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Hepatitis B virus core promoter and precore mutations and their relatedness to genotypes and disease pathogenesis

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ABSTRACT

Despite the successful vaccination programme worldwide, Hepatitis B virus infection remains a global problem. Infection rate in developing countries is higher in comparison to the developed countries. Core promoter region (nts.1591-1822) plays a very important role in HBV replication, morphogenesis and pathogenesis, whereas the precore region (nts.1814 to 1901) contains the start site for synthesis of pregenomic RNA, direct repeat 1 (DR1), and RNA encapsidation signal (ε) region. Mutations in both core promoter and precore regions seem to represent desperate attempts by the virus to escape from host immune surveillance which results in the evolution of new mutated strain which may have altered pathogenicity. It has been demonstrated by many studies that core promoter and precore regions of HBV have direct role in disease pathogenesis. So, in the present review we try to conclude the observations by different groups from all over the world with respect to the influence of core promoter and precore mutations on the HBV-induced disease progression.

Key words: Hepatitis B virus, Core promoter, Precore, mutation

Abbreviations: *AST: Aspartate Transaminase; ALT: Alanine Transaminase; ASC: Asymptomatic carriers; BCP: Basal core promoter region; CH: Chronic hepatitis; C/EBP:CCAAT/enhancer binding protein; DR: Direct repeat; FH: Fulminant hepatitis; HRE: Hepatocyte receptor element; HBcAg: Hepatitis B core antigen; HBsAg: Hepatitis B surface antigen; HBeAg: hepatitis B envelope antigen; HCC: Hepatocellular carcinoma; IFN: Interferon; LEF: Liver enriched factors.*

INTRODUCTION

Hepatitis B virus (HBV), the smallest DNA virus (genome size 3.2 kb) of *hepadna viridae* family of viruses, has infected one-fourth of the world population, 400 million of whom are chronic carriers of the virus[1, 2]. HBV is a non cytopathic, organ specific (hepatotropic),partially double stranded DNA virus which is responsible for nearly 1.2 million death worldwide through uncompensated liver disease or hepatocellular carcinoma each year, making HCC third most deadly cancer worldwide[1-4]. Infection with HBV is globally distributed, however, as compared to Europe and North America, the prevalence of HBV infection in Asia is quite high. India has 15% of total pool of world HBV infection and is responsible for 80-90% of HCC cases [5-7]. HBV is divided into 10 genotypes, from A-J, which is based on an inter-group divergence of 8% or greater in the complete genome nucleotide sequence, or a 4% or greater divergence in the surface antigen gene [1, 2, 8]. It has been shown by worldwide studies that different spectrums of HBV-mediated liver disease depend on the interaction between the host immunological system, viral replication, and genetic heterogeneity of the virus including genotypes and mutations.

MATERIALS AND METHODS

PubMed(www.ncbi.nlm.nih.gov) was searched with keywords like “HBV”, “Hepatitis B virus”, “Hepatitis B virus core promoter mutations”, “Hepatitis B virus precore mutations” and related words, to finalize this review article. One hundred and fifteen research and review articles were selected. The selection criteria, of these research and review articles, were to include the data which represent the global burden of this infection and related disease.

RESULTS AND DISCUSSION

HBV and core promoter/precore mutations

Mutations in HBV genome results in severe health consequences which include appearance of vaccine/drug resistant strains and strains with altered pathogenicity or tissue specificity which may increase the viral persistence which further leads to the hepatocellular carcinoma- the deadly final stage of infection [8]. In a HBV chronic patient up to 10^{13} virions are produced per day. This high replication capacity fuelled by high error rate of viral polymerase resulted in HBV genomes with nearly all possible mutations [9, 10]. Selection pressure by the immune system results in survival of the dominant strains. These dominant strains may be related to increased severity of HBV-related disease. These variants of HBV with point mutations, deletions, insertion or duplication have been described all over the world with their corresponding disease status.

Core promoter mutations

Core promoter (CP) region(nt. 1591-1822) has direct effect on HBV life cycle and pathogenesis. Mutations in this region are related with different clinical outcomes. It has been demonstrated that CP mutations increased the viral replication mainly by two ways: First, they cause a shift change in secondary structure of viral pregenome which leads to enhanced viral replication, and secondly, they create a binding site for HNF which results in increased transcription of pregenomic RNA [11]. Core promoter region (nts.1591-1822) plays a very important role in HBV replication, morphogenesis and pathogenesis. It directs the transcription of both pregenomic RNA (pgRNA) and precore mRNA (PreC mRNA). Pregenomic RNA (pgRNA) is translated into core protein and polymerase enzyme and also serves as a template forreverse transcription. PreC mRNA is translated and modified post translationally to produce hepatitis B virus ‘e’ antigen i.e. HBeAg. It is believed that HBeAg has direct involvement in the induction of immune tolerance. So, any mutation in this region will directly affect the HBV related disease outcomes and this is proved by many studies throughout the world.

1762/1764

The most frequent CP mutation is the double mutation at nt position 1762(A→T) and 1764 (G→A). Both of these nucleotide positions are present within the C/EBP& DR-1 HRE binding region. These mutations decrease the affinity of liver enriched factors (LEF) to this region which results in decrease HBeAg expression [12-14]. In transfection studies it has been shown that presence of this double mutation reduces the levels of preC mRNA and HBeAg secretion, whereas the pgRNA synthesis remains unaffected [13-15]. It has been shown further that presence of this double mutation increases the length of initiation binding site of preC mRNA from nts 1758-1800, whereas in wild type initiation binding site ranges from nts 1786-1800 [16].This double mutation suppresses HBeAg synthesis and hence contributes to hepatocarcinogenesis. There are two favorable explanations for this phenomenon: Firstly, due to presence of identical epitopes of HBeAg and HBcAg, immune-tolerance is induced against both the antigens [17], but HBcAg triggers a more vigorous antibody response in comparison to HBeAg [18]. So, in the presence or absence of reduced serum HBeAg, HBcAg remains the main target for both cellular and humoral immune systems which lead to damage of hepatocytes and ultimately hepatocarcinogenesis [19, 20]. Secondly, immune system directly or indirectly triggers liver damage in response to increase viral load. This damage increase hepatocyte turnover and fibrosis which increases the chance of HCC which is further fuelled by the high regeneration capacity of hepatocytes [21, 22].

1762(T)& 1764(A) mutations convert a nuclear receptor binding site to an HNF1 site [23]. It has been shown that HNF-1 suppresses the transcription of precore RNA, whereas increase the transcription of core-RNA[15, 22]. Additionally, because HBeAg has inhibitory effect on HBV replication [14, 24],the reduced HBeAg expression could be a reason for increased HBV replication. InpgRNA, there is present a secondary structure between nt. 1742-1847 [25, 26]. After consideration of RNA secondary structure, it has been suggested that mutations in the region between 1751-1755 and 1762-1764 have importance at the level of reverse transcription. According to this model, the wild type AGG motif stabilizes DR1 but mutations of AGG into UGA, AGA or AGU creates a break to reverse

transcription by providing 4 primer binding nucleotides in DR1 rather than 3. Similarly, the core promoter deletions may have similar effects i.e they reduced the amount of base pairing in the stem which can increase the rate of reverse transcription [26]. The A1762T and G1764A mutations results in a lysine-to-methionine and valine-to-isoleucine mutation respectively, within a kunitz domain like sequence spanning the (130-**KVFVKGGCRHKLV**-142) HBV X gene [27]. This region has transactivating function and found to interact with proteasomes machinery. Alterations within this important domain may alter the transactivation and protease activity of HBV X-protein-a transactivator for many cellular and viral genes, which is necessary for establishment of a successful HBV infection [28].

This double mutation has been found to be highly prevalent in liver tumour tissues and sera of HCC patients [29, 30]. This double mutation is also reported in FH patients but less often in asymptomatic carriers [14, 31]. The frequency of this double mutation was found to be increased with progressive clinical stages (3%, 32% and 64% in ASCs, CH and HCC group respectively) in patient from Taiwan [32]. Two major reports from China, showed that nearly all HCC patients possessed this double mutation in both serum and tumor tissues [33]and that a presence of this double mutation increased with the advanced clinical status (33%, 56% and 85% in CH, LC and HCC groups respectively) in their patients [34]. In African people, this double mutation is observed more often with HCC patients (66%) compared to asymptomatic (11%) patients [29].In contrast to these findings, a Indonesian study showed that although this double mutation increased from CH (19.7%) to LC (59.7%) patients, but there is a slightly decrease of the double mutation in HCC group in comparison to LC group (54.2% vs. 59.7% respectively) in their study [35].

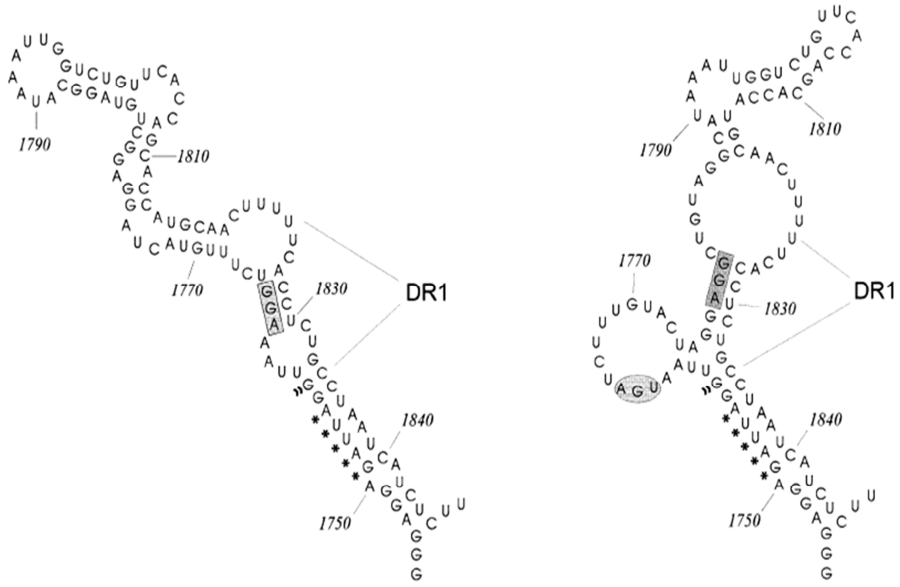

Figure 1 MFOLD predicted structure of folded part of pregenomic RNA (nt 1742 to 1847). This figure is based upon a consensus sequence for genotype B and C strains. (A) Nucleotide region 1762-1764 (AGG) is shown (with a shaded box) to base pair with the centre of DR1. (B) Mutation of positions 1762 to 1764 to either UGA (shaded eclipse) or to AGA/AGU effect structural rearrangement such that region 1778-1780 (AGG) (shown with a shaded box) would base-pair with the centre of DR1 (Adapted from Kidd & Kidd-Ljunggren, 1997)

Further, many studies showed that this double mutation observed higher in genotype C than genotype B [32, 34, 35] and genotype D [36]. Further, it has also been demonstrated that altered HBV genome due to the BCP mutations within the region 1753-1766 are important factors that determined IFN-therapy response [37]. This double mutation has been observed less frequently in genotype D in comparison to genotype C in HBeAg negative patients [38]. Low frequency of this double mutation in genotype D is reported in various studies from Iran, USA and India [39-41].Contradictory to these studies, one of the study from India observed the 1762/1764 mutations significantly more often with genotype D in comparison to genotype A and also it has been observed in one-third of HBV positive patients, but correlation of these mutations with severity of liver disease was not observed [42]. The presence of the sequence AGG (1762-1764) is found to be significantly associated ($P< 0.0001$) with HBeAg positivity[26].One

isolate from a study has been found to have both HBeAg positivity and 1762 (T) and 1764(A) mutation. This phenomenon could be explained by the fact that occurrence of this double mutation decreased the production of HBeAg, but did not totally abolish it [43].

Impact of single 1762(T) or 1764(A) mutation has also been demonstrated in various studies [44]. 1762(T) has been observed to have capacity to prevent binding of LEF to the BCP region which results in suppression of pre-C mRNA. In contrast, 1764(A) alone has some binding affinity for LEF, hence does not suppress RNA transcription of virus and found to only slightly decrease the efficiency of HBV replication [13, 14]. In a study comprising 1526 patients, some rare mutations were also observed in this region (1762/1764=A/A, A/G or A, A/T, A or T/G, G/A, T/G, A or T/C, G/G or A), but could not be correlated with disease status[45].

1753

1753V (V represent C/A/G) mutation among the HBeAg +ve patients has been observed as a predictive factor for hepatocarcinogenesis[46, 47]. Two Japanese studies showed that prevalence of T1753V mutation increased significantly with advancement of liver disease and has been found higher in HCC group (53.2%) compared either with LC (18.8%) or CH (9.8%) patients [34, 48].An Indonesian study demonstrated that a T1753V mutation found more often in LC (46.8%) patients in comparison to HCC (22.9%) and CH (18.0%) patients, which suggest the tight association with LC patients in comparison to HCC [35]. In genotype B of HBV, it has been demonstrated that 1753(C) is directly involved in increased transactivating and anti-proliferative activity of HBV-X protein and this may be responsible for hepatocarcinogenesis *via* integration of HBV genes into host genomes [49, 50]. Further, an*in vitro* study demonstrated a higher replication capacity of viral strains having triple mutation (C1753/T1762/A1764) than individual mutations [51, 52]. These mutations are found to be responsible for increased HBcAg expression and decreased level of HBeAg expression [53-55]. Enhancement of HBV replication due to this triple mutation leads to increased chances of HBV integration into the human host genome, which further accelerates the process of hepatocarcinogenesis [50].These findings are supported by two studies. In those studies, it has been proposed that a secondary structure (1742-1847) in pgRNA have presence of a stable stem hairpin structure comprising nts from 1751-1757 and any mutation in this stem structure would destroy the stability which results in easily opening of stem for reverse transcription and thus enhancement of viral replication [25, 26].

Further, T1753V mutation is reported more often in genotype C and HBeAg +ve patients [32, 34, 35].But contradictory to this, in an another study 1753(C) has been found to be associated with HBeAg negative patients [56]. It is observed that 1757 (A) mutation found mainly in strains having genotype D and T1764/G1766 double mutation [26, 47], whereas 1757(G) found significantly with T1762/A1764 double mutation. 1757(A) mutation has the ability to constrain the binding site for HNF-1 which is maintained by the T1762/A1764 double mutation[40]. A study from eastern India showed that 1757(A) mutation is not specific to genotype D in their study [57].

1809-1812

Translation of a gene by eukaryotic ribosome would be affected, if there are present mutations in the nucleotide sequence immediately preceding that gene [58]. HBV precore region is preceded by such kozak sequence containing important nt position from 1809-to-1812. So, mutations within this region may results in decreased HBeAg expression [59]. These mutations are observed in CH patients [60]. Presence of 'T' at 1809 changes amino acid alanine-to-serine and presence of T at 1812 changes proline-to- serine amino acid and results in replacement of hydrophobic aminoacids with smaller polar amino acids. These mutations are found very commonly (80%) in South African black people [14]. These mutations have role in disruption of base pairing within the secondary structure of pgRNA, but no effect on viral replication [25]. It has already reported that these mutations may change the required optimal sequence for initiation of translation of HBeAg protein by eukaryotic ribosome which results in decrease HBeAg expression [58] and leads to more vigorous response by the immune system. 1809(T), 1810(T), 1811(C) and 1812(T) mutations are found to be present in HBV acute cases[61] and also reported from fulminant hepatitis patients [62]. These mutations have been found to be more frequently associated with genotype A than genotype D. Double or triple mutations at 1809-1812 are observed mainly in subgenotype A1 and not in A2 or other genotypes/subgenotypes. These mutations directly affect the HBeAg expression due to suboptimal initiation of translation. Experimentally, it has been shown that HBeAg expression was adversely affected by the 1809(T)+1811(T)+1812(T) and 1809(T)+1811(C)+1812(T) triple mutations in comparison to the 1809(T)+1812(T) and 1809(A)+1812(T) double mutations. The effect of these double mutations on expression of HBeAg hasfound to be comparable with that of the (1762T/1764A) mutations [59]. These mutations are also described in isolates from other regions of world [60, 62, 63].

1687-1703
Nucleotide region from 1687 to 1703 constitute the δ-box of core upstream regulatory region (CURS), which positively regulate the BCP activity [12, 64-66].

Precore mutations
The precore region (nts.1814 to 1901) contains the start site for synthesis of pregenomic RNA, direct repeat 1 (DR1), and RNA encapsidation signal (ε) region. As for the clinical significance, there is a contradiction in literature regarding the influence of precore mutations on disease outcomes. Some studies revealed that there are no differences in the severity or clinical outcomes of liver disease in term of hepatitis exacerbation, cirrhosis related complications and HCC between patients with precore wild type or with precore mutant [11, 67], while others demonstrate a clear influence of precore mutations on disease outcomes [21, 68-70].These precore mutated HBV strains appear during HBeAg seroconversion and may influence the synthesis of HBeAg synthesis.

1896
One of the most well studied precore mutations is a nonsense G-to-A substitution at nt position 1896.This mutation prevents the HBeAg production due to appearance of a premature stop codon at codon 28 (Tryptophan→Stop) into the open reading frame of the preC region. This results in production of 28 aminoacid truncated precore protein which abolishes the production of complete HBeAg. Because the precore region is neither essential for HBV replication nor for HBcAg expression, the G1896A variant is replication-competent and continues to synthesize viral DNA at sufficient levels to cause progressive liver damage [70]. This non sense mutation is detected in both HBeAg +/- patients from various part of the world but significantly restricted to individuals who are HBeAg negative, HBsAg positive, anti-HBe positive, HBV DNA positive and having elevated serum ALT levels. However, the precore variant is not uniformly pathogenic, and thus, co-mutations or host factors presumably explain the more virulent forms of precore mutant-associated disease[21, 71].1896(A) mutation does not have a prognostic role in predicting progress towards liver disease in eastern part of India [41], but in Brazilian patients this mutation has found to be associated with more severe liver damage as compared to wild one [72]. HCC patients have been found to have significantly higher frequency of 1896(A) mutation [30]. In contrast to these observations, this mutation has been reported equally in both HCC and inactive carriers [73]. Some studies emphasize the role of this mutation in inactivation of chronic liver disease[32, 74]. This precore mutant variant has been found to be associated with a higher incidence of fulminant and subfulminanthepatitis [72, 75]and in acute cases [32, 76, 77].

This mutation may involve in the loss of **'e'** antigen which may help the virus to evade the immune system as HBeAg is main target for both the cellular and humoral immune response [4].In clinical practice, such a mutant is selected during seroconversion from HBeAg to anti-HBeAg and is responsible for HBeAg negative strain in chronic hepatitis B patients [9, 78, 79].So, many author's agree on the point that G1896A mutated strain may represent the fittest strain acclimatize after struggle with host immune selection [80]and may have role in persistence viral replication and the hepatic necroinflammatory activity [78].

The G1896A mutation has been observed more frequently in genotype D than genotype A and there is found no difference in ALT or viral DNA levels between patients infected with this mutation or with the wild type one [72].The 1896 precore mutant has been detected more often in genotype D than in genotype A in south Indian study ($p<0.005$) [81].In agreement with the previous report from eastern part of India which demonstrated that G1896A mutation was found to be less frequent in Indian patient [41], a north Indian study also found less frequency (4.8%) of this mutation in their chronic isolates [3].

This mutation is found frequently among patients infected with HBV genotype B, C, D and E because they have 1858(T) which stabilize the hairpin structure and this is the reason why this mutation is not found significantly in genotype A[82]. This mutation is more prevalent in Asian and the Mediterranean area where genotypes B, C, and Dare predominant, whereas less prevalent in Europe and North America, where genotype A is more common. Presence of precore A1896 strain and HBeAg positivity could be due to the mixed infection of both HBeAg secreting and non-secreting strain with 1896(A). Other explanation comes after studying the full process of seroconversion on some patients in a study and it is demonstrated that process of overtaking the wild type strain by 1896(A) strain may happen earlier than the seroconversion to HBeAg negative/anti-HBV positivity [83].

1858

1858(C) mutation has mainly observed in chronic patients with high level of ALT/AST [56]. Genotype A, some strains of genotype F and genotype C found to possess 'C' at position 1858. This would lead to loss of base pairing and stem stabilization if the G-to-A mutation occurs at position 1896 [84]. So, to avoid the stem disruption in these cases it is speculated that 1858 (C) changes to a U, followed by the 1896 G-to-A mutation, thus protecting the base pairing of the pgRNA stem structure [83]. Hence, Presence of Cat nt. 1858 which prohibits the G→A point mutation at nucleotide 1896 for creating a stop codon in the preC region is found exclusively in genotype A [1]. 1858(T) was found mainly in genotype B, C, and D [85]. Studies revealed that 1858(C) strains developed core promoter mutations more frequently, which have been observed with more severe liver disease [86]. In Asian, African and middle eastern isolates C is present at nucleotide 1858, while North American and European isolates have 'T' at nt. 1858 [78].

1862

1862 position is the most affected position by missense mutations. This G→T transversion mutation is found both in HBeAg +/-ve patients. This mutation is frequently found in HCC patients having genotype A and HBeAg negative marker [21]. This mutation affects codon 17 where aminoacid valine is replaced by phenylalanine. This change affects the signal peptide cleavage site (between residue 19 & 20)and results in non-removal of the first 19 aminoacids of the precore/core protein during its processing into HBeAg protein [87]. This transversion mutation at 1862 may affect this site in such a way that signalase cannot act properly due to which HBeAg is not expressed. Hou*et. al.*[88] confirms the role of this mutation in decrease production of HBeAg under *in vitro* transfection study. G-to-A transition mutation at nt. 1862 which converts amino acid valine to isoleucine is found in a tumorous tissue, but is absent in non-tumorous tissues. Beside this mutation, some HCC patients also shows a G-to-C transversion which converts amino acid valine to Leucine, and they are equally distributed in both HBeAg positive and negative HCC [21. The conversion of G→A/C/T does not influence the encapsidation of pgRNA[89], but found to affect the HBV replication capacity [90].

The missense mutation 1862(G→T) in the precore region has been described in patients with chronic hepatitis [21, 60]and in patients with fulminant hepatitis [88]. Mutations G→A/C at nucleotide position 1862 is observed in genotype/subgenotype A1 [91]. In east India, G1862T mutation was predominantly found in genotype/subgenotype A1, irrespective of HBeAg status. Further, in the same study, this mutation could not be correlated to the clinical outcome [92].In genotype 'A' a rarest mutation 1862(C) was found which was not described in any genotype from A to H [93]. 1862(G) was described as a marker for genotype/subgenotype A2 whereas genotype/subgenotype A1 had 'T' at position 1862 [91].

1888

1888(A) mutation has been observed in three out of four HCC patients from South Africa [21]. This mutation has found to be very prevalent in black Africans HCC patients. The G to A mutation would stabilize the stem structure of the HBV pgRNA[21, 94].Presence of 'A' at nt. 1888 results in a start codon upstream from the core ORF which directly affects the core protein expression and hence decrease the viral load [95]. It is speculated that this mutation stabilize encapsidation signal [96] and hence directly affect the reverse transcription and finally affect the translation of the core protein. It is demonstrated that G→A mutation introduce an out-of-frame start codon that can be potentially translated into a stretch of seven amino acids: "Met-Ala-Leu-Gly-His-Gly-His'. This new start codon may have an important role in the regulation of the translational efficiency of the proceeding start codon [95]. G→A/C/T at 1888 occurred frequently in genotype/subgenotype A1. 1888(G) has been described as a marker for genotype/subgenotype A2 and 1888(A) as a marker for genotype/subgenotype A1[91].

1915

Mutations at nucleotide position 1915 and its relatedness to disease severity and genotype has been described only in few studies so far. 1915(G) has been described in untreated (naïve) patients [97]. A north Indian study demonstrates the prevalence of 1915(A) mutation in chronic symptomatic carrier group, whereas in asymptomatic carriers'T' at 1915 has been observed significantly. 1915 (G) has been reported significantly in genotype A than genotype D in the same study [3].

1802/1803
Initially, 1802(C)& 1803(G) mutations are considered as the mutation of occult infection [98], but now, these are considered as a characteristic of genotype A, E and D, whereas 1802(T) &1803(T) are characteristic of genotypes F, B and C[1, 3, 6, 7].

Deletion/insertion/duplication
Nucleotide region from 1748-1777 of core promoter region is found to be hot spot for deletion in literature. At least 13 types of deletions are defined in literature and one study defines 7 cases of deletion in fulminant hepatitis patients [26, 77]. Many small regions in core promoter have been shown to be favourite site for deletion mutations. They are between nucleotide: 1757-1776, 1758-1777, 1768-1775, 1757-1779, 1760-1763, 1746-1768, 1763-1770, 1765-72, 1763-1770, 1770-1776, 1725-1749, 1753, 1746, 1763-1770, 1753 and 1756 [14, 70]. Most of the patients with advanced liver disease have deletions or insertion or both in CP region and mostly in BCP region [53, 97].The most severe effect was seen if the deletion is present in TA1 and TA2 region and therefore these deletions affect the activity of BCP and there is reduced production of preC mRNA and increased production of pgRNA in medium [16]. Deletion size varies from 8 nucleotides to 21 nucleotides in core promoter region [14]. Deletion mutations were reported in ASCs [99], HBeAg seropositive patients [43]and in HCC patients [21]. It has been proposed that deletions within CP influence the function and expression of X protein by truncating the X protein or by changing its frame [26]. This further result in partial or total removal of the kunitz-type serine protease inhibitor domain, which is very essential for transactivating property of X protein and this may be the reason why deletion is sometimes seen in ASCs patients having low viral load and in patients having no serological markers [100].

Nucleotide deletions and insertions in CP regions were observed during lamivudine treatment. Deletion of 20 base pairs nucleotide (1756-1775) in the CP region has been detected in a patient after 1 year lamivudine therapy. Deletion of nt. region 1758-1777 is also observed after 1 year therapy in chronic patients [101]. Literature has contradiction regarding the lamivudine/Interferon therapy and its response to mutants having deletion or insertion. One study found no correlation of disease status and response to lamivudine therapy and mutants with deletions or insertions [70], while two studies showed that patients with deletion mutations in BCP responds better to IFN-therapy than patients having wild type strain[37]. An 8 bp deletion in genotype D in various regions of core promoter region has also been reported from Pakistan [103]. 42 to 72 nucleotide deletion has been observed in precore region of South African HCC patients [21]. Deletion in precore region may affect the integrity of DR1 and epsilon signal and also produce truncated HBx protein.

CH patients showed insertion in their CP region in some cases [21, 102, 103].Insertions of 11 bp are observed in a fulminant hepatitis (FH) patient and in that patient it was observed that insertion of 11 bp created a binding site of HNF1 and which resulted in exacerbation of HBV infection [104]. Insertion has been also found to be present in patients having advanced liver disease. Insertion of ‘TA’ at 1765, ‘GTT’ at 1768, 15 nt insertion at 1775 are also observed [53]. Insertion of TA at 1764 and 1765 are observed in chronic patients [70]. In an HBeAg positive, duplication of sequence between nts 1641-1666 has been observed [53]. The region between nts 1641-1666 covers the α-box of ENII and CURS to which HNF4 and C/EBP transcriptional factors bind, so it is natural to be the important region of CP activity [14]. This duplication increased the level of preC mRNA, HBeAg, core protein, polymerase enzymes and decrease the level of HBsAg and preS proteins [53]. Duplication of sequence between 1723-1789, 1720-1766, 1641-1668, 1660-1666, 1648-1663, 1644 -1674, 1649-1667, 1635-1644 and 1641-1648 has also been reported but their biological significance are still unknown [53, 105]. One isolate (chronic symptomatic with high ALT/AST) from a north Indian study has been found with a duplication of 54 nucleotides (region between nt. 1628 to 1681) at position 1661 [3]. Deletions/insertions in the X gene/core promoter region has been elucidated in cell culture but the functional importance of these mutations does not appear to be predictable [53, 104]. Deletions/insertions in the core promoter are even found to disappear upon seroconversion to anti-HBe in immunocompetent patients, but selected under immunosuppressive conditions [53, 102, 104]. Literature around the world showed that deletions, duplication, and insertions are being observed in all category of patients like asymptomatic and symptomatic chronic carriers [16, 43, 48, 53, 102], fulminant hepatitis and HCC [102, 106-108].

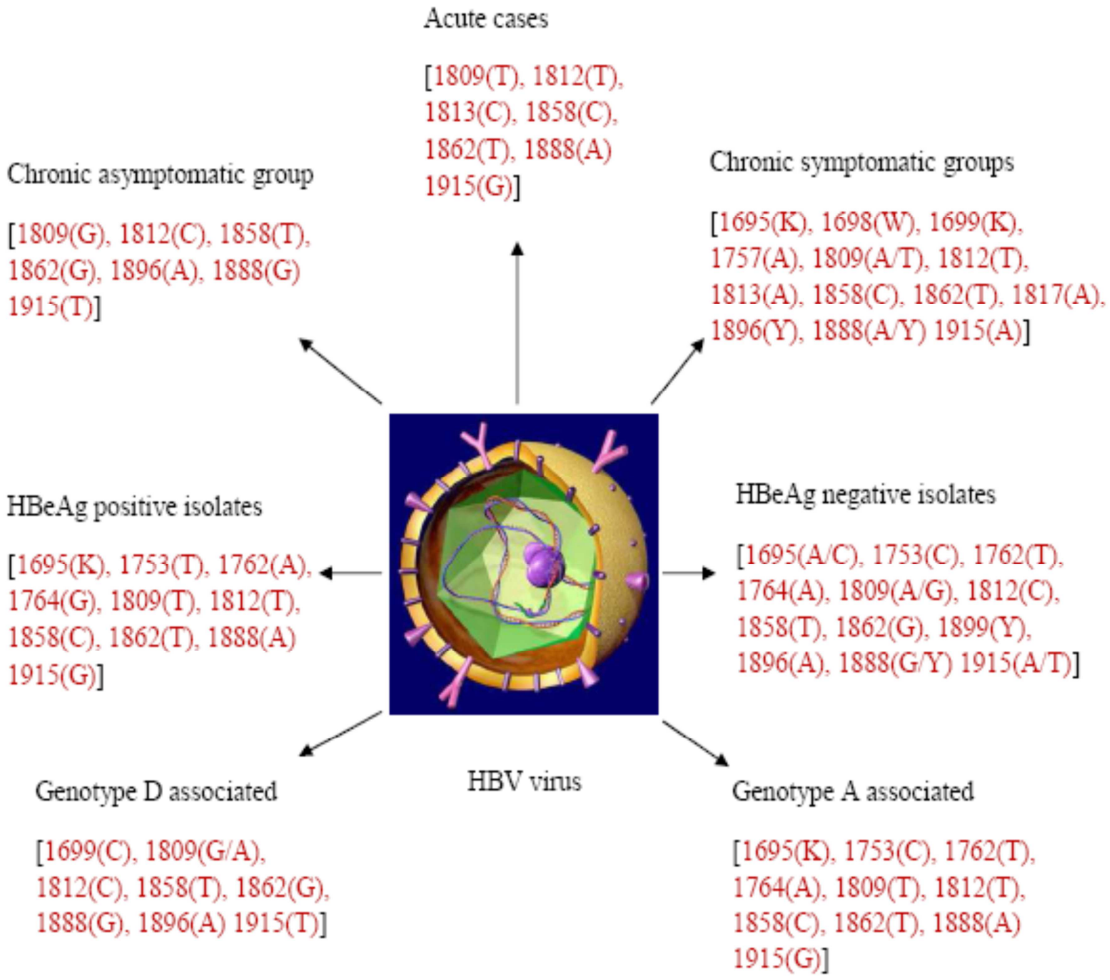

Figure 2 Diagrammatic presentation of association of different set of mutations to a particular HBV infection stage or to a particular HBV genotype, based on the worldwide data

Rare mutations

1766(T) and 1768(A) mutations have been found to be associated with fulminant hepatitis [109].It is observed that 1766(T)1768(A) mutations increased the BCP activity by creating two overlapping low affinity HNF1 sites, which resulted in increase in pgRNA transcription and also enhancement of viral encapsidation. These mutations are observed in isolates showing symptoms of exacerbation of HBV infections. Isolates from Israel with fulminant hepatitis also showed presence of these mutations[14, 107, 109].Rare stop codon C1817T in the preC region has been observed in two studies [110]. A transition at nt. 1898 from G to A of precore region is exclusively found in HCC cells but not in sera. This mutation destabilizes the hairpin structure and therefore it is disadvantageous for viral replication [111]. 1899(A) mutation which changes amino acid Gln-to-Asp affect the precore codon 29 and observed in sera of HCC patients [21].1899(A) mutation has observed significantly in HBeAg negative patients, but with both asymptomatic and cirrhotic patient[112]. This mutation has been observed either alone or with G to T transversion at nt. 1862 or with stop codon at nucleotide position 1896 [21, 53, 113].The three nucleotides of the epsilon (**ε**) bulge (1863 to 1865) also served as a template for HBV –ve DNA strand synthesis and mutation in this bulge of the stem loop may interfere with viral DNA synthesis [78, 114].Nucleotides 1863 to 1865 acts as the priming site for reverse transcription. So, mutation like T1863A may produce viral DNA of reduced size due to translocation.

CONCLUSION

Hepatitis B virus diversifies its genetic information through mutations and recombination. This diversification and the competition to survive under host immune surveillance results in the evolution of dominant strains with specific mutations. This is the principle behind the arrival of the fittest strains of HBV under the host immune surveillance [1, 3, 6, 115].As described earlier, it has been demonstrated successfully that core promoter and precore mutated HBV strains have different pathogenicity, response to therapies and progression to more advanced liver diseases like HCC. At the same point, as discussed earlier [3, 6], it has also been shown that not only the mutated HBV strains but also the host immunogenes polymorphisms have their effect on HBV pathogenesis. This may be the region why some mutations are reported more pathogenic in some ethnic groups and less in others.

So, it is recommended that before drawing the conclusion about the pathogenic capacity of any mutation, the host immunogenes variants must be taken in to account. Secondly, larger studies at national and international level must be needed to integrate the viral and host genetic and clinical data for complete understanding of underlying principle of immunopathogenesis of different mutated HBV strains.

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