ORIGINAL RESEARCH PAPER

Biological Science

DETERMINATION OF VARIOUS CLINICAL STAGES OF CHRONIC HEPATITIS B THROUGH MEASURING VIRUS LOAD DNA BY REAL-TIME POLYMERASE CHAIN REACTION (RT-PCR)

KEY WORDS:

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ABSTRACT

Objective And Aim: There was a paradigm shift of hepatitis B (CHB) diagnosis as clinicians are shifted to molecular diagnostic methods from serological one. Specially in molecular system to determine response of treatment as well as different stages of infection as well as recovery by quantification of viral DNA load through real time polymerase chain reaction (RT-PCR). The main objective of the study is to determine the various clinical stages of chronic hepatitis B through measuring virus load DNA by real-time polymerase chain reaction (RT-PCR)

Material And Methods: This is a retrospective study of those patients whose ALT (elevated) and HBeAg (positive) status is known. Serum fraction were initially obtained after 4 hour centrifugation of blood sample and nucleic acid was extracted at -80 °C. Qiagen DNA extraction kit were used to extract DNA. 48-well MiniOpticon by Bio-red machine and with the help of Geno-sense HBV quantitative PCR kit, real-time polymerase chain reaction (RT-PCR) was conducted.

Result: The study was conducted in 64 patients. It has been found that among this patients inactive carriers that is ALT normal and HBeAg-negative were 27 (42.2%) and rest of the patients had HBeAg-positive or HBeAg-negative with ALT elevated that is they were chronic active hepatitis B patients. HBeAg was negative in 42 (65.6%) and positive in 22 (33.4%) subjects. 15 (23%) patients were infected with Chronic hepatitis B among the patients who were HBeAg-negative. Among 64 subjects, detectable viral load was found in 55 (86%) CHB patients. A significantly lower (median 5.6 × 105) serum HBV DNA load were found in HBeAg-negative16 patients as compare to 26 patients with higher viral load (median 2.5 × 108) and were HBeAg-positive. It has also found that viral load was quite higher (median 1.5 × 103) in 27 inactive carriers. Antiviral therapy was started in HBeAg-negative 6 patients and HBeAg-positive 13 patients based on the viral load.

Conclusion: Stages of CHB can be determined by Quantitation of HBV DNA based on ALT (elevated or not) and HBeAg (positive or negative) status. For those patients who are inactive carriers and HBeAg-negative with respect to viral load it could play an important role in assessment and to decide on antiviral therapy.

INTRODUCTION:

Hepatitis B infection is still a leading global health problem. It has already been estimated by WHO (World Health Organization) that worldwide infected with Hepatitis B virus were more than 2 billion and approximately 240 million individuals mainly from liver cirrhosis and hepatocellular carcinoma (HCC) at risk of serious illness and death and have chronic (long-term) liver infections [1-5]. In a study conducted by Tandon BN et al is had estimated that around 4% estimated carrier rate among pool of approximately 36 southern Indian patients [6]. In different parts of the country differences in HBV carrier rates reported in previous studies and mainly due to variations in economic, social, and health factors in different regions of India [7-13]. Liver damage of caring degree generally found to be different in each clinical stages which once again emphasis the factor that to manage CHB infection asessomint these clinical stages is essential.

Hepatocellular carcinoma (HCC), cirrhosis AND inactive carrier state to chronic hepatitis are the major ranges of chronic infection that are found clinically [14]. Chronic hepatitis B (CHB) infection are progress to 80–90% of infants, 10–25% of young children and Less than 5% of adults [15].

There was a paradigm shift of hepatitis B (CHB) diagnosis as clinicians are shifted to molecular diagnostic methods from serological one. Specially in molecular system to determine response of treatment as well as different stages of infection as well as recovery by quantification of viral DNA load through real time polymerase chain reaction (RT-PCR). In current time the treatment session generally taken based on the clinical status of the patient, HBeAg status, ALT levels, serum HBV DNA and and liver histology [16].

The main objective of the study is to determine the various clinical stages of chronic hepatitis B through measuring virus load DNA by real-time polymerase chain reaction (RT-PCR).

MATERIAL AND METHODS:

This is a retrospective study of those patients whose ALT (elevated) and HBeAg (positive) status is known. CHB patients in whom longer than six months HBsAg value was positive were included in this study. Major three study group which was included in this retrospective study was normal ALT with HBeAg-negative, elevated ALT with HBeAg-negative and elevated ALT with HBeAg-positive. Patients were excluded from the study if they were found to co-infected with hepatitis C and HIV 0r on treatment and pregnant or lactating women.

For HBV DNA quantitation 3–5 ml blood sample were collected from each patients. Serum fraction were initially obtained after 4 hour centrifugation of blood sample and nucleic acid was extracted at -80 °C. Qiagen DNA extraction kit were used to extract DNA. 48-well MiniOpticon by Bio-red machine and with the help of Geno-sense HBV quantitative PCR kit, real-time polymerase chain reaction (RT-PCR) was conducted. Viral load was expressed in IU/ml (1 IU/ml = 5.6 copies/ml) sing the external positive controls in quantification machine.

Statistical software SPSS ver 13.0 (SPSS Inc., Chicago, IL, USA) were used to calculate statistical measurements. Values were considered statistically significant when p value < 0.05.

RESULT:

The study was conducted in 64 patients. Demographic details including subjects age (years), gender (male or female), HbsAg, AbeAg, ALT (IU/L) levels, DNA load number and HBV DNA levels were illustrated in table 1.

Table 1: Characteristics Of CHB Patients

Characteristics	HBeAg Positive (N= 22)	HBeAg Negative (N=15)	Inactive carriers (N=27)
Age (years)	36.25±13.86	37.41±12.26	41.43±12.79
Gender (male/female) (%)	13 (59%) / 09 (41%)	12 (80%) / 03 (20%)	15 (56%) / 12 (44%)
HBsAg	Positive	Positive	Positive
HBeAg	Positive	Negative	Negative
ALT (IU/L)	Elevated	Elevated	Normal
DNA Load (N%)	22 (100%)	15 (100%)	18 (66.7%)
DNA Load	$2.5\times 10^{8\pm}$ 2.2×10^{5}	$5.6\times 10^{5\ \pm\ 1.4}\times$ 10^{5}	$1.5\times 10^{3\ \pm\ 1.7}\times$ 10^{2}

It has been found that among this patients inactive carriers that is ALT normal and HBeAg-negative were 27 (42.2%) and rest of the patients had HBeAg-positive or HBeAg-negative with ALT elevated that is they were chronic active hepatitis B patients. HBeAg was negative in 42 (65.6%) and positive in 22 (33.4%) subjects. 15 (23%) patients were infected with Chronic hepatitis B among the patients who were HBeAg-negative. Among 64 subjects, detectable viral load was found in 55 (86%) CHB patients.

A significantly lower (median 5.6×10^{5}) serum HBV DNA load were found in HBeAg-negative16 patients as compare to 26 patients with higher viral load (median 2.5×10^{8}) and were were HBeAg-positive. It has also found that viral load was quite higher (median 1.5×10^{3}) in 27 inactive carriers. Antiviral therapy was started in HBeAg-negative 6 patients and HBeAg-positive 13 patients based on the viral load.

DISCUSSION:

The main aim of the study is to determine the various clinical stages of chronic hepatitis B through measuring virus load DNA by real-time polymerase chain reaction (RT-PCR). This study has revealed that among participant HBeAg was negative in 42 (65.6%) and positive in 22 (33.4%) subjects. 15 (23%) patients were infected with Chronic hepatitis B among the patients who were HBeAg-negative. There are a quite few studies which confirmed that 83–100% of HBeAg-positive patients have the presence of HBV DNA [17,18]. Studies had already confirmed that, as compare to HBeAg-negative group HBV DNA levels were significantly higher in HBeAg-positive group [19-21]. In the present study, during immune clearance of a wild-type strain HBeAg-negative strain emerges with the highest load was 1.5×103 as compare to HbeAg-negative chronic hepatitis patients with low of 2.5× 108. And it also reveals that HBeAg-negative mutant selection occurred when on the wild-type strain immune pressure increased. During treatment it may occur spontaneously [22].

CONCLUSION:

Stages of CHB can be determined by Quantitation of HBV DNA based on ALT (elevated or not) and HBeAg (positive or negative) status. For those patients who are inactive carriers and HBeAg-negative with respect to viral load it could play an important role in assessment and to decide on antiviral therapy.

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