MINIREVIEWS

Th1/Th2 cytokines and their genotypes as predictors of hepatitis B virus related hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC), the predominant type of primary liver cancer, is one of the most serious life-threatening malignancies, worldwide. In majority of the cases, HCC develops after prolonged and persistent chronic liver disease. hepatitis B virus (HBV) or HCV infection is prominent etiological factors, attributing to this condition. It has been well documented that HBV, being the inducer of chronic inflammation, is the main causative agent in causing HCC, particularly in Asian countries. The HBV infection leads to a wide range of clinical symptoms from carrier state to malignancy. Cytokines being immune-modulatory molecules, are the key mediators in the defense mechanism against viral infection. In this regard, this review will detail the substantial role of key Th1: interleukin 1 (IL-1), IL-2, IL-12, tumor necrosis factor-α, interferon-γ; Th2: IL-4, IL-10 and non Th1/Th2: IL-6, transforming growth factor-β1 cytokines genotypes in analyzing the variability in the clinical manifestations in an HBV-afflicted individual, which might finally, culminates into HCC. Since cytokine production is regulated genetically, the cytokine promoter region single-nucleotide polymorphisms induced changes, greatly affects the cytokine production, thus resulting into differential outcome of immune balance.

Key words: Hepatitis B virus; Hepatocellular carcinoma; Inflammation; Th1/Th1 cytokine; Polymorphism

Core tip: Hepatocellular carcinoma is the prime manifestation of primary liver cancer. Besides, hepatitis B virus (HBV) infection accounts for nearly 50% of hepatocellular carcinoma cases worldwide. The injuries afflicted by HBV infection are predominantly immune-mediated. Th1/Th2 cytokines play a significant role in modulating almost all phases of the host immune response. Moreover, cytokine production and response is genetically controlled. Hence, the population-based variability in patterns of cytokine polymorphisms, might alter the ability of an individual to mount an appropriate immune response, thus causing a differential effect on the progression of the HBV disease pathogenesis.

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INTRODUCTION

Liver cancer includes a wide array of histologically different primary liver cancers comprising hepatocellular carcinoma (HCC), cholangiocarcinoma, hepatoblastoma, bile duct cystadenocarcinoma and haemangiosarcoma. However, out of all these, HCC, a type of hepatocyte epithelial tumor, is the most common, constituting 83% of all the incidences[1,2]. Additionally, HCC is one of the virus-induced human cancers[3].

HCC, poses as a worldwide public health issue, being one of the most widespread and lethal cancers. Accounting for 85%-90% of primary liver cancers[4], HCC is the third most frequent mortality causing malignancy[5]. Roughly 6% of the existing human cancers are HCC induced. The occurrence of over half a million HCC cases, annually worldwide[6], makes it the fifth most widespread cancer (fifth in men and seventh in women), globally[7,8].

The major fraction of the HCC afflicted patients, occur due to infections with hepatitis B virus (HBV) or HCV, constituting the main agents, attributing to this condition. This is primarily due to their role in induction of chronic inflammation. However, out of these two causative agents, HBV is regarded as the predominant causative factor of HCC, worldwide, with the incidence rate of hepatitis B surface antigen (HBsAg) carriers accounting for nearly 2% to 11% of the Indians[9]. The variability in HBV infection induced response, is partly due to different immunological factors like the innate and adaptive immune response against the viral infection. Besides, HBV being a non-cytopathic virus, viral persistence/clearance following HBV infection, occur due to the body's immune response against viral antigens.

HBV: Major causative factor for HCC

HBV infection is considered to be one of the pivotal factor in causation of HCC, with the occurrence of more than 350 million chronic carriers worldwide. HBV has been declared a human cancer causing agent by International Association for Research on Cancer, in 1994. Besides, the recent Asian and Northern-American studies conducted, estimated that the chances of HCC development increases by 25-37 times in HBsAg carriers as compared to control populations[10]. India, one of the most populous developing countries has about 45 million chronic-HBV afflicted people[11]. Numerous reports has suggested that the HBV is not directly cytopathic and hence, any injury to the liver cell is chiefly governed by cytotoxic T cells[12]. A large body of evidence has demonstrated that liver cell injury resulting from chronic immune response triggers the causation of HCC. Moreover, cell-mediated immune responses' induced chronic hepatic inflammation and regeneration,

cause the accumulation of genetic alterations in infected liver cells[3]. Thus, these findings strongly reflect the role of immune responses following HBV infection, in causing the chronic disease to carcinoma. Also, all the other procarcinogenic events leading to HCC, most likely occur due to this process[13]. Therefore, the probability and intensity of the hepatocyte injury and its further progression to cirrhosis and consequently to HCC, is an outcome of the interplay between the host immunity and the virus replication ability[14].

CYTOKINES

Cytokines are proteineous moieties, produced chiefly by immune/non-immune cells[15]. They are potent immune-modulatory molecules and major players in protection against viral infection, by either analyzing the host response pattern or by inhibiting viral replication[16].

Since cytokine production is controlled genetically, variations caused due to single-nucleotide polymorphisms (SNPs) in cytokine genes' promoter region, affect the cytokine production to a great extent, thus affecting the immune balance response. This might hold true for cytokine gene polymorphisms and the HBV related HCC, as liver is an lymphocyte enriched organ, involved in numerous cytotoxic activities and having variable cytokine secretion patterns. Besides, HBV is widely believed to be strongest inducer of HCC, primarily by inducing chronic inflammation. Though, some earlier studies have been carried out in this regard, which have reported variable results concerning association of cytokine polymorphism/expression with HBV-HCC risk in different ethnic groups, but till date, no substantial evidence has been yet obtained from the Indian population.

Though, initial classification divided the cytokines into four large groups, on the basis of their biological functions[17]: (1) Natural immunity mediators: like tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1), IL-6 (minor role), IL-5, IL-8 and the chemokines; (2) Lymphocyte activation, growth and differentiation regulators: like IL-2, IL-4, transforming growth factor-β (TGF-β); (3) Regulators of Immune-mediated inflammation: IL-4, TGF-β, IL-10, IL-1, interferon-γ (IFN-γ), granulocyte macrophage-colony stimulating factor (GM-CSF), macrophage activating factor; and (4) Stimulators of immature leucocyte growth and differentiation: IL-1, IL-3, IL-5, IL-6, granulocyte-CSF, macrophage-CSF, GM-CSF.

However, due to the overlapping and multifunctional nature of many of these cytokines, this classification is considered to be random[15]. So, these are generally catagorized into two groups[18]: (1) Th1 (pro-inflammatory) cytokines: IL-1, IL-2, IL-12 and non-ILs like TNF-α and IFN-γ. These cytokines cause stimulation of virus-specific CD8-positive cytolytic T lymphocytes, leading to viral clearance; and (2) Th2 (anti-inflammatory) cytokines: IL-4, IL-10. They induce Th1 cytokines and stimulate activation/differentiation of B cells. Although

Table 1 Cytokines involved in hepatitis B virus - hepatocellular carcinoma risk

Cytokine genes	Physiological function	Role in viral clearance/persistence	SNP analyzed	Disease association
IL-1	Proinflammatory	Viral clearance	IL-1B (-511 C > T)	NS[28,29,31]; risk[30]
			IL-1RN (VNTR) Intron 2	Risk[28]; protection[29]; NS[30,31]
			IL-1 haplotypes	Protection[28]
IL-6	Pro- as well as anti-inflammatory	Both	-572 C > G	Protection[38]; NS[39]
			-597 G > A	Protection[38]
			IL-6 haplotypes	Risk[38]
IFN-γ	Proinflammatory	Viral clearance	+874 T > A	Protection[42]; NS[43]
IL-10	Anti-inflammatory	Viral persistence	-819 C > T/-592 C > A	Risk[49]; NS[50]
			IL-10 haplotypes	NS[49,51]
IL-12B	Proinflammatory	Viral clearance	+1188 A > C 3'UTR	NS[50,54,55]
TNF-α	Proinflammatory	Viral clearance	-308 G > A	NS[42,60]; risk[59]
TGF-β1	Pro- as well as anti-inflammatory	Both	-509 C > T	Risk[54]; protection[54,70,71]
IL-2	Proinflammatory	Viral clearance	-330 T > G	NS[50,54,77]; risk[78]
IL-4	Anti-inflammatory	Viral persistence	-590 C > T	Protection[54]; NS[50]

NS: Non-significant; TNF-α: Tumor necrosis factor-α; IL: Interleukin; TGF-β: Transforming growth factor-β; VNTR: Variable number tandem repeat; IFN-γ: Interferon-γ; 3'UTR: 3' untranslated region.

several of them do not fit specifically into either category like non Th1/Th2 cytokines; IL-6, TGF-β. Although cytokines act at very low concentrations (pg/mL), their effect is closely related to their circulating levels. Besides, an individual's cytokine production capacity is genetically regulated, which accounts for remarkable variation among individuals[19]. Thus, deregulation of the gene expression that alters the cytokine production may alter the homeostasis of the organism, resulting in organ-specific or systemic failures. This is quite relevant for cytokine gene polymorphisms and HCC[20], as cytokines are key determinants in regulating the immune response during HBV infection.

Role in HBV-HCC pathogenesis

Induction of chronic inflammation creates a tumor-favouring microenvironment that eventually participates in the necroplastic process. Moreover, in immune cell enriched liver, immune responses following hepatitis infection, cause cell damage, regeneration, finally leading to liver cancer due to continued cell proliferation and death[18]. Thus the chances for HCC development in HBV-afflicted individuals increases with up-regulated inflammation and fibrosis[21]. T-lymphocyte immuno-regulatory cytokines are crucial players in regulation of the host response to against HBV infection. In fact, it has been shown that the cell-mediated immunity is responsible for viral recovery[22], while Th2 cytokines actively participate in causing persistent infection[23]. In this context, an HBV-infected individual having down-regulated Th1 and up-regulated Th2 cytokine production, might experience an increased likelihood to HCC development. Hence, polymorphisms in cytokine genes can influence body's immune system; inflammation and tissue injury in HBV related malignancy.

Several studies have documented functional cytokine polymorphisms, associated with varying stages of liver disease. The differences in cytokine expression and the functional consequence of these modifications in HCC,

are primarily the result of the variability in response of the immune system in the presence of primary lesion. However, the genetic make-up of an individual may also alter the immune system and generate tumorigenic effects. The principal cytokines and their genotypes, found to be involved in HBV-HCC development are listed below.

IL-1

This is a multifunctional proinflammatory cytokine. The *IL-1* gene family comprises IL-1α, IL-1β and IL-1 receptor antagonist (IL-1Ra/IL-1RN). It is located on long arm of chromosome 2 (2q13.21)[24] and encodes three proteins namely: IL-α, IL-β (agonists) and IL-1Ra (naturally occurring inhibitor)[25]. An 86-bp variable number tandem repeat (VNTR) polymorphism is present in intron 2 of the *IL-1RN* gene[26]. The *IL-1RN* (VNTR) polymerase chain reaction-analysis, depicted five different allelic combinations (allele 1 - allele 5) of the 86-bp sequence to be present in intron 2 of the *IL-1RN* gene. Pociot *et al*[27] have identified an *IL-1B* biallelic (C/T), promoter region polymorphism (-511), affecting its secretion *in vitro*.

The *IL-1B* (-511) genotypes and HBV-HCC association analysis (Tables 1 and 2), revealed that there was no significant association between the *IL-1B* (-511) heterozygous (CT) and variant (TT) genotypes with HBV-HCC risk, in healthy controls and inactive-HBV carriers[28]. Similarly, a study by Zhang *et al*[29] indicated no change in IL-1B allele/genotype frequencies between hepatitis patients and the controls. These findings, however, differed from a study by Tanaka *et al*[30], where *IL-1B*-511(TT) genotype was potentially in positive association with HCC development (Table 1). Further, we observed that the *IL-1RN* (VNTR) genotypes and the HCC risk association analysis (Tables 1 and 2), revealed a significant positive association between 1/2 genotype with HCC development, among healthy controls and inactive carriers[28]. However, a study by Zhang *et*

Table 2 Association of various cytokine genotypes in progression of hepatitis B infection

Cytokine genes	OR (95%CI)					Ref.
	Control	Inactive HBV-carrier	Chronic-active HBV	HBV-cirrhotic	HBV-HCC	
IL-1RN (VNTR) 1/2	1 (REF)	0.45[a] (0.2-1)	2.70[b] (1.3-5.3)	2 (1-4)	1.90 (1-4)	[28]
	-	1 (REF)	5.80[h] (2.5-13.4)	4.20[f] (1.8-10)	4.12[f] (1.7-10)	
IL-6 (-572 G > C) GC	1 (REF)	3.98[b] (1.5-10.2)	2.50[a] (1.1-6)	0.94 (0.4-2)	0.75 (0.3-1.7)	[38]
	-	1 (REF)	0.63 (0.2-2)	0.24[f] (0.1-0.7)	0.20[f] (0.06-0.6)	
CC	1 (REF)	1.8 (0.6-5.6)	2.54[a] (1.05-6.2)	0.40[a] (0.16-1)	1.50 (0.6-3.8)	
	-	1 (REF)	1.30 (0.3-5)	0.20[c] (0.01-0.6)	0.83 (0.2-3.2)	
IL-6 (-597 G > A) GA	1 (REF)	8.65[d] (3-25)	0.52 (0.2-1.2)	0.63 (0.3-1.5)	2.1 (0.7-6.4)	
	-	1 (REF)	0.06[h] (0.02-0.2)	0.07[h] (0.03-0.2)	0.22[c] (0.06-0.8)	
IFN-γ (+874 T > A) TA	1 (REF)	2.20 (0.6-8)	0.34[b] (0.14-0.8)	0.56 (0.24-1.3)	0.39[a] (0.17-0.85)	[42]
AA	1 (REF)	0.65 (0.26-1.7)	0.78 (0.34-1.8)	0.62 (0.26-1.5)	0.31[b] (0.13-0.72)	
IL-10 (-819 C > T/-592 C > A)	1 (REF)	4.34[d] (1.83-10.3)	ND	2.02[a] (1-4.1)	2.20[a] (1.05-4.5)	[49]
IL-12B (+1188 A > C 3'UTR) CC	1 (REF)	1.44 (0.5-4.1)	3.30[b] (1.3-8)	1.3 (0.5-3.8)	1.80 (0.6-5.3)	[54]
TGF-β1 (-509 C > T) CT	1 (REF)	4.70[d] (1.8-12)	2.20[a] (1-4.5)	2.81[b] (1.3-5.8)	2.10[a] (1-4.2)	
TT	1 (REF)	15.42[d] (5-47.6)	5.87[d] (2.2-15.7)	1.50 (0.4-4.8)	3.72[b] (1.4-10)	
	-	1 (REF)	0.38 (0.13-1.1)	0.10[h] (0.03-0.3)	0.24[f] (0.1-0.7)	
IL-4 (-590 C > T)	1 (REF)	2.26[b] (1.2-4.2)	0.40[b] (0.2-0.7)	0.70 (0.38-1.27)	1.65 (0.9-3)	

OR: Odd ratio adjusted with age, sex, bilirubin, total protein, A/G, aspartate transaminase, alanine transaminase, alkaline phosphatase; ND: Not determined due to a single subject having this genotype. [a]$P < 0.05$, [b]$P < 0.01$, [d]$P < 0.001$ with respect to control; [c]$P < 0.05$, [f]$P < 0.01$, [h]$P < 0.001$ with respect to inactive HBV-carrier. HBV: Hepatitis B virus; IFN-γ: Interferon-γ; IL: Interleukin; TGF-β: Transforming growth factor-β; HCC: Hepatocellular carcinoma.

al[29], documented conflicting results, by showing a significant negative association of the carriage of *IL-1RN* (VNTR) allele 2 with HBV infection. On the contrary, a non-significant association was evident between 2/2 genotype and the liver disease progression in a Japanese study[30], while a potential association was found between the same genotype and cirrhosis development, in our case[28]. Moreover, as reported by Chan *et al*[31], no significant association was found between *IL-1B* and *IL-RN* (VNTR) polymorphisms and liver fibrosis, in Chinese hepatitis patients. Besides, we found that the *IL-1* haplotypes 2 and 3 acted as significant protective factors for hepatitis and subsequently for HCC development (Table 1)[28].

Besides, similar to the Portuguese population[32], the *IL-1B*-511 and *IL-1RN* (VNTR) loci were observed to be in a weak linkage disequilibrium with each other, among controls[28]. Furthermore, on analyzing the effect of *IL-1B* (-511C/T) genotypes on its levels, a substantial decrease in the levels was evident in TT genotyped controls, with respect to those with the heterozygous (CT) genotype but not in HBV-infected individuals. This observation was in line with the previous documentation of an up-regulated IL-1B production due to the presence of C allele[33].

IL-6

This is a 23.7 kDa pleiotropic cytokine, produced by both lymphoid and non-lymphoidcells[34]. This cytokine acts as both pro-as well as anti-inflammatory cytokine and has a key role in growth-promotion and anti-apoptotic activities[35]. The genes involved in processes like differentiation, survival, apoptosis and proliferation are mainly targeted by the IL-6 family[36]. Inter-individual variations at transcription and expression level occur due to IL-6 polymorphisms (promoter region)[37].

Studies conducted so far, have reported three SNPs located in the *IL-6* gene promoter (-597G/A, -572C/G and -174G/C), which result in up-regulation of IL-6 levels and have been observed in chronic hepatitis B patients. The association analysis carried out by us (Table 2), between *IL-6* (-572) genotypes and the HCC risk, showed that in case of GC genotype, a significant negative association was evident for HCC development, among carriers. While the CC genotype, acted as vital protective factor for cirrhosis development[38]. However, a Korean study reported a non-significant association of *IL-6*-572 (G > C) polymorphism with hepatitis outcome, *i.e.*, the occurrence of liver cirrhosis and HCC following hepatitis, in individuals hetero-and homozygotes for G allele, as compared to the CC homozygotes (Table 1)[39]. Further, on associating *IL-6* (-597) genotypes with HCC susceptibility, the heterozygous genotype (GA) was significantly in negative association with HCC risk, among HBV carriers. Besides, when we determined IL-6 haplotypes with the HCC risk, haplotypes 2 (GA) and 3 (CG) were found to be significantly positively associated with HCC development, while the haplotype 4 (CA) acted as a potential protective factor for the same. Additionally, no difference was evident in IL-6 levels in case of *IL-6* (-572) and *IL-6* (-597) genotypes, in our study (Tables 1 and 2)[38]. However, earlier, a study conducted in healthy Spanish population, showed that G allele at -597 is associated with significantly elevated IL-6 circulating levels[40].

IFN-γ

This cytokine has a multifunctional role, produced exclusively by T lymphocytes and natural killer (NK) cells[41]. Several reports have indicated the significance of *IFN-γ* gene polymorphism (+874), situated in its first intron, which coincides with the nuclear factor κB

binding area[41], in modulating HBV infection risk. In our lab, the association analysis conducted between the *IFN-γ* (+874 T > A) genotypes and the cancer risk in Indian population, showed that the heterozygous genotype (TA) was significantly in negative association with hepatitis and later on with HCC development, in healthy controls as well as HBV-inactive carriers. The variant AA genotype was also observed to be in significant negative association with HBV-HCC risk, among controls as reference (Tables 1 and 2)[42]. The results differed from study by Cheong *et al*[43], where no significant association was evident between IFN-γ (+874) polymorphism and susceptibility to HBV infection (Table 1). Studies by several authors carried out in different populations (Colakogullari *et al*[44]; Farhat *et al*[45]; and Forte *et al*[46]), showed that the levels of wild genotype individuals were significantly elevated when compared to TA genotype subjects while, we did not observe such changes in IFN-γ levels among individuals with different genotypes[42].

IL-10
IL-10 is regarded as a pleiotropic Th2 cytokine, mainly involved in regulation of inflammatory responses. It primarily participates in inhibiting cytokine synthesis by Th1 cells[47]. It acts both as an anti-inflammatory (tumorigenic) and anti-angiogenic (anti-tumorigenic) cytokine. Further, as reported by Breen *et al*[48], *IL-10* upstream promoter region has two linked biallelic SNPs at positions -819 (C/T) and -592 (C/A).

Upon studying the association analysis of *IL-10* genotypes with HBV-HCC risk, we found the CC/TA genotype to be in a significant positive association with HBV-HCC development (Tables 1 and 2)[49]. While a study conducted by Nieters *et al*[50], in Chinese population, showed that the wild and heterozygous genotypes shared no significant association with HCC (Table 1). Moreover, the haplotype analysis, revealed a strong linkage disequilibrium between the two studied single nucleotide polymorphisms, consistent to the other studies by Breen *et al*[48]; Shin *et al*[51]; Tseng *et al*[52]; and Gambhir *et al*[53]. However, in case of Indian population, no significant association was found between the 2 haplotypic combinations (CC and TA) observed and HCC risk (Tables 1 and 2)[49]. On the contrary, the CC haplotype was found to accelerate the HCC progression rate in HBV patients in a study by Shin *et al*[51].

IL-12
IL-12, a key Th1 proinflammatory cytokine and is produced chiefly by the antigen presenting cells. This heterodimeric cytokine suppresses the Th2 function and was initially recognized as a connecting link between innate and adaptive immune responses. It's major biological functions include activation of NK and T cells, causing induction of IFN-γ and imparts resistance to tumors, by promoting Th1 adaptive immunity and cytotoxic T lymphocyte responses. Besides, several

molecular epidemiologic studies have stated the functional importance of SNP at +1188 (A/C) in the 3' untranslated region (3'UTR) of *IL-12p40/IL-12B* in immune mediated diseases and cancer risk.

The association study done between the *IL-12B* (+1188 3'UTR) genotypes and HCC risk, revealed no significant association between the AC and CC genotypes with HCC risk (Tables 1 and 2)[54]. Similar observations were reported in two separate studies done in the Chinese population, where these genotypes of *IL-12B* were not found to be significantly associated with HBV induced HCC (Table 1)[50,55]. Another study done in HCV patients, showed that the association of AC genotype with self-limited infection, while the persistent HCV infection was observed to be associated with AA genotype[56]. The presence of "A" allele at *IL-12B* (+1188 3'UTR) resulted in elevated IL-12B production[57].

TNF-α
It is a potent pleiotropic cytokine. It's gene is located on the short arm of human chromosome 6 (6p21.3)[58]. TNF-α is a proinflammatory and an immunomodulatory cytokine. Various studies have shown that TNF-α, along with IFN-γ exerts an antiviral effect, profoundly suppressing *HBV* gene expression in infected hepatocytes noncytolytically. Literature has shown, several functional SNPs in the TNF-α promoter region, which were reported to influence the TNF-α constituitive and inducible expression levels. Till date, however, the best described SNP is at -308 position of the *TNF-α* promoter.

A study conducted by Jeng *et al*[59], showed that the TNF308.2 (A) allele significantly contributes to a higher HCC risk in Taiwanese population (Table 1). However, in our study in Indian population[42] and in a study by Somi *et al*[60] in Iranian population, no such association was observed (Tables 1 and 2). Numerous studies have observed the TNF2 allele(A) to be a stronger transcriptional activator than wild (G) allele[61-64]. On the contrary, no significant difference was evident between the *TNF-α* (-308) genotypes, its serum and *ex vivo* levels in Chilean rheumatoid arthritis patients[65], Taiwanese[66] and the Asian Indians[67].

TGF-β1
TGF-β, a polypeptide growth factor family, being encoded by three different genes-*TGF-β1*, *TGF-β2*, and *TGF-β3*. Among these, TGF-β1 is most frequently up-regulated in tumor cells[68]. TGF-β1, a multifunctional cytokine, acts a potent growth inhibitor in wound healing and differentiation processes. Owing to this, great stress has been laid on studies about impact of TGF-β1 and its gene variations in susceptibility/pathogenesis of various diseases. So far, many *TGF-β1* polymorphisms have been documented *viz*. three variations, located upstream of exon 1 (at positions -988C/A, -800G/A, and -509C/T), an insertion/deletion of cytosine residue in the 5'UTR (at position +72) and three nucleotide substitutions in the gene's coding region[69]. However, the most reported -509 C > T polymorphism in *TGF-β1*

promoter is linked with its increased circulating levels.

The association analysis concerning the *TGF-β1* (-509) genotypes with HBV-HCC risk, revealed that both hetero- and homozygotes for the T allele, acted as vital risk factors for HCC, in Indian healthy subjects. While, the variant genotype acted as a significant protective factor for cirrhosis and the subsequent HCC risk, among inactive carriers (Tables 1 and 2)[54]. Similarly, a study reported significantly lowered HCC risk in hepatitis B patients with variant (TT) genotype, than in those with wild (CC) genotype[70] and another study also reported decreased HCC risk in patients with TT or CT genotypes than in those with the wild genotype[71]. Both the CC and TT genotypes were found to be significant risk factors for cirrhosis in an earlier study done in Italian population (Table 1)[72]. Besides, the -509C allele was also observed to be significantly associated with higher HCV clearance rates (*P* < 0.01), in a study by Kimura *et al*[73]. A Chinese case-control study revealed that both T allele hetero- and homozygotes were significantly associated with decreased colorectal cancer risk[74].

Grainger *et al*[75], have observed that the T allele of -509C/T polymorphism accounts for higher TGF-β1 production. However, our study differed from this finding as no substantial difference in the levels in any of the *TGF-β1* genotypes was observed[54]. Further, a study done by Qi *et al*[70], also did not show any significant difference in TGF-β1 plasma concentration, between CC and TT genotypes among diseased or healthy controls. Ethnic disparity could be the most probable reason for the apparent discrepancy on the genetic control of TGF-β1 production level.

IL-2
IL-2, a proinflammatory and strong immunoregulatory Th1 cytokine, affecting various immune cells. John *et al*[76] had reported two SNPs in *IL-2* gene (-330 and +166). The +166 change occurs in the leader peptide, so no change occurs in amino acid sequence. The SNP at -330 promoter region position produces two alleles (T and G). Since, the -330 promoter region polymorphism consists of two common alleles, so it is regarded as an appropriate marker for association studies.

On associating the *IL-2* (-330 T > G) genotypes with HCC progression in HBV infected individuals, we showed that both the TG and GG genotypes remained largely non-significant in HBV chronicity, among controls and carriers[54]. Similarly, the *IL-2* (-330 T > G) poly-morphism did not appear to modify HBV-HCC risk in the Chinese and American populations[50,77]. On the contrary, a study by Gao *et al*[78] reported, *IL-2*-330 TT genotype to be associated with an increased risk of chronic hepatitis, in case of either HBV or HCV or HBV-HCV coinfection in Chinese population (Table 1).

IL-4
Both IL-4 and IL-10, are cytokines secreted by Th2 cells, and suppress the generation of Th1 response[50]. IL-4, the prime Th2 cytokine, act antagonistically to

various IFN-stimulated functions on Th1 differentiation/stability[79]. *In vitro* and *in vivo* studies had documented the T allele of IL-4 (C-590T) polymorphism, which is in linkage disequilibrium with-33T, to be associated with an increased *IL-4* expression.

The association study showed that the CT genotype was found to be potentially negatively associated with hepatitis B development in healthy Indians (Tables 1 and 2)[54]. On the contrary, in a Chinese cohort study, *IL-4* (-590 C > T) genotypes were not found to be significantly associated with the HCC risk in American population (Table 1)[50]. Besides, in another study, *IL-4* (-590) CT and CC genotype frequencies were significantly higher in chronic hepatitis B patients with abnormal ALT levels, thereby associating them with liver inflammatory injury[78]. Moreover, subjects harboring the *IL-4* (-590) CT genotype, showed significantly raised IL-4 levels, with respect to CC genotype subjects (Tables 1 and 2)[54]. Earlier studies have shown enhanced promoter strength with the variant (T) allele at position -590 due to increased binding of the nuclear transcription factors to the promoter, thus up-regulating IL-4 expression.

CONCLUSION
The association studies carried out with cytokine gene polymorphism and HBV related disease chronicity vary considerably across different populations studied. Due to ethnic variability of the results, it is difficult to conclude the associations based on the available data. In nutshell, on the basis of these observations, it can be said that there is a dire need for analyzing the individual and collective polymorphic forms of various cytokines, both mRNA and the protein expression, the correlation between them, in a larger set of individuals in various set of populations, so as to enhance, not only the diagnostic and prognostic value of such studies, but also for determining an individual's susceptibility to HBV-HCC disease.

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