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A Preliminary Study of Hudu's Score as a Prognostic Scoring System of Predicting the Risk of Liver Cirrhosis in Patients with Chronic Hepatitis B Infection

[1]Shuaibu Abdullahi Hudu, [2]Mohd Taib Niazlin, [2]Syafinaz Amin Nordin,
[3]Soek Siam Tan and [2]Zamberi Sekawi
[1]Department of Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, College of Health Sciences, Usmanu Danfodiyo University Sokoto PMB, 2346 Sokoto State, Nigeria
[2]Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra, Selangor, Malaysia
[3]Department of Hepatology, Selayang Hospital, Lebuh Selayang-Kepong Batu Caves, 68100 Selangor, Darul Ehsan, Malaysia
hudu.shuaibu@udusok.edu.ng, +2348039099312

Abstract: Hepatitis B infection long term prognosis is good in the absence of liver cirrhosis. However, the risk of liver cirrhosis developing in chronic Hepatitis B surface antigen positive varies from 1-5/100 patients per year. This study aimed at developing a novel prognostic scoring system to predict liver cirrhosis in chronic Hepatitis B patients. A total of 82 chronic Hepatitis B patients were recruited retrospectively in a cohort study and their liver Biochemistry, Hepatitis B viral DNA level, viral serology, quantitative Hepatitis B e Antigen (qHBeAg) and development of liver cirrhosis were assessed. The data were included in a multivariate logistic regression analysis based on which this prognostic scoring system was established. A simple scoring system composed of virological and biochemical laboratory parameter was developed to predict liver cirrhosis in chronic Hepatitis B patients. This prognostic score is accurate and reproducible. The prognostic score ranges between 5 and 15. Patients prognostic score of 5 indicates low risk, 10 high risk and 15 very high risk of developing cirrhosis. In conclusion, this study has showed a promising scoring system that can be use in predicting the risk of developing liver cirrhosis in chronic Hepatitis B patients.

Key words: Hepatitis B scoring system, liver cirrhosis, prognostic factors, chronic Hepatitis B patients, viral serology, Hepatitis B

INTRODUCTION

The global prevalence of chronic HBV infection is estimated to be 3.9% in males and 3.5% in females. The global burden of disease, injuries and risk factors study estimated that 1,030,800 and 786,000 people died of liver cirrhosis and liver cancer, respectively. The age-standardised death rate of liver cirrhosis was 15.6/100,000 and liver cancer was 11.5/100,000. Liver cirrhosis ranks 12th and liver cancer 16th amongst 235 causes of death and the latter is the 3rd commonest cause of cancer death, after lung and stomach. HBV infection accounts for 30.3% of liver cirrhosis deaths and 45.4% of liver cancer deaths (Trepo *et al*., 2014). Overall, 1,030,800 people were estimated to have died in 2010 from HBV-related liver diseases including acute/fulminant Hepatitis and liver cirrhosis as well as Hepatocellar Carcinoma (HCC), making HBV infection the 15th cause of mortality. The risk of Hepatitis B infected patients progressing to liver cirrhosis has been found to increase with elevation of Hepatitis B e Antigen (HbeAg) and viral DNA levels (Chen *et al*., 2006). Similar, studies also reported the risk of cirrhosis or HCC increasing when the levels of HBV DNA exceed 2,000 IU/mL (Chen *et al*., 2006; Fattovich *et al*., 2004).

Aminotransferase elevation tends to be modest for chronic Hepatitis but may fluctuate in the range of 100-1000 IU. ALT tends to be more elevated than AST, however, once cirrhosis is established, AST tends to exceed ALT. In severe cases moderate elevations in serum bilirubin upon 10 mg/dL can occur (Limdi and Hyde, 2003). The likelihood of morbidity and mortality of chronic HBV infected patient is directly associated with development of liver cirrhosis while the absence of liver cirrhosis is associated with good prognosis and long-term

Corresponding Author: Hudu Shuaibu Abdullahi, Department of Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, College of Health Sciences, Usmanu Danfodiyo University Sokoto, 840232 Sokoto State, Nigeria, hudu.shuaibu@udusok.edu.ng, +2348039099312

surviva (Pan and Zhang, 2005). Consumption of alcohol also increases the risk of both cirrhosis and hepatocellular carcinoma (Lin *et al*., 2013). Co-infection of HBV with HCV and HDV also increases the risk pf progression to cirrhosis (El-Serag, 2012; Liu and Hou, 2006).

A number of laboratory tests, scores and indices have been proposed as non-invasive predictors of hepatic fibbrosis in chronic Hepatitis C patients (Rosenberg *et al*., 2004). However, for Hepatitis B patients, scoring system such as the Model for End-stage Liver Disease (MELD) score was developed to assess short-term prognosis that predict mortality across a broad spectrum of liver diseases (Malinchoc *et al*., 2000) but not the development of cirrhosis. Identifying prognostic factors associated with development of cirrhosis in chronic Hepatitis B patients is significant for setting treatment criteria. Thus, this study aimed at developing a novel prognostic scoring system to predict liver cirrhosis in chronic Hepatitis B patients.

MATERIALS AND METHODS

Study design: A total of 82 chronic Hepatitis B patients were recruited retrospectively in a cohort study and their liver biochemistry, Hepatitis B viral DNA level, viral serology, quantitative Hepatitis B e Antigen (qHBeAg) and development of liver cirrhosis were assessed. The data were included in a multivariate logistic regression analysis based on which this prognostic scoring system was established.

Ethical considerations: This study was approved by the Human Research Ethics Committee of the Universiti Putra Malaysia with Reference No. UPM/TNCPI /RMC/JKEUPM/1.4.18. 1/F1. Similar approval was also obtain from Malaysia Ministry of Health Medical Research and Ethics Committee with References No. KKM/NIHSEC/P15-63. Consent was also obtained from the patients who volunteered to participate in this study after they were briefed on the benefits of the study and protecting their confidentially.

Sample collection: A total of 82 chronic Hepatitis B patients were recruited retrospectively, between May, 2015 and 2016. The patient's plasma was separated from the blood immediately after collection, frozen and transported on ice to the Laboratory of Clinical and Molecular Virology at Universiti Putra Malaysia for further analysis.

Data collection: Patient biodata, clinical and laboratory information were collected via. a proforma administered by the attending physician, after obtaining patient consent.

Serological method: All Plasma samples were screened for HBV sero markers such as Hepatitis B surface antigen, Hepatitis B e antigen, Hepatitis core antigen and also Hepatitis B surface and e antibodies using ELISA kits. Similarly, all plasma samples were screened for Hepatitis C, D and E virus antibodies (anti-HDV, anti-HCV and anti-HEV) using ELISA kits (Novatein Biosciences, Woburn, MA) and for Hepatitis E virus antibodies (IgG and IgM) (Wantai Bio-pharm., Beijing, China) according to manufacturer's instructions.

Molecular method: Hepatitis B viral DNA was extracted from 200 µL plasma using a QIAamp DNA blood mini kit, according to, the manufacturer's instructions (Qiagen Hilden, Germany). HBV DNA was quantified using the Qiagen artus HBV RG PCR kit Cat. No. 4506366, (Qiagen Inc., Hilden, Germany). The kit is an *in vitro* amplification test for quantifying HBV DNA in human plasma samples it is based on Polymerase Chain Reaction (PCR) and is configured for use with Rotor-Gene instruments (Qiagen Inc., Hilden, Germany). The kits provide five standards for quantifying HBV which enable accurate viral load quantification. It also contains a heterologous amplification system for identifying possible PCR inhibition and is used as internal control in yellow fluorescence channel cycler. In brief, 30 µL of HBV RG/TM and internal control master mix were added into the PCR tubes after which 20 µL of HBV DNA samples and the standards were added for the unknown samples and the standards, respectively. PCR grate water (20 µL) was used as a negative control and one of the standard samples as positive control.

Real time PCR was run for 50 µL reaction mixture using the following PCR condition: initial activation of the hot start enzyme at 95°C for 2 min, denaturation at 95°C for 15 sec, annealing at 55°C for 30 sec and extension at 72°C for 15 sec in a 45 cycles. Data were analysed upon completion of the run. The analytical sensitivity of this kit is 10.22 IU/mL targeting an amplicon of 134 bp region of the HBV surface protein. The detection limit of this kit is 4 IU/mL ($p = 0.05$) implying that the kit has 95% likelihood of detecting 4 IU/mL HBV DNA in 1 µl of plasma. A viral load of less than 2000 IU/mL (10,000 copies/mL) is considered low while above 2000 IU/ml is considered high based on the guidelines by the Asian Pacific for the Study of Liver (APASL) (Sarin *et al*., 2016).

RESULTS AND DISCUSSION

A simple scoring system composed of virological and biochemical laboratory parameter was developed to predict liver cirrhosis in chronic Hepatitis B patients. This prediction score is accurate and reproducible. Patients with a prediction score between 5 and 15 (Table 1) a score

Table 1: Prognostic values of Hudu's scoring system

Factors/units	1	2	3
Serum bilirubin (μmol/L)	≤20	21-40	≥41
Viral load (IU/mL)	≤300	301-9999	≥10,000
qHBeAg (IU/mL)	≤4	5-10	≥11
ALT (μ/L)	≤33	34-68	≥69
Albumin (g/L)	≤34	35-45	≥70
	5	10	15

ALT: Alanine Amino Transferase; qHBeAg: quantitative Hepatitis Be Antigen

Table 2: Hepatitis B viral DNA correlations with quantitative serological markers of infection

	Clinical diagnosis			
Parameters	Non Cirrhotic N = 44%	Cirrhotic N = 18%	Acute Flare N = 17%	HCC N = 3%
HBV DNA (IU/mL)			r (29.304) = 82, p<0.01*	
High (≥2000)	20(45.5)	18(100)	17(100)	3(100)
Low (≤2000)	24 (54.5)	---	---	---
qHBsAg (IU/mL)		r (45.962) = 82, p<0.01*		
High	9(20.5)	18(100)	15(88.2)	3(100)
Low	35(79.5)	---	2(11.8)	---
qHBeAg (IU/mL)		r (79.308) = 82, p<0.01*		
High	---	18(100)	17(100)	2(66.7)
Low	44(100)	---	---	1(33.3)

HBV: Hepatitis B Virus; DNA: Deoxy ribose Nucleic Acid; IU: International Unit; mL: Millilitre; qHBsAg: quantitative Hepatitis B surface Antigen; qHBeAg: quantitative Hepatitis B e Antigen; HCC: Hepato Cellular Carcinoma; N: Total Number; r: Correlation Co-efficient; %: Percentage, p: Probability *significant correlation at 0.01 levels

5 indicates low risk, 10 high risk and 15 very high risk of developing cirrhosis. Our results also showed that high HBV DNA (>300 IU/mL) as associated with cirrhotic ($r = 29.304$; $p<0.01$). Liver cirrhosis was also found to be associated high qHBeAg levels ($r = 79.308$, $p<0.01$) as shown in Table 2.

The likelihood of morbidity and mortality of chronic HBV infected patient is directly associated with development of liver cirrhosis (Shen *et al*., 2017). This study revealed that, high qHBeAg can be considered a significant prognostic factor associated with a high risk of progression to cirrhosis this concurs with a previous study which reported HBeAg seroconversion and a high level of HBV replication associated with development of cirrhosis (Chu and Liaw, 2005, 2007). As HBeAg is released due to hepatic viral replication, so are AST and ALT which is considered the two most important tests in detecting liver injury but ALT is more specific to liver than AST, similarly in this study ALT was found to be elevated in cirrhosis which concurred with the findings reported by other researchers (Hossain *et al*., 2017; Li *et al*., 2017). Similarly, positive correlation was found between serum bilirubin level and development of cirrhosis ($r = 0.543$, $p<0.01$). On the other hand, a negative correlation was found between liver cirrhosis and albumin ($r = -0.464$; $p<0.01$).

The results of the validation cohort showed that cirrhotic patients were found to be having Hudu's score of 10 while two patients with the score of 15 were found to have developed hepatocellular carcinoma. All non-cirrhotic patients were found to have Hudu's score of 5. However, this study is limited by the fact that qHBeAg is not standardised, therefore, caution needs to be taken when comparing with other studies. The result presented is retrospective as such there is a need for it to be independently validated before generalization via. a multi-centered well organized prospective study.

CONCLUSION

We therefore, conclude that this scoring system can be applied to monitor chronic Hepatitis B carries. It also offers a means of predicting the risk of developing liver cirrhosis in chronic Hepatitis B patients.

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