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Hepatitis B: The Past, Present and the Future

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Abstract

Hepatitis B is an acknowledged disease which killed more than 600,000 people yearly due to the HBV related complications. The review discussed the history of HBV, its morphology, and its morphogenesis. It has also discussed the pathogenesis of the virus and the previous and current HBV screening method. Finally, the potential of DNA aptamers as the detection materials for HBV in comparison to antibodies has also been discussed.

Keywords: Hepatitis B; Disease; DNA aptamers; Antibodies

Discovery of Hepatitis B Virus

Hepatitis B is a potentially life-threatening disease which majorly affects the liver, cause by the hepatitis B virus. The disease has been acknowledged as a major health problem which can cause a high risk of death from cirrhosis and liver cancer [1]. It is estimated that 240 million people are chronically infected with hepatitis B, and almost 686,000 people died yearly due to the complications caused by the infection [2]. Hepatitis B virus is also acknowledged as the oldest virus ever found in human, discovered in human skeletons varying from 200 to 7000 years old from Europe and Asia [3]. Fighting the hepatitis B endemic is a main concern of healthcare institutions in many developing countries nowadays, alongside with HIV and cancers.

Despite being notoriously known for centuries, the prominent cause of Hepatitis B was undiscovered up until the World War II period [4]. The clinicians had observed patterns of infection are commonly occur at the unclean and highly populated area with poor living conditions, though the cause of the epidemic which is the virus is still remained unknown at that period. The study on another deadly disease known as the yellow fever had been serendipitous to the discovery of the hepatitis B virus. Yellow fever disease was known to be killing soldier in South America and Africa during the WWII. To combat the disease, a British physician named F.O. MacCallum had produced and injected the yellow fever vaccine on the soldiers to vaccinate them. He noticed that high number of vaccinated soldiers develop hepatitis a few months after vaccination. During the time, inoculation of vaccines was made using human serum thus he hypothesized the hepatitis infection probably caused by virus in the infected serum used for inoculation. MacCallum had also noticed the unhygienic practice of pre-war medicine such as the re-use of non-sterilized needles in the injection procedure which might contain tip of infected blood, thus strengthen his hypothesis. Later MacCallum coined the term Hepatitis B for the infection of hepatitis from blood and serum contaminants and Hepatitis A for hepatitis caused by food and water contaminants.

The Hepatitis B research then stumbled in the 1950's and mid 1960's due to lack of ability for the researchers to isolate and culture the virus *in-vitro*, although the researchers at that time had observed the ability of the culprit to pass through smallest filters pores thus increase their confidence that a specific virus is responsible for the infection. Doctor Baruch Bloomberg, a medical researcher in 1963 had made a breakthrough in Hepatitis B research in 1965 when he and a biochemist named Harvey Alter discovered the Australian Antigen which later be known as Hepatitis B Surface Antigen (HBsAg). The Australian antigen (Aa) was first discover in the blood sample of Australia Aborigines when the antigen were shown to exist in one haemophiliac patient out of 24 haemophiliac samples tested, while it is more common in the leukemia patients with 1 out of 10 patients reacted to the Aa antigen hence earned the name [5]. Deeper in their study they discovered that the patients with Aa antigen are rather linked to hepatitis disease instead of the inherited blood-protein polymorphism, the earlier focus of their study. It was based on their tested subjects, the Down syndrome patients in various residence setting shows that the bigger the institution that the patient resided, the higher the chance of Aa detected in their blood. In addition, one of the Down syndrome patient subjected in the study was initially Aa negative but shows Aa antigen appearance in the blood together with hepatitis-like symptoms when re-tested few months later. The researchers then deducted Aa antigen are more closely related to hepatitis rather than blood-protein polymorphism, the initial focus of the Aa antigen study.

In 1968 Alfred Prince, a virologist later study the relation between Hepatitis B symptoms onset and the presence of Aa antigen. In his observation of the Aa in the blood samples collected from the people subjected to blood transfusion, the antigen starts to appear a few weeks before onset of the illness.

This observation further verified the earlier hypothesis where Aa antigen is related to the Hepatitis B disease. In addition, another independent study by Kazuo Okichi from the University of Tokyo had confirmed that Aa positive blood are more likely to transmit hepatitis B during blood transfusion rather than the negative Aa blood samples. Application of electron microscope had further accelerated the molecular structure aspect of Hepatitis B research where D.S Dane from London and K.E Anderson from New York had discovered the virus particles in the Aa positive sera and around the patient's liver, which would later named Dane particle. Other research on Hepatitis B in the 1970's finally lead to the conclusion that Aa is a part of the Hepatitis B virus. Later the nomenclature of Aa was changed to Hepatitis B surface antigen (HBsAg) due to the protein locality on the surface area of the virus.

Hepatitis B Classification and Morphology

The Hepatitis B virus is a relatively small virus with particle size diameter of 20 nm with 2 nm filaments spread on its spherical surface. As one of the retrovirus similarly to HIV, Hepatitis B virus (HBV) is categorized as *Orthohepadnavirus* species of the *Hepadnaviriae* family [6]. The viruses also recognized in Old World monkeys species and woolly monkeys of the New World monkeys. In addition, other animals such as ground squirrels, woodchucks, heron, Peking ducks and tree squirrel also sharing the similar virus suggesting the ancient origin of this virus in mammals.

Hepatitis B is genetically diverse and can be divided into eight genotypes from A-H, based on its DNA sequence comparison [7]. Newly emerged genotypes, I and J had also been recently identified. At least forty sub-genotypes, emerged from 8 genotypes had also been recognized currently [8]. More than 8% difference in nucleotides sequence categorized the virus into different genotypes, while 4% to 8% difference in nucleotides separates the sub-genotype of Hepatitis B virus. Each Hepatitis B genotype is distributed geographically, where genotype A is highly distributed at the sub-saharan Africa, Northern Europe and Western Africa region. The A type is also found in Japan and The Philippines. Genotype B is highly prevalent in East Asia such as in China, Japan and South East Asia region including Indonesia, Vietnam and The Philippines. It can also found in Brazil and Venezuela. Genotype C can be found predominantly in Vietnam, Myanmar and Thailand, East Asia, and Polynesian Islands. Genotype D can be found in the Africa continent, Mediterranean region and South Eastern European Russia. In India, USA and The Netherlands, genotype D is associated with drug addiction and injection drug users. Genotype E infection concentrated in the Africa continent such as in Western and Central Africa. Further study also suggested that genotype E might arise from the chimpanzee hepadnavirus and been infected human recently, due to the less diversity of genome while covering a large geographical area of infection. Genotype F which diverges 14% of its genomes from other genotype is predominant in Central and South America such as in Venezuela. Genotype G was found in the USA and France, and was discovered to be co-infecting with genotype A in patients. Genotype H which had been isolated in Nicaragua and Mexico differs 7.5% to 9.6% of total genome sequence from genotype F and also predominant in the New World. A clear Hepatitis B genotype-related infection and treatment efficacy in chronic Hepatitis B patients had been observed. Co-infection, which means there is more than one infection of Hepatitis B subtype was also been recorded. Genotype A was detected to have co-infection with genotype G and also with genotype D, compared to other genotype. Therefore for example although genotype A is currently uncommon in South East Asia, there is high possibility of genotype A co-infection exists due to high influx of African immigration to South East Asia in the last 15 years.

The pathogenic differences between various Hepatitis B genotypes had been extensively studied. There are studies which indicate the intracellular levels of HBV DNA and extracellular levels of HBV DNA and Hepatitis B envelope antigen (HBeAg) have been revealed as higher in genotype B and C than in genotype A and D, thus suggesting different detection and treatment procedures in handling each genotypes of Hepatitis B. Hepatitis B viral DNA and intracellular accumulation of viral antigens is potentially play roles in the development of severe damage on hepatocytes. In addition, less Hepatitis B surface antigen (HBsAg) formation in Hepatitis B genotype C compared to genotype B was also observed. While the genotype of Hepatitis B virus is widely diverse, the virus serotypes can be divided into four major serotypes of *adr, adw, ayr* and *ayw*. These serotypes are based on the antigenic epitopes present on the surface protein of the virus. The letter 'a' in each serotype defines the common antigen determinant for all, while *d/y* and *w/r* are two mutually exclusive determinant pairs. Serological subtypes are divided rather on the type of amino acids present on the specific position in the 'a' determinant region rather than categorized into geographical segregations as the genotype. For example at position 122 amino acid Lysine (Lys) determine the specificity '*d*' while Arginine (Arg) determine the specificity '*y*' of serotypes, while specificity are '*w*' given by Lys and specificity '*r*' are given by Arg each at position 160. Additional sub-determinants had divided the serological subtypes into other 9 subtype, namely *ayw1, ayw2, ayw3, ayw4, ayr, adw2, adwq, adr* and *adrq-*.

Morphologically, hepatitis B virion are divided into two types; the infectious Dane particle that is double-layered 42 nm diameter, and the smaller non-infectious smaller spherical with filamentous viral particle around 20 nm to 22 nm. The infectious Dane particle is consists of outer lipid envelope and an icosahedral nucleocapsid core composed of protein, addressed as Hepatitis B core antigen (HBcAg). The nucleocapsid encloses the partially double stranded circular viral DNA genome with length around 3200 base pairs (bp). The 5'end of the DNA minus strand is covalently attached to a DNA polymerase that has reverse transcriptase activity similar to retroviruses. The outer layer of the Dane particle envelope contains embedded proteins known as the Hepatitis B surface antigen (HBsAg) which are involved in viral binding of, and entry into the susceptible cells **(Figure 1)**. The surface and core proteins of hepatitis B virus are consists of molecular recognition patterns that can be detected by the antigen presenting cells such as helper T cells, and triggers the production of the antibodies through B cells. Therefore in addition to the detection of the antigens in

Hepatitis B screening procedure, Hepatitis B core Immunoglobulin M (HBc-IgM) also screened from the suspected serum for the possibility of the virus infection. Similarly to the Dane particle the smaller 20 nm virion particle is encapsulated in spheres and filaments consists of HBsAg and host-derived lipid, however the viral nucleic acids are missing from the structure thus making it non-infectious.

Hepatitis B core antigen (HBcAg) is also playing a crucial part of the morphology of the virus. A comparison study between the wild type and mutations of HBcAg shows that both are similar in the robustness where they are only dissolved at pH 2 or 14, at temperature higher than 75°C or in 0.1% sodium dodecyl sulphate (SDS). There are no significant differences between wild type and mutated HBcAg, thus showing the stability of the structure [9]. Hepatitis B core antibody (anti-HBc) is also considered as one of the sensitive serological markers of Hepatitis B infection and leads to the discovery of "isolated anti-HBc", a condition when the anti-HBc is detected in the absence of HBsAg and anti-HBs in suspected patients [10]. In addition Hepatitis B envelope antigen is also screened positive in the potential infected patient, suggesting an early stage of infection **(Figure 1)** [11].

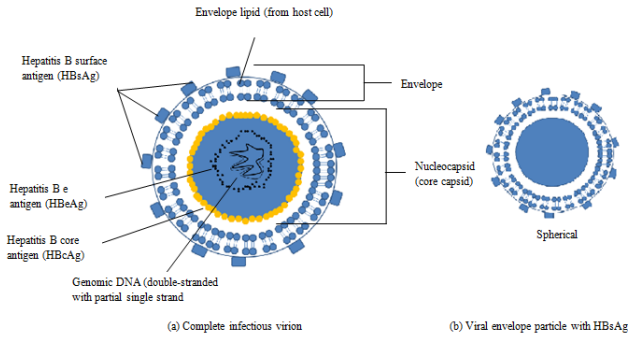

Figure 1: Infectious virion (a) and non-infectious virion (b) of Hepatitis B virus both are encapsulated in HBsAg while only the infectious virion contains the crucial genomic DNA.

Hepatitis B genome is consists of four partially overlapping Open Reading Frame (ORF); *C* gene which encodes core protein and e antigen (HBcAg and HBeAg), *P* gene of polymerase with reverse transcriptase ability (POL) protein, *X* gene which expressed the transcriptional trans-activator protein and *S* gene which expressed the three related S, M and L HBsAg protein. The *C* gene has the ability to encode both HBcAg and HBeAg depending on where the translation process initiated either it is from the core region or the pre-core region of the ORF, respectively. The expressed HBcAg is the core proteins that are able to self-assemble into capsid with the C-terminal that binds to RNA.

The precore ORF are coding the signal peptide that initiate the translation product to the endoplasmic reticulum where the protein is processed to form HBeAg. Although the function of HBeAg is still largely unknown, the protein is suggested to promote persistent infection in the host. Hepatitis B polymerase (POL) is a relatively large protein containing about 800 amino acids are divided into three domain based on its functions. The terminal domain is involved in encapsidation and initiation of minus-strand synthesis while the reverse transcriptase (RT) domain catalyzed the genome synthesis in the virion. The ribonuclease H domain functions as to degrade the pregenomic RNA and facilitates the DNA replication process. *X* gene encodes HBxAg protein which facilitates signal transduction, activation of RNA transcription, inhibition of protein degradation and the DNA repair, though the mechanism of HBxAg and its biological function in the viral-life cycle still unknown. The *S* gene encodes HBsAg that can be divided into three envelope protein namely small, medium and large HBsAg. These proteins surrounded the virion nucleocapsid with lipid bilayer and anchored as transmembrane proteins where they play major roles in Hepatitis B virus morphogenesis and infectivity.

Morphogenesis and Pathogenesis of Hepatitis B

Pathogenesis and morphogenesis of Hepatitis B had been extensively studied. The virus primarily interferes with the liver functions by infecting hepatocytes before accelerates to cause hepatitis and hepatocellular carcinoma. Hepatitis B virus capsid is formed in the cytosol of the infected cell during packaging of an RNA pregenome replication complex by multiple copies of a 21-kDa C protein [12]. The envelope protein (HBcAg) which forming the shell of Hepatitis B virus is highly conserved among the virus isolates, and are able to be expressed in a multiple prokaryotes and eukaryotes cell types hosts. HBcAg are self-assembles to form two types of capsids in hepatocytes; the icosahedral T=3 symmetry of 90 unit C protein dimers and the larger icosahedral T=4 symmetry of 120 C protein dimers. The C protein dimer forms an upside down "T" like structure, where the tip of the spike forms major epitope of HBcAg. The horizontal part of the C protein mediates the inter-dimer contacts with 5 and 6 dimers arranged around the 5-fold and quasi 6-fold symmetry axes respectively. The capsid also had 120 to 150 nm pores on its shells to allow the nucleotides diffusion through the surface during the viral DNA genome synthesis.

The virion will enter the cytosol of the host cell by binding the NTCP on the host cell and being endocytosed. Through the entrance of Dane's particle into the host's cytosol, the viral DNA of Hepatitis B virus will enter the nucleus for DNA copying mechanism through carrier host protein named chaperones. The partially double stranded viral DNA will be converted into a covalently closed circular DNA (cccDNA) which to be transcribed in the pregenomic RNA (pgRNA) around 3500 bp and also in different sub-genomic RNAs which will encodes all the viral proteins necessary for the replication of Hepatitis B virus. Inside the cytosol a single pgRNA molecule and viral reverse transcriptase (RT) will be introduced into the assembling core capsid, where the reverse transcription of the pgRNA into a new genomic DNA will occur. The mature encapsidated genomic DNA then will be enveloped by the expressed surface proteins, thus creating the new generations of infectious virions to be released **(Figure 2)**.

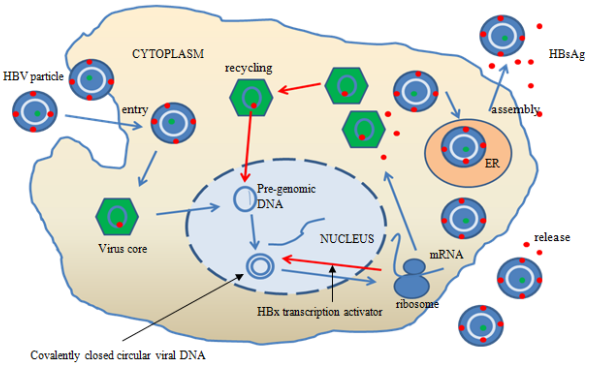

Figure 2: Pathogenesis of Hepatitis B virus in host cell. The virion enters cytosol of host cell, releasing the mRNA to enter the nucleus where the DNA replication occurs with the help of reverse transcriptase-like mechanism.

There are several types of surface protein that are responsible for the encapsulation of core proteins. These three types of envelope protein are known as small (S), medium (M) and large (L) surface proteins, due to the different length of each protein's N-terminal domains. The S protein had the most number of proteins on the surface envelope, while L protein is only available in the virion and on the non-infectious surface envelope of the virus. The distribution of M protein is similar in both infectious and non-infectious particles.

Hepatitis B Detection in the Early Days

In the United States in the early 1960's high percentage of blood for blood transfusions are received from the paid donor, which turns out to be the population highly infected with Hepatitis B virus. Due to that matter high numbers of cases for post-transfusion Hepatitis B had been observed. The medical community in the United States therefore notices the necessities to a proper screening for Hepatitis B for the donated blood. Blumberg and Alter had used an established technique to screen the HBsAg which is the gel diffusion technique. The agar diffusion involves the passive diffusion of proteins and antigen-antibody complexes through gels, where it is based on the ability of the immune system to detect the small differences in protein to produce the antigen-antibody interaction in response to a novel blood protein thus leading to the precipitation of the soluble complexes in the gel matrix. In gel diffusion, the glass slides will be coated with agarose gel where at the middle of the gel the suspected patient's serum will be loaded. The sample then will be surrounded with the gel containing sera of non-transfusion persons, also known as the positive or test sera. All the sera will then diffusing slowly through the gel. If the component from the positive sera reacted with the antibodies in the patient's serum the white lines will appeared on the gel, which is the precipitation of antigen-antibody complexes observant on the gel. However, the detection method was less sensitive for the blood screening procedure and require higher amount of blood from the donor. This had encouraged other methods being developed to increase the sensitivity of the assay.

Later, another method called the radioimmunoassay (RIA) was being utilised to improve the detection of HBsAg in the Hepatitis B suspected patients and blood donors [13]. The radioimmunoassay method that had been developed initially for the insulin detection in the diabetes patients bloodstream, proved to be instrumental in detecting the HBsAg antigen in Hepatitis B suspected patients. At least three variations of the RIA methods had been adopted by researchers, being compared on large population in a study performed in 1975 [14]. Prior to RIA, counter-electrohoresis is a widely accepted method that gained popularity due to its better sensitivity in detecting HBsAg compared to the agar gel diffusions. In RIA, each serum sample for screening will be pipette to an antibody-coated tube and incubated before 125I-labeled anti HBsAg preparation were added into the tube. The mixture then will be incubated before the excess were washed out. Bound antibody then will be determined through the well-type scintillation counter. Results will be count and compared to the negative samples, where the relative mean value will be recorded. RIA method were shown to have sensitivity at least 10 to 200 times better than the counter-electrophoresis, where the detection are able to detect three to eight times more HBsAg carrier compared to the previous method.

Hepatitis B Detection in the present

Mallika Ghosh and colleagues had made an extensive review on the Hepatitis B virus detection method published by the scientific community in the past 15 years [15]. As the method for detection Hepatitis B virus progress, samples were obtained not only from serum, but also from dried blood/plasma spot, hepatocytes, ovarian tissue, cerumen and otorrhoea, saliva, parotid tissue, renal tissue, oocytes and embrios etc. One of the prevalent causes for the improvement in detection for Hepatitis B virus in other samples than serum is based on the advancement of the nucleic acids amplifications technology in the 1990's. The discovery and mapping of the Hepatitis B viral genome sequence had able the scientist to screen the Hepatitis B virus DNA in suspected patients by DNA amplifications and Polymerase Chain Reaction (PCR) method. DNA detection has provided an alternative for the serological detection of Hepatitis B, with higher sensitivity and less sample volume.

Serological detection of Hepatitis B virus had also progress tremendously in the past 30 years. Previously in 1970s and 1980s, HBsAg is the only principle antigen screened for Hepatitis B virus carriers. However since the discovery of other antigens in the virus such as Hepatitis B envelope antigen (HBeAg) and Hepatitis B core antigen (HBcAg), these antigens and antibody against them produced by patient's immune system (anti-HBe and anti-HBc) were also detected for the serological testing. HBsAg is the first marker of acute Hepatitis B infection where it appears as early as 1 week after the initial exposure of Hepatitis B. The chronic infection of Hepatitis B will see a prolonged high level of HBsAg in serum after 6 months of infection. Simultaneously, anti-HBs are also detected in patients. However, depending of the sensitivity of the testing procedure, HBsAg are only become detectable in the serum about 6 to 10 weeks after the exposure. Current method of detecting HBsAg includes the

Enzyme Immunoassay (EIA) procedure, as for anti-HBs titer it is able to detect up to ≥ 10 mIU/mL, which is 97.6% of the immunity [16]. In enzyme immunoassay, the enzymes are attached to one of the reactants in an immunoassay to allow the detection through colour development after addition of chromogenic substrate. Spectrophotometer then utilized to monitor the intensity of the colour produced in relation with substance. However, a variation in sensitivity of the EIA was observed in different Hepatitis B virus genotype and subtypes and other *S* gene mutants [17]. Despite being highly sensitive compared to other method, EIA required high-end equipment's and trained personnel to performed, thus limited its application in the less developed countries with limited access to sophisticated equipment's **(Figure 3)**.

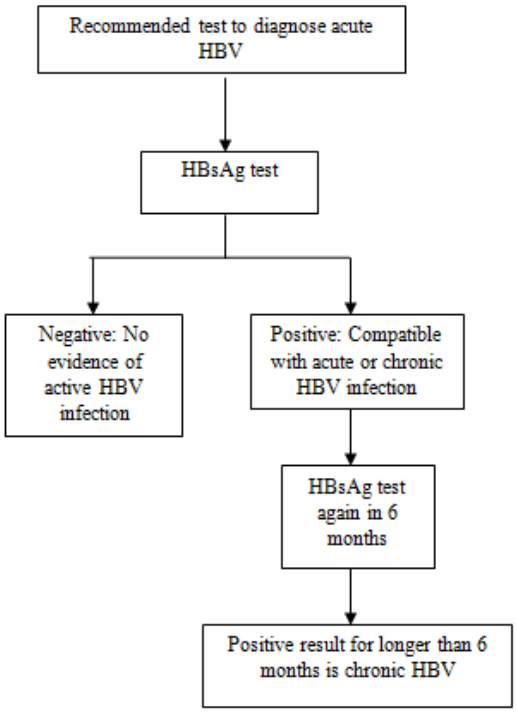

Figure 3: Diagnostic tests for HBV infection.

In the 1990's, another method to detect Hepatitis B virus antigens had been developed by researchers. The rapid, Point-Of Care (POC) method consumed less time to be performed and more practical for the on field application where the suspected patients able to be screened immediately with immediate results. Several assays had been developed for the POC technique, where the principles are based on particle agglutination, immunochromatography, immunodot or immunofiltration. Although the detection able to be performed within minutes in most of the assays, there are weaknesses that requires improvements. In patients infected with several Hepatitis B virus mutants, the assay performance is rather poor. In addition, studies shows that application of rapid detection for HBsAg is better in the developed countries in comparison with the less-developed countries due to the low heterogenicity of the antigen in the developed country. The problem in seroconversion panel also contributed to the low sensitivity of the detection. This shows that necessary improvements are required to address the sensitivity of the POC method, focusing on the biodetecting materials **(Table 1)**.

Table 1: Primary and secondary tests to diagnose/monitor hepatitis B virus (HBV) infection.

Marker	Incubation period	Acute infection	Past/resolved infection	Chronic infection
Primary diagnostic tests				
HBsAg	±	+	-	+
Anti-HBs	-	-	+	-
Anti-HBc total	-	±	+	+
Anti-HBc-IgM	-	+	-	±
Prognostic or monitoring test				
HBeAg	±	+	-	±
Anti-HBe	-	-	±	±
HBV-DNA	±	+	±	+

Conclusion

In the review, I had walking through the history of Hepatitis B virus and its serological and genotypes. I had also discussed on the virus's morphology and morphogenesis. The method of detection of the virus based on its antigens detection and molecular biology methods from the early days to the currently applied had also been discussed. The ability for the virus to mutate and exists in heterogeneity with its subtypes is acknowledged as one of the culprit contributed to the less sensitivity of the assay used to detect the virus. Therefore, further study to develop the highly robust detection method that are able to detect most of the subtypes without discrimination is still a demanding field of research in the Hepatitis B detection area.

The effective treatments for chronic HBV infection are now available in the developed region of the world. The treatment, if properly delivered to the patients will highly reduce the progression of liver disease and complications such as hepatocellular carcinoma. However, the therapeutic measures for chronic HBV infection which normally involves antiviral administration is often require indefinite duration of delivery to the patients. This might come as a threshold to the less developed country where the access to modern and highly potent drugs with high resistance barriers is restricted. Barriers to accessing treatment and care for chronic HBV infection result in poor outcomes for those affected, and ongoing transmission to susceptible contacts.

Therefore, the first line of defense against hepatitis B would be the detection of the virus itself in the potential hosts, proceeded with the localization and the controlled treatment of the infected suspects. To achieve this, detection of hepatitis B in human requires a fast and reliable method without

compromising results precision. Current antigen detections of Hepatitis B involved the application of antibody in the EIA assay of POC procedure. Even though the monoclonal antibodies (MAbs) of anti-HBsAg and anti- HBeAg are currently available in the laboratories, production of hybridomas in having those MAbs requires tedious procedures involving *in-vivo* applications. High contamination risk through dealing with animals, risk of animal dying pre-maturely, and failure to clone the hybridomas which requires special facilities and media for animal cell culture made the development of hepatitis B detection though ELISA and antibody-antigen related procedure are costly in the less developed region of the world. In addition to the economical disadvantages, the chemical structure of antibodies which are consists of proteins, without having proper chemical modifications, are normally subjected the heat-labile properties and is sensitive to the macro and microenvironment. Slight changes in temperature, pH and ionic distribution in the blood might contribute to the dysfunctional of the antibodies. Therefore, application of aptamers which consists of DNA provide a platform of robust detectors which are highly precise and durable to the changes of the environment in comparisons with their antibodies counterpart will be the next focus for the assay development in detection of Hepatitis B virus.

References

1. WHO (2016). Media centre: 1-5.
2. Naghavi M, Wang H, Lozano R, Davis A, Liang X, et al. (2015) Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the global burden of disease study 2013. The Lancet 385: 117-171.
3. Muhlemann B, Jones TC, Damgaard PDB, Allentoft ME, Shevnina I, et al. (2018) Ancient hepatitis B viruses from the bronze age to the medieval period. Nature 557: 418-423.
4. Patlak M (2000) The hepatitis B story. Beyond discovery: the path from research to human benefit. Nat Acad Sci: 1-8.
5. Blumberg BS, Friedlaender JS, Woodside A, Sutnick AI, London WT (1969) Hepatitis and australia antigen: Autosomal recessive inheritance of susceptibility to infection in humans. Proc Nat Acad Sci USA 62: 1108–1115.
6. Liang TJ (2010) Hepatitis B: The virus and disease. Hepatol 49: 1-17.
7. Kramvis A (2005) Hepatitis B virus genotypes. Vaccine 23: 2409-2423.
8. Hundie G B, Raj SV, Michael GD, Pas SD, Koopmans MP, et al. (2017) A novel hepatitis B virus sub genotype D10 circulating in ethiopia. J Viral Hepatitis 24: 163-173.
9. Newman M, Suk FM, Cajimat M, Chua PK, Shih C (2003) Stability and morphology comparisons of self-assembled virus-like particles from wild-type and mutant human hepatitis B virus capsid proteins. J Virol 77: 12950-12960.
10. Wu T, Kwok RM, Tran TT (2017) Isolated anti-HBc: The relevance of hepatitis B core antibody- a review of new issues. Am J Gastroenterol 112: 1780-1788.
11. Krajden M, McNabb G, Petric M (2008) The laboratory diagnosis of hepatitis B virus. The Canadian J Infect Dis Med Microbiol 16: 65-72.
12. Bruss V (2007) Hepatitis B virus morphogenesis. World J Gastroenterol 13: 65-73.
13. Gerlich WH (2013) Medical virology of hepatitis B: How it began and where we are now. Virol J 10: 1.
14. Moraleda MH (1985) Hepatitis B surface antigen testing. Infect Cont 6: 216-217.
15. Ghosh M, Nandi S, Dutta S, Saha MK (2015) Detection of hepatitis B virus infection: A systematic review. World J Hepatol 7: 2482-2491.
16. Servoss JC, Friedman LS (2006) Serologic and molecular diagnosis of hepatitis B virus. Infect Dis Clin North Am 20: 47-61.
17. Villar LM, Cruz HM, Barbosa JR, Bezerra CS, Portilho MM, et al. (2015) Update on hepatitis B and C virus diagnosis. World J Virol 4: 323-342.