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Hepatitis B Virus, an Oncogenic and Mutagenic Virus Emerging More Advanced Vaccines and Treatments in the Future: Updates and Perspectives

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Review Article

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Abstract: Hepatitis B was recognized as infectious disease dating many years ago in human history but its etiology was recently identified, the first discoveries reported a serum protein called Australia antigen which was lately recognized as hepatitis B virus (HBV) surface antigen (HBsAg) and the clinical detection of HBsAg was the first steps allowing the first screening of a strangely infected blood donors for an infectious and dangerous pathogen transmissible to some blood recipients. The development of modern and advanced virus diagnostic tools with high sensitive radio immune assays directed to the deep clinical identification of viral infection markers whereas successful cloning and sequencing of HBV genome allowed the understanding of HBV life cycle, and guided the development of efficient antiviral vaccines and drugs up to nowadays where HBV vaccine was the first vaccine in effective use to be synthesized by genome editing technology. Unfortunately, some current problems such as inaccuracy of recognition of occult HBV infections, the viral potential reactivation, lack of complete protection against mutants and heterologous HBV genotypes by HBV vaccines and therapeutic drugs, and the inability to achieve a complete cure of chronic HBV infections, and its association with hepatocellular carcinoma are still unescapable worldwide health problems.

Keywords: Hepatitis B virus, vaccine, HBsAg, Hepatocellular carcinoma, DNA.

INTRODUCTION

Generally, hepatitis is a dangerous inflammation of the liver cells caused by different major factors such as metabolism, ischemic, autoimmune diseases, genetics and infections; the metabolic causes mainly include drugs, toxins, heavy alcohol and some non-alcoholic fatty liver diseases; some pathogenic infectious microorganisms such as bacteria, parasites and viruses are causative agents of various types of hepatitis[1, 2].

Some hepatic failures may be sometimes resulting from progression of acute hepatitis to chronic hepatitis after failure of resolving on its own dependently to body self-defense mechanisms or from a chronic hepatitis lasting more than six months which may dramatically grow and eventually develop either dangerous chronic liver failure forms or hepatocellular carcinoma (HCC)[2-4].

The viruses as the most striking cause of infectious hepatitis which is among the worldwide emerging health concerns [5], it has awaked researchers up and identified five different genotypes of viral hepatitis namely hepatitis A, B, C, D, and E [1, 2]. Apart from human immunodeficiency virus (HIV) epidemic status, viral hepatitis is among the first following viral infections having easy and wide transmission modes sometimes difficult to control; both hepatitis B and C are mainly sexually transmissible, transferable from mother to baby during pregnancy or childbirth and commonly spread through both infected body fluids such as semen, vaginal secretions, saliva and breastmilk once openly exposed to living body cuts [6], and blood either during needles sharing by intravenous drugs injection or blood transfusions while hepatitis D is caused by incomplete virus and it is presented as hepatitis B virus co-infection only found in hepatitis B patients [2, 89]; high co-infection rate was reported in some countries like Mongolia where 60% of HBV patients have HDV infection and further WHO research reports confirmed that prevention of HBV through vaccination also prevents HDV but the treatments of co-infected patients may differ from single infection cases [90]. The hepatitis A and E do not normally lead to chronic hepatitis; both are mainly transmitted by fecal-oral route and spread by contaminated water, foods and drinks eventually easy to affect children or other people with poor hygienic conditions [6, 12]. Over the lasting time, in adults only 5% of hepatitis B may progress to chronic

complications by which 20-30% develop hepatic cancers and/or liver cirrhosis while hepatitis C is entirely presented as a chronic viral hepatitis infection [8] which is the second common cause of liver cirrhosis following the one caused by alcoholic hepatitis [9].

Nowadays; the therapeutic antiviral medications of viral hepatitis are usefully targeting only to stop the viral multiplication and progress but the complete cleaning of virus and its infectious particles from the infected body is not yet achieved; immunosuppressant drugs are also used for autoimmune hepatitis [10]; liver transplantation was made possible in certain cases [11] but more interestingly hepatitis A, B, and D can be possibly prevented trough vaccinations combined with healthy lifestyle conditions [1, 12]. In 2015, it was worldwidely estimated more than 343 million people infected with chronic hepatitis B and about 142 million with hepatitis C and around 114 million people living with hepatitis A [13]; hepatitis infections lead to more than 1 million deaths per year including liver cancers [14]. This review will be underlining the complexity and virulent viral behaviors of HBV as an emerging public health problem requiring more advanced treatments and adapted vaccines accordingly promising a complete eradication of hepatitis, effective to overcome mutants and oncogenes of HBV genotypes in the future years.

Structural complex and life cycle of hepatitis B virus

HBV is a complex hepadnavirus with virulent virus particle called virions consisting of an outer lipid envelope and an icosahedral nucleocapsid core protein (Fig. 1A) [15]. Its viral DNA and DNA polymerase maintaining reverse transcriptase activity are enclosed in nucleocapsid as an outer envelope containing proteins involving in viral binding and entry into susceptible cell [16], this complex structure of HBV make it one the smallest enveloped human viruses with 30-52 nm having virions with high pathogenicity of infecting liver cells [17]. It was discovered a kind of viral particles called Dane particles without protein core and they are found in the serum of HBV patients with different morphologies [18], these DNA particles consist of viral lipids and proteins from the outer parts of a virion known as surface antigens (HBsAg) which are excessively produced during viral multiplication cycle [19]; inside the HBV core contains the genome of HBV polymerase attached to primase (Pr), the reverse transcriptase(RT) domain ,and cellular protein kinase C alpha (PKC) (Fig.1B) [20].

The complexity of the life cycle of HBV is based on its unusual open circular DNA which is not fully double-stranded with short length-strand of 1700-2800 nucleotides and one end of full-length strand of 3020-3320 nucleotides linked to the viral DNA polymerase [21]. HBV is specially one of the know pararetroviruses which is a non-retrovirus undergoing viral replication using reverse transcriptase [22]. It was shown that only mature core particles containing a complete DNA minus strand and plus strand may interact with the preS domains of the membrane-associated surface protein and develop a real viral envelope [24] which may detach to the viral surface and can function as attachment site and they are secreted as small HBsAg particles ready to be exported by multivesicular bodies following the same mechanism as other enveloped viruses [25].

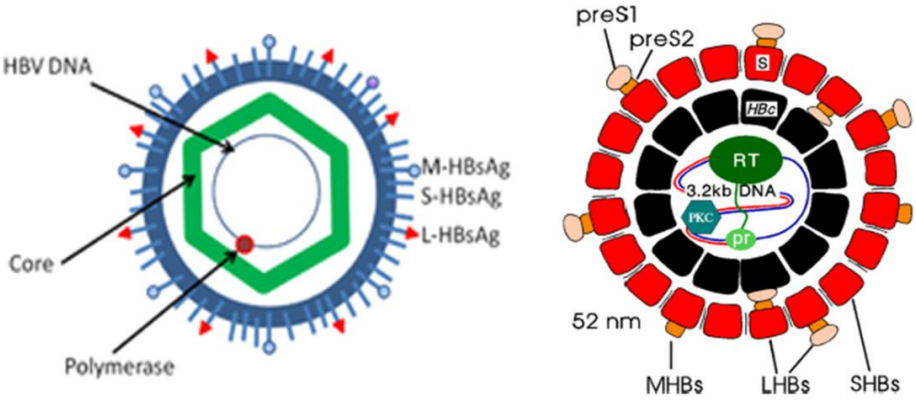

Figure 1: A. Organization of HBV genome **B. Structural components of HBV**

From some years later up to 2013 different teams of researchers have tried to clearly understand the mechanism of attachment of HBV to the liver-specific receptors such as heparansulfate proteoglycan and Na^+-taurocholate cotransporting polypeptide (NTCP) lead to endocytosis of HBV and release of its core particles then transported to the nucleus and arrested at the nuclear pore complex where the HBV genome is

released to the nucleus. The viral DNA is repaired to the covalently closed circular DNA (cccDNA) and complexed with nucleosomes while the interactions of cccDNA with transcription factors is transcribed to the pregenomic and subgenomic messenger ribonucleic acids (mRNAs) which will be transported to the cytoplasm without splicing (Fig.2). Moreover, the two subgenomic mRNAs for the three HBs proteins are translated at the endoplasmic reticulum, assembled to the subviral HBsAg particles and are secreted via Golgi apparatus but also at the same time, the pregenomic mRNA is translated in the cytosol to the HBV core protein and the viral polymerase in case where the three components assemble to the immature viral core particle. The HBV genomes mature in the viral core particles via reverse transcription of the pregenomic mRNA to DNA and these viral particles can migrate back to the nuclear pore complex or some are enveloped by the surface proteins and secreted via multivesicular body [23].

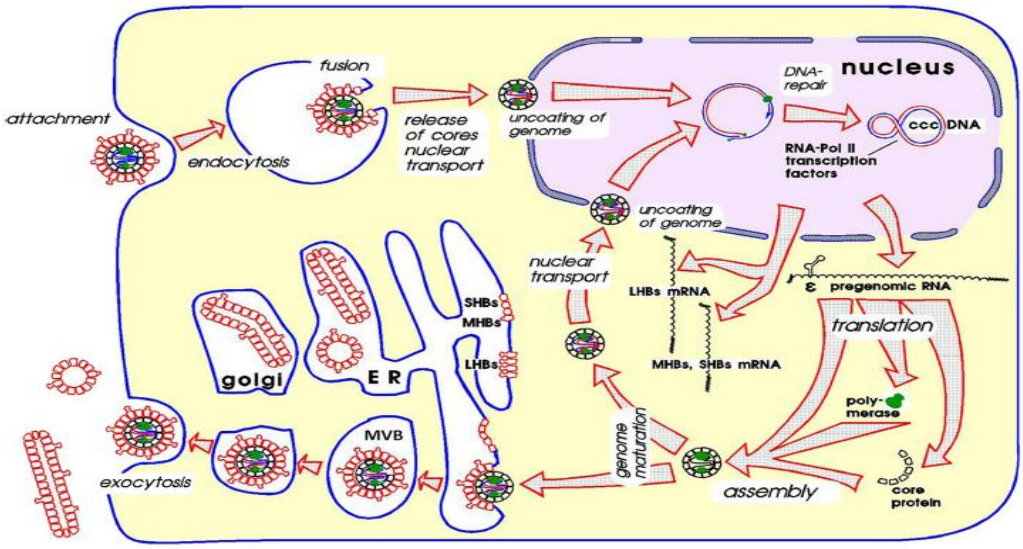

Fig-2: Life cycle of HBV

HBV replication cycle is very complicated and different from other retroviridae, the virus particles contain their genome comprising two copies of RNA and their reverse transcription may only start after entry into the target cells, the tRNA which is considered as the primer for their minus strand DNA intervenes in completing double stranded DNA which is a linearly integrated into the host genome before synthesis of the pregenomic RNA while the progeny virus is assembled and released at the cellular plasma membrane as immature viral particles requiring cleavage for maturation as infectious viral proteins by the viral proteases ready to migrate from cell to another to expand infection .

Epidemiological transmission and pathogenicity of HBV

HBV is highly transmitted though exposure to infectious blood and/or body fluids both by vertical transmission from mother to child and mostly through horizontal transmissions such as sexual intercourses, intravenous drug use, bites or lesions [26, 28]. This virus cannot be spread by holding hands, hugging, kissing, coughing, sneezing, breastfeeding or sharing eating utensils when there has been no blood or body fluids close contacts. The infection can be diagnosed around 30-60 days after exposure [26, 27].In the past decades, there has been HBV transmission to and from health care workers as victims of professional activities after exposures to highly infectious HBV carrier patients and lately in 1970s, there have been many reports of viral HBV transmission from health care professionals with high viremia to patients mainly during surgery where it was reported that in Germany around 50% of all health care workers (approximately 7% of average age population) were HBV positive marker in 1978; consequently, some mandatory protective measures have been taken up to restrictions of HBV positive health care workers [29].

The global distribution of epidemic HBV in 2005 was worldwidely estimated more than 370 million individuals living with the chronic hepatitis B. Many epidemiological reports of prevalence of chronic HBV infection in high endemic areas revealed at least 8-15% prevalence in sub-Saharan Africa, 10% in East Asia, and around 0.5-1% in United States, Northern Europe and Australia (Fig. 3) [26, 30]. Asia as a continent having big population; in 2010, China had 120 million infected people (1/3 of all HBV patients), followed by India and Indonesia with 40 million and 12 million, respectively followed by all sub-Saharan African countries counting more than 100 million of people in total [31]. The complications of chronic HBV result in 15-25% deaths of all HBV infected population including all forms of hepatitis-associated cancers. In 2012 the center for diseases control and prevention concluded that both hepatitis B and C are responsible of

96% of hepatitis-related deaths whereas more than 350 million of worldwide population is currently living with HBV, commonly causing more than 750,000 deaths each year [32].

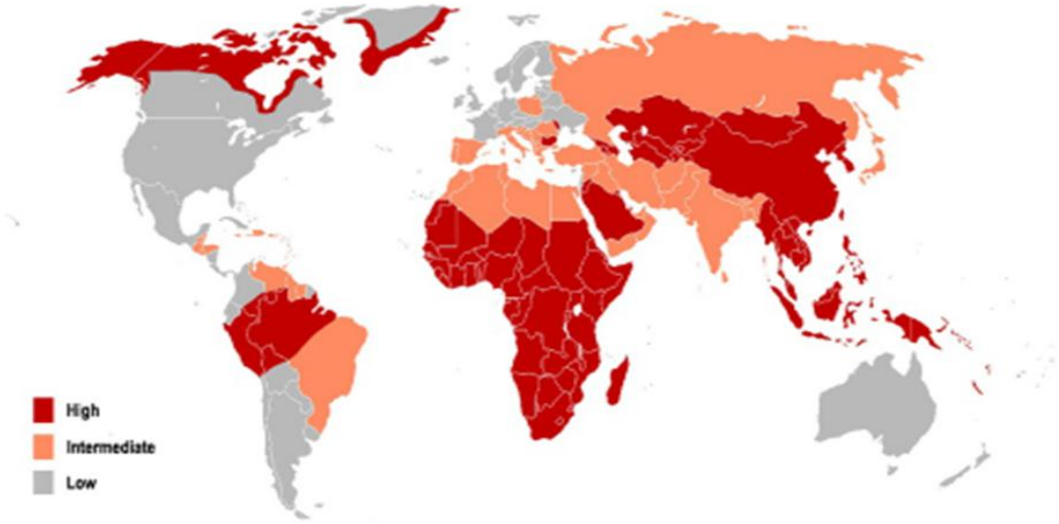

Fig-3: Global prevalence of HBV infections in general population by WHO report 2015

In some cases of immunopathogenesis studies conducted in transgenic mice, chimpanzees and human T cells analysis revealed that in most cases of acute hepatitis B infection, a strong cellular immune system recorganizes the HBV infection after some several months and suppression of viral relpication starts up to a complete elimination of HBV from all hepatocytes [33] either without any or with minor symptoms but with appearance of anti-HBs antibodies preventing new HBV cellular infection [34]. In fact; in few cases, some HBV genomes may persist as cccDNA in serum after exposure even though anti-HBs is negative, and then the HBV reactivation may oftenly be developped either under immunodeficiency cases such as HCV and/or HIV co-infetions [36, 37] or under B-lymhocytes destroying drugs such as rituximab and other immunosuppressing conditions [37]. The HBV DNA particles from an immuno-competent carriers have some low infectivity may be due to the facts that in some phases of infection protective antibodies either may cover up the attachment sites of mutant viral DNA particles or enclose them entirely and make the mjority non-infectious but contrary, they can develop a progressive hepatitis in immunocompromised patients [36].

Therefore; in 1988 some genetics studies conducted on DNA genomics analysis by comparing HBV DNA sequences from different virus strains extracted from patients from different parts of the world established genetic differences and confirmed the existence of four HBV genotypic groups called genotypes mainly A, B C and D with more than 8% of difference in DNA sequences [39]. Lately, further genetics concepts demonstrated by Helene Norder *et al.* revealed the genomic divergence of more than 4% of HBV DNA sequences in different viral strains and extensively proved the existence of genotypes A, B, C, D, E and F; which were attributed to be genetically called sub-genotypes of HBV in 2004 [41]. More interestingly, it was found that there were restriction and geographical distribution of genotypes but with some significant epidemiological dominances and propagations in different areas over the historical periods where genotypes B and C are highly in Asia, E in Africa and F, H in America while some D genotypes were worldwidely distributed (Fig.4). Consequently; a wide spread of different genotypes of human HBV increased with evolution of historic migrations were highly considered and supported by bioinformatics data underlining the effects of alleviating factors of HBV infection possibly resulting from viral mutations or unrecognized co-infections [40, 41].

Thereupon; the anthropologic and epidemiologic studies of clinical interests resulting from experimental data approved that HBV genotypes C, D and F are more pathogenic than others while genotypes A and B respond better to interferons therapy than C and D; and some studies tried to conclude that genotype E has been diffused in America by some immigrants from Africa, subgenotype A2 by North Europe descendants, and subgenotype A1 by human population during slavery movements to Brazil and probably from Somalia through Indian ocean to Asia [42, 43] but, there are no scientifically approved evidence-based facts. Moreover; the confirmation of a particular subgroup C4 in Australian aborigines which never exists in any other parts of the world aroused suggestions of existence of genotype C from ancestors even before many years of modern habitation of Australia [34], and more advanced bioinformatic analysis have confirmed the evolution of HBV subgenotypes to be evolutionally dependent to human

prehistoric migrations [44]. In 2013, it was showed an extension of genotypes A to H (also called putative I genotype) and subgenotypes A1-7, B1-9, C1-16, D1-9, and F1-4 have been identified but some hypothesis are still requiring more thoroughgoing studies [45].

Fig-4: Genotypes and subgenotypes distribution of HBV infections. Note that subgenotype A2 is predominant in low endemic areas of Europe and America, and it is the most used in HBV vaccines.

Chronic HBV infections and immune system

In most cases of patients having chronic HBV infection, the immune defense becomes partially inactivated and the surface antigens (HBsAg) levels and viral load get low (Fig.5) while the destruction of a big number of hepatocytes by HB virus leads to chronic liver inflammations and the infected surface increases significantly, as new liver cells are continuously infected in the absence of immune neutralizing antibodies. When the immune defense become more efficient, the patient may reach the status where no major symptoms due to low production of viral particles production (viremia < 10^4/ml of blood) while the HBsAg are still moderately produced at low quantity in the blood, mostly < 3000IU/ml and no longer any HBeAg (which is known as a non-essential viral antigen which does not intervene in HB virus replication) can be detected but its corresponding antibodies known as anti-HBe are present, and some infectious viral particles such as HBV DNA also still highly presented in the patient's blood even though these HBsAg carriers look like healthy [47].

The significant decrease of HBsAg and a seroconversion of HBeAg to anti-HBe are indicating factors of either a notable therapy improvement or spontaneous response of a great viral elimination by immune defense but consequently, due to high capability of HBV to evade B and T cells immunity capacity, many HBeAg negative carriers may still suffer from progression of chronic hepatitis B infections to hepatocellular carcinoma while high dose of immune tolerance against the viral envelope HBsAg may be induced by a great amount of subviral HBsAg particles and their expression in its immunoprivileged organ (liver), circulation in the blood without development of major symptoms enhance immune tolerance increase [46].

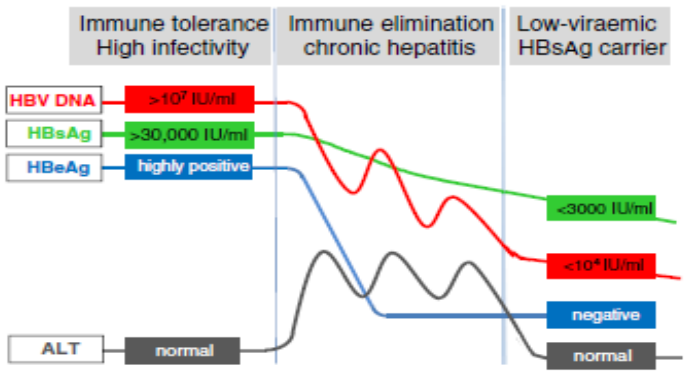

Fig-5: Illustration of three phases of chronic HBV infections

Some researchers systematically studied the infectivity of plasma from an acute hepatitis B by injecting the plasma from infected patients to volunteers, and they showed that even some small dilutions may cause silent HBV infections which may develop into chronic HBV infections, and it was experimentally demonstrated that HBV is both highly species and organ-specific but also extremely infectious to humans if and only once it enters in contact with the blood stream [34]; others tried to use cell lines of human hepatic origin and they showed a very low or no susceptibility for HBV but when primarily differentiated hepatocyte cultures obtained from some surgically exercised human liver portions showed suboptimal susceptibility for HBV but only a kind of a transitory HBV infection has been established after inoculation of a gigantesque number of virus particles [35].

Moreover, some HBeAg negative carriers with a mutated, non-functional preC sequence can even cause fulminant hepatitis B infection [47] and HBeAg acts as an immune modulator capable to suppress the recognition of HBcAg expressing cells by T lymphocytes as the main mechanism of HBV immune control, but a lack of effective immune defenses stimulates the production and secretion of more viruses up to 10^{10} infectious particles per ml without manifestation of clinical symptoms; anti-HBc actually appears with the onset of acute hepatitis or after an unnoticed silent HBV infection while HBsAg is found with anti-HBc and the patient always develop hepatitis. The anti-HBc and anti-HBs usually remain as a sign of immunity control if the HBV infection is completely under immune control while HBsAg and anti-HBc remain positive once the HBV infection becomes chronic [48].

Mutagenicity and Oncogenicity of HBV

The blood plasma contents from HBV infected donors may contain small amount of infectious viral particles leading to an increase of HBV infection noticed after blood transfusions but sometimes small risks from occult infected blood donors can be tolerated and cleaned by body and anti-HBc are markers of occult HBV infection recognized in 1970s [49]. For the nowadays better safety, the blood testing for both HBsAg and anti-HBc was mandatorily implemented before blood donation since 2006 as occult HBV infection can be reactivated in immunosuppressant blood recipients under certain circumstances and may cause severe consequences. This issue was taken into considerations from 1975 but up to now not all hematologists are aware of this HBV reactivation problem [50]. The HBV reactivation can be suppressed from the body with preemptive antiviral therapy only and if the immune system of the blood recipient is not immunosuppressed and when the problem was recognized in advance. This problem is still recognized as a big challenge to start on to overcome HBV high pathogenicity by the fact that up to now it is not possible to reliably infect cell cultures with HBV and experimental injection of cloned HBV DNA into liver of chimpanzees showed a great efficient replication of HBV leading to acute HBV which may possibly develop chronic hepatitis in certain conditions [51].

In fact; in case of acute hepatitis B, HBsAg disappears progressively within 6 months and a longer persistence of HBsAg are defined as markers of chronic HBV infection (Fig. 6) but actually when the HBV infection is from HBV-infected mother to the infants, an effective immune response does not begin for a period of years and it results in a persistent infection while hepatitis B infections of immunocompromised patients typically lead to a persistent HBV infection even if the impairment is mild, it is the same case as in hemodialysis patients. However, after an onset of a long anergic phase of HBV infection, the immune defense may emerge and lead to a selection of escape mutant viral particles and as soon as the cellular immune responses against HBcAg appear, the HBeAg lose its immunomodulatory function [49].

More interestingly, the HBeAg-negative variants with enhanced HBcAg expression and viral replication may usually take over and compensate for loss of destroyed HBV infected cells, and some variants with mutated T cells epitopes of HBcAg and HBsAg may be developed and other non-essential epitopes of the preS domain may be deleted and not expressed [52]. It is most important to know whether the immune response is strong enough to keep HBV DNA replication at low level where expression of HBsAg may still occur but the suppression of HBsAg may be completed by immune control to undetectable levels even in chronic HBV carriers [35]. But contrary, the co-existence of cytotoxic immune responses with ongoing strong HBV DNA replication mainly resulting in progressive liver fibrosis and other inflammatory diseases potentially leading to hepatocellular carcinoma [53].

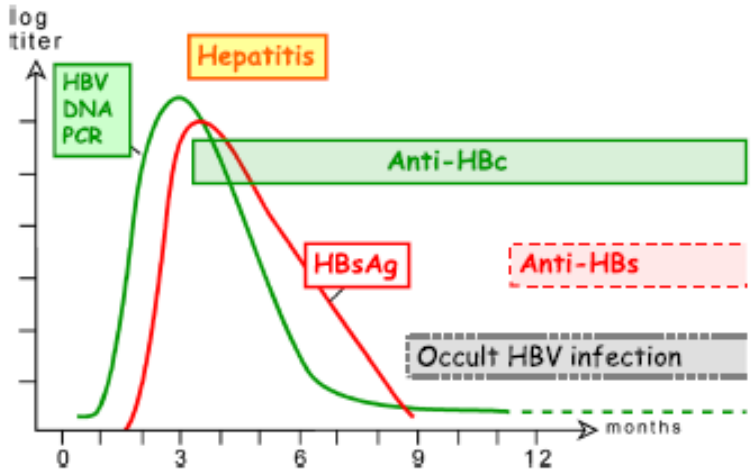

Fig-6: Representative estimation of the makers of acute HBV infections with resolution

After the infection time 0 follows a lag phase of several weeks without detectable markers. The viral interior HBV DNA and HBsAg increase exponentially in the blood, as the HBV DNA can be detectable as it is more sensitive to assay and the peak of HBsAg and HBV DNA is actually reached before outbreak of the acute disease and the both decrease after the physical symptoms onset. At the beginning, the HBV DNA decreases faster because of its shorter half life time in the serum compared to HBsAg and the HBsAg, finally it disappears whereas HBV DNA may remain detectable in small traces. The protective antibodies against HBsAg (anti-HBs) appears very lately after some months of disappearance of HBsAg while antibodies against HBV core antigen (anti-HBc) have already appeared with the onset of clinical symptoms. The HBV often remains in occult form in the liver even after disappearance of HBsAg which is considered as a definitive sign of viral resolution.

Moreover; independently to anterior acute, occult or chronic infection phase there is a high variability of HBV genetic materials such as preC and core gene, HBs antigen loop and preS portion leads to many different defective forms maintaining the viral pathogenicity and many mechanisms of preC sequence may be inactivated by a single point of viral mutation and the most commonly favorable introduction of a stop codon instead of trp codon at the end of preC sequence prevents the translation of the HBeAg precursor and such kind of mutation was recognized to be more in HBV genotype D than in genotype A2 and the HBeAg-negative variants are more prevalent in the Mediterranean than in Central or Northern Europe [41].

The viral mutations through replication by reverse transcription in which a huge number of HBV genomes expressed conduct to mutations of every base more than 2 times per day at rate of 10^{13} per HBV patient, and the RNA editing by cytidine deaminase APOBEC3G which is an interferon induced innate defense factor against retroviruses showed high ability of promoting HBV pregenome over-mutations [54] whereas the immune selection criteria are the same in occult HBV infection and viral strains reactivation under immunodeficiency highly facilitate quick mutations [37, 35] as it is shown in Fig.7 from an unpublished research example conducted by one group of authors from a virology laboratory and it was shown that these highly mutated variants are usually not transmitted due to the lower viremia compared to that of early immune tolerant phases of infection but in case these variants can be transmitted may cause either unapparent or severe infections possibly inducing fatal hepatitis B as these mutant variants lack potential immunomodulators of wild type HBV for viral transmission [38].

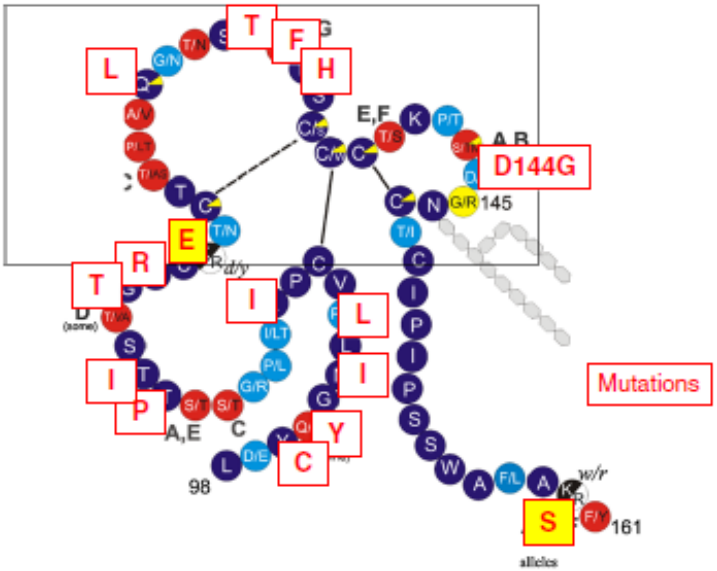

Fig-7: Mutations in the HBsAg loop of a reactivated HBV variant

The complicated folded loop forms the surface of HBV and HBsAg particles but the exact three-dimensional shape of the loop and topology are unknown, each circle represents one amino acid in single letter code of the wildtype HBV and each square shows a mutation while the boxed-in part is called a determinant which is considered as immunodominant but the immune escape induced mutations in the entire HBsAg loop. Yellow shaded squares cause loss of an immunodominant HBsAg subtype determinant. The variant replicated in a patient receiving lymphoma therapy. The patient was anti-HBc and anti-HBs positive before the immunosuppressive lymphoma therapy and developed severe acute hepatitis B after end of the therapy due to immunopathogenesis against the variants which had become abundant under immunosuppression and the serum from acute reactivated hepatitis B phase had a high virus load, but it was HBsAg negative in all assays.

Lately, in 1970s the association between chronic HBV and increase in hepatocellular carcinoma (HCC) incidence was already noted [55] where the area with high prevalence of chronic HBV directly coincided with frequent HCC and the immunopathogenesis of HBV was relatively understood but the one of HBV oncogenicity is not yet understood till now. This step of clarification is still a big challenge for research advance based on the fact that cultivation technologies of hepatoma cells from HBV carriers would lead to a culture system for HBV which finally failed because the cells did not express neither HBV antigens nor HBV DNA but recently, in 1976 the newly established hepatoma cell line PLC PRF-5 known as Alexander cell line successfully secreted HBsAg and some fragments of HBV DNA integrated at several chromosomal sites were identified [53, 56].

Moreover, in 2004 the HBV-related HCC tissue was bio-synthesized from clonal expansion of single cells with aid of one or more chromosomal or rearranged HBV DNA insertions, and after the insertions of HBV genomes ,some integrated HBV DNA elements encoded truncated preS/S proteins that had oncogenic potential *in vitro* and in immunodeficiency mice [57]. It was demonstrated that HBV X proteins were not essential for HBV replication in permanent cell cultures but they have been recognized as essential transcription activators for expression of HBV proteins in differentiated hepatocytes [58] whereas their complex potentiated by preS/S proteins are transcriptional trans-activators possibly activating deregulated cell proliferation and tumors formation [59]; more advanced experimental insertion of HBV DNA promoters may activate cellular oncogenes like *myc* or negatively disrupt proliferation regulators and possibly enhancing activation of fusion proteins as cellular growth factors [60] but there is no typical known site or cellular factor yet identified showing growth of abnormal clonal hepatocytes leading to human hepatocellular carcinoma [61].

In fact; some clinico-epidemiological studies concluded that not only continuous HBV replications are main driving factors of development of hepatocellular carcinoma in HBeAg negative carriers but also high HBV DNA levels in serum is strictly associated with progression of HCC but the replication of HBV DNA in this process is still not clearly understood, and possibly its structure may have a promoting effect on DNA repair in already highly damaged liver cells [62]. The advancement of successful antiviral therapy can stop chronic hepatitis B and the progression of liver cirrhosis but a slowed down risk of HCC may persist.

HBV vaccine development

The researches on HBV vaccines started in 1963 after genetics discoveries of Australian Antigen (lately recognized as HBsAg) in serum of Australian Aboriginal person [63], and this antigen was identified as a part of a viral protein responsible for hepatitis B infections in 1968 [64]. After many years of epidemiological incidence of HBV, famous teams of virologists and microbiologists continued seeking for the trustworthy vaccine of hepatitis B up to where some scientists tried to use three treatments consisting of pepsin, urea and formaldehyde drifted from blood serum after rigorous series of filtration to yield a serum-based product that could be used as vaccine but later, this step to the advanced research idea of collecting from intravenous drug users and gay men with HBV and setting multi-step process of filtration of this blood to remain with only non-infectious hepatitis B surface proteins [11] until a successful development of the HBV diagnostic test and discovery of HBV vaccine by Bruch S. Blumberg in 1976 [65].

The first blood-based HBV vaccine was approved in 1981, but due to its association with increasing transmission of some infectious diseases through blood transfusion, it was withdrawn from the market in 1986 when Pablo DT Valenzuela succeeded in expressing HBV antigen in yeast and launching biotechnological manipulations to produce a non-virulent viral protein serving as the world's first HBV recombinant vaccine [66]. Hepatitis B vaccine (HBsAg) is currently produced by inserting viral HBsAg expressing gene in a transgenic vector cell such as of a power producing yeast like *Saccharomyces cerevisiae* or bacteria like *Escherichia Coli* by recombinant DNA technologies; these biotechnology processes allow microorganisms to produce only the non-infectious surface proteins [67]. The successful development of this recombinant subunit vaccine was an important research step as HBV is unlike other common viruses cannot be grown *in vitro*. In order to achieve this, some more advanced genome editing tools such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) were actually used.

The final protein produced as a HBV vaccine, classified as a subunit vaccine composed of only the surface proteins of the virus, which was previously extracted from the blood serum of chronically infected patients, but now produced by recombination of the viral genes into yeast [23]. Those surface antigen proteins also called "Viral-Like Particles (VLPs)"contain repetitive, high density displays of viral surface proteins that present conformational viral epitopes that can elicit strong T cell and B cell immune responses. Since VLPs cannot replicate, they provide a safer alternative to attenuated viruses [68]. This is the vaccine still in use today but, due to high speed transmission of this viral disease, the current production of hepatitis B vaccine is inefficient to satisfy the worldwide required demand for a secured and complete immunization.

There has been recognized four main genes (S, C, P and X) encoded by the genome in synthesis of HBV vaccine (Fig.8); the S gene codes for the surface antigen protein (HBsAg), C gene codes for core protein (HBcAg) and its start codon AUG which is preceded by an upstream in-frame where pre-core protein is produced and the HBeAg which was identified to be absent in some rare virus strains called HBV pre-core mutants is produced by proteolytic processing of the pre-core protein [69]. The DNA polymerase is encoded by gene P, The HBsAg gene is one long open reading frame but contains three in frame start codons (ATG) that divide the gene into three sections, pre-S1, pre-S2, and S. Because of the multiple start codons, polypeptides of three different sizes called large (the order from surface to the inside: pre-S1, pre-S2, and S), middle (pre-S2, S), and small (S) are produced [70, 71]. The function of the protein coded for by gene X is not fully understood but it is associated with the development of liver cancer. It stimulates genes that promote cell growth and inactivates growth regulating molecules [72]. The S2-S DNA vaccine contains the preS2 and the S HBV domains that are placed under the transcriptional control of the human promoter [72], this allows the expression of both HBV small (S) and middle (preS2 +S) envelope proteins that self-assembled to form protein particles carrying the HBsAg.

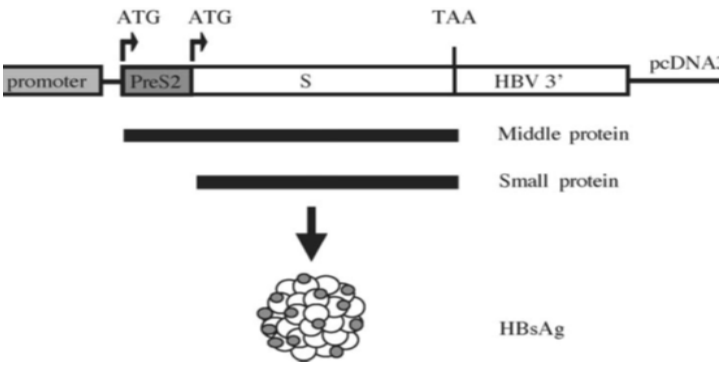

Fig-8: Illustration of genome editing for synthesis of HBV vaccine as HBsAg

S2-S expression vector, the encoded HBV proteins are indicated as thick gray lines below the respective coding sequences. Both HBV envelope proteins self-assemble to form particles carrying the hepatitis B surface antigen (HBsAg). ATG: initiator codon; TAA: stop codon.

From many years ago, thanks to advanced molecular biology for achieving to cloning and sequencing of the HBV DNA [68, 73]. In 2013, the cloned DNA was shown to be indeed circular and encoding the genes for HBsAg, HBcAg, the putative endogenous DNA polymerase and an unexpected X gene conformably to the real copy of HBV genome [74]. The cloning technology of these HBV genetic materials was launched as a relevant biotechnological manufacturing of HBV DNA, HBsAg and HBcAg in unlimited amount by using gene editing instead of extracting them from highly infectious patients' blood, this is greatly promising that these materials would be used soon in large amounts for more advanced diagnostics, drugs and vaccines development.

The CRISPR-Cas9 system is a prokaryotic immune system that confers them resistance to foreign genetic body such as plasmids or phages and provides a form of acquired immunity, its spacers recognize and cut these exogenous genetic materials [75, 76], this system has a significant advantages over ZFN and TALEN as it relies on RNA to home onto DNA whereas ZFN and TALEN depend on custom-making proteins for each specific DNA target. Thus, CRISPR/Cas9 is technically easier to use and is more efficient at cutting targeted DNA [81, 82]. Regarding to advantages and fact that CRISPR/Cas9 system is cost-effective and easily accessible (https://www.addgene.org/), this system has led to an exponential increase in genome engineering of cells and model organisms to study diseases mechanisms in therapeutic studies. It is appreciable to recommend for further research studies on production of HBV vaccines using CRISPR-Cas9 technology as a new and powerful genome editing tool [79, 80], as this method was reported to produce high efficiency for genome editing in most eukaryotic model systems**.** Based on advantages of this system in genome editing and high level of hepatitis, it is wisely useful to use this tool for vaccines production. Its success will adequately promote the low cost and short production time to provide the required quantities of vaccines to overcoming hepatitis and other viral diseases by providing vaccines to population of all ages [79].

Treatments of chronic HBV infections

Generally, treatment of hepatitis A is supportive and includes some measures of providing maintained intravenous hydration and adequate nutrition mainly to reduce hepatic failure even though liver transplantation may be the most supportive therapy [81, 83]. In most cases of patients with acute hepatitis B about 95–99% recover with no long-lasting effects, and antiviral treatment is not warranted but certain patients presenting either severe clinical signs such as ascites, peripheral edema, hepatic encephalopathy or dangerous laboratory parameters such as hypoglycemia, prolonged prothrombin time, very high serum bilirubin and low serum albumin are rarely recommended to be treated with some antiviral drugs similar to those used for chronic hepatitis B such as entecavir or tenofovir even though the use of these nucleoside analogues in such cases have been reported for developing resistance [81]. Chronic hepatitis B therapies aim to control viral replication for slowing down the progression of the disease [82] while the ultimate goal of hepatitis C treatment is prevention of hepatocellular carcinoma [84], but effective treatments of hepatitis D are not yet available, interferon alpha has proven effective at inhibiting viral activity but only on some temporary steps of the disease [85]. In fact, none of the available drugs can totally clean the infection, they can only stop the virus from replicating by minimizing liver damage, and there are seven drug treatments (Table 1) with two immune system modulators mainly alpha interferons and its long acting form bound to polyethylene glycol known as pegylated interferon approved for chronic hepatitis B infection [81].

Table 1: Main drugs used for treatment of chronic hepatitis B infection

Drug treatment	Therapeutic contraindication cases	Resistance possibility
INTERFERON ALPHA (IFN) [82]	-Immunosuppression -Liver failure	None
PEGYLATED INTERFERON (PEG IFN) [82]	-Immunosuppressed patients -Liver cirrhosis -High viral activity	None
LAMIVUDINE [82]	-HIV coinfection(except in multidrug regimen) -HBV reactivation	Very high
ADEFOVIR DIPIVOXIL [82]	-HIV coinfection	Very less
ENTECAVIR [82]	-Lamivudine-resistant patients -HIV coinfection	Very less
TELBIVUDINE [82]	-HIV coinfection (except in multidrug therapy)	High
TENOFOVIR [82]	-HIV coinfection (except in combination)	Very less

The detections of HBV DNA levels, HBeAg status or ALT levels above normal ranges are the main indicating factors to base on for setting treatment initiation; the first line treatments currently used include pegylated interferon, entecavir and tenofovir [82, 86] but multidrug treatment are not actually recommended for long term treatment of chronic HBV as they are not well tolerated compared to single treatment with entecavir or tenofovir but in case of patients with decompensated cirrhosis, treatment and evaluation for liver transplantation are recommended in all cases if HBV DNA is detectable [82]. For the patients with hepatitis C, the best way of reducing long term risks of HCC to the minimum level is determined with achievement to sustained virological response (SVR) which is indicated by an undetectable viral load after 12 weeks of treatment completion, after use of either indirect acting antivirals including pegylated interferon and ribavirin and possibly the combination of these both or direct acting antivirals targeting proteins responsible for viral replication which are mainly grouped in these three following classes [87, 88]:

- NS3/4A protease inhibitors: Telaprevir, Boceprevir, Simeprevir
- NS5A inhibitors: Ledipasvir, Daclatasvir
- NS5Bpolymerase inhibitors: Sofosbuvir, Dasabuvir

These drugs are used in combinations or/and sometimes combined with ribavirin based on virus genotypes, for example genotype 1 which is the most prevalent genotype around the world and USA is better cured with direct antiviral regimen and its first-line therapeutic combination is made of Sofosbuvir and Ledipasvir(SOF/LDV) for 12 weeks in most cases while patient with advanced hepatic fibrosis or cirrhosis require about 24 weeks [88] but high cost of these drugs remains a major limiting factor, particularly in low-resourced areas where the cost of the 12 weeks of regimen of SOF/LDV has been estimated at more than 94,500 US$, which is the main limitation contributing to rapid increase of this epidemic disease [87]. However, high cost limitations of treatments of hepatitis infections is additionally potentiated by antivirals resistance resulting in the fact that HBV present many genotypes developing resistant viral strains, and eventually liver transplantation which is itself also one of the most expensive operations is sometimes applied as last option.

The 2016 HIV report data showed that only not around 50% of HIV infected people were accordingly receiving antiretroviral treatments and this issue obliged WHO to revise the treatment guideline priorities of targeting patients living with VIH in coinfection with chronic HBV infection [91], the recommended use of tenofovir as first-line treatment of HIV-HBV coinfection was reported to be effective in pregnant women having HIV-HBV-coinfection and it allows the prevention of transmission of HBV from mother to child which efficiently reduced new cases of the both viral infections [92]. Consequently, all these above mentioned therapies are limitedly unaffordable to many people, but some of nowadays advanced biotechnology researches are trying to create foods that contain HBV vaccine as a rapid foods regimen therapy to get rid of these clinical problems as one of the most important and quick preventive strategy [3].

Conclusion and Recommendations

The dramatic and global increase of Hepatitis B virus infections do not coincide with current manufacturing technologies of its vaccines; it will not be able to meet the growing demand of hepatitis vaccines based on low production capacity and high cost limitations. Actually, the recombinant hepatitis B vaccine is currently produced using *Saccharomyces cerevisiae* and *Escherichia coli* genome editing for human therapeutic use. Hopefully, there is an upmost need to increase the production by several fold of this biologically active hepatitis B vaccines using other latest novel genome editing tools with efficient technology methods to satisfy the worldwide market demand with affordable cost effectiveness. In fact; not only the mutagenic and oncogenic capacity, and variabilities of HBV genotypes but also high progression of chronic HBV to HCCs and antiviral resistance to the current treatments are a global emerging therapeutic issues to consider in the future clinical researches required for complete cleaning of the infectious viral particles from the patients' blood and reducing mutations of existing HBV infection to achieve a safe and complete eradication of hepatitis B virus infections.

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