Original Article JOURNAL OF CLINICAL AND EXPERIMENTAL HEPATOLOGY

Role of Glutathione S Transferase M1 and T1 Gene Polymorphism in Hepatitis B Related Liver Diseases and Cryptogenic Cirrhosis

Siddharth Kapahtia [*], Rajib K. Hazam [*], Mohammad Asim [*], Vijay K. Karra [*], Soumya J. Chowdhury [*], Bhudev C. Das [†], Premashis Kar [*]

[*]Department of Medicine, Maulana Azad Medical College, University of Delhi, India and [†]Dr B.R.Ambedkar Center for Biomedical Research, University of Delhi, India

Background and aim: **Progression of hepatitis B virus infection (HBV) might be affected by host genetic factors. The present study was undertaken to study the role of glutathione S-transferases (GST)-M1 and T1 gene polymorphisms in different stages of HBV infection: HBV inactive carrier, chronic hepatitis B and cirrhosis, and cryptogenic cirrhosis.** ***Methods:*** **The study population comprised of 170 subjects; 120 cases (HBV inactive carrier, *n* = 30; HBV related chronic hepatitis, *n* = 30; HBV related cirrhosis, *n* = 30; cryptogenic cirrhosis, *n* = 30) and 50 unrelated healthy adults without liver disease as controls. Analysis of GSTM1 and GSTT1 gene polymorphisms was done by multiplex polymerase chain reaction.** ***Results:*** **The GSTM1 null genotype was seen more commonly in hepatitis B cirrhosis (*n* = 21; 70%), chronic hepatitis B (*n* = 19; 63.33%) and cryptogenic cirrhosis (*n* = 17; 56.67%) as compared with inactive carrier (*n* = 9; 30%) and controls (*n* = 13; 26%). The GSTT1 null genotype was seen less frequently in all the groups, the observed frequencies were controls (*n* = 7; 14%), inactive carrier (*n* = 5; 16.67%), chronic hepatitis B (*n* = 8; 26.67%) and hepatitis B cirrhosis (*n* = 7; 23.33%). The difference of GSTM1 null genotype frequencies was statistically significant for hepatitis B cirrhosis vs. controls (*P* = 0.0002), chronic hepatitis B vs. controls (*P* = 0.002) and cryptogenic cirrhosis vs. controls (*P* = 0.01). The GSTT1 null genotype was not found to vary significantly between the groups.** ***Conclusion:*** **The patients with GSTM1 null genotype are at risk of progression of liver disease as the frequency of GSTM1 null genotype was found to be significantly higher in chronic hepatitis B, hepatitis B cirrhosis and cryptogenic cirrhosis as compared with controls. (J Clin Exp Hepatol 2017;xx:1–4)**

Hepatitis B is an infectious inflammatory illness of the liver caused by the hepatitis B virus (HBV). Approximately 2 billion people are exposed to HBV worldwide and more than 350 million suffer from chronic HBV infection. In India, the prevalence of HBsAg ranges from 2% to 8% in general population, which places India in the intermediate endemicity zone for HBV.[1]

Progression in disease is influenced by several factors, like viral genotype, demographic features, concurrent viral infections, and social factors like alcohol abuse and specific mutations. Host genetic factors may also determine the interindividual differences in disease progression from chronic hepatitis to cirrhosis. Genetic polymorphisms have been observed in enzymes that play a role in the detoxification of xenobiotics and they have been linked to various diseases especially cancers.

Glutathione S-transferases (GST) catalyze the conjugation of the reduced form of glutathione to xenobiotic substrates for the purpose of detoxification.[2] α (A), μ (M), π (P), σ (S), υ (T), ϖ (O) and ζ (Z) are the classes of GSTs.[3] The five mu class genes are situated on chromosome 1p13 in a 20 kb cluster (5′-GSTM4-GSTM2-GSTM1-GSTM5-GSTM3-3′).[3,4] GSTT1 and GSTT2, are located on chromosome 22.[5] Of these GSTM1 and GSTT1 subclasses exhibit deletion polymorphisms and the null GSTM1 and GSTT1genotypes have been extensively studied for their role in susceptibility to various cancers like breast cancer,[6] lung cancer,[7] hepatocellular carcinoma (HCC),[8] prostate cancer,[9] colorectal cancer.[10]

GSTM1 and GSTT1 null genotypes are found in the general population and most recent studies have shown the influence of the ethnic component in the distribution of these polymorphisms.[11–13] In a study done in Iran GSTM1 null genotype was found to be more prevalent in cryptogenic cirrhosis than in healthy controls.[14] Later a similar study was undertaken to find the correlation if any between polymorphisms in GSTM1, GSTT1, and GSTP1 in patients with HBV-related, chronic hepatitis, liver cirrhosis and normal carriers, they observed a higher

Keywords: hepatitis B, cryptogenic cirrhosis, GSTM1, GSTT1, null genotype
Received: 11.10.2014; *Accepted:* 24.05.2017; *Available online:* xxx
Address for correspondence: Premashis Kar, Department of Medicine, B.L. Taneja Block, R/N-127, New Delhi 110002, India. Tel.: +91 011 23230132; fax: +91 011 23230132.
E-mail: premashishkar@gmail.com
Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; GST: glutathione S-transferase; HBV: hepatitis B virus
http://dx.doi.org/10.1016/j.jceh.2017.05.208

Please cite this article in press as: Kapahtia S, et al. Role of Glutathione S Transferase M1 and T1 Gene Polymorphism in Hepatitis B Related Liver Diseases and Cryptogenic Cirrhosis. *J Clin Exp Hepatol.* (2017), http://dx.doi.org/10.1016/j.jceh.2017.05.208

frequency of the GSTM1 null genotype in patients with cirrhosis and chronic hepatitis compared to normal carriers.[15]

The present study was undertaken to study the role of GSTM1 and T1 gene polymorphisms in different stages of HBV infection namely HBV inactive carrier, chronic hepatitis B and cirrhosis, and cryptogenic cirrhosis.

METHODS

Study Population

The study was conducted in the Department of Medicine, Maulana Azad Medical College and associated Lok Nayak Hospital, New Delhi, India from September 2010 to April 2012. The study included a total of 170 subjects; 120 cases (HBV inactive carrier, $n = 30$; HBV related chronic hepatitis, $n = 30$; HBV related cirrhosis, $n = 30$; cryptogenic cirrhosis $n = 30$) and 50 unrelated healthy adults without liver disease as controls. Since, it is a pilot study the samples size was not statistically verified. Informed consent was obtained from each subject included in the study and the study protocol confirms to the ethical guidelines of the 1975 Declaration of Helsinki.

Inclusion Criteria

Inactive HBsAg carrier were included when the HBsAg was positive for more than 6 months, HBeAg negative and anti-HBe positive, serum HBV DNA levels less than 2000 IU/ml and persistently normal liver enzymes levels. Chronic hepatitis B was diagnosed when the HBsAg was positive for more than 6 months, serum HBV DNA levels greater than 20,000 IU/ml (10^5 copies/ml) and persistent or intermittent elevation in ALT/AST levels. The diagnosis of cirrhosis was based on clinical, biochemical, and ultrasonography data.

Exclusion Criteria

Cases with viral co-infections (HCV, HAV, HEV, HIV) and alcoholics were excluded. The patients were evaluated on the basis of history, clinical examination, hematological and biochemical profile, LFT profile and serological tests for hepatitis B (HBsAg, HBeAg, Anti-HBe, IgM Anti-HBc and IgG anti-HBc) using commercially available Elisa kits.

Sample Collection

Five ml of blood was collected in ethylenediaminetetraacetic acid vials for genomic DNA extraction. Another Five ml blood was taken in plain vial for serological testing. Blood samples were stored at −70 °C until use.

Genomic DNA Isolation and Polymerase Chain Reaction (PCR)

Genomic DNA was extracted by a salting-out method.[16] Analysis of GSTM1 and GSTP1 gene polymorphisms was done by multiplex PCR simultaneously in the same tube.[17] Genomic DNA was amplified using the set of primers described by Arand et al.[17] Briefly the PCR reaction conditions were initial denaturation at 95 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 58 °C for 1 min and 72 °C for 1 min. The final extension was done at 72 °C for 7 min. GSTM1 and GSTT1 genotypes positive DNA samples yielded bands of 215 bp and 480 bp, respectively.

Statistical Analysis

The data obtained from the study was tabulated and transferred to a personal computer. Allelic frequencies were compared between different study groups and controls using Chi square test or Fisher's test as applicable using OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version 2.3.1. Odds ratio (OR) with 95% confidence interval (CI) were calculated. 2-tailed P value was used to test significance. The data was considered significant when $P < 0.05$.

RESULTS

The mean age of subjects in different study groups were inactive carrier 29.30 ± 13.01 years, chronic hepatitis B 31.67 ± 12.90 years, hepatitis B cirrhosis 40.17 ± 14.35 years and cryptogenic cirrhosis 39.70 ± 14.69 years.

The relative frequencies of GSTM1 and GSTT1 null genotypes are shown in (Table 1). The GSTM1 null genotype was seen more commonly in hepatitis B cirrhosis ($n = 21$; 70%), chronic hepatitis B ($n = 19$; 63.33%) and cryptogenic cirrhosis ($n = 17$; 56.67%) as compared with inactive carrier ($n = 9$; 30%) and controls ($n = 13$; 26%). The GSTT1 null genotype was seen less frequently in all the

Table 1 Genotype Frequencies of Null GSTM1 and GSTT1 Polymorphism.

Gene polymorphism	Controls ($n = 50$)		Inactive carrier ($n = 30$)		Chronic hepatitis B ($n = 30$)		Hepatitis B cirrhosis ($n = 30$)		Cryptogenic cirrhosis ($n = 30$)	
	n	%	*n*	%	*n*	%	*n*	%	*N*	%
NullGSTM1	13	26	9	30	19	63.33	21	70	17	56.67
NullGSTT1	7	14	5	16.67	8	26.67	7	23.33	6	20

Please cite this article in press as: Kapahtia S, et al. Role of Glutathione S Transferase M1 and T1 Gene Polymorphism in Hepatitis B Related Liver Diseases and Cryptogenic Cirrhosis. *J Clin Exp Hepatol.* (2017), http://dx.doi.org/10.1016/j.jceh.2017.05.208