

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

5,300

Open access books available

129,000

International authors and editors

155M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Molecular Variants for HBsAg: Surface and Subtype

Johra Khan

## Abstract

Hepatitis B is a worldwide healthcare problem, especially in developing areas. An estimated one-third of the global population has been infected with this virus; approximately 350 million people are lifelong carriers, and only 2% spontaneously seroconvert annually. Hepatitis B virus (HBV) belongs to the hepadnavirus family of enveloped DNA viruses containing a partially double-stranded genome of  $3182 \pm 3221$  bp depending on the genotype that encodes four overlapping open reading frames. HBV is classified into eight genotypes (A–H) that are geographically dispersed. Genotype A is predominant in North America, Western Europe, and Africa; genotypes B and C in Asia; genotype D in Southern Europe, Africa, and India; genotype E in West Africa; genotype F in Central and South America and Alaska; genotype G has been found in the United States, France, and Germany; and genotype H in Central America. Genotypes A, B, C, and D predominate in the United States, while the other genotypes are less common. Further detailed analysis of these HBsAg variants would provide further understanding of the antigenic structure of HBV.

**Keywords:** genotypes, subtypes, surface antigen, hydrophilic region, HBsAg variants

## 1. Introduction

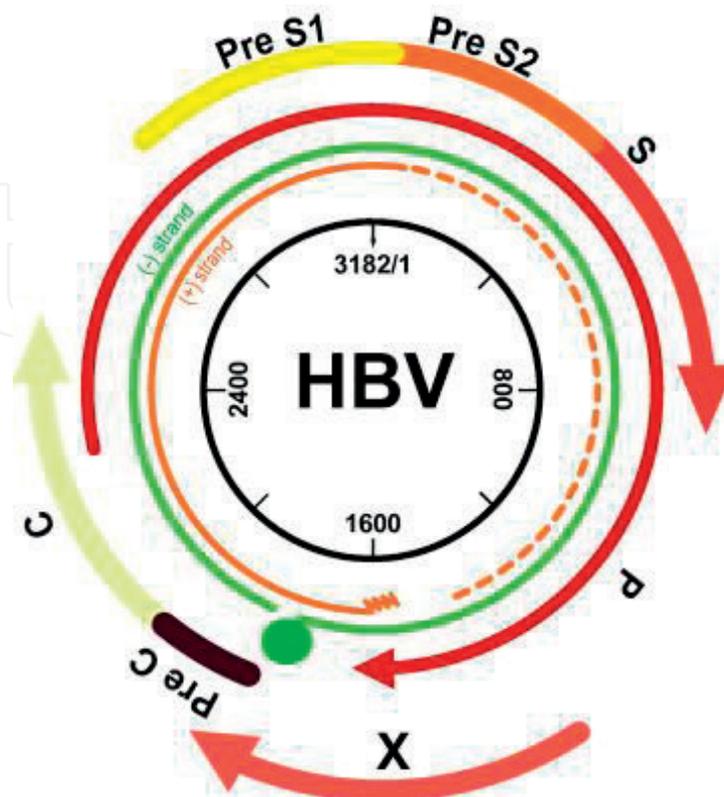
A report by Blumberg in 1965 led to the discovery of hepatitis B surface antigen (HBsAg) which is also known as Australian antigen and its related antibody which is hepatitis B surface antibody or HBsAb [1]. After a gap of 5 years, in 1970, another scientist, Dane, visualized the hepatitis B (HBV). Since that time, a significant development has been made about the epidemiology, virology, natural history, and the treatment of this hepatotropic virus. It is the smallest of the DNA viruses that infect man and cause acute hepatitis of varying severity. It is an extremely resistant strain capable of withstanding extreme temperatures and humidity. It can survive when stored for 25 years at  $-20^{\circ}\text{C}$ , for 24 months at  $-80^{\circ}\text{C}$ , for 6 months at room temperatures, and for 7 days at  $44^{\circ}\text{C}$  [2].

Hepatitis B is a global healthcare problem, especially found in developing countries. As per an estimate, one third of the world population has been infected with this virus which is around 350 million people are enduring carriers and only 2% of them are spontaneously seroconvert yearly [3]. Many current vaccination programs seem to be promising in the effort to reduce the incidence of this disease [4]. HBV is transmitted through hematological and sexual means. The consequence of this infection is a complex viral-host interaction which results in either as an

acute disease with symptoms or an asymptomatic disease. Patients may develop an immunity to HBV or it may enter a chronic carrier state (**Figure 1**). The later consequences of this infection are cirrhosis and then development of hepatocellular carcinoma (HCC) [5]. Hepatitis B virus (HBV) belongs to the hepadnavirus family of enveloped DNA viruses containing a partially double-stranded genome of  $3182 \pm 3221$  bp depending on the genotype which encodes four overlapping and open reading frames which are as follows:

- S for the surface or envelope gene encoding the pre-S1, pre-S2, and the S protein
- C for the core gene, encoding for the core nucleocapsid protein and the “e” antigen
- X for the X gene encoding the X protein
- P for the polymerase gene encoding, a large protein promoting priming, RNA-dependent and DNA-dependent DNA polymerase, and RNase H activities

The genome is read in all three reading frames, and viral regulatory elements are all within coding regions which introduce constraints on the ability of the virus to accept mutations and remain viable [6]. Nevertheless, heterogeneity among the strains of HBV circulating globally is 10%-fold greater than that in the majority of DNA viral genomes. This is explained, at least partially, by the fact that hepadnavirus replication takes place via an RNA intermediate, and reverse transcriptase is known to have a high error rate 16. A nucleotide exchange rate of between 0–1 and 0–7 per year [7] has been estimated for the HBV [8] and woodchuck hepatitis virus (WHV) [9]; genomes, respectively, which is similar

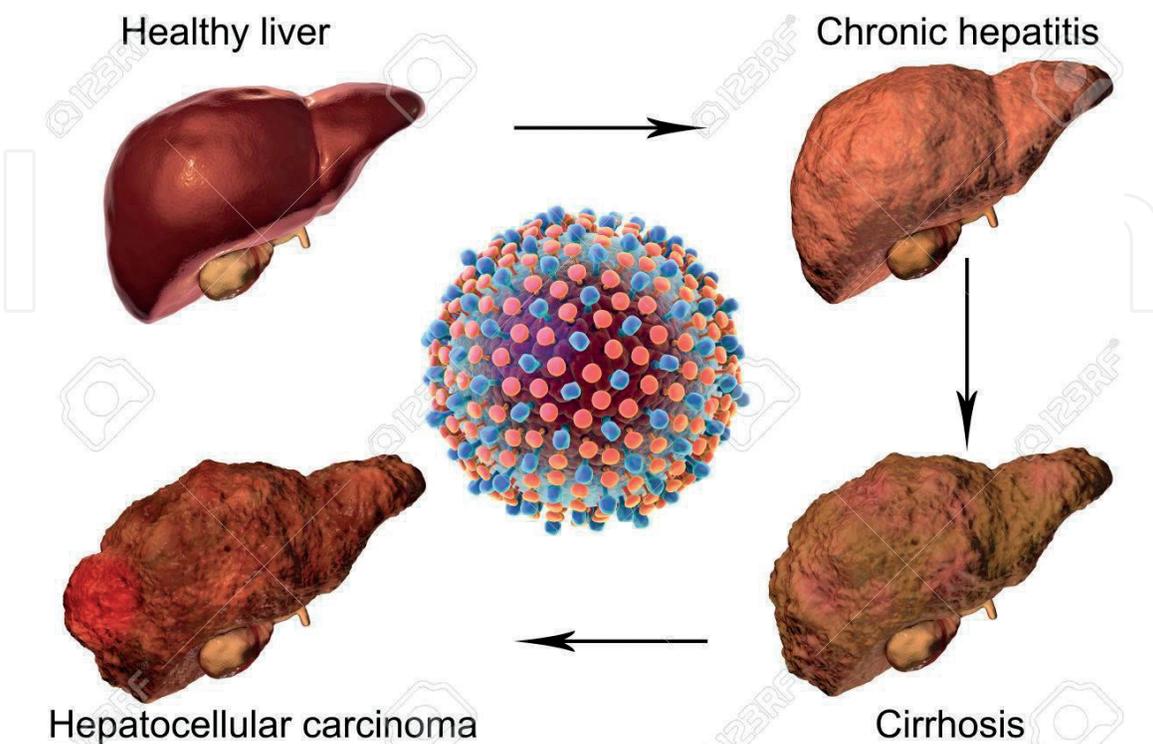


**Figure 1.** HBV genome showing S for the surface or envelope gene encoding the pre-S1, pre-S2, and the S protein.

to the most slowly evolving gene of retroviruses, the gag gene, and one to two orders of magnitude lower than the mutation rates previously calculated for the positive- and negative-strand RNA viruses [10]. The virus persists in 2–10% of adult patients and approximately 90% of infected infants leading to chronic liver disease. In highly endemic areas, infection is predominantly acquired during the perinatal neonatal period or by horizontal transmission in the first few years of life [10–11] which results in a high prevalence of long-term HBV carriers with a low average age at infection [12], the virus has a long time span in which to evolve within its host.

### 1.1 Natural history

Acute infection with HBV in adulthood is rarely associated with the development of potentially fatal fulminant liver failure. Chronic infection, whether acquired in childhood or in adulthood, is associated with progressive liver disease, risk of cirrhosis, liver failure, and HCC and rarely with extrahepatic manifestations. Chronic HBV infection is characterized by four distinct phases: immune tolerance, immune clearance, inactive carrier state, and reactivation [13]. The immune tolerance phase is characterized by detectable HBeAg, high levels of HBV DNA (>10<sup>5</sup> copies/ml), and normal ALT levels. The immune clearance phase, also called CHB, is characterized by detectable or undetectable HBeAg, undetectable or detectable anti-HBe antibodies, lower or fluctuating levels of HBV DNA, high or fluctuating ALT levels, and active inflammation as seen on liver biopsy. The inactive HBsAg carrier state is characterized by detectable HBsAg, undetectable HBeAg, detectable anti-HBe antibodies, low levels of HBV DNA (<10<sup>4</sup> copies/ml), and normal ALT levels. Later in the carrier phase, HBsAg may become undetectable, and anti-HBs antibodies may appear [13], although reactivation can occur in inactive HBV carriers (Figure 2).



**Figure 2.** Different stages of HBV infection. [https://www.123rf.com/photo\\_83543016\\_stock-illustration-liver-disease-progression-in-hepatitis-c-virus-infection-3d-illustration.html](https://www.123rf.com/photo_83543016_stock-illustration-liver-disease-progression-in-hepatitis-c-virus-infection-3d-illustration.html).

## 2. The surface gene and variants

HBV is classified into eight genotypes (A–H) that are geographically dispersed. Genotype A is predominant in North America, Western Europe, and Africa; genotypes B and C in Asia; genotype D in Southern Europe, Africa, and India; genotype E in West Africa; genotype F in Central and South America and Alaska; genotype G has been found in the United States, France, and Germany; and genotype H in Central America. Genotypes A, B, C, and D predominate in the United States, while the other genotypes are less common. 31 Genotype B is associated with less active disease, slower progression, and lower incidence of hepatocellular carcinoma (HCC) than genotype C. Genotypes A and B generally respond better to treatment with interferon than do genotypes C and D. No relation between HBV genotypes and response to nucleos(t)ide analogue-based therapies has been demonstrated 31.

Originally, four genotypic groups of HBV (A–D) were defined, based on an inter-genotypic divergence score of  $8 \pm 5$ – $10 \pm 0\%$  between 18 complete genomes, as compared to a score of  $1 \pm 1$ – $2 \pm 7\%$  between isolates within the same genotype [14]. This genotypic classification was extended to six genotypes (A–F) by phylogenetic analysis of 122 surface antigen (HBsAg) genes [15]. The genotypic groups are geographically arranged [16] with genotypes B and C confined to Asia, while genotype A predominates in Northern Europe giving way to genotype D as one moves toward the Mediterranean region. Genotype E is mainly found in parts of East, Central, and West Africa, and genotype F is only found in the New World and the Pacific which is also home to the Cq—subgroup of genotype C [17]. Two subgroups of genotype A, subgroups A and A, were found in approximately equal amounts in an urban population from South Africa together with 10% of genotype D [17].

In the recent advances in molecular diagnostic techniques, it was found that HBV envelop is made of host-derived bilayer of phospholipids encoded by S gene. c1.

## 3. The surface gene variants

The mutation in HBV depends on the high rate of replication of this virus which in turn depends on RNA- dependent DNA polymerase. In highly variant individuals, the mutation of genome can be up to  $10^{10}$  per day. Whereas most of the variants produced are defective, some of them can cause infection or reason for treatment failure.

### 3.1 HBsAg variants

In the structure of virus, the coat of the outer surface is made up of hepatitis B surface proteins which are made in larger amounts than needed by the virus to its reproduction process. The excess amount of these surface proteins clusters together into some spherical units of size between 17 and 25 nm of diameter, and sometimes it also forms rods of different lengths. It is found that in some studied cases, these units condense as a core particle to produce a whole and infectious virus unit that passes into the blood stream and can also infect many other healthy liver cells. All the different structures like extra spheres, rodlike and also sometimes complete viral particles easily move in the blood stream in large amount and can be easily detected; only it takes little long time for these protein particles to appear in blood.

The incubation time for this hepatitis B Virus (hepatitis B) can be between 6 and 25 weeks. It was found that after infection for up to 1 to 6 weeks before symptoms start occurring, HBsAg appears. To confirm the presence of hepatitis B infection, the positive result for the presence of hepatitis B surface protein (HBsAg) is the

available standard test to indicate current infection. If the hepatitis B surface protein (HBsAg) remains present for a time of more than 6 months, it is generally considered as an indicator for chronic infection.

It is reported that if excess of HBs proteins is produced, it may allow infectious hepatitis B virus particles to leak the immune system by mopping some low levels of surface antibodies that may be produced by the immune system due to its 145 amino acid which is glycine that changes to arginine (G145R) [18].

### **3.2 Hepatitis B core protein (HBcAg)**

The HBc proteins link together to form the hepatitis B core that encapsulates HBV DNA and DNA polymerase; this core is in turn encapsulated by HBs proteins.

The core protein (HBc) is not detectable in the bloodstream; however it can be detected in the sample of liver cells taken after a liver biopsy.

### **3.3 HBe protein (HBeAg or “e” antigen)**

The Hepatitis B “e” antigen (HBeAg) is a peptide and normally detectable in the bloodstream when the hepatitis B virus is actively reproducing; this in turn leads to the person being much more infectious and at a greater risk of progression to liver disease. The exact function of this nonstructural protein is unknown; however it is thought that HBe may be influential in suppressing the immune system response to HBV infection. HBeAg is generally detectable at the same time as HBsAg and disappears before HBsAg disappears. The presence of HBeAg in chronic infection is generally taken to indicate that HBV is actively reproducing and there is a higher probability of liver damage. In acute infection HBeAg is generally only transiently present [19].

However mutant strains of HBV exist that replicate without producing HBeAg. In many cases infection with these mutant strains is more aggressive than HBe producing strains [20].

## **4. Variants associated with antiviral therapy**

Polymerase variants are another class of variants detected during therapy with nucleoside analogues which become the reason for drug resistance. The most recognized mutation affecting lamivudine drug is due to change in 204 amino acid from methionine to valine or isoleucine (M204 V/I) which sometimes occurs with another change at 180 amino acid position (L180 M). This change helps in replication and healthy survival of the mutant.

In HBV-infected patients during liver transplant therapy, antiviral prophylaxis is combined with HBIG drug as the polymerase gene and surface gene in HBV have the same regions of the genome; although read in a different frame, the mutation in one can force the other for it. This could lead to complications in selection of mutants for both HBsAg and HBV polymerase [21].

### **4.1 HBV variants associated with active immunization**

Active immunization is the most effective way to control the prominent cause of hepatitis, human hepatitis B virus (HBV). The highly antigenic hepatitis B surface antigen (HBsAg) is directly related to induce the humoral immune response, which on the other hand provides immunity against HBV infection. Neutralizing B cell epitopes are believed to be due to changes in position 124 to 147 of HBsAg, defined as the “a” determinant [22].

In recent years, HBV variants with mutations on the “a” determinant have been recognized following vaccination. These mutants are proficient in independent replication and lead to active infection [23–25]. These mutations have been identified at various positions on the “a” determinant, the most often identified being the glycine-to-arginine change at position 145 (G145R) of HBsAg [25–31].

The most important is that some of the mutants existed before the vaccination program is introduced. These include changes of amino acids at positions 126, 129, 133, and 145 [29, 31]. Some of these are naturally occurring HBsAg variants which are transmissible and are able to infect even the vaccinated population [29, 31]. HBsAg variants have recently been recognized on the major hydrophilic loop of HBsAg (aa 100–160) but outside the conventional “a” determinant [32]. These new mutants can occur in both the vaccinated and unvaccinated individuals in a population and are not able to be neutralized by the presently available antibodies.

## 5. HBsAg in liver transplantation

The patients suffering with chronic HBV contagion are having high risk of developing cirrhosis and hepatic liver failure and having hepatocellular carcinoma. The well-known orthotopic liver transplantation (OLT) procedure is a well-established treatment for liver failure and other hepatocellular carcinoma patients. The occurrence of HBV infection creates many exceptional issues with patients undergoing orthotopic liver transplantation treatment. The inability to get treatment and HBV reinfection occurs in almost 75–80% of persons who undergo OLT treatment. Recurrence of HBV infection even after OLT repeatedly followed by aggressive clinical treatments and also related with a major decrease in successful graft and the rate of patient recovery. In recent times some new antiviral therapies and prophylactic schemes using hepatitis B immune globulin (HBIG) have been established to decrease the risk of recurrence of HBV infection in OLT patients.

### 5.1 Liver transplantation after treatment of HBV infection

There are numerous aims of providing antiviral therapy to patients with cirrhosis which is secondary to prolonged HBV infection. The patients who have with compensated cirrhosis, they can get antiviral therapy which can inhibit development to decompensated cirrhosis and eradicate the possible requirement for OLT treatment. The antiviral therapy can effectively decrease the risk of advancement to hepatocellular carcinoma.

For patients having decompensated cirrhosis, antiviral therapy can improve liver function which can also delay the need for transplantation. If the patient's condition progresses to the point that transplantation is needed, the aim of antiviral therapy is to minimize the HBV risk at the time of transplantation, thereby controlling the risk of recurrence of HBV infection after OLT.

#### 5.1.1 Major therapies for minimizing HBV infection or recurrence

**IFN- $\alpha$ :** Before starting treatment with IFN- $\alpha$ , the hazards and uses related to IFN- $\alpha$  therapy must be carefully assessed in patients having cirrhosis with chronic HBV infections. IFN- $\alpha$  treatment is related with a burst in serum aminotransferases in 35–55% of already treated patients. For patients having progressive liver disease, IFN- $\alpha$  rehabilitation may increase the hepatic breakdown, so it has to be avoided. IFN- $\alpha$  treatment can aggravate cytopenia and additional intensification the risk of severe bacterial toxicities. IFN- $\alpha$  treatment can be provided safely by keeping

close monitoring system for the patients having compensated cirrhosis [33]. Some researchers prove that up to 31% of the cirrhotic HBV patients cured using IFN- $\alpha$ -2b had showed seroconversion of antibody related to hepatitis B antigen, and they lose of measureable HBV DNA, in response rates which are similar to that of the non-cirrhotic patients. Various studies had shown that the cirrhotic patients who have lost HBeAg show superior up to 10 years of survival rate in comparison to the patients who had shown no response to IFN- $\alpha$  therapy [34]. Some of the researchers had shown in their research that the rate of incidence of hepatocellular carcinoma may be lesser in patients who had received treatment using IFN- $\alpha$ , generally in the subcategory of patients who had cleared HBV DNA from their blood serum [35, 36]. Generally, the risks related to IFN- $\alpha$  treatment and the occurrence of harmless and well-tolerated oral antiviral therapies have reduced the usefulness of IFN- $\alpha$  treatment in patients having cirrhosis. Pegylated IFN- $\alpha$ -2a was newly accepted by the US Food and Drug Administration to be used for the treatment of chronic hepatitis B. A few related data is presently available showing the use of pegylated IFN- $\alpha$  in relation to cirrhotic patients having hepatitis B.

**Lamivudine:** It is based on nucleoside analogue which is an effective inhibitor of HBV DNA replication process. It was one of the first accepted orally administered medications to be used for the treatment of patients having chronic HBV infection. It has also confirmed an exceptional safety picture in both the compensated and uncompensated patients of cirrhosis. In patients suffering from decompensated cirrhosis because of HBV infection, lamivudine is shown to be a safe and effective drug therapy. Previous researches having uncontrolled case studies show the conflicted results for lamivudine treatment which was shown to delay advancement to death or toward liver transplantation [37, 38]. Liaw et al. [39] stated the results related to a large, potential, multicenter randomized trial based on the study of 651 patients who had chronic hepatitis B, and bridging fibrosis was randomized to obtain any of the lamivudine or placebo. The basic points of this research was time essential for the disease development, which is also known as hepatic compensation, hepatocellular carcinoma, spontaneous bacterial peritonitis, variceal bleeding, or death associated with liver disease. After they receive a treatment for a period of 32.4 months which can range from 0 to 42 months in which a substantially larger percentage of patients in the placebo group in contrast to the lamivudine group developed disease advancement (17.7% vs. 7.8%; Pp. 001), because of which the study was finished early. Moreover, hepatocellular carcinoma arose in a less percentage of patients in the lamivudine-treated individual than in the placebo group (3.9% vs. 7.4%, Pp .047). The results show that based on lamivudine is more effective in reducing the occurrence of hepatic decompensation and hepatocellular carcinoma in patients who have chronic HBV infection and also progressive fibrosis or cirrhosis. The main aspect reducing the use of lamivudine is that it causes the increase of mutations in the YMDD motif of the HBV DNA polymerase gene, which develops resistance against lamivudine. YMDD mutations are emerging with a rate of  $\sim$ 20% every year against lamivudine treatment which are related to the return of active viral replication [40]. The findings of lamivudine resistance in clinical setting vary according to the rigorousness of underlying liver damage. The initial sign related to resistance usually occurs as a recoil in the HBV DNA level, lacking any other irregular biochemical or clinical results. However, advanced liver failure has been defined in connotation with the occurrence of YMDD mutations.

So, patients with cirrhosis need closely observing during their receiving prolonged lamivudine therapy.

**Adefovir dipivoxil:** An oral prodrug of adefovir which is a nucleotide analogue of adenosine monophosphate that inhibits HBV DNA polymerase. Many studies show that adefovir has tremendous activity against wild-type as well as

lamivudine-resistant HBV strains. Researchers evaluated the safety and efficacy of adefovir dipivoxil (10 mg daily) in 128 patients having lamivudine-resistant HBV and are waiting for liver transplantation. Treatment for a period of 48 weeks managed to cause an average decrease in HBV DNA titer of 4.1 log<sub>10</sub> copies/ml, where as in non-detectable HBV DNA (by PCR; lower limit of detection, 400 copies/ml), it was in 81% and showed better Child-Pugh score which is up to 92%. Adefovir dipivoxil treatment can generally be accepted very finely with an increase in their serum creatinine level up to 10.5 mg/dL from baseline in around 12% of the patients. No patient is required to stop adefovir dipivoxil therapy due to nephrotoxicity. The survival rate after 48 weeks was 84% which is significantly better than the rate for historical control subjects.

**Other oral agents:** Entecavir a new guanosine nucleoside analogue which was recently accepted by the US Food and Drug Administration for the treatment of chronic hepatitis B. Although no publication is done, but a huge phase III research has proven that HBeAg-positive patients provided with entecavir (0.5 mg daily for 48 weeks) showed a mean change in HBV DNA titer of  $-6.98$  log<sub>10</sub> copies/ml, which was considerably better than that was found with lamivudine [33]. Entecavir has also revealed activity against lamivudine- and adefovir dipivoxil-resistant HBV strains. Still there are no exact data available on the use of entecavir in patients with decompensated cirrhosis or in association with liver transplantation.

## **6. Conclusion**

HBsAg variants with changes outside the “a” identification of these new HBsAg variants and their functional analysis may provide further understanding of the antigenic structure of HBV; also liver transplantation for hepatitis B is having complication of the risk of recurring HBV infection. It significantly reduces the rates of graft success and patient survival after transplantation. Effective therapy with OLT recipient patients having chronic HBV infection involves management of antiviral therapy before OLT to reduce the hepatitis B viral titer at the time of transplantation.

## **Acknowledgements**

I am grateful to Deanship of Scientific Research, Majmaah University, Al Majmaah 11952, Saudi Arabia, for providing support for this research.

## **Conflict of interest**

I declare that there is no conflict of interest with any or all the above writing.

IntechOpen

IntechOpen

### **Author details**

Johra Khan  
College of Applied Medical Sciences, Department of Medical Laboratories,  
Majmaah University, Al Majmaah, Saudi Arabia

\*Address all correspondence to: [j.khan@mu.edu.sa](mailto:j.khan@mu.edu.sa)

### **IntechOpen**

---

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

## References

- [1] Blumberg BS, Alter HJ, Visnich S. Landmark article Feb 15, 1965: A "new" antigen in leukemia sera. By Baruch S. Blumberg, Harvey J. Alter, and Sam Visnich. *Journal of the American Medical Association*. 1984;**252**(2):252-257
- [2] Dane DS, Cameron CH, Briggs M. Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. *Lancet*. 1970;**1**(7649):695-698
- [3] Pungpapong S, Kim WR, Poterucha JJ. Natural history of hepatitis B virus infection: An update for clinicians. *Mayo Clinic Proceedings*. 2007;**82**(8):967-975
- [4] Shen LP, Yang JY, Mo ZJ, Li RC, Li YP, Wei ZL, et al. Variations of hepatitis B virus infection epidemic pattern after long-term HBV vaccine immunization. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi*. 2007;**21**(3):253-255
- [5] Natural history of chronic hepatitis B virus infection in adults with emphasis on the occurrence of cirrhosis and hepatocellular carcinoma. *Journal of Gastroenterology and Hepatology*. 2000;**15**(Suppl):E25-E30
- [6] Yang Z, Lauder IJ, Lin HJ. Molecular evolution of the hepatitis B virus genome. *Journal of Molecular Evolution*. 1995;**41**:587-596
- [7] Boyer JC, Bebenek K, Kunkel TA. Unequal HIV-1 reverse transcriptase error rates with RNA and DNA templates. *Proceedings of the National Academy of Sciences, USA*. 1992;**89**:6919-6923
- [8] Gunther S, Fischer L, Puli I, Sterneck M, Will H. Naturally occurring variants of hepatitis B. In: *Advances in Viral Research*. 1999. pp. 25-137
- [9] Okamoto H, Imai M, Kametani M, Nakamura T, Mayumi M. Genomic heterogeneity of hepatitis B virus in a 54-year-old woman who contracted the infection through materno-fetal transmission. *The Japanese Journal of Experimental Medicine*. 1987;**57**:231-236
- [10] Girones R, Miller RL. Mutation rate of the hepadnavirus genome. *Virology*. 1989;**170**:595-597
- [11] Beasley RP, Hwang LY. Epidemiology of hepatocellular carcinoma. In: *Viral Hepatitis and Liver Disease*. 1984. pp. 209-224
- [12] Botha JF, Dusheiko GM, Ritchie MJ, Mouton HWK, Kew MC. Hepatitis B virus carrier state in black children in Ovamboland: Role of perinatal and horizontal infection. *Lancet*. 1984:1210-1212
- [13] Edmunds WJ, Medley GF, Nokes DJ, O'Callaghan CJ, Whittle HC, Hall AJ. Epidemiological patterns of hepatitis B virus (HBV) in highly endemic areas. *Epidemiology and Infection*. 1996;**117**:313-325
- [14] Yim HJ, Lok AS. Natural history of chronic hepatitis B virus infection: What we knew in 1981 and what we know in 2005. *Hepatology*. 2006;**43**(2 suppl 1):S173-S181
- [15] Chandra R, Kapoor D, Agarwal SR, Malhotra V, Sakhuja P, Sarin SK. Profile of asymptomatic chronic HBV infection in India. *The Indian Journal of Medical Research*. 2002;**116**:50-57
- [16] Satapathy SK, Garg S, Chauhan R, Malhotra V, Sakhuja P, Sharma BC, et al. Profile of chronic hepatitis B virus in children in India: Experience with 116 children. *Journal of Gastroenterology and Hepatology*. 2006;**21**(7):1170-1176
- [17] Nayak NC, Dhar A, Sachdeva R, et al. Association of human hepatocellular carcinoma and cirrhosis

with hepatitis B virus surface and core antigens in the liver. *International Journal of Cancer*. 1977;**20**:643-654

[18] Tandon BN, Acharya SK, Tandon A. Epidemiology of hepatitis B virus infection in India. *Gut*. 1996;**38**(suppl 2):S56-S59

[19] Alter HJ, Holland PV, Purcell RH, et al. Posttransfusion hepatitis after exclusion of commercial and hepatitis B antigen-positive donors. *Annals of Internal Medicine*. 1972;**77**:691-699

[20] Liu C-J, Chen D-S, Chen P-J. Epidemiology of HBV infection in Asian blood donors: Emphasis on occult HBV infection and the role of NAT. *Journal of Clinical Virology*. 2006;**36**(Suppl. 1):S33-S44

[21] Margolis HS, Alter MJ, Hadler SC. Hepatitis B: Evolving epidemiology and implications for control. *Seminars in Liver Disease*. 1991;**11**:84-92

[22] Lok AS, McMahon BJ. Chronic hepatitis B. *Hepatology*. 2007;**45**(2):507-539

[23] Okamoto H, Tsuda F, Sakugawa H, Sastrosoewignjo RI, Imai M, Miyakawa Y, et al. Typing hepatitis B virus by homology in nucleotide sequence: Comparison of surface antigen subtypes. *Journal of General Virology*. 1988;**69**:2575-2583

[24] Norder H, Hammas B, Lee SD, Bile K, Courouce AM, Mushahwar IK, et al. Genetic relatedness of hepatitis B viral strains of diverse geographical origin and natural variations in the primary structure of the surface antigen. *Journal of General Virology*. 1998;**74**:1341-1348

[25] Samuel D, Muller R, Alexander G, et al. Liver transplantation in European patients with the hepatitis B surface antigen. *The New England Journal of Medicine*. 1993;**329**:1842-1847

[26] Kim WR, Poterucha JJ, Kremers WK, et al. Outcome of liver transplantation for hepatitis B in the United States. *Liver Transplantation*. 2004;**10**:968-974

[27] Perrillo RP, Schiff ER, Davis GL, et al. A randomized, controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. The hepatitis interventional therapy group. *The New England Journal of Medicine*. 1990;**323**:295-301

[28] Lau DT, Everhart J, Kleiner DE, et al. Long-term follow-up of patients with chronic hepatitis B treated with interferon alfa. *Gastroenterology*. 1997;**113**:1660-1667

[29] Lin S-H, Sheen I-S, Chien R-N, et al. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. *Hepatology*. 1999;**29**:971-975

[30] Ikeda K, Kobayashi M, Saitoh S, et al. Significance of hepatitis B virus DNA clearance and early prediction of hepatocellular carcinogenesis in patients with cirrhosis undergoing interferon therapy: Long-term follow up of a pilot study. *Journal of Gastroenterology and Hepatology*. 2005;**20**:95-102

[31] Villeneuve JP, Condreay LD, Willems B, et al. Lamivudine treatment for decompensated cirrhosis resulting from chronic hepatitis B. *Hepatology*. 2000;**31**:207-210

[32] Fontana RJ, Keeffe EB, Carey W, et al. Effect of lamivudine treatment on survival of 309 north American patients awaiting liver transplantation for chronic hepatitis B. *Liver Transplantation*. 2002;**8**:433-439

[33] Liaw YF, Sung JJ, Chow WC, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. *The New England Journal of Medicine*. 2004;**351**:1521-1531

[34] Leung NW, Lai CL, Chang TT, et al. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: Results after 3 years of therapy. *Hepatology*. 2001;**33**:1527-1532

[35] Carman WF, Zanetti AR, Karayiannis P, et al. Vaccine induced escapemutan of hepatitis B virus. *Lancet*. 1990;**336**:325-329

[36] Ogata N, Zanetti AR, Yu M, Miller RH, Purcell RH. Infectivity and pathogenicity in chimpanzees of a surface gene mutant of hepatitis B virus that emerged in a vaccinated infant. *The Journal of Infectious Diseases*. 1997;**175**:511-523

[37] Carman WF, Korula J, Wallace L, MacPhee R, Mimms L, Decker R. Fulminant reactivation of hepatitis B due to envelope protein that escaped detection by monoclonal HBsAg ELISA. *Lancet*. 1995;**345**:1406-1407

[38] Hsu HY, Chang MH, Ni YH, Lin HH, Wang SM, Chen DS. Surface gen mutants of hepatitis B virus in infants who develop acute or chronic infections despite immunoprophylaxis. *Hepatology*. 1997;**26**:786-791

[39] Protzer-Knolle U, Naumann U, Bartenschlager R, et al. Hepatitis B virus with antigenically altered hepatitis B surface antigen is selected by high dose hepatitis B immune globulin after liver transplantation. *Hepatology*. 1998;**27**:254-263

[40] Carman WF, Trautwein C, Van Deursen FJ, et al. Hepatitis B virus envelope variation after transplantation with and without hepatitis B immune globulin prophylaxis. *Hepatology*. 1996;**24**:489-493