Association of Interleukin-1β and Gene Polymorphisms with Liver Pathogenesis in Hepatitis B Virus Infection among Eastern Indian Population

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Background: **Interleukin-1β (IL-1β) is an important member of the family of the proinflammatory cytokines that modulate outcome of hepatitis B virus (HBV) infection.** ***Objectives:*** **This study was designed to investigate the relationship between the polymorphic genotypes of the interleukin-1β (IL-1β) promoter region and the interleukin-1 receptor antagonist gene (IL-1RN) and disease outcome in HBV-infected individuals.** ***Methods:*** **DNA was extracted from 395 study subjects including HBV carriers with varying clinical presentations, as well as healthy controls and spontaneously recovered cases (SRC). Polymorphisms in IL-1β (at position −511) and IL-1RN (variable nucleotide tandem repeats, VNTR) were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and PCR based assay respectively.** ***Results:*** **Among the study subjects, different IL-1β (at position −511) (CC, CT and TT) and IL-1RN (1/1, 1/2, 2/2 and 1/3) polymorphic genotypes were found at variable proportions. Logistic regression analysis revealed, no notable difference at the level of IL-1β promoter (*P* = 0.244; OR = 0.78; 95% CI = 0.52–1.18) or IL-1RN genotype polymorphism (*P* = 0.840; OR = 1.03; 95% CI = 0.78–1.36) among the HBV carriers and controls or SRC cases. Pairwise proportion testing showed, IL-1β −511 genotype CC was significantly higher among asymptomatic carriers (ASC) in comparison with liver cirrhosis (LC) patients (*P* value = 0.028) and healthy control group (*P*-value = 0.036). IL-1RN genotype 2/2 was considerably higher in LC group than SRC as well as control group. Combinations of IL-1β (−511) and IL-1RN polymorphisms were associated with disease progression, such as CC-1/2 with ASC and TT-2/2 with LC.** ***Conclusion:*** **IL-1β polymorphisms are found to be associated with disease severity. Different polymorphic combinations are associated with degree of disease severity. Overall this is the first report from Eastern India, which shows association of IL-1β polymorphisms with HBV-related hepatic complications. (J Clin Exp Hepatol 2013;3:281–287)**

Worldwide Hepatitis B virus (HBV) associated liver disease is a common health problem with 360 million suffering from chronic infection.[1] HBV infection can lead to a broad spectrum of clinical manifestations ranging from asymptomatic and self limiting infection to chronic liver disease, acute hepatitis, fulminant hepatitis, cirrhosis and hepatocellular carcinoma (HCC).[2,3]

The natural history of chronic HBV infection and disease outcome is variable and complex and is still not well understood. A careful understanding of the clinical outcomes and factors affecting disease progression is thus important for better HBV management. Since the discovery of HBV variants, several studies has been carried out that focused on the viral factors affecting disease outcome.[4–7] On the other hand, some family studies have examined the role of host factors by characterizing the genetic basis for susceptibility to HBV infection.[8] In the last decade several studies have established the significance and influence of host genes on the outcome of viral hepatitis. Host genetic factors that play an important role in the pathogenesis of HBV infection were the main objective of these studies. Among all these factors, cytokine interleukin-1 (IL-1) has been shown to be the key regulator. IL-1 is an important member of the family of the

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Abbreviations: IL-1β: interleukin-1β; HBV: hepatitis B virus; IL-1RN: interleukin-1 receptor antagonist gene; SRC: spontaneously recovered cases; VNTR: variable nucleotide tandem repeats; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; ASC: asymptomatic carriers; LC: liver cirrhosis; HCC: hepatocellular carcinoma; SNP: single nucleotide polymorphisms; CLD: chronic liver disease; USG: ultrasonography; LR: logistic regression
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proinflammatory cytokines that modulate acute-phase response proteins and thus regulates inflammation. IL-1β and IL-1 receptor antagonists (IL-1RN or IL-1RA) both are very important members of the IL-1 family.[9] Both IL-1β and IL-1RN molecules bind to IL-1 receptors. IL-1β is a potent proinflammatory cytokine, while IL-1RN acts as an anti-inflammatory cytokine that competes with IL-1β for binding to IL-1 receptors without intrinsic effects.[10]

Unlike gene mutations, polymorphisms in different genomic regions are common genetic events. At varying nucleotide positions of IL-1β, biallelic C/T single nucleotide polymorphisms (SNP) are reported.[11,12] Both IL-1β and IL-1RN are located on the 430 kb region of chromosome 2q13-21, where IL-1RN contains an 86-basepair variable number tandem repeat (VNTR) polymorphism, which results in 5 different alleles. These are allele 1 (four repeats), allele 2 (two repeats), allele 3 (three repeats), allele 4 (five repeats) and allele 5 (six repeats).[11,13] All these polymorphisms are situated within the regulatory regions of the IL-1 gene and exert significant effect on the regulation of IL-1 activity. Polymorphism in the IL-1β −511 promoter region and IL-1RN gene VNTR are reported to be associated with varying degree of HBV associated clinical prognosis.[11,14] Another study from Iran found no association of IL-1RN gene polymorphism with the development of chronic HBV infection.[15] Hence the association of the above mentioned polymorphic genetic factors with HBV associated clinical outcome seems to vary from one population to another.

Recent studies from Eastern India confirmed that HBV genotypes and even subgenotypes of the same genotype can differ significantly from the perspectives of prevalence, mutational patterns, recombination and thus in the clinical manifestations.[16,17] But the knowledge about the role of host genetic factors influencing disease outcome in the said population is lacking. Hence, this retrospective study was designed to determine the effect of genotype and allele frequencies of IL-1β −511 and IL-1RN VNTR polymorphisms as host genetic factors with an increased risk of developing HBV-related advanced liver complications in Eastern Indian population.

MATERIALS AND METHODS

Study Subjects

Individuals who attended different voluntary blood donation camps and patients referred to our unit from different out patient clinics for HBV diagnostic purposes have been included in this study only after obtaining informed written consent of participation. The study was approved by the institutional ethical committee. After blood collection, the serum samples were stored at −70 °C, until further use. After serum separation, the rest amount of sera and whole blood cells were used for genomic DNA isolation.

All the samples were tested for HCV and HIV serological markers and those found to be positive were excluded from the study. Subjects with any other potential etiology of liver diseases such as autoimmune liver disease or Wilson's disease were also excluded. Samples were also tested for HBV markers, HBsAg, anti-HBc and HBV DNA. Total 164 HBsAg positive cases, 79 HBsAg negative but anti-HBc positive cases and 144 cases negative for any HBV, HCV and HIV markers are included in the study. Thus, a total of 395 cases were included in the study.

The HBsAg positive samples (n = 164; considered as HBV infected or HI group) were categorized into 3 different clinical categories namely asymptomatic carrier group (ASC, n = 69), chronic liver disease or chronic hepatitis group (CLD, n = 52) and liver cirrhosis group (LC, n = 43), based on the criteria described earlier.[18] ASC was mostly incidentally detected HBsAg positive voluntary blood donors with normal ALT parameters (<40 IU/L; evaluated 2–3 times at 3 months interval) and normal liver ultrasonography (USG) findings. CLD cases were symptomatic or asymptomatic patients, with persistently or intermittently elevated ALT (1.5 times upper limit of normal) for more than 6 months and liver biopsy showing necro-inflammatory changes and fibrosis. LC cases were considered with clinical features of liver cell (hepatocytes) failure and liver biopsy showing fibrosis with nodule formation. Another category included with self limiting HBV infection namely spontaneously recovered cases/group (SRC, n = 79). This SRC group was defined based on previous and resolved HBV infection indicated by HBsAg negative but anti-HBc positive state with or without the presence of anti-HBs, without further virologic, biochemical or histological evidence of active virus infection or disease. A total of 144 ethnically and geographically matched healthy blood donors were included as control group.

Serological and Biochemical Assays

All sera were tested for HBsAg (Hepanostika, Boxtel, The Netherlands), anti-HBc (Biomerieux, Boxtel, The Netherlands), anti-HBs (Biomerieux, Boxtel, The Netherlands), anti-HIV (Biomerieux, Boxtel, The Netherlands) and anti-HCV (Ortho-Clinical Diagnostics, NJ, USA). Anti-HBc and anti-HBs were determined for all HBsAg negative subjects.

The ALT levels were measured by using commercially available kits (Ecoline ALAT; Merck Specialities; Ambernath; India).

DNA Extraction, HBV DNA Detection and Quantification

Genomic DNA was extracted from peripheral blood cells using the QIAamp DNA blood Mini Kit (Qiagen, Hilden, Germany). HBV DNA was extracted from serum using the same kit.

HBV DNA detection and HBV Viral load quantification was done from surface gene region as described earlier.[19,20] Guidelines for avoiding false positive results were followed strictly.[21]

Genotyping of IL-1β Promoter Polymorphisms and Detection of Penta-Allelic VNTR Polymorphism in Intron 2 of the IL-1RN gene

Polymorphism at position −511 in the promoter region of IL-1β was detected by PCR-restriction fragment length polymorphism (PCR-RFLP) method.[22] By a single round PCR amplification, 304 b.p. long DNA fragment was amplified spanning the promoter region of IL-1β. The primers used in the PCR amplification are; pil-1βf (5′-TGGCATTGATCTGGTTCATC-3′) and pil-1βr (5′-GTTTAGGATCTTCCCACTT-3′). Briefly 5 μl of extracted DNA was amplified in a 25 μl reaction volume containing 1.5 mmol/L $MgCl_2$, 200 μmol/L of each dNTP, 1 U of hot start Taq polymerase (AmpliTaq Gold DNA polymerase, Applied Biosystems, Foster City, CA, USA) and 10 pmol of each primer. The thermal cycling parameters were as follows: initial denaturation and Taq activation at 94 °C for 10 min, 35 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s, and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. After that, 15 μl of amplified products were digested with 5U of AvaI (New England Biolabs Inc.) restriction enzyme in a final reaction volume of 20 μl at 37 °C for 3 h. The digested PCR products were then analyzed by 3% agarose gel electrophoresis stained with ethidium bromide and MspI digested pUC19 vector was used as molecular weight marker. Digestion of the 304 b.p. with AvaI yields two fragments of 114 b.p. and 190 b.p. in case of CC at −511, three bands of 304 b.p., 190 b.p. and 114 b.p. in case of CT and the amplicons remained intact in case of TT at −511.

The penta-allelic VNTR of IL-1RN intron 2 gene was assessed by amplicon size estimation with another single round PCR based assay. The primers used to amplify the target region were IL-1RN-F (5′-CTCAGCAACACTCC-TAT-3′) and IL-1RN-R (5′-TCCTGGTCTGCAGGTAA-3′).[9] The fragment sizes are: 410 b.p., 240 b.p., 325 b.p, 500 b.p. and 595 b.p. for allele 1 to 5 respectively. The PCR conditions, reagent and enzyme concentrations were same as for IL-1β promoter region amplification. The amplified products were then separated in 2% agarose gel and the same marker was used.

Statistical Analysis

All comparisons have been made using frequency χ^2 Fisher′s *t*, two sample *t*, Mann–Whitney and Levene′s tests, wherever necessary. Logistic regression analysis has also been carried out to study the association as necessary. A *P*-value <0.05 has been considered to indicate statistical significance. The package used for analysis is MINITAB release 13.31.

RESULTS

Demographic, Biochemical and Virological Characteristics

Table 1 shows the characteristics of the study population. The proportion of female subjects was low in all the study categories as well as in controls. The ALT values of different groups were compared by carrying out pair-wise tests, no significant differences were observed among ASC, SRC and control group, but showed significant difference when compared with CLD and LC groups.

IL-1β Promoter (−511) Polymorphisms

Table 2 shows the distribution of genotype and allele frequency of the promoter region of IL-1β. Overall, in all patients category, SRC and control group genotype CT was most common with CC being the least prevalent genotype. No significant difference in the distribution of genotypes or allele frequencies (genotypes CC, CT and TT; allele C or T) in different clinical groups (ASC, CLD and LC) was

Table 1 Demographic and biochemical properties of the different clinical groups and control groups.

Characteristics ↓	Group →				
	ASC (69)	CLD (52)	LC (43)	SRC (87)	Control (144)
Age in years[a]	30.0 ± 11.81	31.0 ± 10.10	42.43 ± 15.29	32.53 ± 14.56	32.06 ± 12.28
Male/Female	56/13	43/9	27/16	61/26	115/29
ALT (IU/l)[a],*	31.54 ± 9.66	68.80 ± 61.20	87.0 ± 108.70	36.35 ± 17.10	29.88 ± 12.38

*ASC/CLD—*P*-value = 0.0005 (Mann–Whitney test) (less than type test).
*ASC/LC—*P*-value = 0.0122 (Mann–Whitney test) (less than type test).
*CLD/LC—*P*-value >0.05 (Mann–Whitney test) (not equal to type test).
*CLD/SRC—*P*-value = 0.0028 (Mann–Whitney test) (greater than type test).
*CLD/Control—*P*-value = 0.0022 (Mann–Whitney test) (greater than type test).
*LC/SRC—*P*-value = 0.0183 (Mann–Whitney test) (greater than type test).
*LC/Control—*P*-value = 0.0162 (Mann–Whitney test) (greater than type test).
[a]Mean ± standard deviation.

Table 2 Comparison of IL-1β (−511) promoter polymorphism among different groups of clinical categories with hepatitis B infection and healthy controls.

	IL-1β (−511 promoter polymorphism)					
Genotype frequencies	**ASC (69)**	**CLD (52)**	**LC (43)**	**SRC (87)**	**Control (144)**	***P* value**
CC (%)	14 (20.3)[a]	9 (17.3)	3 (6.9)[a,b]	15 (17.2)	16 (11.1)[b]	0.028[a], 0.036[b]
CT (%)	29 (42.0)	24 (46.2)	22 (51.2)	40 (46.0)	72 (50.0)	NS
TT (%)	26 (37.7)	19 (36.5)	18 (41.9)	32 (36.8)	56 (38.9)	NS
Allele frequencies						
C (%)	57 (41.3)	42 (40.4)	28 (32.6)	70 (40.2)	104 (36.1)	NS
T (%)	81 (58.7)	62 (59.6)	58 (67.4)	104 (59.8)	184 (63.9)	NS

NS: not significant (no significant difference was observed among clinical groups and with SRC/Control).
[a]*P*-value for comparison of IL-1β CC genotype frequency between ASC and LC group.
[b]*P*-value for comparison of IL-1β CC genotype frequency between ASC and Control group.

observed by logistic regression (LR) analysis (P = 0.244; OR = 0.78; 95% CI = 0.52–1.18). However, pair-wise comparison showed that genotype CC was in significantly higher proportion in ASC patients in comparison with LC patients (P = 0.028) and control group (P = 0.036). No significant differences with respect to proportions of CT and TT genotypes were observed in other different clinical categories.

When the three clinical categories i.e. ASC, CLD and LC, were considered together as HI group, and compared with SRC as well as with control group respectively, no significant differences in genotype frequencies or allele frequencies were observed.

The ALT value of CT genotype category was highest 109.0 ± 130.4 IU/l, having a considerable difference with CC genotype category 48.8 ± 32.2 IU/l, but statistically insignificant. The ALT value for TT genotype category was 89.3 ± 122.0 IU/l.

IL-1RN Gene Polymorphisms

Table 3 shows the distribution of IL-1RN polymorphic genotypes. In our study population we found 4 different genotypes, namely 1/1, 1/2, 1/3 and 2/2. Genotype 1/1 was most common followed by 1/2 and 2/2 with 1/3 being the least. Allele 1 was most predominant and allele 3 being the least frequent. There was no significant difference in the distribution of IL-1RN genotypes and allele frequencies among patients of ASC, CLD, LC group (P = 0.840; OR = 1.03; 95% CI = 0.78–1.36) by LR analysis, as well as SRC and control groups. However, pair-wise comparison showed that the percentage of IL-1RN genotype 1/2 was significantly lower in LC patients than in controls (P = 0.024) as well as SRC group (P = 0.023). Genotype 2/2 was considerably higher in LC group than SRC as well as control group. Also when HI group was compared with SRC as well as control group separately, no significant difference was found. Notably, none of the ASC cases was of genotype 1/3.

The ALT level was considerably higher in 2/2 category (151.6 ± 223.5 IU/l), followed by 1/2 category (106.0 ± 136.5 IU/l) in comparison with 1/1 and 1/3 category (74.5 ± 75.1 IU/l and 80.7 ± 18.5 IU/l), but the differences were not statistically significant.

Differential Combination of IL-1β (−511) and IL-1RN Polymorphism Associated with Disease Outcome

All possible combinations of IL-1β (−511) and IL-1RN polymorphisms are tested to asses their combined influence on disease progression. The combination of CC-1/2

Table 3 Comparison of IL-1RN polymorphisms among different groups of clinical categories with hepatitis B infection and healthy controls.

	IL-1RN polymorphisms					
Genotype frequencies	**ASC (69)**	**CLD (52)**	**LC (43)**	**SRC (87)**	**Control (144)**	***P* value**
1/1 (%)	32 (46.4)	25 (48.1)	23 (53.5)	39 (44.8)	65 (45.2)	NS
1/2 (%)	26 (37.7)	19 (36.5)	11 (25.6)[c,d]	37 (42.6)[d]	61 (42.3)[c]	0.024[c] 0.023[d]
2/2 (%)	11 (15.9)	4 (7.7)	8 (18.6)	9 (10.3)	14 (9.7)	NS
1/3 (%)	0 (0)	4 (7.7)	1 (2.3)	2 (2.3)	4 (2.8)	NS

NS: not significant (no significant difference was observed among clinical groups and with SRC/Control).
[c]*P*-value for comparison of IL-1RN 1/2 genotype frequency between LC and Control group.
[d]*P*-value for comparison of IL-1RN 1/2 genotype frequency between LC and SRC group.

was significantly higher among the ASC (8/69; 11.59%) cases than CLD (2/52; 3.85%) and LC (1/43; 2.32%) cases (P = 0.049 and P = 0.019 respectively). Combination of TT-1/2 was significantly higher in CLD (10/52; 19.23%) cases than among LC (3/43; 6.98%) cases (P = 0.034). The combination of TT-2/2 was significantly associated with LC cases (6/43; 13.95%) in comparison to CLD cases (1/52; 1.92%) (P = 0.016). Interestingly, when ASC and CLD cases were taken together and compared with LC, the frequency percentage of TT-2/2 was found to be significantly higher in LC cases (P = 0.041).

Association of IL-1β (−511) and IL-1RN Polymorphism with HBV DNA Status

For CC, CT and TT genotype categories the average viral load (log10 copies/ml) were found to be 3.01 ± 0.49, 4.21 ± 1.59 and 4.79 ± 1.84 respectively, with significantly lower viral load in CC category than that of CT and TT (P = 0.036 and 0.012 respectively) (Figure 1).

The viral load for each of the IL-1RN polymorphic genotypes namely 1/1, 1/2, 1/3 and 2/2 were 4.49 ± 1.83, 4.17 ± 1.58, 5.15 ± 2.73 and 3.77 ± 1.32 respectively. The viral load for 1/3 was highest and 2/2 was lowest, but no significant difference was found.

DISCUSSION

With the growing knowledge about the factors influencing severity of viral infections, the importance of host genetic polymorphisms became pertinent. Several studies reported that allelic variants of different cytokine genes influence the regulation of cytokine expression, which in turn affect the degree of susceptibility to various infectious diseases.[23]

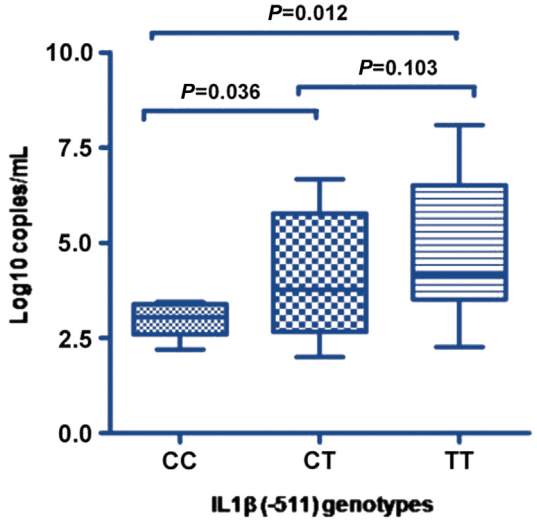

Figure 1 Correlation between HBV viral load and IL-1β (−511) genotypes are presented in the figure. Boxes indicate upper and lower quartiles; horizontal lines inside boxes indicate median values.

IL-1β is a proinflammatory cytokine with several reports suggesting its role in tumor growth development.[24–26] Also previous reports suggest that IL-1β may regulate the production of other cytokines such as IL-2, IL-6, and TNF-α that trigger the complex immunological processes to eliminate the pathogens.[11] To the best of our knowledge this study is the first to illustrate the association between host genetic polymorphisms and HBV related disease complications in the Eastern Indian population, apart from few studies from Northern and Southern India.[27–29]

Interestingly, the frequency of genetic markers in an ethnic population varies from the other. According to Tanaka et al (2003) the prevalence of these specific polymorphisms within certain ethnic populations indicates the influence of past selective pressures and varies in different geographic regions.[30] In this study we included subjects from a single ethnic background. It is reported that different caste subpopulations of West Bengal (Eastern India) shares same allelic or genotypic distributions of various cytokine gene polymorphisms.[31] Previous studies showed that in Eastern Indian population host genetic polymorphisms (IL-1β, IL-6, IL-8, TNF-α), especially IL-1β promoter region determines the degree of susceptibility to *H pylori* associated duodenal ulcer,[32,33] which in turn confirm the importance of studying the possible effect of these polymorphisms in the context of infectious diseases.

The role of IL-1β promoter (−511) polymorphism in the pathogenesis of HBV infection remains controversial. Some studies showed that IL-1β −511 C haplotype is associated with advanced liver disease.[14,34] In the present study, genotype CC was found to be higher in asymptomatic cases, no significant difference in the proportions of CT or TT genotypes in other clinical categories was observed. Interestingly, it was observed that genotype CC category was associated with significantly lower HBV titer (Figure 1) as well as with relatively low ALT. Thus, it can be stated that asymptomatic stages of HBV infection can be correlated with lower HBV viral load and low ALT, which indirectly supports the probable association of CC genotype with ASC. Additionally, no particular difference was noted at the level of viral loads among the cases with differential clinical presentations with CC genotype (viral load 2.91 ± 0.89 log10 copies/ml for ASC, 3.32 ± 0.21 log10 copies/ml for CLD and 3.09 ± 0.61 log10 copies/ml for LC).

It is reported that IL-1RN has been shown to inhibit the effects of IL-1 both *in vivo* and *in vitro*.[35] Different IL-1RN polymorphic genotypes are associated with different rates of IL-1 inhibition and hence are associated with degree of susceptibility to individual diseases. After studying the IL-1RN gene polymorphisms in the study population, genotype 2/2 was found to be considerably higher in LC group than in control group as well as in SRC. A recent study from India reported 2/2 genotype as a potential

risk factor for hepatitis and subsequent cirrhosis development.[36] Recently, allele 2 of IL-1RN intron 2 has been reported as a resistant marker of HBV infection, suggesting the role of IL-1 polymorphisms in the pathogenesis of developing chronic hepatitis B.[11] This allele (allele 2) is associated with enhanced IL-1β production *invitro* and *in vivo*.[37,38] Also the role of heightened secretion of IL-1β leading to the appearance of fibrosis and ultimately the formation of tumor tissue has been suggested.[11,34] This hypothesis is supported by the observation that IL-1β level is increased in the liver surrounding the tumor tissue.[39] It was observed that IL-1RN polymorphic genotype 2/2 was associated with relatively lower viral DNA level. This observation might be due to the fact that, as this genotype leads to heightened IL-1β productions resulting in higher immune response, which may be correlated with viral load suppression.

IL-1β promoter (−511) polymorphisms regulate IL-1β production.[40] It is reported that the degree of IL-1β production depends on the allelic interaction between the −511 and −31 polymorphic sites that determines the overall strength of IL-1β promoter.[32] Also a previous study revealed that high level of IL-1β in blood plasma is associated with IL-1β −511TT and −31CC genotype.[41] According to Tanaka et al (2003) IL-1β −511 proinflammatory TT genotype might be a factor for higher incidence of HCV associated HCC in Japanese population.[30] Another study from Eastern India showed that IL-1β −511TT and −31CC genotypes were significantly associated with *Helicobacter pylori* infected individuals with duodenal ulcer, and also stated that T/C haplotype at the IL-1β −511/−31 loci is mostly prevalent in Indian population.[32] Interestingly the frequency of proinflammatory −511TT genotype is widespread in the study population (38.23%, data not shown). The higher occurrence of the said genotype in the study population might be a crucial factor that influence poorer clinical outcome. Among the 164 HBsAg positive subjects HBV genotype could be determined for 114 cases among which 61 were HBV genotype D (53.5%), 29 were of genotype A (25.4%) and 24 were of genotype C (21.1%) (data not shown). Notably, in this part of India HBV genotype A are found to be involved with advanced liver disease stages.[18] Hence it will be interesting to study the coordination of −511TT genotype and HBV genotype A regulating clinical outcome. Among the HBV carriers, genotype 2/2 of IL-1RN was highly associated with IL-1β −511TT genotype (47.83%, data not shown) and it will be interesting to follow up the patients bearing this genotypic combination (TT-2/2) to asses their clinical progression. In this study also, the combination of TT-2/2 was found to be associated with advanced liver disease (LC) state, which further confirms the importance of the said genotypic combination determining the disease status in the study population. Also it can be hypothesized that proinflammatory TT genotype with favorable allelic combination (position IL-1β −31 and genotype 2/2 of IL-1RN) that up regulates expression of IL-1β and results in higher immune response might be among the important factors contributing towards the evolution of wide range of molecular variants of HBV circulating among Eastern Indian population.[17,42]

One limitation of the present study is that, we could not eliminate the potential confounding effects of IL-1β −31 polymorphism, which could not be determined. However, it should be mentioned that the degree of gene expression is organ and tissue specific. Hence an assessment of IL-1β level in the liver tissue is needed for better understanding of the functional study of liver pathogenesis by HBV. Additionally, this study was done with relatively lower sample size in some clinical groups and thus in future similar studies with larger study population should be done to get a more clear idea about the overall scenario.

Overall this study indicates that IL-1β gene polymorphisms are associated with the clinical outcomes of HBV infections in Eastern Indian population. The genotype of IL-1β and IL-1RN can be used as a marker for better monitoring of patients who are at higher risk of developing advanced liver diseases. Studying the correlation between host genetic makeup and immune response in relation to viral variants will certainly help to develop appropriate strategies for controlling viral hepatitis in near future.

CONFLICTS OF INTEREST

All authors have none to declare.

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