

An Overview of Hepatitis B Virus Surface Antigen Mutant in the Asia Pacific

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Abstract

Hepatitis B virus infection is a serious health problem worldwide, and more than 350 million people are chronic carriers, constituting a major global threat. Southeast Asia and the Western Pacific have the highest levels of endemicity in the world, with an estimated seroprevalence ranging between 2% and 31%. Mutations in the hepatitis B surface antigen (HBsAg) have been reported in many parts of the world but are most common in Asian infants; such mutants have several clinical effects, such as the development of hepatocellular carcinoma. Diagnostic failures by commercial assays have reduced the diagnostic effectiveness of HBsAg detection. For example the substitution of an amino acid in the major hydrophilic region of the S gene reduces the binding of hepatitis B surface antibodies leading to immune escape. The safety of blood transfusion may be compromised by current screening tests due to escape from being neutralised by antibodies induced by HBsAg mutants, and undetectable levels of viral surface protein. Data on the epidemiology of HBsAg mutation in Asia Pacific are scant; however, this manuscript has reviewed the available information on the epidemiology of HBsAg mutation in Asia Pacific.

Introduction

Asia Pacific is made up of countries in Southeast Asia, East Asia and Oceania (World Macro Regions and Components, 2009). Hepatitis B is a global health problem with about 350 million chronic carriers constituting a major global threat (McMahon, 2005). According to the World Health Organisation (WHO), the western pacific region accounts for about 50% of the world's chronic hepatitis B infection (Clements et al., 2006). Although hepatitis B global endemicity can be divided into high (>8%), intermediate (2-7%) and low (<2%) (Chen Chien-Jen, 2000), there is substantial variation amongst countries of the same continent. Asia Pacific was previously categorised as a high endemic region, but now only a few countries like Vietnam and Laos remain in this category in the region with a prevalence of 8.8% and

8.7%, respectively (Jutavijittum et al., 2007). Taiwan, Japan, Australia, New Zealand, Thailand and Malaysia were also categorised as intermediate endemic areas, but due to the introduction of vaccination with effective coverage, they are now considered low endemic areas (Chen et al., 2010; Clements et al., 2006; Ng et al., 2005; Olinger et al., 2008), while China, Indonesia, Pakistan, Singapore and Cambodia are classified as intermediate endemic areas in the region (Abbas et al., 2004; Hong et al., 2010; Lu et al., 2010; Soeung et al., 2009). Although the majority of the countries in Asia Pacific fall within the low endemic (<2%) area as shown in figure 1, the region has a mean prevalence of 3.6% (SD 2.9), indicating that Asia Pacific is now an intermediate endemic region. Table 1 shows the prevalence of the hepatitis B surface antigen in the general population of some Asia Pacific countries with the highest rates in Vietnam and Laos. Some Asia Pacific countries remain the location for the majority of HBV infections in the world, with Vietnam, South Korea and the Philippines accounting for 75% of chronic HBV infections worldwide (Clements et al., 2006). The predominant mode of transmission in Asia Pacific is perinatal, and the disease is transmitted vertically during early childhood from the mother to infant (Dwivedi et al., 2011; Shao et al., 2011); however, both horizontal and vertical transmission have been reported in sub-Saharan Africa (Botha et al., 1984).

In Southeast Asia, about 100 million people are chronic carriers of HBV, with an estimated annual mortality of

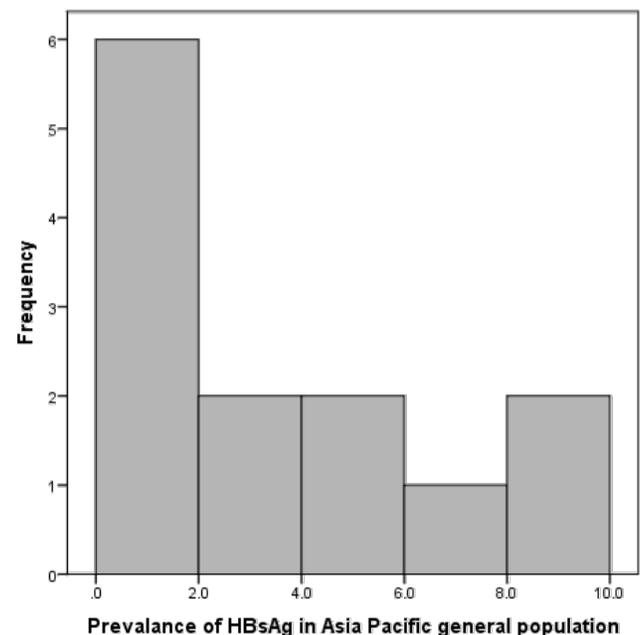


Figure 1. The distribution of hepatitis B surface Antigen positive individuals in the Asia Pacific population.

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Table 1: Prevalence of HBsAg in the Asian general population

Countries	HBsAg in general population (%)	Reference
Vietnam	8.8	Jutavijittum et al., 2007
Laos	8.7	Jutavijittum et al., 2007
China	7.2	Liang et al., 2006
Indonesia	4.6	WHO 2009
Pakistan	4	Abbas et al., 2003
Cambodia	3.5	Soeung et al., 2009
Singapore	2.8	Hong et al., 2010
Japan	<2	Clements et al., 2006
New Zealand	<2	Clements et al., 2006
Taiwan	1.1	Chen et al., 2011
Australia	0.8-1.1	Cowie et al., 2010; O'Sullivan et al., 2004
Thailand	0.7	Chongsrisawat, 2006
Malaysia	0.3	Ng et al., 2005

300,000, mainly due to hepatocellular carcinoma (HCC) and liver cirrhosis. Therefore, all countries in the Asia Pacific Region view hepatitis B as a serious public health issue and maintain policies, goals and plans, targeted at the prevention and control of hepatitis B. However, in most countries, implementation is not sufficient, sometimes following a series of uncoordinated programmes rather than a consistent strategic approach (WHO 2011).

History of Hepatitis B

Hepatitis B Virus (HBV) can be dated back to 1967 with the discovery of an unknown antigen in Australia which was recognised to be associated with hepatitis B. It was first called "Australia antigen" but later referred to as the hepatitis B surface antigen (HBsAg) (Blumberg B. S., 1967).

Hepatitis B Virus

Hepatitis B virus is a Hepatotropic DNA virus belonging to the genus Orthohepadnavirus, family of Hepadnaviridae, and is composed of an envelope, a core, a DNA genome, and a viral polymerase. It has a circular form of partially double-stranded DNA that is approximately 3200 nucleotides in length (Lee and Ahn, 2011; Scaglioni, 1996). The full grown, infective virion of HBV is spherical in shape and 42-45 nm in diameter (Dane particle), meaning that it can be visualised under an electron microscope. It has two-layered shells: the outer shell is the envelope protein referred to as the hepatitis B surface (HBs) protein, which is further divided into small, middle, and large HBs proteins (SHBs, MHBs and LHBs proteins, respectively), and the inner shell is a core protein referred to as the hepatitis B core protein, in which the viral polymerase and the HBV genome are enclosed (Juergen Beck, 2007).

HBV Genotype

HBV genotypes can be localised into different geographical areas and are classified into 10 recognised genotypes (A-J) (Tatematsu et al., 2009; Yu et al., 2010). Genotype A has been found to be dominant in Northern Europe and

North America while genotype D is typically found in the Mediterranean region (Kreutz, 2002). In Asia, genotype A can be found at a high frequency in India, Indonesia and the Philippines (Weinberger et al., 2000). Genotypes B and C are found mainly in the Far East, such as in China, Japan and the South-East Asia region (Huy and Abe, 2004), genotype I is found in Vietnam, Laos, North western China and isolated valleys of northern India, while the 10th genotype, J, has been reported in a Japanese patient (Tatematsu et al., 2009). Therefore, in the Asia Pacific region, four genotypes (B, C, I and J) largely dominate. These genotypes have discrete biological distributions with subtype *adw* found in genotypes A, B, C, F and G, while both *adr* and *ayr* occur in genotype C alongside *adw* (Echevarría and Avellón, 2006).

Pathogenesis of Hepatitis B infection

The pathogenesis of hepatitis B is due to the interaction of the virus and the host immune system in which the immune system attacks HBV and causes liver injury. Activated CD4⁺ and CD8⁺ lymphocytes recognise various HBV-derived peptides located on the surface of the hepatocytes, leading to immunologic reaction, impaired immune reactions (e.g. cytokine release, antibody production) or a relatively tolerant immune status, resulting in chronic hepatitis. In particular, a restricted T cell-mediated lymphocytic response occurs against the HBV-infected hepatocytes (Yang et al., 2008). The first step in HBV infection involves a specific non-cell type primary attachment to the cell-associated heparin sulphate proteoglycans (Schulze et al., 2007). This first reversible attachment step is then followed by an irreversible binding of the virus to a specific, unknown hepatocyte-specific receptor (Urban et al., 2010). This step requires activation of the virus, resulting in exposure of the myristoylated N-terminus of the L-protein. This is a vital determinant for infectivity within the HBV envelope proteins (Engelke et al., 2006). Potential HBV receptor candidates have been described in the past, but none has been confirmed in a functional assay (Glebe and Urban, 2007). However, recent studies indicated that cell polarisation, in addition to the differentiation status of the hepatocytes, plays an important role in the infection process (Schulze et al., 2012).

Diagnosis of hepatitis B

The detection of HBsAg remains the principal diagnostic test for hepatitis B, both in the routine virology laboratory and for blood donation screening; most infected individuals are also positive for antibodies to the nucleocapsid protein (anti-HBc). However, not all carriers of HBsAg have a substantial level of viraemia, this is because the number of infectious virus particles (viremia) is usually higher during the hepatitis B e antigen infection phase and decreases during hepatitis B e antibody (anti-HBe) phase, and subsequently disappears during anti-HBs. The clinical implication of this non viremic hepatitis B infection includes: contribution to progression of liver disease; may cause cryptogenic liver disease; and may contribute potential risk of HBV transmission through blood transfusion, organ transplant and haemodialysis. HBsAg may be produced from HBV DNA and get incorporated into the genomes of the hepatocytes without virus replication. However, a single change in the immunodominant region of HBsAg demonstrates the inconsistency whereby a polyclonal antibody may be more likely to fail to detect

variant HBsAg with single amino acid substitutions than monoclonal antibody-based assays (Bruce and Murray, 1995), and multiple substitutions in HBsAg elucidate the real threat to surface antigen testing. The concern is that a failure to diagnose HBsAg will not only lead to diagnostic dilemmas, but also to transfusion-associated hepatitis B infection. The detection of hepatitis B viraemia is eased by an additional protein, the hepatitis B e antigen (HBeAg), which is secreted by the infected hepatocytes. The presence of HBeAg in serum consistently indicates active virus replication in the liver. Normal HBeAg synthesis is usually altered by presence of mutation in the core and precore region of the HBV DNA (Tong et al., 2005). The most commonly reported mutation in precore involves G to A base pair substitution at nucleotide 1896 region of the HBV genome (Funk et al., 2002). The diagnosis of HBeAg negative in hepatitis B chronic infection is by identification of high HBV DNA load. Precore and core-promoter mutations usually occur spontaneously or treatment-induced clearance of HBeAg. Patients with HBeAg negative hepatitis B chronic infection tend to be older than patients with wild-type infection (Hadziyannis and Vassilopoulos, 2001).

Therefore, screening before vaccination is recommended in areas of high prevalence of HBV infection; although no potential damage can occur when a carrier is vaccinated, it also confers no protection. Besides, these carriers may be miscounseled about their HBV status and to believe that they have been protected while they are not and continue to be a source of infection to others. For those that are immune, vaccination would enhance their antibody levels. Hepatitis B surface antigen and antibodies are widely adopted as a screening test. Antibody to hepatitis B core antigen (anti-HBc) when present indicates past infection but does not distinguish carriers from those who have recovered from an acute infection. A previous study showed that the proportion of patients with prior exposure to HBV was similar whether anti-HBc was measured alone or when HBsAg and anti-HBc were measured simultaneously (Liew et al., 2010).

Treatment

The currently approved hepatitis B infection therapies consist of interferon therapy and an antiviral composed of nucleotide and nucleoside analogues, which potentially provide long-term suppression of viral load; unfortunately, these are only associated with low rates of complete cure. The treatment of chronic hepatitis B is aimed at preventing transmission by eliminating infectivity and the spread of HBV, stopping the progression of liver disease and preventing the development of HCC. Interferon is used in the treatment of Chronic hepatitis B (Parkin et al., 2001). Interferon has antiviral, immunomodulatory, and anti-proliferative effects and is available in forms of pegylated (pegINF) and non-pegylated interferon. Pegylated interferon has a longer half-life than its non-pegylated counterpart, but they enhance T-cell helper activity, enhance the maturation of B lymphocytes, inhibit T-cell suppressors, and enhance human leukocyte antigen (HLA) type I expression.

Hepatitis B Vaccine

There are two types of HBV vaccine: plasma-derived vaccine, which has been in circulation since 1982, and recombinant yeast-derived vaccines which became available in 1986

(Francis et al., 1986). Hepatitis B vaccine containing yeast-derived recombinant HBsAg protein provides an effective means of preventing HBV infection. A seroconversion rate up to 95% has been reported among vaccinees (Emini et al., 1986) and in a study in Thailand, where a yeast-derived vaccine was shown to have protective efficacy of 95% in infants of HBeAg positive mothers (Poovorawan et al., 1990). The HBsAg protein is a highly conformational and cysteine rich "a" determinant (Bertoletti and Gehring, 2006). Antibodies stimulated by active hepatitis B vaccination and anti-HBs antibody present in HBIg are mainly directed to this region of the HBsAg protein (Xu et al., 2010). Conversely, other relevant antigenic epitopes outside the "a" determinant and situated on the surface of the virus have also been described (Chen et al., 1996) Some of these epitopes are situated downstream of the "a" determinant and may be potential neutralisation domains (Irving et al., 2001). Mutations within the "a" determinant can alter the antigenicity of the HBsAg protein which could lead to a failure of anti-HBs antibodies to neutralise HBV (Carman et al., 1997; Hsu et al., 1999). It has been estimated that vaccine-escape mutants will become the dominant HBV quasi species globally (Wilson et al., 1999). Serologic tests carried out after immunisations are intended to evaluate immunity, identifying non-responsive or weak-response individuals. A response is considered absent whenever anti-HBs titres are less than 10 IU/L and ineffective when titres are less than 100 IU/L at least one month, post hepatitis B vaccine booster dose.

Hepatitis B surface antigen

Hepatitis B surface antigen particles consist primarily of a glycoprotein with 226 amino acids that carry the B-cell epitopes essential for the induction of protective antibodies in humans and which confer immunity against hepatitis B infection. It has been clearly shown that the region between amino acids 124 and 147 of the S protein represents the "a" determinants (Zheng et al., 2004) common to all HBV variants and is exposed on the surface of the HBV particle. Anti-"a" antibodies protect adults against the majority of infections irrespective of the subtype of the wild-type virus (Zheng et al., 2004).

Hepatitis B Surface Mutation

Mutations in the HBsAg, also called surface (S) mutations, have several clinical effects, including: (i) reduced sensitivity to available diagnostic tests, (ii) a lack of immunity following vaccination with non-mutant HBV variants (vaccine escaped mutant) and (iii) a failure of passive immunisation with HBV IgG (El Chaar, 2010). The failure of a diagnostic assay may be a major threat to recipients of blood transfusions or organ transplants (Levicnik-Stežinar, 2004; Thakur et al., 2005). Hepatitis B surface mutants are stable and can be spread through either vertical or horizontal transmission (Hunt et al., 2000). In Asian infants, vertical transmission is the most common (2% to 3% of vaccine recipients) (Hunt et al., 2000). This may be due to vaccination and administration of hepatitis B immunoglobulin (HBIg) at birth, exerting evolutionary pressures to select mutants (Wu et al., 2010). However, research has shown that vaccinated children are more likely to harbour mutations than unvaccinated children, possibly due to the fact that neutralisation-resistant HBV variants may be selected

as a result of pressure from the vaccine-induced immune response (Carman et al., 1990). On the other hand, a delay of three months before administration of the vaccine to the child will allow replication of the hepatitis B virus to proceed in the liver, with the mutant emerging possibly even before vaccination. The antibody response to vaccination would then have imposed the selection pressure necessary to allow the "escape" mutant to replicate in an unrestricted manner. However, there is contradictory evidence as to whether escape mutants arise *de novo* in infected infants (in which instance vaccination would have played a direct role in inducing the emergence of the mutants) or whether the mutants, having pre-existed maternally, subsequently undergo selective replication in the infant under immune pressure, hence the need for further study to ascertain the claims.

The common immunodominant B-cell epitope cluster shared by different subtypes of HBV situated within the "a" determinant region (124–147 amino acid), is regarded as the neutralising epitope of the S protein. Single or multiple mutations occurring within the Major Hydrophilic Region (MHR) could lead to a conformational change of this epitope which can affect the antigenicity (Ma and Wang, 2012). Several notable mutations: T/I126S, Q129H, G130N, S143L, D144A, G145A, and G145R (Figure 2), are involved in diagnostic failure, and escape from being neutralised by antibodies induced by available vaccines as well as resulting in a failure of HBVg therapy (Jolivet-Reynaud et al., 2001). On the other hand, mutations outside the "a" determinant region are located primarily at positions 120 and 123. The Pro at position 120 can mutate to Gly/Thr/Ser/Asn/Gln (Jolivet-Reynaud et al., 2001), and mutation at position 123 gives rise to T123N, which is responsible for both diagnostic and HBVg therapeutic failure (Ly et al., 2006).

In Cambodia, the majority of the mutations occur outside the "a" determinant region resulting in amino acid substitutions (Srey et al., 2006). In Vietnam, three M133L mutations have been found in the first loop of the "a" determinant, no mutation was identified within the second loop, while T126M was found in one sample (Thuy et al., 2005). In Singapore, HBsAg mutation was found in neonates of HBsAg positive mothers; 16 (39%) of 41 neonates showed breakthrough infection despite HBVg and a full course of a plasma-derived vaccine being given (Chong-Jin et al., 1999). However, in Thailand, 22.4% of vaccinated and unvaccinated children were found to have varying mutations in the "a" determinant region of HBV (Poovorawan et al., 2012). In Malaysia, mutations were observed in 11% of patients receiving antiviral therapy, of which, 8% carried mutants previously described as vaccine-associated escape mutants and seven strains (9%) carried other types of mutants (Meldal et al., 2011). Although the introduction of hepatitis B vaccination program in Asia Pacific countries has been effective, the emergence of anti-HBc in vaccinated individuals suggested the possibility of contact with hepatitis B infection with mutant strains subsequent to vaccination (Huang et al., 2009; Xu et al., 2010).

Mechanisms of HBsAg mutation

Numerous mechanisms can lead to mutations in the HBV genome. However, the viral polymerase lacks a proofreading function and, together with the cellular RNA polymerase II, are the main driving forces for the emergence

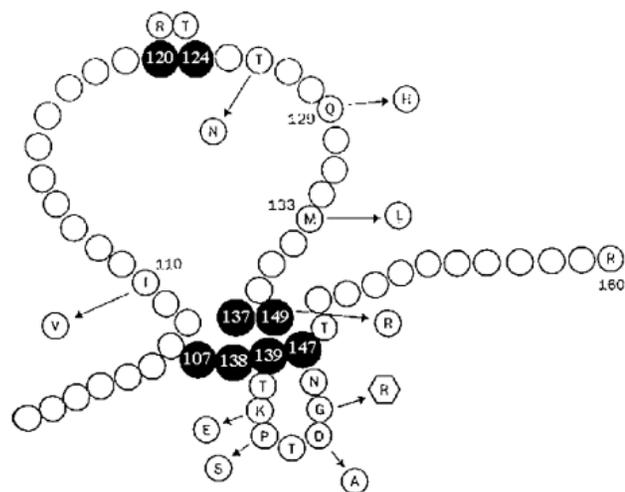


Figure 2. Molecular structure of the major hydrophilic region of the "a" determinant of HBsAg between amino acids 100–160. Letters in circles are viral variants associated with vaccination; most are clustered in the region around amino acids 137–147. The most common and most stable mutation is G145R. Shaded areas indicate disulphide bonds (source: Zuckerman and Zuckerman, 2003).

of point-mutations in the HBV genome (Steinhauer and Holland, 1986). Viral mutations are controlled by functional limitations (Mizokami et al., 1997) and, therefore, variation is not random within the HBV genome (Yang and Summers, 1995). Likewise, selective pressure and interaction between hosts and viruses, imposed exogenously by vaccination and antiviral treatments with hepatitis B immunoglobulin (HBVg), nucleoside analogues or interferon alpha, as well as endogenously by the host immune system, can also affect the mutation selection balance of the HBV genome and apply selective pressures on HBV in infected individuals, leading to the generation and build-up of mutations in the S gene. Most of these mutations occur in the MHR of the S gene, leading to alterations in the structure of the hepatitis B surface antigen, which can disrupt the binding of polyclonal antibodies (Kreutz, 2002). These alterations consist of mutations to the cysteine residue involved in the formation of the disulphide bridges that are responsible for the double-loop structure of the MHR (Seddigh-Tonekaboni et al., 2001; Wakil et al., 2002) and their modifications to protein acidity, electric charge and hydrophobicity (Thakur et al., 2003). Some mutations involve amino acid insertions into the "a" determinant (Lazarevic et al., 2010; Weinberger et al., 2000) or through the creation of stop codons at an inappropriate position, leading to premature termination of the amino acid sequence (Thuy et al., 2005), while other mutations eliminate the glycosylation site at position 146 of the protein (Roznovsky et al., 2000) or create potential new glycosylation sites in the first loop of the MHR (Koyanagi et al., 2000). However, not all mutations in the MHR lead to escape mutants (Avellón and Echevarria, 2006; Kazim et al., 2006). The administration of antiviral drugs, like lamivudine, which inhibits viral polymerase activity, can lead to

mutations in the polymerase gene. Consequently, because of the overlap of the polymerase and S genes, a change in the polymerase has the potential to cause mutations in the S gene. Thus, lamivudine therapy is associated with the generation of escape mutants (Kazim et al., 2006); these are HBV mutants carrying amino acid sequence variations within the "a" determinant and are not neutralised by antibodies induced by the recombinant vaccine, therefore causing infection in vaccinated individuals.

HBsAg mutation and blood donation

Transfusion-associated hepatitis B signifies a global public health problem. Routine screening of HBsAg among blood donors implemented in the early 1970s has significantly enhanced transfusion safety and has steadily reduced the incidence of transfusion-transmitted hepatitis B over the last four decades (Niederhauser et al., 2008). On the other hand, it was demonstrated that HBV transmission by blood components that are negative for HBsAg can still occur (Kuhns et al., 2004) and HBV transmission remained the most frequent transfusion-transmitted viral infection (Liu et al., 2006). Subject to how blood donors were selected and screened, it has been shown that intermediate to high levels of circulating HBV in Asia Pacific is increasing the likelihood of contamination of blood transfusion (Xu et al., 2010). Although prevention and control is the most effective approach to assist in decreasing the spread of hepatitis B infection, an increase in the number of infected individuals similarly creates a circumstance in which evolutionary progressions can lead to the emergence of new HBV isolates that are capable of evading detection, thereby entering the blood circulation (Liu et al., 2010). An assessment of screening techniques might provide insights into strategies that could possibly make blood transfusion safe. To reduce the levels of post-transfusion hepatitis in the Asia Pacific region, the feasibility and effectiveness of using multiple immunoassays or nucleic acid-based amplification assays such as PCR, DNA dot-blot hybridisation assays and Nucleic acid amplification test (NAT) should be evaluated for the effective prevention of post-transfusion hepatitis, especially those cases that are due to HBsAg mutants. NAT complements serological screening for blood donors and reduces the rate of transfusion-associated infection because it has the ability to detect the presence of infection by directly testing viral nucleic acid rather than antibodies. It is capable of detecting small amounts of DNA or RNA; this is beneficial during the window period and for occult hepatitis. However, in most Asia Pacific countries, this method is not cost-effective (Liu et al., 2006). Therefore, screening for HBsAg and total anti-HBc is important in the prevention of post-transfusion hepatitis. In Malaysia, it was found that the presence of HBsAg and anti-HBc in blood donors from the Kuala Lumpur hospital was 5.5%, and 50.1%, respectively (Ton et al., 1979). However, the HBV infection rate has been significantly reduced since the introduction of a nationwide vaccination program in 1989 (Ng et al., 2005). In blood donors in Indonesia, analysis of antigenic marker and *de novo* prediction of tertiary conformation of the three HBsAg variants (T123A, M133L, and T143M) revealed that T143M will alter antigenicity in comparison with the other two mutation patterns (Ie Susan I, 2010). The safety of blood transfusion may be compromised as a result of donors that are infected with HBsAg mutants, as well as those with

circulating undetectable levels of viral protein, which can escape detection by common screening tests as a result of alterations in the "a" determinant region of the S gene (Qiu et al., 2008). However, more accurate and cost-effective tests are increasingly required to prevent the risk of contracting an infection by receiving a contaminated blood unit (Kosan et al., 2010).

HBsAg mutation and hepatocellular carcinoma

Hepatitis B surface antigen mutants are asymptomatic and would only be detected by routine screening. No available guidelines are provided for categorising those who should be screened for surface antigen mutants. Nevertheless, such screening should be considered in the following situations: blood donors, subjects with unexplained liver diseases and subjects who are HBsAg negative but anti-HBc and/or anti-HBs positive. This is further elucidated by Candotti et al., who showed that surface mutants are commonly seen in habitual donors, with almost 100% carrying anti-HBc, and 50% anti-HBs, signifying the occurrence of surface mutations mostly in individuals that recovered from infection but were unable to develop effective immune control (Candotti et al., 2008).

Hepatocellular carcinoma (HCC), accounts for 85-90% of liver cancers worldwide (McMahon et al., 2009). Between 500,000 and 1 million new HCC cases are diagnosed annually, with an age-adjusted annual rate of 14.9 per 100,000 in men, and 5.5 per 100,000 in women (Gomaa et al., 2008; Jolivet-Reynaud et al., 2001). In the highly endemic Asia-Pacific region, HBV infection accounts for 17% of HCC cases in Japan (Lemon et al., 2000), but is associated with up to 80–90% of HCC cases in Singapore and Vietnam (Pokorski and Ohlmer, 2001). HBsAg mutants have been shown to aid in the development of hepatocellular carcinoma through the capability of independent replication, infectivity in chimpanzees and participation in acute human hepatitis (Hunt et al., 2000; Ogata et al., 1997). The occurrence of hepatitis B surface mutants in HCC patients, using variations within the "a" determinant region of HBsAg that are comparable to those described in vaccine escape, were reported in Singapore (Oon et al., 1995). Similarly, 20 cases of HBsAg mutants detected in Singapore among patients with HCC were found to have mutations at positions G145A and M133T (Chemin et al., 2001). The highest rates are in Southeast Asia and sub-Saharan Africa, with the HCC incidence being more than 50/100 (Chemin et al., 2001). The introduction of hepatitis B vaccination worldwide has demonstrated effectiveness in the prevention of HCC, as supported by several studies in Asia Pacific (Li et al., 2004). In Taiwan, it has been reported that the rate of HCC in children aged 6 to 14 years has significantly dropped from 0.70 per 100,000 children in 1981 to 1986 to 0.57 from 1986 to 1990, and to 0.36 from 1990 to 1994 ($P < 0.01$) in the vaccinated cohort (Li et al., 2004).

Conclusion

In conclusion, this review sheds light on fundamental aspects of HBV surface antigen mutations that are essential for the diagnosis of HBV infection, which can lead to the development of severe diseases such as HCC, and pose a risk of transmission through blood donation. However, good knowledge of detailed structural and physiochemical changes as a result of mutations within the S gene is required to define which mutants regulate S gene antigenicity,

as well as the need for guidelines on the diagnosis and management of HBsAg mutant infection. A country-based research on the type of prevailing mutants will no doubt help in elucidating the perspective of HBsAg mutants in South East Asia to make diagnostics and blood transfusion safe.

Authors' contributions

This information was compiled as part of the literature review of my Master of Science thesis entitled "Molecular detection of hepatitis B surface antigen, mutant and evaluation of post hepatitis B vaccination" which was substantially revised by ZS, YAM, NMT and NSH. All authors read and approved the final manuscript.

Conflict of interest

We express no competing interests.

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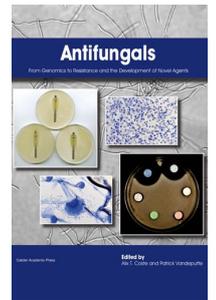
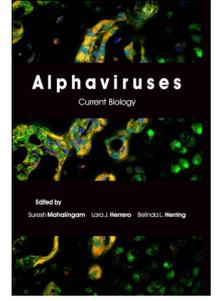
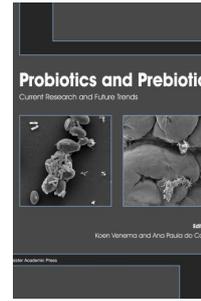
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