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HOST AND VIRAL FACTORS ASSOCIATED WITH HEPATITIS B CLINICAL OUTCOMES IN CHRONIC INFECTION - REVIEW ARTICLE

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ABSTRACT.

Viral and host factors have been implicated in persistence of HBV infection to chronicity and perhaps to liver cancer. Fortunately 90-95% of those who get infected in adult hood clear the virus and remain with antibodies suggesting previous exposure to HBV. The underlying reasons as to why majority of the patients with acute infection clear the virus while a small proportion progress to chronic infection lies in the difference in host immunological and genetic factors. The immune determinants of complete clearance are not fully understood but both innate and adaptive are paramount in this response. Similarly, the role of the host genes in the pathogenesis of the virus are not fully elucidated but polymorphisms in genes encoding for the HLA, cytokine and vitamin D receptor (VDR) have been highlighted in influencing both disease clearance and progression to chronicity. In this review, the host and viral factors responsible for differential clinical presentation of hepatitis B are discussed.

Introduction

Hepatitis B virus (HBV) is the causative agent for liver diseases, including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (Matsuura, K., Tanaka, Y., Hige, S. et al., 2009). Hepatocellular carcinoma is one of the most common cancers globally with high incidents in Eastern Asia and Sub-Saharan Africa (Jemal, Bray, Center, Ferlay, & Ward, 2011). Most patients who get infected with hepatitis B virus go into an acute phase during when the virus is cleared. None the less, in some patients the virus persists resulting into chronic hepatitis B infection which may later progress to liver cancer. Immune responses and genetic factors of the host along with the viral characteristics remain pivotal in determining the course of the disease

Hepatitis B genotypes and subtypes

The HBV genome is composed of approximately 3,200 nucleotides (Matsuura et al., 2009). Arauz-Ruiz, Norder, Robertson, & Magnius, (2002) previously classified HBV into 8 genotype identified as A-H based on an intergroup divergence of 8% or more in complete nucleotide sequence whose geographical distributions was previously extensively studied by Sanchez-Tapias, Costa, Mas, Bruguera, & Rodes, (2002) who documented that genotype A is pandemic, B and C are predominant in Asia, D in southern Europe, E in Africa, F in United States of America, G in France while H in Central America. However recent studies by McMahon (2009), Cao (2009), Kurbanov, Tanaka, & Mizokami, (2010) have introduced two new genotypes designated as I and J giving a total of 10 genotypes together with several sub-genotypes. With the exception of the newly identified genotypes, the other genotypes and sub-genotypes have well characterized ethnic and geographical distribution (Lin & Kao, 2011).

In Africa, many genotypes have been characterized including A, B, C, D, E & F together with some sub-genotypes having unique regional and sub-regional distribution (table 1).

Region	Genotypes	Sub-genotypes	Reference
Central Africa	A, D, E	A1, D4	(Komas et al., 2013)
Egypt	D	D1	(Ragheb et al., 2012)
Gambia, Nigeria, Congo, Rwanda, Cameroon	A	A4, A5, A6, A7	(Shi, 2012)
Morocco	D, A	D1, D7, A2	(Baha et al., 2012)
South Africa	D	D3	(Yousif & Kramvis, 2013)
Tunisia	D, F	ND	(Ayari et al., 2012)
Uganda	A, B, C, D, E	ND	(Zirabamuzaale & Ocama, 2016)

According to the study by genotype A has three sub-genotypes (A1-3) with A1 having prevalence in Sub-Saharan Africa whereas A3 located in West Africa. Genotype B has six sub-genotypes B1-6 with none of them isolated in Africa. Genotype C has five sub-genotypes C1-5 and none of them in Africa. Genotype D, with sub-genotypes D1-5, with only D1 identified in Africa. Genotype E has no reported sub-genotype up to date and it is restricted in West Africa. Genotype F has four sub-genotypes and none of them is distributed in Africa. The other genotypes G- J have no sub-genotypes and none of them has been reported in Africa.

Hepatitis B viral characteristics and clinical manifestation of the disease Many studies have indicated that disease exacerbation is a function of the infecting genotype, sub-genotype, the level of viremia as well as viral mutations in any of the four open reading frames of the viral genome (Kobayashi et al., 2002, Tanaka et al., 2004, Toan et al., 2006, Matsuura et al., 2009). This review will focus only on the effect of infecting genotype, viral mutations and the resultant viral load on the clinical profile of hepatitis B.

Genotype mono infection and disease outcome

Although most of the severe disease manifestations are not limited to genotype C, most studies have implicated this genotype as the key culprit in progressing to liver cirrhosis and subsequent hepatocellular carcinoma (HCC). The association of genotype C with the worst prognosis has been reported in many studies including Ming-Whei Yu et al., 2005 (n=4841, genotyped by qPCR) and Henry Lik-Yuen et al., 2008 (n=1006, genotyped by sequencing using ABI 3100 genetic analyzer) (Table 2). These studies have implicated genotype C in poor prognosis increasing the chances of progressing to HCC. In the study by Henry Lik-Yuen and co-workers, genotype C was more correlated with HCC than B with a hazard ratio of 3.83 (P<0.0001). Ming Whei Yu and colleagues (Table 2) established that genotype C was associated with high HBV DNA and increased risk of progressing to HCC in patients infected with this genotype (P<0.001, OR=5.11). Surprisingly, the study by Henry Lik- Yuen and colleagues (2008), reported that within genotype C infection, sub genotype Ce had a higher risk of HCC than Cs with a hazard ration of 2.75, P<0.0001 (Table 2). This suggests a strong correlation between sub-genotype and hepatitis B disease profile given the large sample size used in this study (n=1006) that has given the findings a strong statistical power. Studies by Ming Shi et al., 2012 (n=89, genotyping by qPCR) reported that patients with genotype C had higher HBeAg positive rate than patients with genotype B (Table 2) confirming the earlier studies that implicated genotype C in severe disease exacerbation. This is in concordance with the earlier study by Chu, Hussain & Lok (2002) who showed that patients with genotype B achieve HBeAg sero-conversion earlier than those infected with genotype C suggesting less severe liver damage in HBV/ B infection than HBV/C infection.

Interestingly some studies have implicated genotype B in causing more severe liver disease than genotype C. Man-Fung and co-workers, 2003 (n= 318, genotyping using INNO-LiPA HBV genotyping) found out that patients with genotype B had more severe exacerbation as compared to those with genotype C (Table 2). Their finding is consistent with the recent study by Ren X and colleagues, 2010 (n=559, HBV DNA determined by qPCR, genotyping by sequencing) who established that patients with chronic hepatitis B had higher risk of developing liver failure as compared to those with genotype C (Table 2). Related studies by Yuen et al., 2003 and Kobayashi et al., 2002 established that patients with HBV genotype B had more severe presentation of the disease and are at a more risk of hepatic decompensation as compared to HBV/C infection.

These contradictions in the available literature on the clinical outcome of hepatitis B genotypes C and B infection are fundamental issues that justify more studies to give conclusive clinical outcomes as per the infecting genotype.

HBV DNA levels (viral load) and disease outcome

Growing evidence provides a strong correlation between HBV DNA levels (viral load) with genotype both in mono and mixed infection and hence resulting into interesting differences in disease prognosis. High HBV viral loads > 104 copies/ml are associated with high risks of HCC as well as post HCC treatment recurrence. Surprisingly, some studies have found no association between viral load and genotype. Toan et al., 2006 (n=375, genotyped with modified RFLP PCR & quantified HBV DNA with qPCR) established that HBV was higher in mono infection with HBV genotype A and C than D (P=0.0002 & P=0.0001 respectively) (Table 2). In mixed infection, viral load was higher in C/D infection than in patients singly infected with C and D genotypes (P=0.019 & P<0.0001 respectively). They concluded that the course of CHB and the subsequent development of HCC is influenced by HBV genotype and by the occurrence of mixed infection (Table 2). Their report is in agreement with studies by who reported high viral loads in patients infected with genotype mixtures as compared to those infected with a single genotype.

Table 2: Correlation between genotype diversity and the risk factor development of HCC

Reference	Methodology	Results	Conclusion
Ming-Whei Yu et al., 2005	• Cohort of 4841 male HBsAg+ ≥30years. • Viral load determined by qPCR and HBV DNA transformed to Log10. • OR determined for noting risk ratio of genotype C to B	• Plasma DNA≥4.23log10 copies/ml (P<0.001). OR=5.11 (95%CI)	• Genotype C associated with High HBV DNA than B and associated with higher risk of HCC
(Toan et al., 2006)	• 375 HBV+ Vietnamese. • All HBsAg+ by EIA, HCV & HIV negative. • Genotyping done with a modified RFLP PCR & HBV DNA was quantified with qPCR. • Statistical analysis performed at 95% CI	• HBV DNA higher in mono infection with HBV/A and C than D (P=0.0002 and P=0.0001 respectively). • VL was higher in mixed infection than mono infection (P=0.019). • VL was higher in C/D mixed infection than in single infection with C & D (P<0.0001)	• Clinical course of CHB and the development of HCC is influenced by HBV genotype and by the occurrence of mixed genotype infection
Henry Lik-Yuen et al 2008	• Prospective cohort study on 1006 CHB patients followed for 3 years • HBV DNA detected and quantitated by TaqMan qPCR • Sequencing done by ABI 3100 genetic analyser	• Genotype C was associated with higher risk of HCC than B: hazard ratio 3.83 (95% CI 2.15-6.81; P<0.0001) • HBV/B genotype Ce had a higher risk of HCC than Cs: hazard ratio 2.75 (95% CI 1.66-4.56; P<0.0001)	• Viral factors have an important impact on the risk of HCC development compared with other factors
Richard S. Tedder et al., 2012	• 558 patients, aged ≥18 with CHB regardless of the antiviral treatment history	• HBV genotype was associated with ethnicity & country of birth (p<0.05) • The e status was associated with the genotype (p=0.001) • Patients with genotype A & C were more likely to be HBeAg+ compared to B, D & E	• There was no significant association between VL & genotype in HBeAg+ (P=0.30) or the anti-HBe+ (P=0.10)

Table 2 continued

Reference	Method	Results	Conclusion
Man-Fung et al., 2003	• 318 patients were used segregated as follows; • 73 patients (group I) with severe exacerbations of hepatitis all HBsAg+ for >6 months (ALT>10x the ULN) • 44 patients with moderate exacerbation (group II)-ALT levels 5-10x the UNL • 80 patients with mild exacerbation (group III)-ALT levels 2-5x the UNL • 121 patients with no exacerbation (group IV)-ALT levels <2x the UNL • Genotypes were determined with the line probe assay (INNO-LiPA HBV genotyping)	• All patients with exacerbation (groups I-III) were categorized into genotypes B & C • No significant differences in the prevalence of genotypes B & C between the 4 groups (all P=NS) • Patients with genotype B had higher medium ALT level, high medium bilirubin level and lower medium albumin during periods of exacerbation as compared with patients with genotype C	• Patients with genotype B had more severe exacerbations as compared to those with genotype C

Ming Shi et al, 2012	• 89 patients were recruited in a longitudinal study • All HBeAg+ for ≥ 6months & had detectable serum HBV-DNA but lower than 20,000IU/mL by rtPCR • Genotyping was done using rt-PCR	• Patients with genotype B had lower viral load, higher HBeAg negative rate and older than genotype C	• Patients with genotype B had lower viral load, higher HBeAg negative rate and older than genotype C
Ren. X et al., 2010	• Sera from 559 patients • HBV DNA determined by using rt-PCR • Genotypes determined by sequencing	• Genotype B had lower frequency of BCP mutations than CP mutations as compared to C	• Patients with CHB infected with genotype B have higher risk of developing liver failure compared to genotype C

statistically significant association between plasma DNA and viral load (DNA≥4.23log$_{10}$, P<0.001, OR =5.11, 95%CI). They concluded that genotype C was associated with higher HBV DNA levels (viral load) that B and is associated with higher risk of HCC (Table 2). Mendy ME et al., 2010 (n=242 HBeAg+, HBV DNA determined & quantified by qPCR) reported that cirrhosis & HCC cases were more common in higher HBV DNA compared asymptomatic carriers (P<0.01) and thus HBV-DNA levels are strongly associated with HBV infection independent of HBeAg status (Table 3).

Table 3: Correlation between HBV DNA levels and the risk of progressing to HCC

Reference	Method	Results	Conclusion
Mendy M.E et., al 2010	• 242 HBeAg+ were used. • HBV DNA was determined and quantified by qPCR. • HCC diagnosed by pathology/ ultrasonographic evidence & serum AFP. • Quantitative cirrhosis scores of ≥ 7 were used to define cirrhosis	• Cirrhosis & HCC cases were more common in higher HBV DNA comapared to ASY carriers (P<0.01). • High-level HBV viremia (>10 000 copies/ml) was strongly associated with both HCC and cirrhosis, conferring significant 17.3- and 38.8-fold increased risks of cirrhosis and HCC respectively	• HBV-DNA levels are strongly associated with HBV infection, independent of HBeAg status.
Mendy M.E et al 2008	• 190 HBV chronic carriers age between1-19 followed for 19 years in a cross-section study with longitudinal evaluation. • HBs Ag, HBeAg, ant HBc Ab, HBV DNA levels and LFTs were done at base line. • Patients were followed for 19 years. RIA was used for HBsAg & HBeAg. • HBV DNA was determined and quantified with qPCR	• HBeAg+ carriers had high DNA levels (> 105 copies/ml) and decreased during the years of follow up with significant decrease in young patients (p<0.001). • There was 1log10 drop in HBV DNA after 5 years and 4log10 drop over the next 10 years	• HBV DNA levels decrease with age
Henry Lik-Yuen et al 2008	• Prospective cohort study on 1006 CHB patients followed for 3 years • Patients aged 40-70 years • AFP monitored every 6 months • AFP >20µg/L was used to confirm HCC • HBV DNA detected and quantitated by TaqMan qPCR	• Higher HBV DNA associated with HCC, hazard ratio 1.38 (95% CI. 1.23-1.55; P<0.0001)	• Viral factors have an important impact on the risk of HCC development
Tran et al., 2015	• Retrospective analysis of data from 355 female CHB patients aged 18-69 years. • Women of child bearing age were of age 18-44 years • At baseline, patients were assessed for HBeAg status, HBV DNA levels, genotype and race. HBV DNA was measured using Roche Cobas Taq-Man PCR • Association between HBV genotype and VL were examined using Fisher's exact test	• 355 female patients (56.9% aged ≤44years) with CHB; 197 HBeAg negative & 157 HBeAg positive. HBeAg status of one individual was unknown • Women in the young cohort ≤ 45 years were significantly more HBeAg positive (P<0.0001) & had significantly higher HBV DNA (P<0.0001) • Most patients were infected with genotypes C &D though genotypes A & B were identified	• There was no obvious relationship between genotype and viral load

In a related study, Mendy ME et al., 2010, correlated HBV DNA levels with age (n=190 prospective cohort CHB carriers, HBV DNA quantified by qPCR). They reported that HBeAg+ carriers had high HBV DNA levels (>10^5copies/mL) and decreased with years of follow up; significantly decreasing in younger patients (P<0.001). Further, they established that there was 1 log drop in HBV DNA after 5 years and 4 log drops over the next 10 years (Table 3).

Consequently, from their results, HBV DNA levels decrease with age. Henry Lik-Yuen et al., 2008 quantitated HBV DNA using TaqMan qPCR from a sample of 1006 CHB patients (n=1006) and confirmed HCC by monitoring AFP levels for 6 months (Table 3). They found out that higher HBV DNA is associated with HCC (hazard ratio 1.38, P<0.0001). Consequently, viral factors have an important impact on the risk of HCC development. Several other studies have findings in concordance with the aforementioned studies. Yu et al., 2005, Oommen, Wirth, Wintermeyer, & Gerner, 2006, and Yousif, Mudawi, & Bakhiet, 2013 have reported that infection with genotype C is associated with higher HBV DNA than B, D higher than A and E higher than D. Viral specific differences have been hypothesized to have an impact on viral replication and hence influencing viremia.

Interestingly, in a study by Tedder RS et al.,2012 (n=558 ≥18 years with CHB), there was no significant association between viral load and genotype in HBeAg positive and anti-HBe positive (Table 2). This is in agreement with the study by Tram TT et al., 2015 (n=355 CHB prospective cohort, HBV DNA quantitated using Roche Cobas Taq Man PCR) who reported HBeAg positivity with high HBV DNA (P<0.0001) but no obvious relationship between genotype and viral load (Table 3).

These contradicting findings justify the need for more studies to characterize genotype diversity and viral load in order to predict the likelihood of HBV infected patients progressing to HCC in order to provide cost-effective ways in the management of hepatitis B virus infection.

Host factors and viral hepatitis B clinical profile

Cytokines and disease profile

Studies by Katia et al., 2006 (n=20 CHB; HIV & all forms of hepatitis negative sex, age, ethnicity matched to health controls; IFN- , TNF-α, IL-2, IL-4, IL-10, and IL-6 evaluated by CBA, IL-18 measured by ELISA) reported higher levels of TNF-α in HBV + than the control group (P<0.0001). Again TNF-α and IL-2 were higher in HBV+ than the control (P<0.005). Only in HBV+ IFN- & IL-2 exceeded the normal (Table 4). Similarly IL-4 & IL-10 were higher in HBV+ than the control group (P<0.01) but the levels were always in normal range. Plasma IL-6 was higher in the HBV+ than the control (<0.04). Plasma levels of IL-18 were higher in the HBV+ patients than the control group (P<0.0001) (Table 4). Katia and co-workers reported a strong correlation between high plasma levels of IL-6 and IL-18 with the hepatic index of disease, illness duration, viral load as well as serum ALT/AST activity.

In the study by Yong-Qiong Deng, et al., 2015 (n=235, prospective cohort, HBsAg+ for ≥6months, treatment naïve, HCV/ HIV negative and no evidence of any liver disease. HBV DNA assayed by COBAS and cytokines assayed by Human cytokine/chemokine panel 1) reported a correlation of cytokine/chemokine with HAI scores (Table 4). Also patients with moderate or severe inflammation had higher levels of IL-2R than patients with no/mild inflammation (P<0.001). The authors reported a strong correlation between fibrosis and levels of IL-8 (P=0,027), TGFα (P=0.0011), IL-2R (P=0.002). Thus IL-2R and TGFα are independent predictors for fibrosis (Table 4). Thus Cytokine profiles can be used as markers of disease progression and liver damage and should be evaluated as plausible alternatives to the current gold standard of liver biopsy which is an invasive mechanism and associated with potential complications. An IL-2R and TGF-α-based score fib-index was superior to the existing scores APRI and FIB-4 for predicting significant fibrosis in chronic HBV infected patients.

This represents a promising tool for noninvasive diagnosis of fibrosis in patients with normal and mildly elevated ALT levels.

Studies by Ge et al., (2009) have correlated host immunological factors including both pro and anti- inflammatory cytokines with liver disease progression during HBV infection. During normal physiological conditions, a homeostatic regulation of both cytokines occurs (Racanelli & Rehermann, 2006).

Table 4: Role of cytokines in disease profile

Reference	Method	Results	Conclusion
Katia F. et al., 2006	• 20 subjects with CHB & 20 healthy controls matched for ethnicity, sex, and age, were recruited. • Subjects were HIV- & all other forms of viral hepatitis. • IFN- , TNF-α, IL-2, IL-4, IL-10, and IL-6 were evaluated by CBA assays. • IL-18 levels were measured by ELISA assay. • A p value of <0.05 was required. • Spearman's correlation coefficients between plasma IL-18 and IL-6 levels and disease duration, viral load, serum AST, and serum ALT were computed.	• Th1 cytokines • HBV infection showed higher values of IFN-α levels than the control (p <0.0001). • TNF-α and IL-2 were higher in patients with HBV+ than the control (p <0.005). • Th2 cytokines • IL-4 and IL-10 levels were higher in HBV+ than the control (p <0.01), but the levels were always within normal range. • Inflammatory cytokines • IL-6 & IL-18 were higher in HBV+ group than the controls (p <0.05)	• Strong correlations between high plasma levels of IL-6 and IL-18 and the hepatic index of disease. • IL-6 correlated with illness duration (p <0.001) and viral load (p <0.001). • IL-18 correlated with serum ALT activity (P<0.001) and AST activity(P<0.001)
Yong-Qiong Deng, et al., 2015	• 235 patients were prospectively enrolled in the study. • Inclusion criteria: • HBsAg+ for ≥ 6months, treatment naive; age between 18 and 65 years; negative serum levels for anti-HAV IgM, anti- HCV, anti-HEV IgM/IgG, anti-EBV IgM, and anti-CMV IgM; and off potential transaminase-lowering agents such as bicyclol for at least 2 weeks prior to blood sampling biochemistries. • Exclusion criteria: • HCV/HIV coinfection; presence of other causes of chronic liver diseases, HCC & decompensated liver • HBV-DNA assay: • Serum HBV DNA was measured by COBAS TaqMan. • Cytokine/Chemokine assay • IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, IL-17A, CCL-2, IL-12 p70, CCL-3, IFN-g, TNF-a, TGF-a, and granulocyte monocyte colony stimulating factor) were measured by Human cytokines/Chemokine panel I. • CXCL9, CXCL-10, CXCL11, IL-2R, IL-33, and IL-34 were measured by Luminex screening system (LXSAHM-6, R&D, Minneapolis, MN). • Statistical analyses were performed using SPSS ver. 16.0	• Cytokines and chemokine levels correlated with HAI scores. • Patients with moderate or severe inflammation had significantly higher levels of CXCL-1 and IL-2R than the patients with no/mild inflammation (all P<0.001). • IFN- and IL-17A levels showed a declining trend with rising HAI scores (P=0.066 and 0.076, respectively). • Patients with significant fibrosis had higher levels of IL-8 (P=0.027), TGF-α (P=0.011), CXCL-11 (P=0.032), and IL-2R (P=0.002) than patients with no significant fibrosis. • CXCL-10 showed an increasing trend in patients with significant fibrosis (P=0.097 • Multivariate analysis showed that • levels of TGF-α (P=0.005, OR=1.064), IL-2R (P=0.008, OR=1.002), CXCL-10 (P=0.038, OR=0.990), and PLT (P<0.001, OR=0.983) were independently associated with significant fibrosis • Multivariate analysis showed TGF-α, IL-2R, platelets, and HBeAg/HBV DNA were independent predictors for significant fibrosis in patients with ALT<2XULN	• IL-2R and TGF-a were independent predictors for significant fibrosis. • An IL-2R and TGF-α-based score fib-index was superior to the existing scores APRI and FIB-4 for predicting significant fibrosis in chronic HBV infected patients. • This represents a promising tool for noninvasive diagnosis of fibrosis in patients with normal and mildly elevated ALT levels.

Variation in pro-inflammatory and anti-inflammatory cytokine expression levels in HBV infected patients and health controls have been reported in earlier studies (F. Wang & Zhang, 2009). Similarly, variation in expression levels both cytokines with HBV DNA viral load have also been reported (Dunn, Maurizia, Gary, Theodoros, & Patrick, 2007). A decreased ability to produce a pro-inflammatory cytokine IL-2 in HBV infected patients with higher viral loads have been observed (Das et al., 2008, Fierro et al., 2011)

This is consistent with the findings reported by Arababadi, Pourfathollah, Jafarzadeh, & Hassanshahi, (2010) and Arababadi, M. K. Pourfathollah, A. A Jafarzadeh, Hassanshahi, G Daneshmandi, S

Shamsizadeh, & Kennedy, (2011) who established a down regulation of the inflammatory cytokines in the serum of HBV-infected patients. However, the findings of Das and colleagues (2008), Fierro and colleagues (2011), Alababadi and co-workers (2010 & 2011) contradicted an earlier finding in the study by Dunn et al., (2007) which showed up regulation pro-inflammatory cytokines IL-8 and TNF-α in patients with higher viral load. So, more studies are warranted to illuminate on the variation in the levels of cytokines in HBV infected patients and normal subjects which is pivotal in understanding the immunopathology of this viral infection.

HLA polymorphisms and HBV disease profile

The contribution of the host genetic factors to the pathogenesis of hepatitis B is poorly understood. Dengming et al., 2015 (Table 5) in an analytical observational case-control study on 1464 CHB infected Han people; China reported that TLR-IFN pathways are associated with susceptibility to CHB infection. They further elucidated that TLR-INF SNPs may be involved in the mechanism behind the observed association between HLA polymorphism and CHB infection (OR: 0.55, P<0.0001). In an earlier study by Fretcher GJ, et al 2011(Table 5), no association of class I MHC alleles were found between individuals with SR and C-HBV but the distribution of class II alleles were significantly higher in CHB compared with SR (OR; 3.76, P<0.006). The HLA class II polymorphisms are pivotal population specific host genetic factors that influence HBV clinical manifestation (Fretcher GJ, et al 2011).

Table 5: HLA polymorphisms and clinical manifestation of hepatitis B

Reference	Methodology	Results	Conclusions
Dengming et al., 2015	• Analytical observational case-control. • 1464 CHB infected Han people, China • HBV DNA was quantitated using TaqMan PCR (Roche, Basel, Switzerland). • 23 genes belonging to the TLR-IFN pathway were used as targets • Gene-gene interaction analysis was done by MDR, V 3.0.2 & GMDR, V 0.7 • SNPStats & SPSS 18.0 were used to obtain OR at 95%CI & P<.05 • Multiple logistic regression models were used in analysis of genotypes	• 39 SNPs were identified. • Significant association between SNP and susceptibility to CHB infection. • (OR=0.55, P<0.0001)	• TLR-IFN pathways are associated with susceptibility to CHB infection • TLR-INF SNPs may be involved in the mechanism behind the observed association between HLA polymorphism and CHB infection
Fretcher GJ, et al 2011	• Case-control study: • SR- group (n=150), CHB group (n=137) • HBV DNA detected by real-time PCR (Artus HBV RG PCR Kit; Qiagen) • HLA typing of class I done & class II alleles was done by AllSet+ Gold SSP (Invitrogen, Brown Deer, WI, USA) • Multivariable logistic regression analyses were perfomed for each of the three genetic models (codominant, dominant & recessive) to estimate the independent effect of polymorphisms on disease phenotypes • Statistical analysis was done using STATA 10 (Statacorp, College station, TX, USA)	• No association of class I (A & B) MHC alleles were found between individuals with SR and C-HBV • The distribution of class II (DR) alleles (HLA-DRB1*07:01) were significantly higher in CHB compared with SR (OR; 3.76, P<0.006) • The allelic frequency of HLA-DRB1*03:01 was higher in SR than in CHB group (OR: 0.08, P=0.007)	• The HLA-DRB1*07:01 allelic polymorphism is strongly associated with CHB infection in the South Indian population
Tao Xu et al., 2017	• Meta analysis on studies; • Evaluating the association between HLA-DQ polymorphisms rs285671 or rs7453920 • Case-control • Having detailed genotype data needed to calculate OR • Having genotype distribution and control group in Hardy-Weinberg equilibrium • Data collection • The first author, year of publication, country, genotyping method & genotype numbers of HBV infection & control group were noted • Statistical analysis • STATA version 12.0 was used • X2 & I2 used for heterogeneity tests • Publication bias was assessed using Begg's and Egger's test • All analyses done at P<0.05	• 9 articles were used for rs2856718 SNP of G allele • 7 articles met the criteria for rs7453920 SNP for A allele • Pooled risk estimate indicated that G alleles were associated with decreased risk of HBV infection (G vs A: OR=0.65, P<0.001) • For HBV clearance G allele had significantly lower chance of natural clearance upon HBV infection (G vs A: OR=0.75, P=0.002) • Pooled risk estimate indicated that A alleles were associated with decreased risk of HBV infection (A vs G: OR=0.66, P<0.001) • For HBV clearance A allele had significantly lower chance of natural clearance upon HBV infection (A vs G: OR=0.61, P<0.001)	• HLADQ rs2856718-G is beneficial against HBV infection • HLADQ rs2856718-A serves as a risk factor in HBV infection. • The HLA-DQ rs7453920-A allele was a protective factor for chronic HBV infection, • HLADQ rs7453920-G serves as a risk factor in HBV infection.

Understanding the influence of HLA polymorphisms on clinic manifestation of HBV will provide better understanding of the molecular mechanisms of HBV persistence and thus provide novel therapeutic potential to manage CHB.

In a recent meta-analysis by Xu, Sun, & Wang,(2017), they reported that polymorphisms in G (rs2856718) and A (rs7453920) alleles of HLA-DQ had different effect on the risk of HBV infection compared to the controls. Allele G was significantly associated with decreased risk to HBV infection whereas allele A was not associated with risk of CHB. This contradicted an earlier finding by Al-Qahtani, Al-Anazi, & Abdoet, (2014). Many studies posit that SNPs in HBV related genes can contribute to increased risk to HBV infection by altering gene expression (L. Yu, Cheng, & Cheng, 2015, L. Wang, Zou, & Wang, 2016). However, in the meta-analysis by Xu et al., (2017), HLA DQ- A allele was found to confer protection against progressing to CHB.

VDR gene polymorphism and in HBV infection

Table 6: Effect of vitamin D receptor gene polymorphisms and HBV infection and severity of the disease

Reference	Methods	Results	Conclusion
Pathokamuri V.S et al 2006	• Case-control (n=622) • Analysis: X2 and logistic regression • All analyses were done by SPSS version 12.0 at p<0.05	• VDR genetic polymorphism were significantly more common in patients with; • severe liver disease compared to mild disease (P<0.05) • high viral load (P=0.002)	• polymorphisms in VDR genes are associated with; • susceptibility, • severity • and viral persistence
Peng, Q et al 2014	• Case-control (n=660) • The SNPs of VDR rs2228570 , rs3782905 & rs11568820 were detected • Method: RFLP-PCR & SSP-PCR • Analysis: NOVA & X2 at P<0.05	• The rs22285570 T allele was associated with significant increased HCC risk as compared with C allele (OR=1.49, P=0.022) • The rs2228570 TT and the rs2228570 TT/TC genotypes were correlated with significant increased HCC risk when compared with the wild type CC homozygote (OR=1.82, P=0.039)	• VDR rs2228570 polymorphism is associated with a significantly increased risk of HBV-related HCC in the Chinese population
Xing Yao., 2013	• Hospital based case-control • Patients: (n=968 CHB; 436 HCC & 532 non HCC) • Controls: (n=132 HCC with no HBV) • VDR polymorphisms determined by RFLP-PCR • Statistics: • HWE analyzed by X2 • OR estimated by MLR at 95% CI, P<0.05 • SAS version 9.1 used for data analysis	• The genotype frequencies of all SNPs in the control patients were in HWE equilibrium • The genotype frequency of VDR FokI C>T polymorphism were significantly different between HCC and non HCC groups (P<0.05) • For SNPs of BsmI, ApaI & TaqI, the genotype and allelele frequencies did not significantly differ between HCC & non HCC group (all P>0.05)	• Only FokI C>T polymorphisms were associated with HCC susceptibility in the study population ; other SNPs were not related with the development of HCC

VDR, vitamin D receptor; HBV, hepatitis B virus; SNPs, single nucleotide polymorphisms; RFLP-PCR restriction frafment length polymorphism- polymerase chain reaction Many studies have linked VDR gene polymorphisms to susceptibility, severity and persistence of CHB including Parthokamuri and co workers (Table. 6), Peng, Q et al (n= 660, SNPs of VDR rs2228570, rs3782905 & rs11568820; Method: RFLP-PCR & SSP-PCR; Analysis: ANOVA & X^2 at P<0.05) and Xing Yao et al (n=968 CHB; 436 HCC & 532 non HCC-patients and n=132 HCC with no HBV-controls; VDR polymorphisms determined by RFLP-PCR; HWE analyzed by X2, OR estimated by MLR at 95% CI, P<0.05).

Pathokamuri and colleagues reported that VDR gene polymorphisms are more prevalent in CHB patients with severe liver disease as compared to those with mild disease presentation (P<0.05) as well as those manifesting with high viral load (P=0.002) increasing the risk of progressing to HCC. The association of the SNPs in VDR gene to carcinoma has been reported in other studies; breast cancer (Curran et al., 1999), multiple myeloma (Shafia, Qasim, Aziz, Bhat, & Nisar, 2013) and epithelial ovarian cancer (Mohapatra, Saxena, Gandhi, Koner, & Ray, 2013).

The study by Peng, Q et al., 2014, reported that VDR gene polymorphism in the rs22285570 T allele was associated with significant increased HCC risk as compared with C allele (OR=1.49, P=0.022). They further highlighted that VDR polymorphisms in the genotypes rs2228570 TT and rs2228570 TT/TC were correlated with significant increased HCC risk when compared with the wild type CC homozygote (OR=1.82, P=0.039). The rs2228570 polymorphism is proximal to the 5' terminus of the VDR gene and it has been implicated in the formation of a variant protein with three additional amino acids at the amino terminus of the polypeptide chain (Uitterlinden, Fang, Van Meurs, Pols, & Van Leeuwen, 2004). This altered VDR protein conformation affects the biological function of vitamin D and is the most likely cause of cancer as reported in an earlier study by Arai, Miyamoto, Taketani, Yamamoto, & Iemori, (1997).

The role of rs2228570 VDR gene polymorphism in hepatocellular carcinogenesis reported in the study by Peng, Q and colleagues (Table 6) has been highlighted in other studies including multiple myeloma reported by Shafia et al., (2013) and epithelial ovarian cancer reported by Mohapatra et al., (2013). This is further supported by the study by Xing Yao., 2013 (Table 6) who reported that only FokI (rs2228570) C>T polymorphisms were associated with HCC susceptibility in the study population; other SNPs were not related with the development of HCC. However these studies were hospital based which guaranteed selection bias, so studies with large number of study subjects including sampling from households are warranted.

CONCLUSION

This review article has underlined the host immunological and genetic factors as well as the viral factors influencing the fate of chronic hepatitis B infection. The article has quoted studies which has left several questions unanswered hence leaving knowledge gaps. Many studies posted contradicting findings warranting more studies in this area. The paper has outlined the methodology used in the various studies and has questioned the methodology used in some of the studies giving opportunity for researchers to replicate the studies with improvements. We declare that none of the authors has any competing interest

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