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Hepatitis B virus genotypes/subgenotypes: Epidemiology, pathogenicity and response to therapies

Vikas Pahal[*], Jasbir Singh[†] and Krishan Swaroop Dadhich[*]

[*]*Department of Microbiology, Dolphin (PG) College of Science and Agriculture, Chunnikalan, F. G. Sahib, Punjab, India*
[†]*Department of Biochemistry, Kurukshetra University, Kurukshetra, Haryana*

ABSTRACT

Hepatitis B virus has been divided into ten genotypes, A-J, and in several respective subgenotypes on the basis of phylogenetic analysis of whole genome. Different geographical region has been dominated by different genotypes. It has been proposed by many studies that different genotypes have been found to have different pathogenic behavior and respond differentially to different therapies. Similarly, several other studies correlate the differential clinical outcomes of HBV-induced liver disease with respect to genotypes. So, in the present review we try to club as maximum as possible information regarding the Hepatitis B virus genotypes/subgenotypes epidemiology, characteristic pathogenic behavior and response to various interferon's and nucleos(t)ides analogues.

Key words: Hepatitis B virus, Genotypes, Subgenotypes.

Abbreviations: *AST: Aspartate Transaminase; ALT: Alanine Transaminase; Bp: base pair; BQW: best quality water; CHB: chronic hepatitis B; ELISA: Enzyme linked immunosorbent assay; HBsAg: Hepatitis B surface antigen; HBeAg: hepatitis B envelope antigen; HBV: hepatitis B virus; HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma; HEV: Hepatitis E virus; HIV: Human immunodeficiency virus; Nt: Nucleotide; RFLP: Restriction fragment length polymorphism; SNP: Single nucleotide polymorphisms*

INTRODUCTION

Hepatitis B virus (HBV), a non-cytopathic & organ specific (hepatotropic) virus, is one of the five distinct families of viruses that cause different spectrum of liver disease (from self-limiting acute stage to hepatocellular carcinoma) in humans. Despite success with the development of vaccines and antiviral drugs [1], it remains a main cause of morbidity and mortality worldwide, particularly in Asia [2, 3]. It is estimated that nearly one-fourth of the world's population has serological evidence (HBsAg/anti-HBsAg marker) of past or present infection with this virus [4, 5] and out of which 400 million are chronic carriers of the virus [4, 6]. HBV infection is responsible for nearly 1.2 million deaths per year worldwide due to HBV-related liver disease (including HCC), making HCC third most deadly cancer worldwide [7, 8].

MATERIALS AND METHODS

To make this review article PubMed(www.ncbi.nlm.nih.gov) was searched with keywords like "HBV", "Hepatitis B virus", "Hepatitis B virus genotypes", "Hepatitis B virus subgenotypes", "Hepatitis B virus and response to therapy" and related words. One hundred and thirty-nine research and review articles were selected. While selecting these research and review articles, it was kept in mind that the data must represent the reports throughout the world.

RESULTS AND DISCUSSION

Epidemiology

HBV is distributed worldwide but its prevalence and patterns of transmission vary greatly in different geographic region of world and in different population subgroups [9]. Whole world is divided into three regions with respect to rate of prevalence of (HBsAg as a seromarker) HBV infection [10].

First region comprises of high endemicity region with equal to or more than 8% population having HBsAg positive infection marker. It has been estimated that nearly 45% of the total global pool of HBV-infected population lives in high endemicity region. This area includes most of the Asia, sub-Saharan Africa, Middle East region, South America's Amazon basin, the pacific islands, and other special population groups such as primitive tribes of Andaman and Nicobar islands of India (HBsAg positivity ranges from 11.6% to as high as 65% in Jarawa tribes), native Alaskans, Australian aborigines, and New Zealand's Maoris tribe. Second region is considered as the region of moderate endemicity in which 2% to 7% population has HBsAg infection marker. 43% of the global population lives in this area of moderate endemicity which includes, North Africa, some of the Middle East, the Southern parts of Eastern and central Europe, the USSR, Indian subcontinent and some part of Brazil. Third region is the region of low HBV endemicity in which HBsAg carrier rate is less than 2% which includes Northwest Europe, Australia, and North America [10, 11, 12, 13].

Transmission of HBV

Blood is the most infectious source of HBV infection but virus particle may be found in other various body fluids like saliva, semen, breast milk and menstrual fluid. The risk of chronicity due to HBV-infection is found to be inversely proportional to age at which the viral infection is acquired. For neonates and children less than 1 year the chances of chronicity has been observed upto 90%, for children aged between 1-5 years it is about 30% and for the children older than 5 years and for adults, the risk is nearly 2% [14, 15]. There are mainly two mode of HBV transmission: horizontally, the virus transmitted by exposure of percutaneous or permucosal area to the infectious blood or body fluids, and perinatally the transmission route is from an infected mother to her infant during the perinatal period [10, 16]. In high endemic regions as described earlier, the most common source of infection is through perinatal transmission or through infection acquired horizontally during early period of childhood. It has been estimated that the life time risk of HBV-infection in highly endemic areas are greater than 60% [10, 11]. In intermediate zone of HBV infection routes remain the same but nosocomial transmission, tattooing and body piercing transmission is also observed. The life time risk of HBV infection in these area remains between 20%-60%. Finally, in low endemic areas, beside the above mentioned routes of transmission, high risk sexual behaviors or intravenous drug users is the dominant route of HBV transmission. The life time risk of HBV infection in low endemic area of HBV infection is less than 20% [10, 11, 17].

HBV Polymorphism

HBV is a partially double stranded DNA virus having genome size of 3.2 kb- the smallest known DNA virus. It uses reverse transcriptase enzyme for replication which is an error generating process because reverse transcriptase lacks proof reading activity. So, sequence heterogeneity is one of the main features of HBV virus. HBV mutation rate has been estimated to be 1.4×10^{-5} to 5×10^{-5} substitutions/site/year which is equivalent to some retroviruses mutation rate but 100 folds lower than that of envelope gene of HIV [18]and 10^4 times greater than that of other DNA viruses [19]. In patients who are in immunocompromised state due to liver transplantation there is a further 100 times more mutation rate than normal patients [20]. Beside these mutations generating pathways, the selective pressure imposed by host immune system, vaccination and antiviral treatments can also be responsible for generation of mutated strains [21]. These mutations become clinically significant with time if they do not have any deleterious effect on various viral processes. In HBV infected individuals, presence of HBV quasispecies (mixture of HBV mutant species) can contribute to the selective advantage over a wide range of pressures as described earlier in this paper.

HBV genotypes/subgenotypes characteristics and geographical distributions

A complex interaction between viruses and host defense system resulted in the evolution of a new strain. Selection pressure by the host immune system results in the survival of only those strains which are replication competent, and hence stabilized them within the host and this constitute the genotype of a virus [20]. HBV is classified into ten genotypes (A-J) which are defined by an intergroup nucleotide divergence >7.5% in the complete genome or >4 % at the level of HBV surface antigen protein [21]. Subgenotypes are defined as the subgroups within the same

genotype having nucleotide divergence >4% and less than 7.5% over complete genome with high phylogenetic bootstrap support [22, 23].

Genotype A

Till date, genotype A family has been found to have six subgenotypes. Genotype A1 is found in South Africa, Malawi, Somalia, Brazil, Nepal, Yemen, India [5, 24-27]. With the exception of few A1 which were isolated from Philippians which were *ayw*, the majority of A1 belongs to *adw*2 serotype [25, 28]. Subgenotype A2 has been observed in Northern Europe, the U.S.A, Alaska and Greenland [22, 29-30]. Subgenotype A3 is found in West Africa [31].Subgenotypes A4 and A5 has been observed in Mali and Nigeria [32]and subgenotype A6 has been found to be circulating in African-Belgian patients [33]. The classical precore mutation, which consists of G to A substitution at 1896 which creates a stop codon is not found in genotype A. Presence of C at position 1858 which stabilize the precore loop is commonly found in genotype A. Carboxyl terminal of HBV core gene contains a 6 nucleotide insert in genotype A, and this insert was found to be absent in other genotypes [24].

Genotype B

Genotype B is divided into 7 subgenotypes from B1-B9 [34]. The subgenotype B1 is found in Japan and it is considered as a pure strain as there is no recombination in this subgenotype. Subgenotype B6 is a newly discovered subgenotype found in Alaska, Canada and Greenland [35]. Phylogenetically B6 is related to B1 and it is proposed that it might be removed from Japan many centuries ago [35]. The precore/core ORF of the subgenotypes B2-B5 have a genotype C recombinant region [26]. Subgenotype B2 is found mainly in China and is widely distributed in Asian countries while others are found restricted to a particular country. Subgenotype B3 is found mainly in Indonesia, subgenotype B4 in Vietnam and subgenotype B5 in Philippines. Subgenotype B7, B8 and B9 has been isolated from Indonesia [34, 36]. Subgenotype B1 has presence of G at nucleotide 1838 exclusively while subgenotype B2 has found A at nucleotide 1838. Serotype *adw* was exclusively found among subgenotypes B1, B2 and B3, while serotype *ayw* was found among the subgenotypes B4 and B5 [37].

Genotype C

HBV genotype C is a dominant genotype of Eastern and Southeastern portions of Asia (in Indian subcontinent it is restricted to eastern India and Bangladesh) and also observed in pacific islands as well as in the immigrants from these areas in the U.S.A, Australia, New Zealand and other European countries [38, 39]. Subgenotype C1 has been observed mainly in Vietnam, Myanmar and Thailand. Whereas, subgenotype C2 dominates the Japan, Korea and China and subgenotype C3 dominates the Micronesia, Melanesia and Polynesia [38-40]. Subgenotype C4 is found in Australia while subgenotype C5 is detected in Vietnam and Philippines. Subgenotype C6 was discovered in Papua (Indonesia) [41]. Subgenotype C7 to C16 also has been discovered from Indonesia [42, 43]. Genotype 'C' has been found to have largest number of subgenotypes. It has been observed that genotype C with 1858(C) mutation instead of usual 1858(T) mutation has been observed in 10-25% of genotype C carriers of East Asia [44, 45].

Genotype D

HBV genotype D has worldwide distribution and found throughout the Northeren Asia, Middle East, Russia, Eastern Europe and Mediterranean region and North Africa. It is also commonly found in U.S.A, Alaska and Greenland [21]. Genotype D is divided mainly into 6 subgenotypes D1-D6. Subgenotype D1 is mainly found in Europe, Middle East, Egypt, India, and Pakistan. Subgenotype D2 is mainly found in Europe, Japan and India. Subgenotype D3 is distributed in Europe, South Africa, United States, India and Pakistan whereas subgenotype D4 is present mainly in Australia, Japan and Papua [21, 46]. Subgenotype D5 has been reported from India [47]. Serological subtype *ayw*2 was well represented in subgenotype D1 whereas majority of subgenotype D2 isolates were of *ayw*3 [21]. Subgenotype D6 has been observed in the population of Kenya, Russia & Indonesia, D7 in the population of Morocco and Tunisia. Subgenotype D8 and D9 are found in India and Nigeria. Subgenotype D9 was a recombinant of genotype D & C [48, 49]. The distinctive feature of genotype D is the presence of 33 nucleotide deletion at the N-terminus of the preS1 region [21].

Genotype E

HBV genotype E is the main dominant genotype of West and central Africa [21]. It has also reported from India [50]. This genotype is characterized by very low genetic diversity [51, 52]. This genotype is not divided into any subgenotype yet. This genotype is characterized by a 3 nucleotide deletion at the N-terminal region of preS region. Serotype *ayw*4 is characteristics of genotype E. This genotype along with the genotypes F, G & H has presence of a nucleotide motif 'CCAGCTTCC' which is present 18 nucleotides upstream from the stop codon of core gene. This

genotype is further characterized by the presence of golgi peptidase motif Asn-Thr-Trp-Arginspite of usual motif Thr-Thr-Trp-Arg[53]. HBV genotype E is isolated from chimpanzee and other non-human primates and this has been speculated that genotype E may have been entered in human population from there recently [54].

Genotype F

Genotypes F along with genotype H are considered as "new world" genotypes and have been found to be 14% divergent from other genotypes. This genotype may represent the first split from the human hepadnaviral ancestor. Genotype F is found mainly in indigenous population of North & South America. This genotype has four subgenotypes F1-F4 [55]. All genotype F isolates belonged to serological subtype *adw*4 [21]. Subgenotype F1 is found mainly in Alaska, Argentina and Bolivia. Subgenotype F2 is observed in Venezuela and Brazil. Subgenotype F3 is found in Venezuela, Columbia and Panama whereas subgenotype F4 is found restricted to Argentina and Bolivia [55, 56]. Subgenotype F2 codes has 1858(C) whereas F1 possess 1858(T) (Schaefer, 2005). A study also proposed the presence of Thr45 in subgenotype F1 and Leu45 in subgenotype F2 in the S gene [57].

Genotype G

Genotype G is found in France, United States and Vietnam and it is found almost exclusively with genotype A and in some cases with genotype C [58, 59]. There is difficulty in establishing an infection in hepatocytes cell line on monoinfection with genotype G, but upon superinfection with genotype A infection with genotype G occurs [60]. Genotype G genome has been found to be least divergent from the genotype E (11%) and most divergent (15%) from the genotype F [59]. Some unique features of genotype G like 36 bp insert at 3' position of 1905 & 3 bp deletion in the N-terminal of preS1 region have also been observed. Like other genotypes, it also lacks the 6 bp insert on the C-terminal end of the core ORF that is found only in genotype A. The Precore/core region of this genotype has 2 stop codons at position 2 and 28 and therefore expression of HBeAg protein does not takes place but if HBeAg is present in serum it may be due to the coinfection with other genotypes, mainly genotype A [61]. This genotype is not divided into further subgenotypes. More isolates having genotype G are needed for their subclassification into subgenotypes.

Genotype H

HBV genotype H has been found in Mexico, Central America and Nicaragua. Genotype H is most closely related to genotype F but divergence of 7.5%-9.6% on the basis of whole genome from genotype F makes it new genotype [62]. This genotype is also not subclassified as there are fewer isolates in comparison to the other genotypes like A, B, C and D.

Genotype I

Recently, HBV isolate from Vietnam was reported to have ninth human genotype called genotype I. However, this genome differed from genotype C by only 7.0%+/- 0.4% on the basis of whole genome. It has been proposed as a complex recombinant of genotype C, A andG along with seven unique conserved amino acid residue not present in any of the known genotypes [63]. Genomic length of subgenotype I is 3215 nucleotide. It possesses*adr*serotype. Very similar to Taiwanese strain, genotype I has been also reported from a primitive tribe Idu-Mishmi of northeast India. Use of bootscan analysis revealed that all the isolates of genotype I from the Indian tribe are found to be the recombinants of genotypes G, C and A [64].Yu *et.* al. (2010) also reported the presence of genotype I in Taiwanese population with serotype *adw*2 and *ayw*2. More studies are needed to establish it as a new genotype [65].

Genotype J

Tatematsu*et.* al. (2009) isolated a novel genotype from a Japanese patient with HCC [66]. It has been proposed that this genotype is neither a recombinant of nine genotypes (A-I) nor a recombinant from four Apes (Gorilla, Chimpanzee, Gibbon and Orangutan). This isolate differs from genotype I by 9.9 %-16.5% on the basis of whole genome. It has a genomic length of 3182 nucleotide and shows *ayw* serotype.

Recombination between different genotypes of HBV

Because of the presence of several different genotypes in the same geographical area, recombination between genotypes is possible in a host following simultaneous transmission of several genotypes (co-infection) or from sequential infections with different genotypes (superinfection) [67, 68]. Recombination between different strains of HBV leads to a large evolutionary jumps and can provide a mechanism by which virus can improve their fitness by fighting with the host immune response which further results in the evolution of drug/vaccine resistant virus strain or strain with enhanced transmissibility and pathogenicity [28, 69].Template switches between minus and plus strand

of HBV-DNA during genome synthesis provides a good chance for recombination but not at the transcription level because reverse transcription occurred only after the encapsidation of a single RNA molecule. It is speculated that recombination is likely to be occur in nucleus where genomic segments can be exchanged between DNA strands derived from different coinfected genotypes [61, 70, 71].

The first report of HBV recombination was first observed in serum samples of HCC patients[72, 73, 74]. Different regions in the HBV genome have different capability of recombination process and the break points for recombination are found to be located frequently near gene boundaries [70]. There are four hot spot region in HBV genome where recombination occurs and are well defined. The first and most important hot spot region is found in the vicinity of the DR1 region (nt 1640-1900). It has been shown that the recombination site density in this region was almost five fold higher than that of the remainder of the viral genome [75]. It has been further further demonstrated that region between nts 1885-1915 is a hot spot and indispensable for enhancement of *in vitro* recombination. This region facilitates the intragenic recombination between different genotypes of HBV like A/D, A/B/C, A/E, B/C, C/F, C/G genotypes [71, 76, 77]. The preS1/S2 region (from nts 3150-100) constitutes the second hot spot region for recombination. Intergenotypes recombination like A/D, C/D, A/B/C, A/C and A/G hybrids showed break point in this region. Genotype G is found to have mosaic area of genotype A in this region [61] and recombination breakpoints in genotypes B and genotype C is found to be located in this region [78, 79].

The third hot spot region found to span the region from nts 2330- 2485, which is located near the 3'end of the C gene. It is demonstrated that recombinants with breakpoints in this region also have breakpoint in DRI hot spot region. Intergenotypes recombination like A/D, A/B/C, B/C and C/G hybrids showed break point in this region [71]. The fourth hot spot region spans the region between nts 650-830 which covers the 3' end of the surface gene. Intergenotypes recombination like A/D, C/D, A/E and B/C hybrids showed break point in this region. Some A/D hybrids had breakpoint at 6 bp insertion of genotype A (nts. 2356-2361) or at the 33 bp deletion (nts. 2855-2887) of genotype D [71].

Some genotypes like A, D, F & H showed great tendency of recombination in comparison to other genotypes like B, E & C, whereas genotype H has not shown recombination with any other genotype [70]. The observed low recombination in B and C genotypes may be due to the resistant towards reinfection in Asian adults because of the reason of perinatal (Mother to child) transmission of infection in early life. A study showed that genotype A and C have a higher recombination tendency in comparison to other genotypes [71]. Another study speculates the higher recombinant frequencies observed in genotypes A and D due to the horizontal transmission of reinfection sub Saharan Africa [70, 80, 81].

Some intergenotype recombinants become prevalent in certain areas and represent the main HBV type in that area like B/C hybrids of genotype B (B2-B5) in Asia excluding Japan [77]and C/D hybrid of genotype C in north West China and Tibet. In Tibet C/D hybrid is present in 96% of the total isolates hence this hybrid is the major HBV type in that region [82, 83].

Hepatitis B virus genotypes and clinical outcomes

Medical reports from all over the world have shown that a different genotype has different effect on severity of liver disease. A case control study of HBsAg positive patients from Africa shows that there is a 4.5 fold increase in risk of developing cancer in patients who were infected with genotype A when compare with the other genotypes [84]. Kumar *et. al.,* (2005) showed that in Indian patients genotype A is more often associated with ALT elevation, HBcAg positivity, absence of anti-HBeAg in patients aged more than 25 years and also it is found more frequently associated with patients of LC than to the patients infected with genotype D[85]. Similarly, genotype A has been found to have more strong relation with severe liver disease in Pakistani patient as compared to genotype D [86]. But, Thakur *et*. al. (2002) reported that in north Indian patient's genotype D has been found to be associated with more severe liver disease including hepatocellular carcinoma in younger patients as compared to genotype A[87]. Similarly, HBV associated vasculitis (polyarteritisnodosa) is significantly found associated with genotype D in comparison to genotype A2, B6, C2 and F1 [88].

In contrast to these studies, two studies from Spain showed that genotype A has been associated with a higher cumulative rate of sustained biochemical remission, HBsAg clearance, viral DNA clearance in comparison to genotype D. Genotype A2 has been found less associated with HCC patient when compared to genotype D, C or F1. Same study showed that genotype F associated death rate was more rapid than those infected with A and D

genotypes [89, 90]. Furthermore, genotype F mediated liver cancer has been detected in young people as compared (22.5 years vs. 60 years) to those who were infected with genotypes/subgenotypes A2, C2 or D [90].

Genotype B has been found to have lower HAS score and normal ALT level in comparison to genotype C infected isolates [91]. On the same line of agreement, a Japanese study showed that in genotype B infected patient liver cirrhosis process is low when compared to the genotype C. Further, same study also showed that HBeAg seroconversion was found at a younger age in genotype B infected isolates in comparison to genotype C [92]. Same conclusion has been drawn from several studies from China and Japan that genotype C is more prevalent in patients with active liver disease than genotype B [93-95].Chu *et. al.,* (2003) reported in their study that patients with genotype B infection were less likely to develop more liver damage in comparison to genotypes A, C, and D[30]. Same study demonstrated that genotype A has been associated with higher prevalence of HBeAg as compared to genotype B and D. Similarly ALT level, HBV DNA and HBeAg positivity has been found to be significantly higher in genotype C infected patients than genotype B infected carriers in Thai patients[69].

A study from Taiwan contradicts the belief of less harmful effect of genotype B. Their study revealed that genotype B was more frequently associated with the patients with HCC aged less than 50 years. Mean age of those genotype B infected HCC patient had been found to be less than or equal to 35 years[96]. Hepatitis B splice protein (HBSP), a Pro-apoptotic protein has been found to induce apoptosis in hepatocytes. It has been observed that genotype B express more HBSP as compared to other genotypes and hence caused more intensified apoptotic effect. This intensified apoptotic effect along with the great regeneration capacity of hepatocytes results in accumulation of deleterious mutations and may ultimately contribute to final stage- the HCC[97].

Lin et. *al.,* (2007) demonstrates the clinico-pathological differences between the genotypes B and C related resectable hepatocellular carcinoma[98]. They found that genotype B patients have a higher rate of solitary tumors and satellite nodules compared to genotype C patients. Genotype C infected patient's shows fivefold increased risk of HCC and if the viral load is higher than the risk become 26.5 times higher when compared with all other genotypes. Genotype C has been found more prevalent in LC patient in comparison to other genotypes [96]. Genotype C relation to more severe liver complications is further confirmed by the findings of highest HBV DNA levels in cell lysates for genotype C which is followed by genotype B and genotype D/A. Genotype A(A1) cell lysates showed the lowest HBV ($P<0.01$) DNA [99]. Lamivudine resistant mutations were found higher in genotype C when compared to genotype B. It is now universally accepted that genotype C is associated with an increased risk of liver inflammation, hepatic flares, fibrosis and LC condition as compared to other genotypes [100, 101, 102]. Reason behind the more deleterious effect of genotype C may be due to the presence of prolonged viremia, which may increase the chances of HBV integration to host genome and hence increased opportunity for liver inflammation and fibrosis. Second reason of this effect is that core promoter mutations which are independently associated with higher risk of HCC, appeared to be occur more frequently in genotype C which further increases the risk many fold. There are a few studies on effect of different subgenotypes of genotype C on disease progression although, in Hong Kong a study demonstrated that risk of developing HCC is higher in subgenotypes C2 followed by subgenotype C1 and genotype B [102].

In Asian patients, it has been shown that HBeAg is more prevalent in genotype C when compared with genotype B, and seroconversion of HBeAg is faster in genotype B than genotype C [91, 92, 101, 103, 104].There are contradictory results when HBV DNA level is measured against genotypes. Kao *et. al.,* (2002a) reported that in blood donors genotype C infected patients have higher HBV DNA levels than those infected with genotype B[100]. In HBeAg positive patients, genotype C infected patients have higher viral DNA as compared to others genotypes, but in HBeAg negative patients HBV viral DNA is higher in patients infected with genotype D compared to others genotypes [105]. But in contrast to these findings a nationwide study from United States showed no such differences in the level of HBV DNA between different HBV genotypes A, B, C and D [30].

Precore/core region of HBV plays a very crucial role in HBV mediated liver complications. Mutations within this region affect the disease progression. Some studies shows the relationship between the genotypes and the mutations in precore/core region of viral genome. The predominant mutation in the precore is presence of A at position 1896 instead of G, which results in the premature termination of translation. Presence of A always favored the presence of T at nt. 1858 which further favored the stable stem loop structure. HBV genotype A almost always has a 1858 (C) mutation. This may explain why this common precore mutation is not found commonly in Northeren Europe, U.S.A. and in India [106]. Genotype B, C and D frequently harbors T at position 1858 in Asia and Mediterranean basin

where these genotypes predominates[107]. Precore mutations are found most commonly in genotype D (65-75%) infected patients and least common in genotype A (9%-18%) infected patients in France and Spain [106, 108].Genotype B more commonly have precore variant than those with genotype C (48% vs. 5%) [107]. A nationwide study revealed that precore variations are found more frequently in genotype D (73%) followed by B (46%), C (24%) and A (3%) in U.S.A [30].

Two most studied mutations in CP region are "A1762T and G1764A" and found to be associated significantly with higher risk of HCC and are found more frequently associated with genotype C in comparison to genotype B [107]. Further, it was found that these two mutations in HCC patients are found more frequently in subgenotype B2-B5 in comparison to subgenotype B1 [26], whereas acute and FH condition occurs more frequently with subgenotype B1 compared to other subgenotypes of genotype B [109].

HBV genotypes/subgenotypes and response to antiviral therapy

Total eradication of the virus from the host is never achieved and loss of HBeAg along with undetectable viral DNA is considered as the standard therapeutic endpoints in clinical trials [14]. There are two modes of HBV treatment- Immunomodulation (Interferon therapies) and viral suppression or eradication with nucleos(t)ide analogues.

Influence of HBV genotypes on IFN-based therapies

It has been shown by many studies worldwide that different HBV genotypes respond differentially to the interferon therapy. Genotype B showed higher rate of HBeAg seroconversion as compared to genotype C (41% vs. 15%) in patient from Taiwan and Hongkong[101, 110]. Sanchez-Tapias*et. al.*, (2002) reported in their study that genotype A infected patients has a much quicker rate of HBeAg seroconversion than genotype D infected patients in Spain[89]. A placebo-controlled study with PEG-IFNα-2b alone and in combination with Lamivudine confirmed the high rate of HBeAg seroconversion in genotypes A (47%) and genotype B (44%) as compared to genotype C (28%) and genotype D (25%). The same study also showed that HBsAg seroconversion is found to be higher in genotype A than genotype D (13% vs. 2%). So, they found PEG-IFNα-2b therapy best to achieve HBsAg and HBeAg seroconversion in patient having genotype A infection [111]. A study from Germany revealed that interferon induced seroconversion is higher in genotype A when compared to genotype D (49% Vs. 26%), and also the sustained response favors the genotype A when compared with genotype D (46% Vs. 24%) in HBeAg+ve patients. In HBeAg-ve patients again genotype A has better response to interferon (59% vs. 29%) when compared with genotype D infected patients [112]. Response to Thymosin α-1 therapy is found higher in genotype B infected patients as compared to patient having genotype C infection [113]. In HBeAg positive individuals, seroconversion of HBeAg after IFN based therapies are significantly more often observed in patients infected with genotype A and genotype D and lesser extent to genotype B and genotype C infected patients.

In HBeAg positive individuals, genotype B has been found to be associated with better response to interferon therapy as compared to genotype C but failed to do better response in HBeAg-ve patients. Spontaneous HBeAg seroconversion also occurs more often in genotype B than in genotype C, although these spontaneous seroconversion rates are lower than after IFN therapy [98]. Combination therapy of PEG-IFN-α2a plus Lamivudine showed a reduced efficacy than monotherapy with PEG-IFN-α2a in both HBeAg+/- patients infected with genotype A[114]. Study on effect of IFN-α therapy on genotype E, F, G and H, demonstrated that sustained virological response varied considerably. It is found that genotype E, F and H showed 36%, 50% and 50% sustained virological response whereas lesser is shown by genotype G (20%). The lower sustained virological response for genotype G is explained by the frequent double infection with genotype A or C and the inability of the genotype G to secrete HBeAg [61, 115].

In treatment of patient with genotype E there are two main problems. First, because this genotype transmitted vertically hence due to immunotolerence it is difficult to eradicate the infection [116]. Second, African origin patients have inherently hypo-responsiveness to Interferon's [117]. Till date small preliminary data of response of IFN- α therapy to genotypes E, F, G and H suggest that IFN-α can be considered as a treatment option for genotypes E, F, and H, whereas, for genotype G nucleos(t)ides can be a first line treatment option [118].

Influence of HBV genotypes on nucleos(t)ides analogues therapies

Lamivudine

Lamivudine- a negative enantiomer of the deoxycytidine analogue 2'-deoxy-3'-thiacytidine, is the first nucleoside analogue used for the treatment of HBV infection. Histological study showed that lamivudine has the ability to

reverse the process of liver fibrosis in most patients [119]. Many studies analyzed the influence of lamivudine on genotype A vs. genotype D group and genotype B vs. genotype C group. Endpoints of the treatment in this therapy are: HBeAg seroconversion, loss of HBsAg or loss of HBV DNA. These studies do not show any treatment difference between genotype A vs. genotype D group and genotype B vs. genotype C group neither in HBeAg+ve patients nor in HBeAg-ve patients [120, 121, 122, 123].Contradictions are also present regarding the lamivudine treatment response in different genotypes. Two reports revealed that good response and sustained virological response are found better in genotype B in comparison to genotype C infected patients. The post-treatment relapse rate has been observed higher in genotype C infection than genotype B [100, 124].Patients with genotype D infection has shown higher sustained viral response after lamivudine therapy when compared to genotype A[125], and also lamivudine-resistant mutants emerged more spontaneously in genotype A as compared to genotype D in German patients [126]. Two studies on Japanese and Chinese CHB patients with lamivudine treatment demonstrated no significant difference in virologicalresponse between genotype B and genotype C, but the emergence of the YMDD mutation has been found to be associated more firmly with genotype C infected patients than genotype B. Overall, it has been demonstrated that lamivudine is safe and effective in treatment of patients with decompensated liver disease [127, 128].

AdefovirDipivoxil

AdefovirDipivoxil (ADV) is an acyclic analogue of deoxyadenosine monophosphate which inhibits HBV DNA amplification but not *de-novo* formation of cccDNA in HBV infected hepatocytes. It has been observed in a study that 10 mg of adefovir given daily for 48 weeks found to be associated with an improved histological results, a higher rate of HBeAg seroconversion, greatly reduction in viral DNA concentrations and with higher chance of normalization of ALT level [129]. Westland *et. al.,* (2003) in their study find no difference in the virological response between genotypes A, B, C, or D in a combined analysis of both HBeAg +/- patients within 48 weeks of ADV therapy, when HBV DNA reduction is considered as one of the end point of treatment[105]. Opposite to this, Shiffmanet. *al.,* (2004) reported that HBsAg seroconversion occurs preferentially in genotype A infected patients (6/9) after 72 weeks of ADV therapy[130]. Adefovir has been found to be active against lamivudine resistant YMDD mutant HBV strains treatment [131].

Entecavir

Entecavir is a guanosine analogue which inhibits the viral DNA synthesis by affecting the HBV polymerase [132]. Entecavir induced resistant strains were not detected in wood chucks after 3 years of treatment. It has been observed that a 0.1-0.5 mg daily dose of entecavir is more effective than 100 mg of lamivudine in suppression of viral replication [103]. It is also found to be effective against YMDD mutants, if given 1 mg dose daily. Till date with this polymerase-inhibitor results are available which shows loss of HBV DNA in both HBeAg +/- patients. There is found equal reduction of HBV DNA in genotype A, B, C and D after 52 weeks of therapy [122].

Telbuvidine

Telbuvidine is an L-nucleosides, and have specific HBV inhibitory activity. It has been observed that telbuvidine has almost no side effects and it is better than lamivudine in suppressing the HBV virus. Till date there are no such studies which differentiate the effect of telbuvidine with respect to genotype [133].

Beside these nucleos(t)ide analogue, other analogues are also tested for HBV eradication like tenofovirdisoproxil fumarate which is structurally similar to adefovir, emtricitabine-a pyrimidine analogue which is structurally similar to lamivudine. Neither of these analogues is found to be effective against YMDD mutants. There is also paucity of information regarding their effect in correlation with genotype/subgenotype [134, 135].

CONCLUSION

Evidence are accumulating which showed that genotype A and B response better to interferon as compared to genotype C or D, whereas lamivudine showed better and sustained response in genotype B and D as compared to genotype A or C. Genotype A has been shown to have greater tendency in accumulating lamivudine-resistant mutations more quickly than other genotypes. HBV genotypes should be determined prior to antiviral therapy in CHB patients, especially if IFNs are considered as treatment option. This is truer if the patient is genotype A infected and also holds true for genotype B infected patients who are HBeAg positive. More and more studies all over the world are needed before genotypic specific therapy included in clinical evaluation and decision making in the treatment of chronic HBV patients.

Many studies throughout the world agreed unanimously that different spectrums of HBV-induced liver disease depend not only on the genotypes but also on the genetic makeup of the host. Several SNPs mutations in various immunogenes have been shown to have direct effect on the HBV-induced disease resolution[136-139]. As described earlier in the present paper, it is the viral-host interaction which is responsible for the evolution of genotypes with new mutations. These mutations may increase or decrease the pathogenicity of virus. More pathogenic genotypes/subgenotypes may have a more severe effect on the health of the host having weak allele of certain immunogenes and may have less profound effect on the health of the host having strong allele. Similarly, a less pathogenic genotype/subgenotype may be wipe out from the host having strong alleles but remain persistent in those who have weak ones. Different ethnic groups have different alleles of immunogenes due to presence of SNPs and same is true between two individuals of same ethnic group. Hence in the generation of new viral strains host immune system has an important role. This may be the reason that why some studies describe one genotype in their population more pathogenic in comparison to others. So, through this review article we want to suggest some points: 1) before concluding about the pathogenecity of HBV genotypes/subgenotypes host immune factors should be considered in the respective ethnic group; 2) HBV genotypes/subgenotypes classification should be reclassified. Only those genotypes having functional mutations (mutations which have direct effect on the pathogenicity of virus and hence on disease progression) should be classified as new genotype. Subgenotypes having same pathogenic behavior should be grouped together; 3) Drug resistant strains should be classified separately under the respective genotypes.

Last but not the least, data from large studies at national and international level from various ethnic groups must be analyzed to find out the pathogenicity of different strains and also to find out the disease-susceptible and disease-resistant alleles of various immunogenes. This will help us in designing of the new effective measures against HBV-infection in future.

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