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RESEARCH ARTICLE

Status of TNF-α polymorphism in different phases of Chronic Hepatitis B

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Abstract

Chronic hepatitis B is a severe form of liver infection. The host immune response plays an important role in the progression of the disease. This host pathogen interaction results into different phases of chronic hepatitis B. In this study we have tried to analyze the role of TNF-α polymorphism in the four phases of chronic hepatitis B to see if the polymorphism has got any role in the disease progression. We found that TNF-α 308 homozygous GG genotype was frequently associated with immune reactive state and progressed to chronic hepatitis in HBeAg positive subjects whereas heterozygous AG genotype was associated with non reactive subjects.

Introduction:-

Chronic hepatitis B is still a public health problem worldwide. Such inflammatory response is co-ordinated by cytokines. The natural outcome in hepatitis B infection varies greatly among individuals. The gene polymorphism determines the outcome of a disease to a great extent. The difference in disease outcome is due to the variance in the host immune response. In this short study we have focused on one of the important cytokines TNF- α. TNF-α is one of the important cytokines involved in non-cytotoxic antiviral mechanism and participates in the host immune response and viral clearance of HBV. The capacity for the cytokine production in individual largely depends on genetic polymorphism in the promoter region. TNF-α can suppress the replication and multiplication of HBV. It inhibits replication through post translational mechanism that accelerates the degradation of HBV mRNA. It is synthesized as a membrane protein, which is cleaved to produce its soluble 17Kd form. TNF-α is a principal mediator of cellular immune response. It might have an important function in non-cytolytic and cytolytic clearance of HBV. Th1 cytokine TNF- α exert an antiviral effect profoundly suppressing HBV gene expression. The way TNF-α inhibits HBV replication differs from other cytokine inhibitors because it targets the stability of nascent nucleocapsids. The maintenance of the cccDNA pool is thought to be critical for HBV persistence in infected hepatocytes. TNF-α has a number of sites to be studied, however locus -308 is much more considered than any other. Polymorphism in the TNF-α promoter region has been shown to affect cytokine transcription rate and release. The G to A nucleotide substitution at position -308 in the promoter region of the TNF-α gene was associated with elevated TNF-α gene transcriptional activity. Thus it can be hypothesized that the TNF-α promoter alleles -308A is associated with higher plasma levels and is strongly associated with the resolution of HBV infection. The polymorphism at this locus was studied in all the four phases of chronic hepatitis B using allele specific primers [Rad IA et al, 2012]. Figure 1 shows the gel picture of the desired band obtained after allele specific PCR. Presence of bands for both the alleles indicates heterzygosity.

Our study attempts to analyze the pattern of polymorphism in different clinical expression of chronic HBV infection. The four phases of chronic hepatitis B have been described as Immunotolerant (IT), Immune reactive HBeAg positive (IA+), Immune reactive HBeAg negative (IA-) and Inactive carriers (IC).

On single nucleotide polymorphism it was observed that heterozygous genotype AG was the most commonly distributed genotype in case of immunotolerant and inactive carrier group i.e. asymptomatic subjects without any evidence of liver injury. This heterozygous AG genotype prevalence was higher than homozygous AA prevalence in all the groups. However in IA- subjects it failed to reach statistical significance. In asymptomatic individuals with

normal liver status i.e. immunotolerant and inactive carrier group the heterozygous AG genotype was significantly higher than homozygous GG genotype but among IA+ subjects dominant genotype was homozygous GG. In IA- group there was no characteristic pattern in genotype distribution i.e. all the genotypes were equally distributed (Table 1).

On calculation of odds ratio we found that G allele has high frequency in case of IA+ subjects having the following ratio A:G::1:3 whereas in IA- group the frequency of both the alleles was similar. G allele was noted to be relatively more frequent than A allele in case of asymptomatic group with normal liver status. In case of inactive carrier group it was 2 times more prevalent (OR=0.26, $p<0.001$). However in immunotolerant group its frequency was marginally higher i.e. 1.4 times which failed to reach statistical significance (OR=0.5, $p=0.06$).

TNF-α 308 heterozygous AG genotype subjects remained more frequently associated with immunologically non-reactive state and did not progress to chronic hepatitis. Whereas homozygous GG genotype was frequently associated with immune reactive state and progressed to chronic hepatitis in HBeAg positive subjects. This substantiate that G to A substitution is associated with resolution of HBV. In absence of this G to A substitution probably the progressive liver disease results in the setting of homozygous GG status. However in HBeAg sero converted, immune reactive subjects no such relationship could be observed. Moreover the allele G was associated with significantly higher risk factor for the progression of hepatitis B infection in case of HBeAg positive immune reactive subjects, but failed to show such association in case of HBeAg sero-converted immune reactive chronic hepatitis B subjects in conformity to our afore said observation. However inactive carriers also had more frequent distribution of allele G which needs further evaluation.

There are several studies which supports this view. For example Cheong JY et al, 2006 conducted a study on Korean patients with HBV infection and recovered subjects. They found that -308G allele were associated with HBV persistence. They found that genotype distribution of both gene promoters in inactive carriers were similar to those in patients with chronic progressive liver disease.

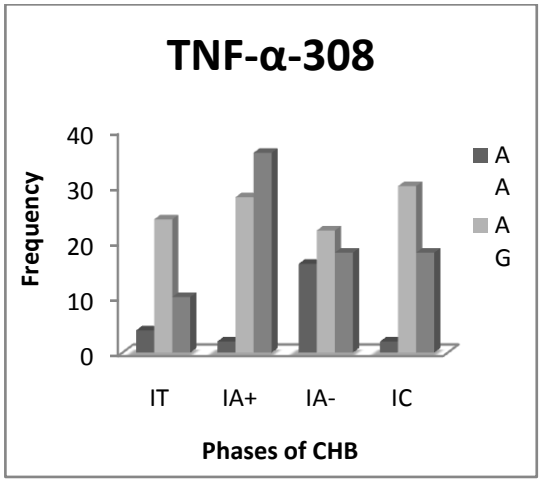
In conformity to our observation, study by Niro et al, 2005 on 184 CHB carriers Hepatitis B surface antigen positive cases and 96 controls with sero clearance (HBsAg negative and AntiHbs and Anti HBcIgG positive) reported that TNF-α promoter polymorphism did not appear to be determinant of HBV sero-clearance in Southern Italians whereas the genotype -308G/G was associated with prognosis in patients with chronic HBV infection.

Similarly a study by Kim YJ et al, 2003 showed that spontaneous HBV clearance was associated with the presence of TNF-α 308 A allele. The study was performed on a large number of Korean population i.e. 1400. The study cohort comprised of two groups: chronic carrier group and subjects who spontaneously recovered.

However another study on Iranian population performed by Somi et al, 2006 failed to find any association between TNF-α promoter polymorphism -308A with development of chronic HBV infection. The study cohort comprised 100 CHB infected subjects, 91 spontaneously recovered HBV infected and 89 healthy controls. He used simple PCR-RFLP method. They found that -308A is common in Iranian population but had no association with the development of disease.

Similarly Yang et al., did not find statistical difference in TNF-α -308G/A alleles or genotypes frequencies between Chinese HCC patients (n=772) and healthy controls (n=852). Although they also found that greater TNF-α production up to five-fold of the basal level and induction of mRNA expression have been associated with the TNF-α -308*A allele, with elevated serum TNF levels being observed even in heterozygous patients [Abraham et al, 1999].

There is still no worldwide agreement on association between polymorphisms and TNF-α production. However its importance in the disease progression cannot be ignored.

a) 

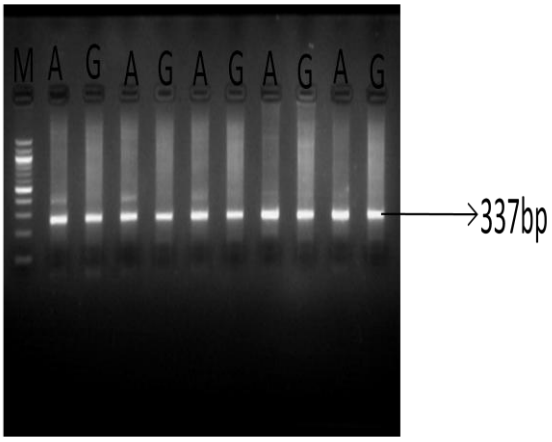
b) 

Figure 1: a) shows the distribution of genotypes of TNF-α-308 between different groups in the form of a bar graph. b) the figure shows the gel image of the desired band obtained after allele specific PCR. Presence of bands in for both the alleles indicates heterzygosity.

Table 1: Genotype frequency of the cytokines TNF-α, IL-4 and IL-8.

Cytokines and Clinical status	**No. (%) of patients (frequency)**			**P value**		
TNF-α 308 A/G	**AA (1)**	**AG (2)**	**GG (3)**	**1 vs 2**	**1 vs 3**	**2 vs 3**
IT	4 (10.5)	24 (63.2)	10 (26.3)	<0.001*	0.07	<0.01*
IA^+	2 (3)	28(42.4)	36 (54.5)	<0.001*	<0.001*	0.16
IA^-	16 (28.6)	22 (39.3)	18 (32.1)	0.23	0.68	0.43
IC	2 (4)	30 (60)	18 (36)	<0.001*	<0.001*	<0.05*
χ^2 =31.75, p value =0.001*						

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