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Methodology Article

Analysing Liver Histology in Relation to Elevated HBV DNA Levels in Immuno-Tolerant Phase of Chronic Hepatitis B Viral Infection Patients

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Abstract

Introduction: Chronic Hepatitis B viral infection is associated with significant health and financial challenges. An estimated 15-40% of the Chronic Hepatitis B infected patients would develop liver Cirrhosis and a quarter of which may result in Hepatocellular cancer. In Africa, about 65 million people are said to be chronically infected with HBV. Thus, Africa with 12% of the world's population carries approximately 18% of the global burden of HBV infection. An elevated level of ALT is an important component in the consideration for treatment of CHB, however, the presence of normal level ALT levels with discordantly elevated HBV DNA levels may requires further evaluation with Liver histology. *Method*: All patients who were newly diagnosed with CHB infection and above 18 years of age were consecutively recruited from December 2019 to October 2020, into the study. Blood samples were taken for the Liver Function Test, HBV immunologic panel and HBV DNA levels. Liver biopsy was performed for 84 patients with variance elevated serum HBV DNA (>2000 IU/mls) and normal serum ALT defined as level <40 Unit/mls. The Data collected was entered and analyzed using the SPSS version 20. The clinical profiles and HBV DNA level of the respondents were presented using mean and standard deviation while frequencies, percentages and charts was used to summarize qualitative variables. Chi-square test and Fisher's exact test were used to analyze factors associated with respondent's HBeAg status and liver histology. *Result*: The Liver biopsy histology shows, mild inflammation (A1) was observed in 50 (60%), and moderate inflammation (A2) in 12% while the remaining 10 patients (28%) showed no inflammatory. Similarly, 20 patients (24%) had no fibrosis (F0), 8% had portal fibrosis without septa (F1), 24% had portal fibrosis with rare septa (F2) while the remaining majority (44%) had numerous septa (F3). *Conclusion*: Chronic HBV infection is Endemic in our region and studies had shown the importance of adequate evaluation and early treatment to avert the associated high morbidity and mortality. Also, significant number of patients with normal ALT levels had fibrosis on liver histology. Liver histology remains an important parameter in the evaluation of patients with CHB, as 57 patients would have missed the opportunity of early treatment. These findings are expected to provide a basis for an informed planning of national and International CHBV treatment guidelines.

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Keywords

Chronic HBV Infection, ALT, HBV DNA, Necro-inflammation, Fibrosis, Cirrhosis, Hepatocellular Cancer

1. Introduction

Chronic HBV infection is a major health challenge particularly in the Tropics. The estimated world prevalence is about 2 billion and 240 million people with evidence chronic carriers of HBV [1]. The South-east Asia, China, Sub-Sahara Africa and Amazon basin accounted for 75% of chronic carrier state [2]. About 15-40% of the CHB infected patients would develop liver Cirrhosis and a quarter of which may result in Hepatocellular cancer [3].

In Africa, an estimated 65 million people are said to be chronically infected with HBV. Hence, Africa with about 12% of the world's population carries approximately 18% of the global burden of HBV infection [4]. The pooled prevalence estimate for Nigeria was 13.6% [5], and the higher prevalence rates were in older children which further supports the established fact that most HBV transmission in Africa occur in early childhood via contact rather than vertically.

It is therefore pertinent to have an early evaluation of patients with CHB infection and consider treatment where necessary, to significantly reduce the diseases burden and associated morbidity and mortality. The thorough evaluation to determine those who might benefited from the treatment based on the guidelines, factors including the level of ALT, HBV DNA, presence or absence of HBeAg and/or Liver histology need to be considered.

The common pathway in the development of CLD is fibro-genesis which is initially a physiological response, and beneficial to the host but subsequently becomes pathological if viral infection and chronic hepatocellular injury persist. After considering viral HBV DNA levels, precise definition of liver fibrosis stage, as well as inflammation, are essential for management of the patients with chronic viral hepatitis in clinical practice [6]. In order to achieve liver tissue diagnosis, liver biopsy must be resorted to; therefore liver biopsy remains probably the most important diagnostic tool in CLD therapeutic decision [7].

HBV infection is a fluctuating process with replicative and non-replicative/low replicative phases. The presence of circulating HBsAg and the HBeAg and high levels of HBV DNA characterize the immune-tolerant phase. Where patients have no symptoms, normal or slightly increased ALT levels, and minimal histological activities, this imply that there is a lack of or a very weak immune response against the infected hepatocytes. The second phase of chronic HBV infection, patients may, is the Immune-active phase, which is associated with a slightly decrease in HBV DNA concentrations but markedly elevated ALT levels and increase histological activity, this is due to immune-mediated lysis of infected hepatocytes. In the low or non-replicative third phase, seroconversion from HBeAg to HBeAb (anti-HBe) occurs. This phase is usually preceded by a marked reduction of HBV DNA to levels that may not be detectable in the serum followed by normalization of ALT levels and resolution of liver necro-inflammation. This phase is also referred to as the Inactive HBV Carrier state. There is a fourth phase of the disease called Immnuo-reactivation stage, it occur when previously inactive carrier patients become active due to either change in host immunity or virulent factors of the infection [8]. Anti-viral therapy are mostly indicated in Immuno-active and Immuno-reactive phases of the CHB infection.

However, all these recognized Chronic HBV infection phases according the guidelines significantly played down the importance of the liver histology in the evaluation of the HBV infection management by using ALT level as a surrogate. Earlier studies had shown that normal ALT level in patients with CHB diseases is significantly associated with low prevalence of liver injury histologically [9-11], other studies have however reported significant liver injury in a fair proportion of these patients [12, 13].

Therefore, the aim of our study is to determine the relationship between the elevated HBV DNA levels and the liver histology in a patients with normal serum ALT level.

2. Method

The study was conducted at the Gastroenterology out-patient Clinic of Aminu Kano Teaching Hospital (AKTH) Kano, Nigeria. It a cross-sectional descriptive study of patients that was newly diagnosed with HBsAg positive result referred to the Out-patient Clinic. All patients newly diagnosed with CHB infection without co-infection with HCV, HIV and above 18 years of age were consecutively recruited from December 2019 to October 2020, into the study after signing the informed consent form for the study. And 10 mls of blood samples were taken for the Liver Function Test, HBV immunologic panel and HBV DNA level.

The immunologic markers (HBeAg, anti-HBcAb, anti-HBeAb and anti-HBsAg) were done using commercially available enzyme-linked immunosorbent assay (ELISA) kits, while biochemical (LFTs) tests were performed using routine automated analyzers. The serum HBV DNA levels was quantified using ABI 7300 machine with LIFERIVER PCR kit with the lower limit of detection being < 5 IU/ml.

Liver biopsy was done for all patients with discordant elevated serum HBV DNA (>2000 IU/mls) with normal serum ALT defined as level <40 Unit/mls [14]. All the liver biopsies were done after obtaining of written informed consent and 84 patients were found to have normal ALT level with elevated HBV DNA and were further evaluated for the Liver biopsies. The liver tissues obtained was transferred into a well-labelled formalin container and sent to histopathology laboratory for analysis.

Ethical clearance was obtained from the Ethics and Research committee of AKTH for the study. Ref. No. NHREC/21/08/2008/AKTH/EC/2612 dated 9th September, 2019.

The provision of the Helsinki declaration was duly observed. Data collected from the patients were kept confidential.

Data collected was cleaned, entered and analyzed using the SPSS version 20. The clinical profiles, serum ALT; and HBV DNA levels of the respondents were presented using mean and standard deviation while frequencies, percentages and charts was used to summarize qualitative variables. Chi-square test and Fisher's exact test were used to analyze factors associated with respondent's HBeAg status and liver histology. In all tests of significance, P<0.05 was considered statistically significant.

3. Result

Demographic pattern of the study population: Liver biopsy was performed on 84 patients with elevated HBV DNA but normal ALT of which 71 were males, and 13 females with M:F 5.5:1 and the mean age was 29.5 ± 6.4 years as shown in Table 1.

Table 1. *HBV DNA vs Gender and Age distribution.*

Variables	HBV-DNA (IU/mL) n (%)		
	2000-19,999	≥ 20,000	p-value
All	26 (31.0)	58 (69.0)	
Sex			0.03
Male	19 (22.6)	52 (61.9)	
Female	7 (8.3)	6 (7.2)	
Age group			
≤ 20	2 (2.4)	6 (7.1)	
21 to 30	9 (10.7)	26 (31.0)	
31 to 40	12 (14.3)	23 (27.4)	
> 40	3 (3.6)	3 (3.6)	

*Significant at p< 0.05

Figures 1 and 2 shows the distribution of liver inflammation and fibrosis respectively. Using METAVIR scoring system, mild inflammation (A1) was observed in 50 (60%) of patients and moderate inflammation (A2) in 12% while the remaining 10 patients (28%) showed no inflammatory activity. Similarly, fibrosis score shows that, 20 patients (24%) had no fibrosis (F0), 8% had portal fibrosis without septa (F1), 24% had portal fibrosis with rare septa (F2) while the remaining majority (44%) had numerous septa (F3). No patient had cirrhosis.

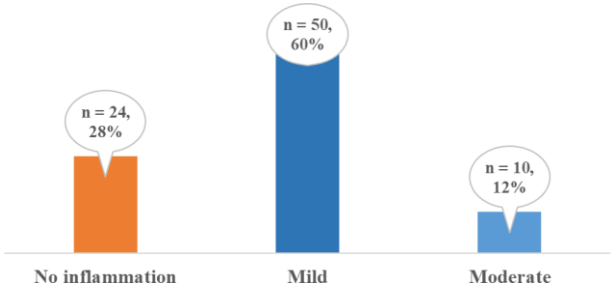

Figure 1. *Distribution of liver inflammation in biopsy indicated patients.*

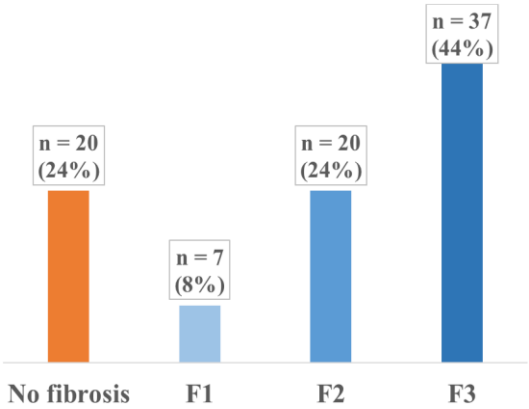

Figure 2. *Distribution of liver fibrosis in biopsy indicated patients among treatment naïve subjects with chronic HBV infection.*

4. Discussion

Hepatitis B viral infection has the tendency for chronicity with significant morbidity and mortality. Nigeria being in a hyper-endemic region, with a huge burden of CHB patients in its population, robust studies are needed as to identify patients who are eligible for treatment. The eligibility for treatment is defined as individual with HBV DNA count >2000 IU/ml with F2 and/or A2 on liver histology.

4.1. Gender Distribution of the Study Population

Of the 84 CHBV patients with elevated HBV DNA and

liver biopsies, 71 patients (84.5%) were males and 13 patients (15.5%) were female, with a mean age of 29.5 ± 6.4 years. This was similar to one of the Iranian studies by Mansour-Ghanaei et al [8] where male patients was 34 (63%) and female was 20 (37%) but mostly elderly population, mean age of 41.89±11.54. Similarly in India study [13], of the 157 patients (M:F =123:34) However, In another Iranian study by Esmaeelzadeh et al [15] where total of 150 patients were biopsied, female patient slightly dominated, 72 (48%) were male and 78 (52%) were female M;F ratio 1:1.1.

4.2. Liver Histology

The study showed that a significant number of patients (60%) had mild to moderate necro-inflammation. In a similar study done in Netherland by Sonneveld et al [16] there is significant inflammatory activity (mild to severe) in 83.6% while only 16.4% had no inflammation on liver biopsy. Also, this difference is may likely be due to the predominant genotypes in the Western Europe compared to sub-Saharan Africa.

Also, our study showed that a fairly large number (68%) of biopsied patients had ≥ F2 on liver histology. A study done in China by Da-Wu et al [17] reported significant fibrosis in a slightly higher number of patients (77.2%) and cirrhosis was seen as compared to our study, this therefore, could explain the slight variation observed in the two studies.

Several other similar studies had also demonstrated serious association between high level of HBV DNA and Liver histology. Zacharakis et al [18] reported that liver fibrosis is significantly related HBV DNA level in HBeAg-negative patients. Also, Mommeja-Marin et al [19] showed correlation between viral load and histology grading similar to our findings. Additionally, Changjiang and his team performed liver biopsy in 396 patients with CHB infection and the results show significant correlation between HBV DNA level and liver fibrosis and cirrhosis. The serum HBV DNA levels was correlated with mild fibrosis, moderate to severe fibrosis and cirrhosis ($P = 0.009$, $P<0.001$, and $P<0.001$ respectively) [20].

However, Martinot-Peignoux et al [21] study observed that there is no significant correlation between the total histological score, staging or grading and elevated HBV DNA titre. Also Shao et al [20] studied 178 patients with markedly elevated HBV DNA levels greater than 10^5 and found that there is no correlation between the HBV DNA and histology grade and stage of the liver disease. This contrary observations might however be due to the difference in the disease pattern and HBV genotype between Africa/Middle East/Far East and Europe-France.

5. Conclusion

Chronic HBV infection is Endemic in our region and the study had shown the importance of adequate evaluation and early treatment necessary to avert the associated high morbidity and mortality. The shows that younger age group are particularly affected by the disease and predominantly the male patients. These findings further confirmed the importance of the CHB infection in contributing to the African economics losses and the resultant sequel.

Furthermore, the significant number of CHB patients with normal ALT levels had fibrosis on liver histology. Thus, patients who have elevated HBV DNA (>2000 IU/ml) but low or normal ALT might be considered for treatment if liver histology result is not available as significant number of them had active histological findings.

Therefore, Liver histology remains an important parameter in the evaluation of patients with CHB, as 57 patients would have missed the opportunity of early treatment.

6. Recommendation

These findings are expected to provide a basis for an informed planning of national CHBV treatment guidelines.

Abbrevations

A1	Mild Inflammation
A2	Moderate Inflammation
AKTH	Aminu Kano Teaching Hospital
CHB	Chronic Hepatitis B
CHBV	Chronic Hepatitis B Virus
CLD	Chronic Liver Disease
DNA	Deoxyribonucleic Acid
F0	No fibrosis
F1	Portal Fibrosis Without Septa
F2	Portal Fibrosis with Rare Septa
F3	Numerous Septa Fibrosis
ELISA	Enzyme-linked Immunosorbent Assay
HbcAg	Hepatitis B Core Antigen
HbcAb	Hepatitis B Core Antibody
HbeAg	Hepatitis B Envelop Antigen
HBeAb	Hepatitis B Envelop Antibody
HBsAg	Hepatitis B Surface Antigen
METAVIR	Meta-Analysis of Histological Data in Viral Hepatitis
SPSS	Statistical Package for the Social Sciences

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Author Contributions

Nurudeen Olalekan Muhammad: Conceptualization,

Funding acquisition, Methodology, Project administration, Writing – original draft

Yussuf Maisuna Abdulkadir: Data curation, Formal Analysis, Project administration, Supervision, Writing – review & editing

Yusuf Musa: Investigation, Methodology, Supervision, Writing – original draft

Yusuf Ibrahim: Conceptualization, Formal Analysis, Software, Supervision, Visualization, Writing – review & editing

Adamu Alhaji Samaila: Conceptualization, Formal Analysis, Project administration, Supervision, Visualization, Writing – original draft

Muhammad Musa Borodo: Conceptualization, Methodology, Project administration, Supervision, Visualization, Writing – original draft

Conflicts of Interest

The authors declare no conflicts of interest.

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