing 27 with a detection limit of 16 IU/ml as measured by the local laboratories. The baseline clinical characteristics and accumulative incidence of CVR were compared between patients with and without HS.

Results: A total of 91 CHB patients were enrolled from June 2019 to June 2020. 43 (47%) of them had histological evidence of HS. Patients with chronic hepatitis had significantly higher body mass index (BMI) compared to patients with chronic infection. Patients with HS had higher HBV deoxyribonucleic acid (DNA) but without statistical significance. The severity of fibrosis was greater in HS group but without statistical significance. CVR at 6, 12, and 24 months after treatment onset was significantly higher in patients without HS (p = 0.01. 0.000,0.06, respectively).

Conclusion: HS is highly prevalent in CHB patients. HS is not associated with HBV DNA or the severity of fibrosis. The rates of CVR are higher in CHB patients without HS.

INTRODUCTION

Despite the existence of an appropriate vaccination, hepatitis B virus (HBV) infection continues to be a serious health issue, with an estimated 296 million patients of chronic hepatitis B (CHB) **[1]**. Patients were classified according to the phase of CHB infection into: hepatitis B e antigen (HBeAg) positive chronic HBV infection, HBeAg-positive chronic hepatitis B, HBeAg-negative chronic HBV infection, and HBeAg-negative chronic hepatitis B **[2]**. Owing to the increased global incidence of obesity and metabolic syndrome, non-alcoholic fatty liver disease (NAFLD) has emerged as a significant contributor to chronic liver disease. The overall prevalence of NAFLD is increasing over time at an alarming rate with an estimated worldwide prevalence of 37•8% in or after 2016 compared to 25•5% in or before 2005 **[3].** As a result, the co-existence of NAFLD and HBV infection is frequently observed in healthcare settings Data on the prevalence of NAFLD in CHB patients is widely variable due to the variability of research cohorts and differences in NAFLD diagnostic techniques such as liver biopsy, transient elastography, and ultrasound. The estimated prevalence of NAFLD in CHB patients ranges from 14% to 70% **[4,5]**.

Because both NAFLD and CHB infection share the ability to cause progression of the hepatic inflammation and fibrosis, the interaction between these two diseases has become more and more interesting to research **[6,7].** However, the effect of NAFLD on the outcome of CHB infection is not well established, and various studies showed conflicting results. Most of the previous studies concluded that hepatic steatosis (HS) was associated with hepatitis B surface antigen (HBsAg) seroclearance **[8-12]** and low HBV deoxyribonucleic acid (DNA) serum levels **[13, 14]**. However, other studies found that HS did not affect HBV viral load **[15,16]**. HS was found to be a contributing factor in liver fibrosis progression and HCC development **[12, 17-19]**. On the contrary, multiple studies have revealed that HS is not a risk factor for fibrosis progression or HCC development in CHB patients **[6,20, 21]**. According to a recent study, CHB cases with high controlled attenuation parameter (CAP) measures had incomplete response to antiviral medications **[22]**. Other studies, however, revealed no connection between NAFLD and long-term biochemical or virological response to antiviral therapy **[20]**. To our best knowledge, data about the effect of HS on CHB patients in the Middle East are scarce; therefore, we conducted this study aiming to assess the associations between biopsy-proven HS and HBV viral load, liver biochemistry, and liver fibrosis and inflammation in CHB patients. This study also aims to evaluate the effect of HS on the complete virological response (CVR) within 2 years of follow-up.

PATIENTS AND METHODS

Study subjects and design:

This hospital-based longitudinal cohort study included all CHB patients who were subjected to liver biopsy between June 2019 to June 2020. CHB was defined as the HBsAg's continued existence for at least six months **[23]**. Patients were recruited from the outpatient clinic of the Tropical Medicine and Gastroenterology Department, Sohag University Hospital. The inclusion criteria were: (a) age ≥18 years; (b) treatment naïve; (c) detectable HBV DNA at baseline. The exclusion criteria were as follows: (a) decompensated cirrhosis; (b) HCC; (c) serological evidence of hepatitis C virus (HCV) infection; (d) human immunodeficiency virus (HIV) co-infection; (e) other causes of chronic liver diseases as autoimmune hepatitis, primary biliary cirrhosis, Wilson's disease, hemochromatosis, or drug-induced chronic hepatitis; (f) alcohol consumption; (g) current pregnancy; (h) any contraindications to liver biopsy, such as unwilling patients, prothrombin time (PT) >4 seconds more than control; international normalized ratio (INR) greater than 1.6; platelets count <100.000/mm^3. Patients were classified according to the phase of CHB infection into: hepatitis B e antigen (HBeAg) positive chronic HBV infection, HBeAg-positive chronic hepatitis B, HBeAg-negative chronic HBV infection, and HBeAg-negative chronic hepatitis B **[2]**. We categorized the patients into two groups: patients with chronic infection (including HBeAg positive and HBeAg-negative chronic HBV infection), and patients with chronic hepatitis (including HBeAg-positive and HBeAg-negative chronic hepatitis B).

Clinical and Laboratory Evaluation:

Baseline data included: demographic data, history of diabetes mellitus (DM) or hypertension, and body mass index (BMI) which was assessed as weight (kg) divided by height squared (m^2). Liver biochemistry, HBV DNA, HBV serological markers, and a complete blood count (CBC) were also done. Abdominal ultrasound was done to evaluate liver echogenicity, and splenic size, and to exclude hepatic focal lesions.

Liver Biopsy and Histopathological Assessment:

Ultrasound-guided percutaneous liver biopsy was done using a 16G semi-automated tru-cut needle to obtain two cores of tissue, 10 mm in length each **[24]**. All specimens were sent to the Pathology Laboratory of the same hospital. Formalin-fixed paraffin-embedded tissue sections were prepared and processed to be stained using hematoxylin and eosin (H&E) stain. The specimens were assessed by one experienced histopathologist who is blinded to participant data. Specimens were subjected to histopathological evaluation to determine both the stage of fibrosis and the grade of inflammation, using the METAVIR classification system **[25]**, which evaluates the fibrosis stage on a scale from F0 to F4. The necro-inflammatory activity was scored on a

scale from A0 to A3. A standard pathological method, including H&E, was used for examination. At least 6 portal tracts were analyzed. We classified fibrosis according to the METAVIR staging system, where F0= absence of fibrosis, F1= portal fibrosis, F2= portal and septal fibrosis, F3= nodular fibrosis, and F4= cirrhosis. Patients were divided into 2 groups according to fibrosis level: early fibrosis (mild to moderate) was defined as F0-F2; while advanced fibrosis was defined as F3-F4. NAFLD and CHB use different scoring systems for histological examination. Histologic grading of steatosis was evaluated according to the NAFLD activity score (NAS) which includes 3 components: steatosis, lobular inflammation, and ballooning degeneration. This system considers only macrovesicular steatosis and assesses the percentage of hepatocytes with steatotic vacuoles (Figure 1). The degree of steatosis was determined as follows: Score 0= <5%, Score1= 5-33%, Score 2= 34-66%, and Score 3= >66%. S0, S1, S2, and S3 were designated as minimal, mild, moderate, and severe steatosis, respectively. Lobular inflammation was scaled on a 0–2 scale, and ballooning on a 0–2 scale **[26]**.

Follow UP:

Patients were followed up for liver biochemistry and HBV DNA at six months intervals for two years. Patients who were eligible to therapy treated with the available oral nucleotide analogue. CVR was defined as undetectable HBV DNA by polymerase chain reaction (PCR) testing **[27]** with a detection limit of 16 IU/ml as measured by the local laboratories.

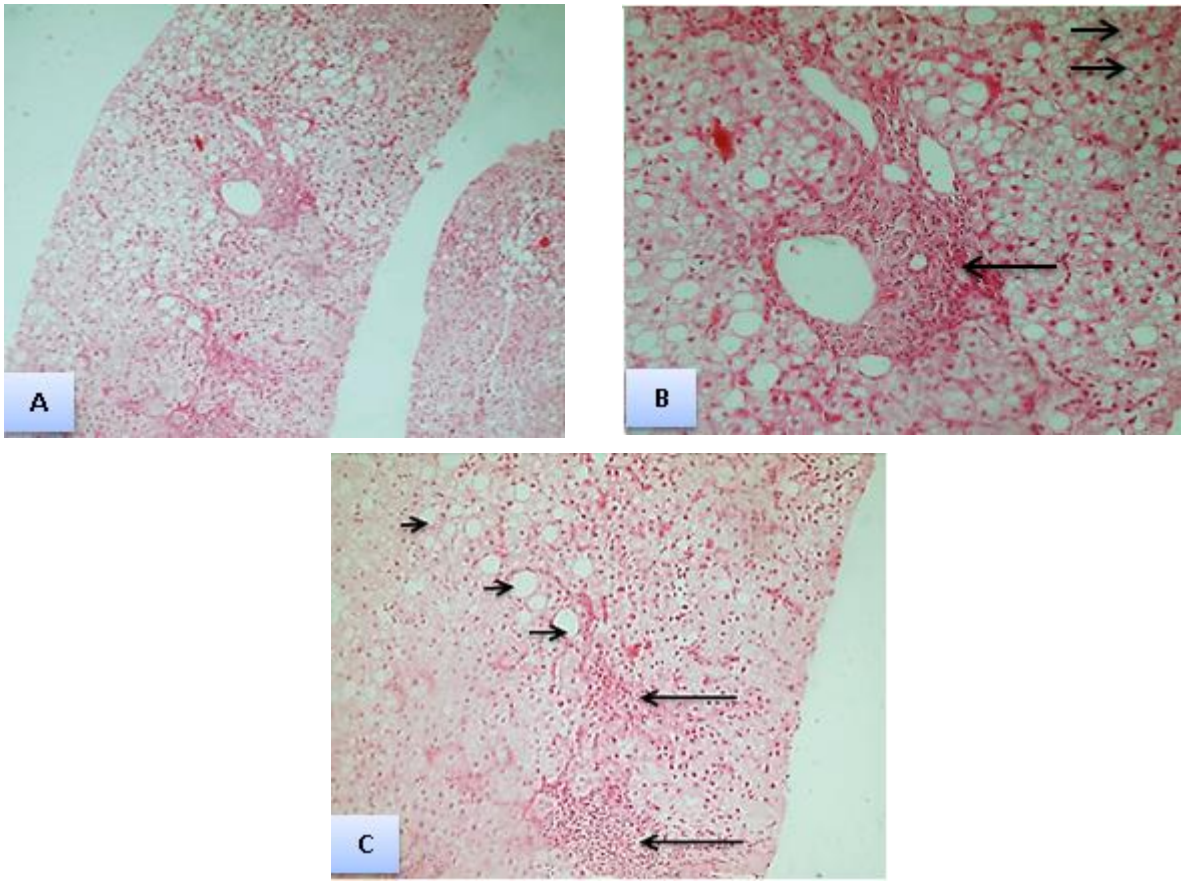

Figure 1: (A)&(B) Liver biopsy showed an area surrounding the central vein region with steatosis, inflammatory cells (long arrow), and ballooning of hepatocytes with enlarged rarefied cytoplasm (short arrow) (H&E stain, X100 & X200, respectively). (C) Hepatocytes containing large fat droplets occupying the cytoplasm and displacing the nucleus to the periphery (short arrows), foci of lobular inflammation (long arrow) composed predominantly of lymphocytes and Kupffer cells (H&E stain, X200).

Statistical analysis

SPSS version 16 (SPSS Inc., Chicago, USA) was used to assess the data. Categorical data were expressed as numbers and percentages, whereas continuous data were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR). For continuous data, examination of the differences between two groups was done using the *Student t-tes*t or *Mann-Whitney U* test, while the *Chi-square* test was used to analyze categorical data. The complete virological response over time was assessed using the *Kaplan-Meier* survival analysis test. *Pie and bar* charts were expressed by excel.

RESULTS

The current study was conducted on 91 CHB patients. Their mean age was 36±9.7years with an age range of 20-60 years. Of them,84 patients (92%) were HBeAg negative and only seven (8%) patients were HBeAg positive. Sixty patients (69%) had positive hepatitis B e antibody (Anti-HBe). When evaluating the studied population according to the CHB stage **[23]**, we found that only one patient (1%) had HBeAg positive chronic HBV infection, seven patients (8%) had HBeAg positive chronic hepatitis, 29 patients (32%) had HBeAg negative chronic HBV infection, and 54 patients (59%) had HBeAg negative chronic hepatitis (Figure1). Patients with chronic hepatitis had significantly higher BMI and alanine transaminase (ALT) levels compared to patients with chronic infection (p = 0.003, 0.01, respectively). The prevalence of HS was higher in patients with chronic hepatitis compared to patients with chronic infection but without statistical significance. Patients with chronic hepatitis had significantly higher scores of lobular inflammations compared to those with chronic infection *(p= 0.02)* (Table 1). Forty-seven percent of the studied cohort (43 patients) showed HS on liver biopsy (Figure 2). A mild degree of HS was reported in 28 CHB patients (31%), a minimal degree of HS was reported in 11 patients (12%), and a moderate degree of HS was reported in 4 CHB patients (4%). However, a severe degree of HS was not reported (Figure 3). According to the presence of steatosis on liver biopsy, we divided our population into two groups: the first group included CHB patients with HS (n=43), while the second group included CHB patients without HS (n=48). The age and gender distribution had no significant difference between the two groups. The BMI, aspartate transaminase (AST), total bilirubin, and splenic size were significantly higher in CHB patients with HS than those without (p = 0.02, 0.03,0.001, respectively). Moreover, patients with HS had higher HBV DNA and ALT levels but without statistical significance. The severity of hepatic fibrosis was greater in patients with HS than those without steatosis but without a statistical significance (p = 0.08) (Table 2). High lobular inflammation scores had a strong significant association with advanced fibrosis stages *(P=0.000)* (Table3).

All patients with HBeAg positive and negative hepatitis (61 patients) received medical treatment in the form of lamivudine 100 mg tablets once daily. During follow up six patients were excluded due to poor compliance. Fifty-five patients were followed for 2 years to evaluate the virological response. CVR at 6 months, 12 months, and 24 months after treatment onset was significantly higher in CHB patients without HS compared to those with HS (p = 0.01. 0.000,0.06, respectively). As CVR at 6^{th} month of treatment was reported in 88% of CHB patients without HS versus 57%of those with HS. Moreover, after 1 years of follow up 100% of CHB without HS achieved CVR, while only 56.7% of those with HS achieved CVR with one patient reported virological breakthrough (Table 4).

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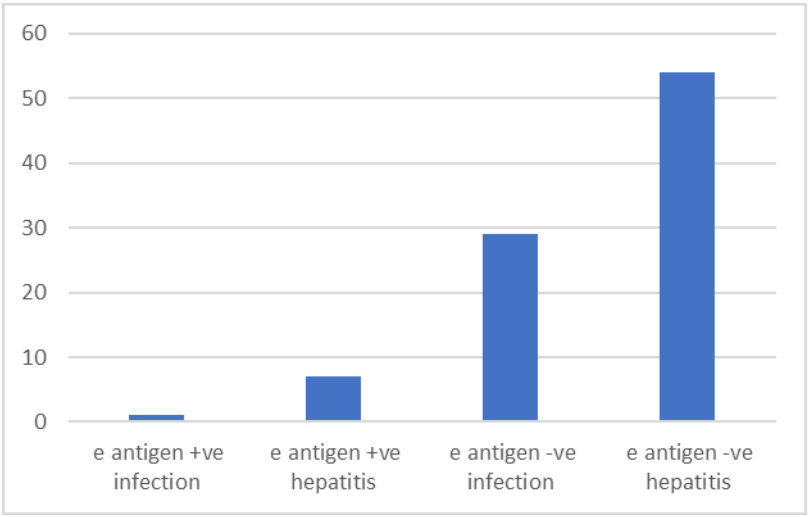

Figure 2: Stages of CHB infection in the studied population (Bar chart).

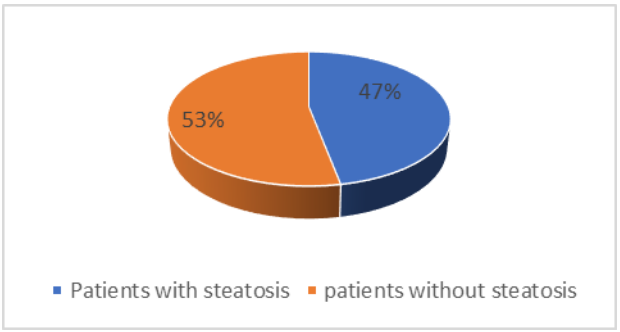

Figure 3: Percentage of CHB patients with steatosis on liver biopsy (Pie chart).

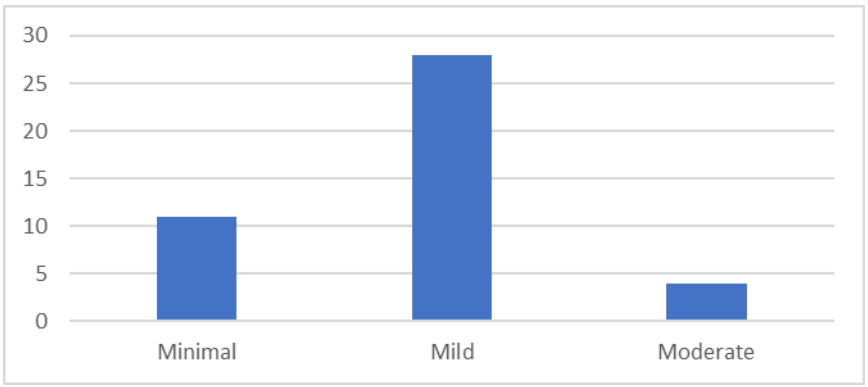

Figure 4: Different degrees of steatosis in the studied population (Bar chart).

Table 1: Baseline clinical, laboratory and histopathological characteristics of the studied population according to CHB stage (N=91).

	Chronic infection* N=30	Chronic hepatitis** N=61	P-value
Age (years), mean±SD	36±11.2	35.7±9	0.6
BMI, median (range)	22.7 (20.5-30)	25.7 (23.5-30)	0.003
Male, n% Female, n%	24 (80%) 6 (20%)	45 (73.8%) 16(26.2%)	0.5
HBV DNA (IU/L), median (IQR)	8700 (5324-53469)	15000 (4530-91395)	0.2
ALT (IU/L), median (IQR)	21.5 (17.3-27)	29 (20-46.5)	0.01
AST(IU/L), median (IQR)	17.8 (22.5-28)	24.6 (18-38)	0.3
Albumin(g/dl), mean±SD	4.3±0.4	4.3±0.5	0.2
Total bilirubin (mg/dl), mean±SD	0.72±0.27	0.74±0.22	0.6
PT, mean±SD	12.9±0.8	12.7±1	0.5
Prothrombin concentration, mean±SD	88.9±17.5	92±11.6	0.2
INR, mean±SD	1.05±0.12	1.04±0. 1	0.3
Steatosis	12 (40%)	31 (50.8%)	0.3
Fibrosis F0	 9(30%)	 0 (0%)	0.000

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F1 F2 F3 F4	21 (70 %) 0(0%) 0(0%) 0 (0%)	15 (24.6 %) 35 (57.4%) 6(9.8%) 5(8.2 %)	
Mild to moderate fibrosis (F0, F1, F2) Advanced fibrosis (F3, F4)	30 (100%) 0(0%)	50 (82%) 11(18%)	0.000
Cirrhosis	0(0%)	5(8.2 %)	0.01
Inflammation A0 A1 A2 A3	 3(10%) 24 (80%) 2(6.7%) 1(3.3%)	 0(0%) 12(19.6%) 40(65.6%) 9(14.8%)	0.000
Lobular inflammation 0 1 2 3	 12 (40%) 12 (40%) 5 (16.7%) 1 (3.3%)	 8 (13.1%) 26 (42.6%) 17 (27.9%) 10 (16.4%)	0.02
Hepatocyte ballooning 0 1	 15 (50.0%) 11 (36.7%)	 24 (39.3%) 24 (39.3%)	0.5

2	14 (13.3%)	13 (31.3%)	

Chi-square test was used to analyze categorical data
*Student t-tes*t or *Mann-Whitney U* test to examine of the differences between two groups

*(1 patient had HBeAg positive infection and 29 patients had HBeAg negative infection)

**(7 patients had HBeAg positive hepatitis and 54 patients HBeAg negative hepatitis)

ALT: alanine transaminase, AST: aspartate transaminase, HBV: hepatitis B virus, DNA: deoxyribonucleic acid, INR: international randomized ratio, IQR: interquartile range, BMI: body mass index, PT: prothrombin time.

Normal range of laboratory data: HBV DNA less than 20IU/L, ALT (0-40 IU /L), AST (0-40 IU /L), Albumin (3.5-5.2), T.bilirubin (0.2-1 mg/dl), PT (11-12.5), INR less than 1.1

Table 2: Baseline clinical, laboratory, and histopathological characteristics of the studied population according to the presence of steatosis (N=91).

	CHB patients with HS N=43	CHB patients without HS N=48	P-value
Age (years), mean±SD	36±8	36±10	0.9
BMI, median (IQR)	26.9 (23.8-30)	23.6 (21.3-27)	0.02
Male,n% Female, n%	29 (67%) 14 (33%)	40 (83%) 8(17%)	0.08
DM	3 (7%)	6(13%)	0.4
Hypertension	2 (5%)	1(2%)	0.4
Abdominal ultrasound measurements, mean±SD Liver size (cm) Splenic size (cm)	13.8 ±1.5 11.4±1.8	14±1.6 10.3±1.5	0.4 0.001
Hepatic echo-pattern Normal Bright	23(53.5%) 9(20.1%)	32(66.7%) 0(0%)	0.02

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Coarse	11(25.6%)	16 (33.3%)	
HBV DNA (IU/L), median (IQR)	17137 (5880- 70692)	9500 (3639-65872.5)	0.2
ALT (IU/L), Median (IQR)	29 (20-44)	22.1 (16.5-48.5)	0.07
AST(IU/L), Median (IQR)	27 (20-33)	21.3 (16-27.5)	0.03
Albumin(g/dl), mean±SD	4.2±0.58	4.3±0.34	0.5
Total bilirubin (mg/dl), mean±SD	0.79±0.23	0.68±0.23	0.03
PT, mean±SD	12.8±1	12.8±0.98	0.9
Prothrombin concentration, mean±SD	92±16.4	90±11	0.7
INR, mean±SD	1.03±0.09	1.05±0.12	0.3
Fibrosis F0 F1 F2 F3 F4	 0 (0%) 19 (44.2%) 17 (39.5%) 3 (7%) 4 (9.3%)	 5 (10.4 %) 15 (31.2%) 24(50 %) 3 (6.2 %) 1(2.1 %)	0.08
Mild to moderate fibrosis (F0, F1, F2) Advanced fibrosis (F3, F4)	36 (83.7%) 7(16.3%)	44 (91.6%) 4(0.4%)	0.2

Cirrhosis	4 (9.3%)	1 (2%)	0.1
Inflammation			0.8
A0	2(4.7%)	2(4.2%)	
A1	16(37.2%)	19(39.6%)	
A2	19(44.2%)	23(47.9%)	
A3	6(14%)	4(8.3%)	

Chi-square test was used to analyze categorical data
Student t-test or *Mann-Whitney U* test to examine of the differences between two groups

ALT: alanine transaminase, AST: aspartate transaminase, HBV: hepatitis B virus, DM: diabetes mellitus, DNA: deoxyribonucleic acid, INR: international normalized ratio, IQR: interquartile range, BMI: body mass index, PT: prothrombin time.

Normal range of laboratory data: HBV DNA less than 20IU/L, ALT (0-40 IU /L), AST (0-40 IU /L), Albumin (3.5-5.2), T.bilirubin (0.2-1 mg/dl), PT (11-12.5), INR less than 1.1

Table 3: Relationship between inflammation and ballooning and the stage of fibrosis in the studied population (N=91)

Stage of fibrosis	**Lobular inflammation**				**p-value**	**Ballooning of hepatocyte**			**p-value**
	0	**1**	**2**	**3**		**0**	**1**	**2**	
F0 N=5	4 (80.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	0.000	5 (100.0%)	0 (0.0%)	0 (0.0%)	0.1
F1 N=34	8 (23.5%)	19 (55.9%)	5 (14.7%)	2 (5.9%)		13 (38.2%)	12 (35.3%)	9 (26.5%)	
F2 N=41	7 (17.1%)	18 (43.9%)	13 (31.7%)	3 (7.3%)		19 (46.3%)	17 (41.5%)	5 (12.2%)	
F3 N=6	1 (16.7%)	0 (0.0%)	2 (33.3%)	3 (50.0%)		1 (16.7%)	4 (66.7%)	1 (16.7%)	
F4 N=5	0 (0.0%)	0 (0.0%)	2 (40.0%)	3 (60.0%)		1 (20.0%)	2 (40.0%)	2 (40.0%)	

Chi-square test was used to analyze categorical data

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Table 4: The accumulative incidence of virological response at 6 months, 1 year, and 2 years after the start of therapy (N=55).

	CHB patients with HS N= 30	CHB patients without HS N=25	P-value
CVR at 6 months, N (%)	17 (56.7%)	22 (88%)	0.01
CVR at 12 months, N (%)	16 (53%)	25(100%)	0.000
CVR at 24 months, N (%)	26 (68.7%)	25 (100%)	0.06

Kaplan-Meier survival analysis test
CVR: Complete virological response, HS: hepatic steatosis.

DISCUSSION

The increasing global prevalence of NAFLD raised the interest of researchers to study the interaction between NAFLD and other causes of chronic liver diseases. Our biopsy-based longitudinal cohort study evaluated different aspects of the relationship between NAFLD and CHB infection, including HBV viral load, liver biochemistry, liver fibrosis, inflammation, and the effect of HS on virological response to antiviral therapy. We found a high prevalence of biopsy-proven HS (about 50%) in CHB patients. This is consistent with previously published data from Turkey **[28,29]**, Italy **[30]**, Taiwan **[31]**, and China **[32]**. A low prevalence of HS in CHB patients was found by other investigators (5% to 34%) **[33-35]**. However, these studies were based on imaging modalities rather than liver biopsy, which is the gold standard for the diagnosis and grading of HS. The effect of HBV on lipid metabolism is complex. Animal studies showed that both lipid degradation and lipid synthesis were enhanced by HBV infection **[36]**. HBV X protein was found to be a key factor in regulating HS. HBV X protein enhances the gene expression of different lipogenic enzymes leading to fat deposition in hepatocytes and consequently HS **[37-39]**. Moreover, HBV DNA was found to be a contributing factor to HS by interfering with Sterol regulatory element binding proteins-1c (SREBP-1c) expression **[40]**. However, host factors such as metabolic syndrome have a predominant role in the induction of HS and cannot be neglected. In our study, we found that CHB patients with HS had a higher BMI, which is in agreement with many authors **[28,31,33,41]**. In addition, we found that CHB patients with active disease had a significantly higher BMI compared to patients with stable disease, while no significant difference was found between the two groups as regards HS frequency. This finding confirms the proposed role of obesity in the progression of fibrosis. Adiponectin, a powerful adipose-derived anti-inflammatory mediator, is frequently deficient in obesity. Adiponectin deficiency contributes to hepatic stellate cell (HSC) activation, fibrosis, and increased production of inflammatory mediators. Animal models showed that adiponectin knockout mice kept on a high-fat diet had more severe fibrosis than wild controls **[42]**, whereas carbon tetrachloride 4 induced fibrosis is reduced in mice after receiving adiponectin **[43]**.

Our data showed that CHB patients with HS tended to have higher HBV DNA levels than those without HS, but without statistical significance. Previous literature investigated the relationship between HBV DNA and HS with conflicting results. Two retrospective studies from China showed that HS was associated with metabolic factors rather than viral factors; namely HBV DNA and HBeAg status **[44,45]**. Moreover, many other studies did not find any relation between HBV DNA levels and NAFLD in CHB patients **[33,46,47]**. On the other hand, Xiaoyan et al **[48]**. found that HBV DNA levels were significantly higher in lean CHB patients with minimal HS compared to those without HS. However, the proposed protective effect of HS in CHB patients was attributed to the inverse relationship between HS and HBV DNA documented by many authors **[28,49,50]**. Nevertheless, the diagnosis of HS in many of these studies was imaging-based; using abdominal ultrasound or CAP measurements which is less accurate than liver biopsy; and this is a major strength in our study. Furthermore, the mechanism of the inverse relationship between HS and HBV DNA is still unclear. To our knowledge, few studies investigated this possible mechanism, and it was claimed that HS suppresses HBsAg and HBV DNA secretion directly through induction of hepatocyte endoplasmic reticulum (ER) stress **[51]**, or indirectly through induction of hepatocyte apoptosis [10]. Undoubtedly, more thorough research is required to uncover the relationship between HS and HBV DNA and its possible mechanisms.

The current study found that the frequency of advanced fibrosis (F3 and F4 METAVIR score) was higher in HS group but without a statistical significance. HS has been identified by many authors as a factor contributing to the burden of liver fibrosis in CHB patients. Two cross-sectional studies from China reported that severe steatosis measured by CAP score was associated with severe fibrosis in both treatment naïve CHB patients and those who received treatment **[14,19]**. A possible explanation of the association of HS with the progression of fibrosis is NAFLD-related lipotoxicity, with the production of excess free radicals through endoplasmic reticulum stress **[52]**. On the contrary, many authors did not find a relation between HS and the stage of fibrosis **[4,53-55]**. In addition, Bondini et al **[56]**. reported that liver fibrosis in CHB patients was related to viral and metabolic factors rather than HS. Moreover, we found that the severity of fibrosis was linked to biopsy-proven lobular inflammation rather than the presence of steatosis itself, and this is partially consistent with the results of Huang et al **[57]** who reported a significant association of the severity of fibrosis with both lobular inflammation and cytological ballooning rather than steatosis. An interesting finding was documented by a retrospective biopsy-based study from China, as they found that HS had an inverse relationship to the grade of inflammation and the stage of fibrosis **[44]**. Thus, we can say that convincing evidence linking HS to hepatic fibrosis is still lacking.

Another major finding of the current study is that HS had a negative impact on the virological response to oral antiviral therapy in CHB patients. We observed significantly higher rates of CVR in the non-steatotic group at 6 months, 12 months, and 24 months after the onset of lamivudine therapy. The negative impact of HS on the treatment response was also observed by Zhu et al **[41]**. They found that HBeAg positive patients with HS had a lower total virological response rate at 12 weeks of entecavir therapy compared to those without HS. This is consistent with Chen et al **[57]**. who observed a poor response to antiviral therapy in CHB patients with high CAP measurements at 12, 24, and 48 weeks of therapy compared to those with normal CAP measurements. Another prospective study observed that the rate of HBV-DNA clearance was significantly higher at 24, 48, and 96 weeks of entecavir therapy in CHB patients without HS **[58]**. On the contrary, Li et al **[33]**. observed comparable cumulative rates of complete viral suppression in CHB NAFLD and CHB non-NAFLD groups at 12, 24, 36, 48, and 60 months of oral antiviral therapy. Despite the advantage of long follow-up duration, the study of Li et al.

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used radiological studies as the main methods for diagnosis of HS, and only a few cases underwent liver biopsy. Moreover, DNA quantification was done using PCR testing with a relatively high detection limit (20-100 IU/ml) compared to that of the testing used in the current study (16 IU/ml), making the definition of CVR variable in the two studies. The possible negative effect of HS on treatment response could be attributed to hepatocellular fat droplet accumulation, which reduces the bioavailability of nucleoside analogues' intrahepatic metabolites **[59]**. The effectiveness of therapy may also be impacted by decreased hepatic cytochrome activity in steatotic hepatocytes, insulin resistance, and obesity coexisting with HS, which results in dysfunction of cellular immunity **[58]**.

Despite our best efforts, this study has some limitations. First, it is a single-center study with a small sample size. However, it was difficult to readily increase the sample size due to the biopsy-based nature of the study; which would be challenging to approach in future prospective studies given the emergence of noninvasive techniques. Undoubtedly, we performed a liver biopsy only when it was indicated. Secondly, a small proportion of our cohort had positive HbeAg. Thirdly, we evaluated the virological response to lamivudine only, which was the only available oral antiviral drug in our locality at the time of data collection. Lastly, we followed our cohort for 24 months only after the start of therapy, which is a relatively short period to detect treatment resistance.

To conclude, the results of this longitudinal cohort biopsy-based study of CHB patients confirmed the high prevalence of HS among CHB patients. We also detected that CHB activity is related to BMI rather than HS. We did not find a significant association between HS with HBV DNA and the severity of fibrosis. The rates of CVR were higher in the non-HS group, emphasizing the need for further studies with large sample sizes to confirm the results and to identify if combined CHB-HS necessitate any special considerations when choosing the suitable CHB treatment line.

Ethical statement: This study conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the Scientific Research Ethical Committee, Faculty of Medicine, Sohag University (IRB Registration number: Soh-Med-22-12-26).

Conflict of interest: The authors declare that there are no conflicts of interest.

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Authors' contribution: Conceptualization, formal analysis, and original draft writing; Mona Mohammed Abdelrahman. **Resources provision, data curation management, and supervision;** Amira Maher, Nagwa Abd El-sadek Ahmed**. Methodology, review, and editing;** all authors.

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Abbreviations: ALT: alanine transaminase; AST: aspartate transaminase; BMI: body mass index; CAP: controlled attenuation parameter; CHB: chronic hepatitis B; CVR: complete virological response; DNA: deoxyribonucleic acid; HBV: hepatitis B virus; HBsAg: hepatitis B surface antigen; HCC: hepatocellular carcinoma; HS: hepatic steatosis; INR: international randomized ratio; NAFLD: non-alcoholic fatty liver disease; PT: prothrombin time.

HIGHLIGHTS:

- Because both NAFLD and CHB infection share the ability to cause progression of the hepatic inflammation and fibrosis, the

interaction between these two diseases has become extremely interesting to research.

- There was high incidence of hepatic steatosis among patients with CHB.
- Patients with chronic hepatitis had significantly higher BMI compared to patients with chronic infection.
- The rates of CVR are higher in CHB patients without HS.

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