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# Histopathological features of chronic hepatitis B reactivation

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**Abstract**

It was aimed to investigate histopathological changes in acute hepatic exacerbations. A total of 20 patients, who developed acute hepatic exacerbation on the base of chronic hepatitis B infection, were enrolled in the study and liver biopsy was performed. Histological activity index (HAI) and its components, fibrosis scores and total HAI score were calculated according to the Ishak scoring system. All of the patients enrolled in the study had portal inflammation and spotty necrosis. Fibrosis was present in 17 patients. The median grade, stage and total HAI scores of the patients were 7 (2-14), 2 (0-3) and 8.5 (4-16), respectively. Positive correlation was determined between necroinflammation and ALT. While grade and total HAI score did not show significant difference among genders, fibrosis was significantly higher in males (p=0.008). The findings of this study showed that there are significant correlation between histopathologic changes (grade and total HAI score) in liver biopsy and plasma ALT, AST and Total bilirubin levels during acute hepatic exacerbation in patients with chronic hepatitis B infection.

**Keywords:** Liver biopsy, hepatic exacerbation, Chronic hepatitis B infection.

## Introduction

Chronic hepatitis B infection is a dynamic event with interaction observed between hepatitis B virus (HBV), hepatocytes and immune cells of the host [1]. Acute hepatic exacerbations that appear over the course of natural history of chronic HBV infections are characterized by abrupt elevation in serum ALT level [2,3]. Clinical spectrum of hepatic exacerbation may range from totally asymptomatic clinical picture to typical symptoms of acute hepatitis, hepatic decompensation and liver insufficiency [4,5]. It may cause changes in many biochemical parameters primarily in aminotransferase levels [3].

Liver biopsy is the traditional gold standard to evaluate the degree of liver injury, including both inflammatory activity and fibrosis stage [6] Acute hepatitis B is characterized by lobular disarray, ballooning degeneration, Kupffer cell activation, numerous apoptotic bodies, and lymphocyte-predominant lobular and portal inflammation. The similar pattern of this injury may also be seen in chronic hepatitis B with acute flares of disease [6].

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The present study investigated the histopathological changes caused by acute hepatic exacerbations, which occurred on the base of chronic hepatitis B infection, and relation of these changes with patient age, gender, aminotransferase level, alpha fetoprotein (AFP) and viral burden.

## Materials and Methods

### Patients and Study Design

This prospective study comprised the patients that developed acute hepatic exacerbation on the base of chronic hepatitis B infection. Demographic data, laboratory values and virological markers of these patients were obtained. They underwent liver biopsy and histopathological findings were recorded.

All of the participants had chronic hepatitis B infection with known HBsAg positivity for at least 6 months. Diagnosis of acute hepatic exacerbation was made based on aminotransferase activity, which is intermittently elevated over fivefold the upper limit of normal or increased over twofold the baseline value [1].

The study was formed by performing liver biopsy in 20 patients that met the eligibility criteria for acute hepatic exacerbation among those who had chronic hepatitis B infection and applied to

our clinic for elevated liver transaminases. Dicle university school of medicine ethics committee approved this study.

Causes of similar biochemical profile such as hepatitis A, C and D, as well as drugs, alcohol, herbal medicines, ischemia, pregnancy, etc. were excluded. In addition, other non-hepatotropic viruses (HIV, HSV, CMV, EBV) were also excluded. There was no cirrhosis in these patients included in the study. Demographic data and biochemical, virological and histopathological findings of the cases with acute hepatic exacerbation, which was developed in the patients with chronic hepatitis B, were analyzed.

**Data collection**

As the laboratory data; complete blood count was studied in the tubes containing EDTA via automatic optic laser impedance system using CELL-DYN 3700 device. Prothrombin time was studied by Siemens CA7000 Sysmex model device using Thromborel S'kit. Glucose, urea, creatinine, ALT, AST, ALP, gamma glutamyl transferase (GGT), total bilirubin and albumin were studied in serum gel biochemistry tubes by enzymatic method using Architect C 1600, Anti-nuclear antibody (ANA) and Anti-smooth muscle antibody (ASMA) were studied in serum gel biochemistry tube by immunofluorescent method using ASTRA kit, whereas HSV, CMV and EBV panel was studied in serum gel biochemistry tubes by ELFA method. Enzyme immunoassay (EIA) was used to analyze HBV viral markers and COBAS® AmpliPrep/COBAS® TaqMan® HBV Test version 2. Roche diagnostics was used to analyze HBV-DNA. Second generation EIA test was used to analyze anti-HCV and EIA was used to analyze total anti-delta.

A prediagnosis was written and US was performed in the patients by a blinded researcher using TOSHIBA SSH-140A USG device with 3.5-7.5 probes.

Liver biopsy: Liver biopsy was done percutaneously under local anesthesia by Menghini needle. Histological findings were scored semiquantitatively according to Knodell et al. Grade (necroinflammation) was scored from 0 to18 and stage (fibrosis) was scored from 0 to 6. Total scores (HAI) were calculated by the sum of grades and stages. All liver biopsies were reviewed by a single pathologist who was blinded to the clinical data.

**Statistics**

Data were analyzed by SPSS program. Whether numerical parameters distributed normally was tested by Kolmogorov Smirnov and Shapiro tests. Comparison of normally distributed parameters was done by Student t-test; whereas, comparison of non-normally distributed parameters was done by Mann Whitney U test. Chi-square test and Fisher's exact test were used for the comparison of categorical variables. Correlation between numerical variables was determined using Pearson test for normally distributed parameters and using Spearman's test for non-normally distributed parameters. $P < 0.05$ was considered statistically significant.

**Results**

A total of 20 patients were enrolled in the study. The mean age of the patients that have been included in the study due to acute hepatic exacerbation was 30.5 ± 8.9 years (median 29.00 min 18-max 51 years). The number of male patients was 14 (70%) and the number of female patients was 6 (30%) with a male/female ratio of 2.33. ALT level was over 10 fold the upper limit of normal in 13 (65%), 5-to-10 fold in 6 (30%), and 1-to-5 fold in one (there was three times increase as compared to baseline ALT level) of the patients. Total bilirubin was high in 16 (80%), GGT was high in 18 (90%), ALP was high in 8 (40%), ferritin was high in 15 (75%) and AFP was high in 7 (35%) patients. Demographic and laboratory characteristics of the patients are demonstrated in Table 1.

**Table 1.** Demographic and laboratory data of the patients with acute hepatic exacerbation

| Variables | Patient number (n), percentage (%) | Mean±SD, (Max-Min) |
|---|---|---|
| **Age** | | 32.15±10.76 (18-60) |
| **Male** | 14, 70% | |
| **Female** | 6, 30% | |
| **White blood cell (K/UL)** | | 6655.0±1625.38 (4030 - 10800) |
| **Thrombocyte (K/UL)** | | 214390.0±68511.31 (95800- 377000) |
| **Prothrombin time (sec)** | | 13.60±1.87 11-17 |
| **ALT (U/L)** | | 1204.30±1029.88 (211- 3311) |
| **AST (U/L)** | | 859.80±808.21 (113-2966) |
| **Total bilirubin (mg/dl)** | | 5.94±5.92 (0.3-17.5) |
| **ALP (U/L)** | | 152.0±98.54 31-419 |
| **GGT (U/L)** | | 195.45±204.33 39-907 |
| **Albumin (g/dl)** | | 3.61±0.61 2.2-4.6 |
| **AFP** | | 7.23±8.69 0.77-28.80 |
| **HBeAg positive** | 10 (50%) | |
| **HBV-DNA copy/ml** | 16 (80%) | 1.88x108±3.62x108 |

All of 20 patients had HBsAg and anti-HBc IgG positivity. Anti-HBc IgM positivity was present in 5 (25%) patients. Ten patients (50%) were HBeAg-positive and 10 patients (50%) were HBeAg-negative. HBV-DNA level was found to be negative under detectable value in 4 (20%) and positive at detectable value in 16 (80%) patients. Mean HBV-DNA level was 3.23x107 ± 6.22x107 IU and 1.88x108 ± 3.62x108 copies. Virological characteristics of study participants are demonstrated in Table 3. Anti-HVC was negative in all patients. Exacerbation was spontaneous in 17 (80.8%) of 20 patients, whereas one patient developed exacerbation due to immunosuppressive therapy, one patient developed exacerbation due to therapy withdrawal, and one patient developed exacerbation under treatment.

Data of liver biopsy: all patients had lymphocyte infiltration in portal areas (portal inflammation) and hepatocytes one by one infiltrated by lymphocytes (spotty necrosis). Piecemeal necrosis was present in 18, whereas confluent necrosis was present in 9 patients. Fibrosis was present in 17 of the study participants. Bridging necrosis was observed in only five patients. Median grade, stage and total HAI scores of the patients were 7 (2-14), 2 (0-3) and 8.5 (4-16), respectively. Characteristics of the data of liver biopsy, which was performed in 20 patients that developed hepatic exacerbation, are demonstrated in Table 2. No correlation was determined between fibrosis score and ALT (rp:0.067), (p: 0.778) and HBV-DNA values (rp: -0.139), (p: 0.559). No correlation was observed also between grade and fibrosis (rp:0.156), (p:0.512). Positive correlation was determined between necroinflammation and ALT, AST and total bilirubin. There was no correlation between viral burden and grade (rp: -0.084), (p: 0.725) and fibrosis score (rp: -0.139), (p: 0.559). Correlation of histological liver findings with viral burden and age is given in Table 3. Whilst grade and total HAI scores of the patients showed no significant change among genders, fibrosis was found to be significantly higher in males (p=0.008). Mean total HAI scores in males and females were 10.07 ± 3.45 and 7.0 ± 2.44, respectively (p= 0.065). Correlation of histopathological liver findings and their mean values with gender, HbeAg and HBVDNA are demonstrated in Table 4.

**Table 2.** Histological findings of all patients with acute hepatic flare

| Patient | Portal inflammation score | Spotty necrosis score | Piecemeal necrosis score | Confluent necrosis score | Grading score (0-18) | Fibrosis score (Staging) (0-6) | Total HAI score (0-24) |
|---|---|---|---|---|---|---|---|
| **N1** | 3 | 2 | 2 | 0 | 7 | 1 | 8 |
| **N2** | 3 | 3 | 3 | 1 | 10 | 1 | 11 |
| **N3** | 3 | 3 | 2 | 1 | 9 | 2 | 11 |
| **N4** | 1 | 1 | 0 | 0 | 2 | 2 | 4 |
| **N5** | 4 | 4 | 3 | 3 | 14 | 2 | 16 |
| **N6** | 3 | 3 | 3 | 2 | 11 | 3 | 14 |
| **N7** | 2 | 3 | 2 | 0 | 7 | 2 | 9 |
| **N8** | 2 | 1 | 1 | 0 | 4 | 0 | 4 |
| **N9** | 2 | 3 | 2 | 2 | 9 | 2 | 11 |
| **N10** | 2 | 2 | 1 | 0 | 5 | 0 | 5 |
| **N11** | 1 | 2 | 2 | 0 | 5 | 3 | 8 |
| **N12** | 3 | 3 | 2 | 1 | 9 | 3 | 12 |
| **N13** | 3 | 2 | 3 | 1 | 9 | 3 | 12 |
| **N14** | 1 | 2 | 2 | 0 | 5 | 2 | 7 |
| **N15** | 4 | 4 | 4 | 0 | 12 | 2 | 14 |
| **N16** | 2 | 2 | 1 | 1 | 6 | 0 | 6 |
| **N17** | 1 | 2 | 0 | 0 | 3 | 3 | 6 |
| **N18** | 2 | 2 | 2 | 0 | 6 | 2 | 8 |
| **N19** | 2 | 2 | 2 | 1 | 7 | 3 | 10 |
| **N20** | 2 | 2 | 1 | 0 | 5 | 2 | 7 |
| **Median value (min-max)** | 2 (1-4) | 2 (1-4) | 2 (0-4) | 0 (0-3) | 7 (2-14) | 2 (0-3) | 8.5 (4-16) |

**Table 3.** Correlation of histopathological changes with HBVDNA, biochemical values and age

| Histopathological variables | Correlation coefficient (rp ) | P |
|---|---|---|
| **Portal inflammation –HBVDNA** | 0.177 | 0.456 |
| **Grading – HBVDNA** | -0.084 | 0.725 |
| **Staging – HBVDNA** | -0.139 | 0.559 |
| **Total score – HBVDNA** | -0.67 | 0.779 |
| **Portal inflammation –Age** | -0.264 | 0.260 |
| **Grading – Age** | 0.265 | 0.259 |
| **Staging – Age** | 0.292 | 0.211 |
| **Total score– Age** | -0.164 | 0.489 |

**Table 4**. Correlation of histopathological changes with gender, HBVDNA and HbeAg

| Histological finding | Mean ±SD | Male | Female | P | Hbe Ag+ N=10 | Hbe Ag- N=10 | P | HBVDNA< 105 N=10 | HBVD-NA>105N=10 | P |
|---|---|---|---|---|---|---|---|---|---|---|
| **Portal inflammation** | 2.30±0.9 | 2.36±1.00 | 2.17±0.75 | 0.728 | 2.30±0.82 | 2.30±1.05 | 0.811 | 2.27±1.10 | 2.33±0.70 | 0.749 |
| **Spotty necrosis** | 2.40±0.82 | 2.57±0.85 | 2.0±0.63 | 0.152 | 2.40±0.69 | 2.40±0.96 | 0.743 | 2.64±0.92 | 2.11±0.60 | 0.161 |
| **Piecemeal necrosis** | 1.90±1.02 | 2.0±1.10 | 1.67±0.81 | 0.383 | 2±0.66 | 1.80±1.31 | 0.689 | 1.91±1.22 | 1.89±0.78 | 0.872 |
| **Confluent necrosis** | 0.65±0.87 | 0.86±0.94 | 0.17±0.40 | 0.090 | 0.70±0.82 | 0.60±0.96 | 0.644 | 0.91±1.04 | 0.33±0.50 | 0.205 |
| **Grading** | 7.25±3.09 | 7.79±3.33 | 6.0±2.19 | 0.197 | 7.40±2.50 | 7.10±3.72 | 0.760 | 7.73±3.71 | 6.67±2.17 | 0.460 |
| **Staging** | 1.90±1.02 | 2.29±0.82 | 1.0±0.89 | 0.008 | 1.70±1.16 | 2.10±0.87 | 0.444 | 2.18±0.87 | 1.56±1.13 | 0.181 |
| **Total score** | 9.15±3.43 | 10.07±3.45 | 7.0±2.44 | 0.62 | 9.10±3.21 | 9.20±3.82 | 0.950 | 9.90±3.78 | 8.22±2.90 | 0.287 |

## Discussion

Hepatitis B virus (HBV) infection is a challenging health problem. [7] Acute hepatic exacerbation is a well-known complication of chronic HBV infection. These flares may develop spontaneously and may lead to a misdiagnosis of acute hepatitis B. As per definition; acute hepatic flare indicates an abrupt elevation in serum transaminases in a patient with chronic HBV infection, with or without symptoms of hepatitis. Majority of these flares are caused by an alteration in balance between immune response against HBV and viral replication [8-11].

During the natural course of chronic HBV infection, hepatitis B flares start to occur during the HBeAg positive immune clearance phase [1,12-15]. Hepatitis B flares also occur in the HBeAg negative reactive phase, but less frequently than during the HBeAg-positive phase [1,16-18]. In a hospital based study of CHB, the annual incidence of hepatitis flares was calculated to be 27% in 358 HBeAg-positive patients and 10% in 279 HBeAg-negative counterparts during a mean follow-up period of 2 years after entry [1]. Different from this information, prevalence of hepatic exacerbation was found to be equal in HbeAg-positive and HbeAg-negative patients. However, it should be kept in mind that this may change in larger patient groups.

The biochemical changes, including serum AST, ALT and total bilirubin of hepatitis B flares are similar to but in general less severe than those of acute hepatitis or acute superinfections [1,4]. In the present study, ALT was over tenfold the upper limit of normal in 16 of the patients, and the mean value was 1204.3 ± 1029.88. Moreover, it was determined that total bilirubin was 5.94 ± 5.92 with higher values reaching to 17.5. These results of the present study support that acute hepatic exacerbation shows similar biochemical changes to those of acute hepatitis and acute superinfections.

Chronic hepatitis B is characterized by the grade (the degree of inflammation and hepatocellular injury) which is thought to lead to the fibrosis stage. The end stage of chronic hepatitis is cirrhosis with clinical decompensation [19,20]. Hepatitis activity index (HAI) or grading and fibrosis stage or staging are two important points that determine the mild, moderate or the severity of the disease [21]. Several scoring systems have been developed and among them Ishak scoring system is more popularly used [22].

In chronic hepatitis B, There is a varying degree of predominantly lymphocytic portal inflammation with interface hepatitis and spotty lobular inflammation in chronic hepatitis B. Investigating liver biopsy findings in the patients with acute hepatic exacerbation that developed on the base of chronic hepatitis B infection, it was observed that all patients had lymphocyte infiltration in portal areas (portal inflammation) and each of the hepatocytes has been infiltrated by the lymphocytes (spotty necrosis). Piecemeal necrosis was present in all patients except two, whereas severe illness-associated confluent necrosis was detected in only 9 of the patients. These findings suggest that portal inflammation and spotty necrosis are constant histopathological changes in acute hepatic exacerbations. Whilst piecemeal necrosis was present in the majority of patients, confluent necrosis, which is the indicator of severe illness, was observed in less than half of the patients. These histopathological changes that develop during chronic hepatitis B infection appear to support the similarity with the changes seen in acute hepatitis.

Piecemeal necrosis shows a diagnostic criterion in chronic active hepatitis that was seen more in those who had higher viral load [19]. In a previous study [23], mean piecemeal necrosis and grading scores were found to be higher in those with viral burden $>10^5$. Moreover, other studies determined positive correlation between the degree of histological inflammation and HBVDNA [23-25]; likewise, other studies also found the prevalence of liver injury, morbidity and cirrhosis to be higher in those with viral burden $>10^5$ [23,26,27]. In the present study, piecemeal necrosis, grading score and fibrosis score were not statistically significantly different between those with viral burden $>10^5$ and $<10^5$. Likewise, different from these studies, no correlation was determined between necroinflammation and fibrosis and HBVDNA.

As is known, presence of confluent necrosis is the indicator of severe activity and potential clinical outcomes should be taken into account [28]. In the present study, confluent necrosis was present in 9 of the patients and the score was higher than 2 in only one patient. In addition, whilst confluent necrosis showed positive correlation with HAI (grading) and total HAI score, it showed no correlation with fibrosis. Fibrosis was present in 17 of the study participants with a mean fibrosis value of 1.90 ± 1.02. Fibrosis showed no correlation with age, ALT and HBVDNA values.

In another study, bridging hepatic necrosis (BHN) is evident

in more than 80% of the patients with AFP >100 ng/ml during hepatitis B flares [29]. It was further demonstrated that patients with high AFP or bridging hepatic necrosis during the hepatitis flare had a high degree of AFP-producing oval cell activation, in contrast to 2.4 – 5.6% in patients with APF <100 ng/ml or no BHN [1]. Bridging necrosis was present in only five patients participated in the study. Mean AFP value was 7.23 ± 8.69 and was <100 in all patients. AFP values were found to be correlated with grade and total HAI score. These results appear to support the above mentioned study.

Similar with the study that indicates the absence of correlation between inflammatory activity in the liver and clinical, biochemical and virological parameters, other studies as well determined no significant correlation between HBVDNA levels and liver histology. Whilst liver histology shows correlation with viral burden in HBeAg-negative patients, such a correlation was not observed in HBeAg-positive patients [26,30,31]. Results of a large-population histological study [32] suggested poor correlation between liver disease and ALT in HBeAg-positive and HBeAg-negative patients. Supporting this finding, another study [33] demonstrated that ALT is not an accurate indicator of liver histology. Contrary to these studies, multivariate analyses in different cohorts have shown an association of age over 40 years, male sex, and high HBV DNA levels and ALT values, with significant necroinflammation and advanced fibrosis [34].

According to the results of the present study; whilst ALT is correlated only with spotty necrosis, AST was found to be correlated with portal inflammation, piecemeal necrosis, spotty necrosis and confluent necrosis. Both ALT and AST showed positive correlation with grade and total HAI score. However, correlation with AST was stronger. No correlation was determined between ALT, AST and fibrosis. These results suggest that AST is more valuable parameter than ALT in indicating the degree of necroinflammation in hepatic exacerbation. Moreover, it appears to partially support above-mentioned studies.

In the present study, which evaluated histopathological changes in acute hepatic exacerbation, no correlation was determined between grade and total HAI score and HBVDNA and age. In the analysis of HBVDNA$<10^5$ and $>10^5$, no significant relation was found between elevation in HBVDNA and histopathological changes in the liver (portal inflammation, piecemeal necrosis, spotty necrosis and confluent necrosis). Likewise, HBVDNA elevation showed no statistically significant correlation with grade, total HAI score and fibrosis. Moreover, no significant difference was determined between HBeAg-negative and HBeAg positive patients in terms of grade, total HAI score and fibrosis scores. Whilst there was no difference between grade and total HAI scores of males and females, fibrosis score was found to be higher in males.

## Conclusion

In conclusion, number of studies that demonstrate histopathological changes in acute hepatic exacerbations is limited. In the present study, there was correlation between grade and total HAI score and ALT, AST and total bilirubin, whereas no correlation was found with viral burden, age and gender. Moreover, no significant difference was determined between HBeAg-negative and HBeAg-positive patients in terms of grade, total HAI score and fibrosis scores. Fibrosis score was higher in male patients. AST appears to be a better indicator of necroinflammation than ALT in hepatic exacerbations. There is no significant relation between viral burden and histopathological liver changes, grade, stage and total HAI score.

**Conflict of interests**
*The authors declare that they have no conflict of interest and any financial disclosures.*

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**Ethical approval**
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