Summary

Viral hepatitis remains a major public health problem and the most common cause of liver diseases worldwide. Hepatitis B virus, the causative agent for hepatitis B, is mainly transmitted through contact with infected body fluids. Hepatitis B infection in people leads to a wide variety of symptoms in different individuals depending on their age, sex and other factors. The carrier status for infection established in childhood is through horizontal and neonatal transmission. These carriers range from a healthy carrier state to patients with active chronic disease. Some of the chronic patients develop hepatocellular carcinoma (HCC) also. Infection during adult stage results in acute symptoms that vary from mild to fulminant disease status. Invariably, after acute disease, the patients show clearance of the virus, however, about 5% of the patients with acute disease result in a chronic infection. The basis for this differential development of the disease is not fully understood. But, clearly, the host factors have an important role to play. The host factors or genes, which are the mediators of viral pathogenesis or host survival, significantly affect the outcome of the disease in different individuals.

This study makes an attempt to study the role of genetic polymorphisms in some of the host genes such as, Fas ligand (Fas L), Fas, Transforming growth factor-beta1 (TGF-β1), Tumor necrosis factor-alpha (TNF-α) and Interleukin-6 (IL-6), which could influence the outcome of the hepatitis B virus infection in the background of different genotypes. Primers were designed to amplify the specific regions of the chosen candidate genes and a PCR-SSCP based approach was adopted to explore the novel and the known polymorphisms in the selected regions, which were later confirmed by automated sequencing.

An absence of mutation or polymorphism was observed in the selected regions of the Fas L (-406 to −105) and exon 9 of Fas (+10 to +287, +676-70 to +882 and +831 to +1136). The known polymorphisms: G>A at position −1378 in Fas promoter; G>A at −1640, C>T at −1349, T>C at +29, G>C at +74 in TGF-beta1; G>C at −236 in IL-6; G>A at −488 and −418 in TNF-α (according to HUGO nomenclature) were observed in patients as well as controls. Novel variations, T>G at +29 position in two and insertion 36-37Ins(CTG)2 in one individual, were also observed.

The genotyping and the statistical analysis revealed that high Fas producing genotype G/G and intermediate TGF-beta1 producing genotype T/C were associated with protection and low Fas
producing genotype G/A and high TGF-beta1 producing genotype C/C were associated with risk to hepatitis. Since, Fas has a role in cell lysis mediated control of virus and TGF-beta1 has a role in immunosuppression, it is logical to associate high Fas producing genotypes with protection and the high TGF-beta1 producing genotypes with risk to the disease. Further, it was interesting to associate high Fas producing genotype G/G and intermediate TGF-beta1 producing genotype T/C with the retention (non-clearance) of HBeAg and the clearance of HBsAg. This indicated a role of some other interacting factors in determining the outcome of the disease.

In hepatitis B, a complex infectious disease one or more genes acting alone or in concert may increase or decrease the risk. Keeping this in mind, this study also investigated the influence of gene-gene interactions on the risk of hepatitis and inhibition of viral clearance using logistic regression analysis to implicate a number of genes as risk factors for hepatitis. An analysis of the risk for the disease with high Fas producer G/G genotype in the background of low producer T/T genotype of TGF-beta1 and G/G genotype at -236 position in IL-6 showed an increase in risk when compared to the Fas genotype background of G/G alone. This probably suggests an antagonistic role of Fas and TGF-beta1 and IL-6 as also observed in the in vitro studies where TGF-beta1 and IL-6 have been shown to inhibit Fas mediated apoptosis.

The high Fas producing genotype -1378 G/G and low TNF producing genotype -418 G/G apparently interacted to favour complete viral clearance; whereas, on the other hand, the high Fas producing genotype -1378 G/G and high TNF-alpha producing genotype -418 G/A apparently interacted to provide protection to hepatitis suggesting the possible activation of both cytolytic and non cytolytic pathways in providing protection against hepatitis. It is hypothesized that Fas, which is responsible for cytolytic killing of viral-infected cells and TNF-α, which is responsible for non-cytolytic removal of the virus, may act antagonistically or synergistically depending on the presence of other interacting genetic backgrounds and factors.

The carriers of high Fas producing genotype -1378 G/G (OR=0.61) and a combination of Fas G/G and low TGF-beta1 producing genotype +29 T/T (Fas G/G-TGF T/T, bicarriers) apparently provided protection against hepatitis (OR=0.51) when compared to the carriers with TGF T/T, showing no significant association. Interestingly, carriers of Fas G/G, TGF T/T and low IL-6
expressing genotype G/G showed greater protection (OR=0.39) which decreased marginally on addition of low TNF expressing genotype -418 G/G (OR=0.41). This observation supports the fact that Fas-mediated apoptosis and probably the viral removal is inhibited by the suppressive properties of the TGF-beta1 and IL-6 and enhanced by TNF-alpha. Thus, individuals with the combination of high Fas, low TGF-beta1 and low IL-6 producing genotypes are protected from hepatitis. These observations found further support in the analysis of the carriers of a combination of high TGF-beta1 producer genotype +29 C/C, low producer genotypes of IL-6 -236 G/G, TNF -488 G/G and Fas -1378 G/A gave a very high risk to hepatitis (OR=4.97). This observation suggested that the presence of low producer genotype of Fas, IL-6, TNF-alpha and a high producer genotype of TGF-beta1 could generate an immunocompromised state and a risk to suffer from hepatitis. It was interesting to observe that IL-6 G/G genotype behaved differently in the presence of other genotypes as it could be associated with the risk or protection depending on the background genotypes of Fas, TGF-beta and TNF-alpha, which supports the reported observation that IL-6 enhances as well as suppresses the immune response.

The genotype background of -1378 G/A of Fas in combination with TNF -418 G/A and IL-6 G/G gave a very high risk to hepatitis (OR=6.6). For all combinations and single genotypes studied, this was the one of the two highest risk genotype combinations, the other one being the combination of TGF-beta1 +29 C/C and TNF-418 G/A. The low Fas levels generated by Fas G/A in the presence of high TNF and low IL-6 levels is again expected to provide risk because of the suppressed immune responses. The high TGF producer genotype C/C in combination with high TNF producing genotype G/A was also found to give a very high risk, probably due to high immunosuppressive milieu generated by high TGF and TNF producing genotypes. The analysis of genotype interactions indicated that the contradictory observations made for isolated single genotypes in association with the disease could be resolved depending on the number of potentially interacting genes analysed.

The important observation of this study was that some genotypes like low Fas producing -1378 G/A and high TGF-beta producing +29 C/C genotypes always associated with risk; whereas high Fas producing genotype G/G associated with the protection to hepatitis. Also, the same genotype of IL-6 and TNF-alpha were associated with the risk or protection depending on the background of Fas and TGF-beta1 genotype background at the studied positions. A maximum
risk for HBeAg retention was provided by a combination of high Fas expressing genotype – 1378 G/G, an intermediate TGF expressing genotype +29 T/C and low expressing genotype of TNF –418 G/G (OR=4.2) suggesting the influence other genotype backgrounds could have on the cytolytic activity of Fas. Apparently the intermediate TGF-beta levels are sufficient to suppress Fas activity of apoptosis and thereby the retention of virus, which is further strengthened by low TNF levels. It is difficult to say whether the effect of the combination: Fas G/G-TGF T/C-TNF –418 G/G, seen here was due to Fas, TGF or TNF independently or due to a synergistic effect of all these.

For HBsAg clearance, a combination of Fas genotype G/G, TNF-418 G/G and IL-6 G/G apparently favoured HBsAg clearance (OR=0.18). This association is in agreement with the synergistic roles of high Fas, low TNF and low IL-6 levels in immune-system mediated clearance of the virus. Since Fas is involved in CTL mediated killing of the target cells and TNF and IL-6 may be involved in immunosuppression, association of high Fas and low TNF and IL-6 producing genotypes with HBsAg clearance can be explained. This genotype background for the candidate genes suggests that the cytolytic activity of Fas through CTL mediated killing of infected cells and the clearance of HBsAg is more effective in reduced TNF and IL-6 background.

An association of polymorphisms in TGF-β1 with clinical parameters showed an increase in the frequency of low TGF-beta1 producing genotype T/T at position –1349 and intermediate TGF-beta1 producing genotype T/C at +29 in patients with decrease in liver span. Thus, it can be hypothesized that the polymorphisms in TGF-β1 could be important determinants of a decrease in liver span. Association of high producing genotypes of TGF-β1 with increase in frequency of symptoms like abdominal discomfort, fever, purpurae, kidney dysfunction and raised levels of ALP and globulin suggested a pathological role of high expressing genotypes of TGF-β1 in hepatitis patients. Similarly, high producer IL-6 genotypes G/C and C/C were found to be associated with raised levels of SGOT, SGPT and ALP in hepatitis patients. This suggested a role of increased IL-6 levels in liver pathogenesis, suppression of Fas/FasL mediated apoptosis and inflammation of the liver. The frequency of individuals with high TNF-alpha producing genotypes at position –488 was found to be higher in patients with an increase in liver span. Since, TNF-α is a known positive regulator of liver regeneration, it is logical to conclude that
TNF-α polymorphisms, which might influence its expression may influence change in liver span during hepatitis.

In conclusion, the present findings provide an insight into the complex genetic interactions, which play a role in influencing the differential susceptibility to hepatitis or the disease progression in HBV infection. The study also highlights that the isolated assessment of the genotypes of single candidate genes in association studies conducted in hepatitis B could be misleading, since the same genotype which independently acts as a protector could turn out to be a risk factor if analysed in the background of another interacting genotypes. Nevertheless, genotype backgrounds of the candidate genes such as TGF-beta1 apparently seem to be less influenced by the other genotypes, which are confounding for other candidate genes. It is suggested that in future the studies should be designed to include a large number of potentially interacting genes and their genotypes to understand the complex disease processes in complex system such as humans.