Introduction

Hepatitis B virus (HBV) infection and its sequalae; including chronic liver disease, cirrhosis and hepatocellular carcinoma are a major global health problems. It is estimated more than 350 million suffering from chronic HB hepatitis worldwide ***(Marcellin, 2009; El Bahnasy et al., 2016)***. They represent the primary reservoir of infection ***(Kao and Chen, 2000)***.

Egypt is considered an area with moderate endemicity for HBV as the prevalence of HBV chronic carries among adults in the general population range from 2-7% ***(CDC, 2008)***.

Exposure to blood and body fluids is a major risk factor for development of HBV infection and it is a well-established fact that in an unvaccinated individual; the risk of blood acquisition of HBV infection after single exposure of HBV infected blood or body fluid range from 6-30%, therefore, health care workers are at high risk of HBV infection due to repeated exposure ***(Rosea et al., 1999; Talaat et al., 2003; Vipul et al., 2012)***.

Talaat et al. (2003) reported that 5% of Egyptian health-care workers are infected each year with either HCV or HBV.

With the availability of HBV vaccine since 1982, the decline in the incidence of HBV infection and associated

morbidity and mortality was reported ***(Ni et al., 2001; Gunson et al., 2003)***.

Therefore, in 1997 CDC recommended that all health care workers should be vaccinated against HBV ***(CDC, 1999)***. ***Chen and Gluud (2005)*** mentioned that promotion of HB vaccination in health care workers is an important component of HBV infection control strategy. Despite the recommendation and excellent protection profile among post-vaccinated personnel, compliance to this recommendation remained poor in various health care settings ***(Shrestha and Bhattarai, 2006)***.

Hepatitis B protection means that, the anti-HBs level of at least 10 mIU/mL following three doses of recombinant HBV vaccines has been reported to provide 90-95% protection ***(CDC, 2006)***. Routine serologic testing to assess immune status of HBV vaccine is not recommended; however, testing is recommended for health care workers at high risk continued percutaneous or mucosal exposure to blood or body fluids to determine the need for revaccination and to guide post-exposure prophylaxis ***(CDC, 2001; Zeeshan et al., 2007; El Bahmasy et al., 2016)***.

Aim of the Work

The aim of the study:

1- To estimate the prevalence rate of non-responder to the hepatitis B vaccine

2- To identify possible risk factors of non-response.

Chapter 1

Hepatitis B Virus

Introduction:

Hepatitis B is a major global health problem and a potentially life-threatening liver infection caused by the hepatitis B virus (HBV). Hepatitis B virus belongs to the hepadnaviridae class of viruses ***(Littlejohn et al., 2016)***.

The hepatitis B virus was discovered in 1965 by Dr. Baruch Blumberg who won the Nobel Prize for his discovery. Originally, the virus was called the "Australia Antigen" because it was named for an Australian aborigine's blood sample that reacted with an antibody in the serum of an American hemophilia patient. Working with Dr. Blumberg, microbiologist Irving Millman helped to develop a blood test for the hepatitis B virus. Blood banks began using the test in 1971 to screen blood donations and the risk of hepatitis B infections from a blood transfusion decreased by 25 percent. Four years after discovering the hepatitis B virus, Drs. Blumberg and Millman developed the first hepatitis B vaccine, which was initially a heat-treated form of the virus ***(Blumberg and Alter, 1965).***

It is transmitted by direct percutaneous or permucosal exposure to infected blood. The hepatitis B infection occurs in adolescents and adults and can lead to acute hepatitis, subclinical infection, or the development of chronic infection.

The incubation period ranges from 45–160 days, with an average of 75 days, followed by an insidious onset of acute disease ***(Kwon and Lee, 2011)***.

Chronic hepatitis B virus (HBV) is the ninth leading cause of death, with An estimated 257 million people are living with hepatitis B virus infection (defined as hepatitis B surface antigen positive) ***(Bhat et al., 2012)***. In 2015, hepatitis B resulted in 887000 deaths, mostly from complications (including cirrhosis and hepatocellular carcinoma ***(Papatheodoridis et al., 2015)***.

Hepatitis B is an important occupational hazard for health workers. However, it can be prevented by currently available safe and effective vaccine ***(Sheth et al., 2016)***.

A vaccine against hepatitis B has been available since 1982. The vaccine is 95% effective in preventing infection and the development of chronic disease and liver cancer due to hepatitis B ***(Edward, 2015)***.

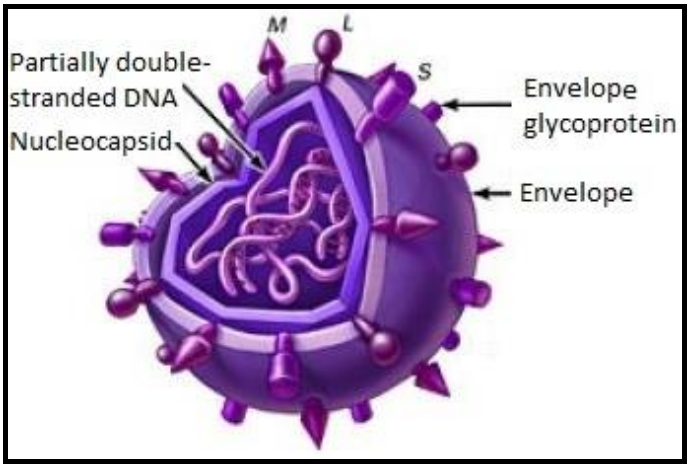

Figure (I): Morphology of hepatitis B virus ***(Kaito et al., 2006)***.

Virology:

The hepatitis B virus (HBV) is a small DNA virus with unusual features similar to retroviruses ***(Hollinger and Liang, 2001)***. It is a prototype virus of the Hepadnaviridae family ***(Ganem and Schneider, 2001)***.

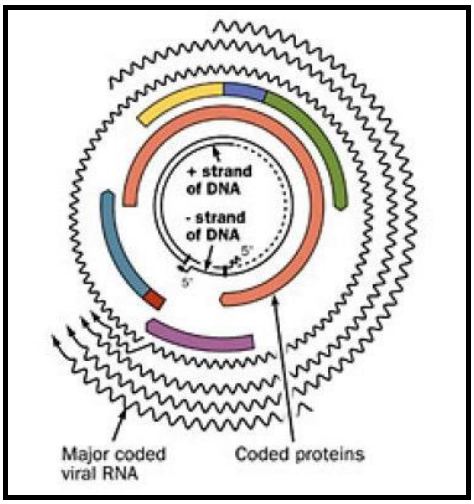

Figure (II): Genomic organization of hepatitis B virus ***(Datta et al., 2012)***.

HBV is differentiated into many genotypes, according to genome sequence. To date, eight well-known genotypes (A-H) of the HBV genome have been defined. Moreover, two new genotypes, I and J, have also been identified. Some HBV genotypes are further classified as sub-genotypes ***(Cooksley, 2010)***.

HBV sequence is characterized by > 8% nucleotide differences for genotype, and 4%-8% nucleotide differences for sub-genotype. Over 30 related sub-genotypes belonging to HBV genotypes have been determined to date, but the mechanisms of different pathogenic characteristics of HBV genotypes are not known for certain ***(Huang et al., 2013)***.

Many studies have reported that different genotypes and sub-genotypes show different geographical distribution, and are related to disease progression, clinical progression, response to antiviral treatment, and prognosis. A-D and F genotypes are divided into various sub-genotypes; no sub-genotypes have been defined for E, G and H genotypes ***(Biswas et al., 2013)***.

Genotype A is widespread in sub-Saharan Africa, Northern Europe, and Western Africa; genotypes B and C are common in Asia; genotype C is primarily observed in Southeast Asia; genotype D is dominant in Africa, Europe, Mediterranean countries, and India; genotype G is reported in France, Germany, and the United States; and genotype H is commonly encountered in Central and South America. Genotype I has

recently been reported in Vietnam and Laos. The newest HBV genotype, genotype J, has been identified in the Ryukyu Islands in Japan ***(Allain, 2006)***.

Geographic distribution of HBV genotypes may be related to route of exposure. For example, genotypes B and C are more common in high-endemic regions of perinatal or vertical exposure, which plays an important role in viral transmission. Other genotypes are primarily observed in regions of horizontal exposure ***(Moura et al., 2013)***.

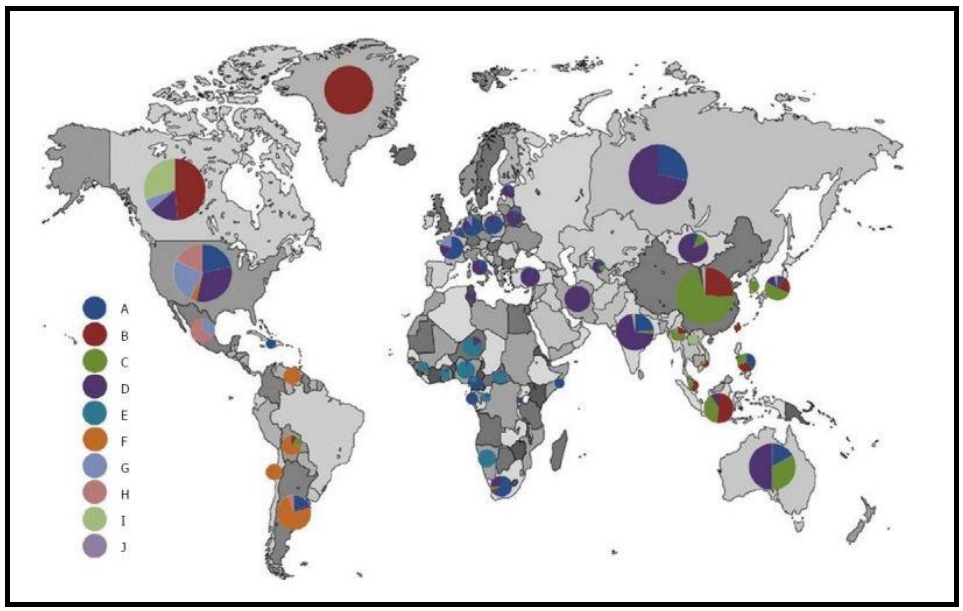

Figure (III): Geographic distribution of hepatitis B virus genotypes worldwide ***(Shi et al., 2013)***.

Three types of viral particles are visualized in infectious serum by electron microscopy. Two of the viral particles are smaller spherical structures with a diameter of 20 nm and filaments of variable lengths with a width of 22 nm. The

spheres and filaments are composed of hepatitis B surface antigen (HBsAg) and host-derived lipids without viral nucleic acids and are therefore noninfectious ***(Gavilanes et al., 1982)***.

The infectious HBV virion (Dane particle) has a spherical, double-shelled structure 42 nm in diameter, consisting of a lipid envelope containing HBsAg that surrounds an inner nucleocapsid composed of hepatitis B core antigen (HBcAg) complexed with virally encoded polymerase and the viral DNA genome. The genome of HBV is a partially double-stranded circular DNA of about 3.2 kilobase (kb) pairs. The viral polymerase is covalently attached to the 5′ end of the minus strand ***(Gerlich and Robinson, 1980)*.**

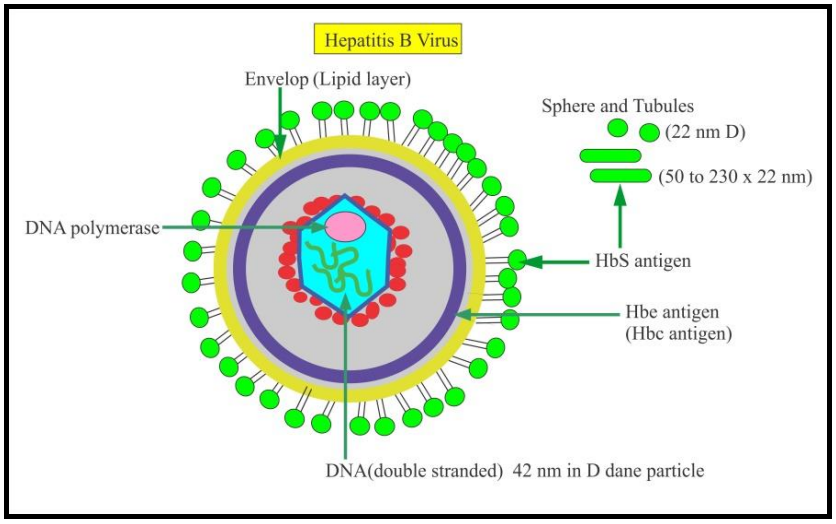

Figure (IV): Electron micrograph of circulating forms of HBV particles in the blood is shown at the top and a schematic drawing of Dane particle, the infectious HBV particle, is shown at the bottom with various structural features ***(Vos et al., 1979)***.

The core protein has the intrinsic property to self-assemble into a capsid-like structure and contains a highly basic cluster of amino acids at its C-terminus with RNA-binding activity ***(Hatton et al., 1992)***. The function of HBeAg remains largely undefined, although it has been implicated as an immune tolerogen, whose function is to promote persistent infection ***(Milich and Liang, 2003)***. The polymerase (pol) is a large protein (about 800 amino acids) and is functionally divided into three domains: the terminal protein domain, which is involved in encapsidation and initiation of minus-strand synthesis; the reverse transcriptase (RT) domain, which catalyzes genome synthesis; and the ribonuclease H domain, which degrades pregenomic RNA and facilitates replication ***(Zhang et al., 2001***).

The HBV replication pathway has been studied in great detail and is summarized in the figure shown below. The initial phase of HBV infection involves the attachment of mature virions to host cell membranes, likely involving the pre-S domain of the surface protein ***(Klingmuller and Schaller, 1993)***. Various cellular factors have been proposed to be the viral receptors, but only carboxypeptidase D has been shown to play an essential role in viral entry for the duck HBV ***(Breiner et al., 1998)***. Mechanisms of viral disassembly and intracellular transport of the viral genome into the nucleus are not well understood and probably involve modification of the nucleocapsid core protein ***(Kang at el., 2006)***. After entry of the

viral genome into the nucleus, the single-stranded gap region in the viral genome is repaired by the viral pol protein, and the viral DNA is circularized to the covalently closed circular (cccDNA) form. This form of HBV DNA serves as the template for transcription of several species of genomic and sub-genomic RNAs and is the stable component of the replication cycle that is relatively resistant to antiviral action and immune clearance ***(Kock and Schilicht, 1993)***.

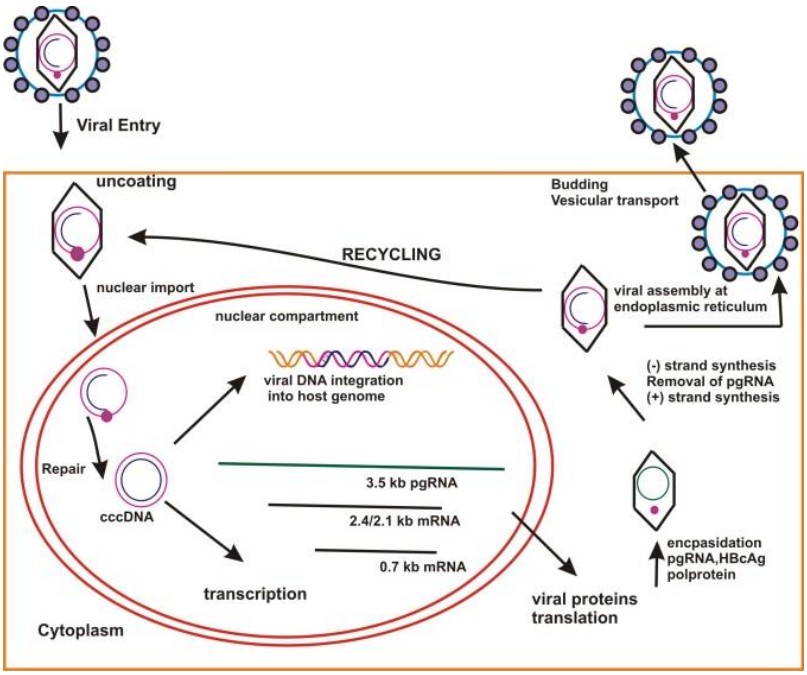

Figure (V): The replication cycle of HBV ***(Jayalakshmi et al., 2013)***.

Mode of transmission:

The hepatitis B virus can survive outside the body for at least 7 days. During this time, the virus can still cause infection if it enters the body of a person who is not protected by the vaccine. The incubation period of the hepatitis B virus is 75

days on average, but can vary from 30 to 180 days. The virus may be detected within 30 to 60 days after infection and can persist and develop into chronic hepatitis B ***(Prince et al., 1974)***.

HBV is spread through contact with infected body fluids and the only natural host is human. Blood is the most important vehicle for transmission, but other body fluids have also been implicated, including semen and saliva ***(Scott et al., 1980)***. Currently, three modes of HBV transmission have been recognized: perinatal, sexual and parenteral/percutaneous transmission. There is no reliable evidence that airborne infections occur and feces are not a source of infection. HBV is not transmitted by contaminated food or water, insects or other vectors ***(Bancroft et al., 1977)***.

Perinatal Transmission

Transmission of HBV from carrier mothers to their babies can occur during the perinatal period, and appears to be the most important factor in determining the prevalence of the infection in high endemicity areas, particularly in China and Southeast Asia ***(Sevens et al., 1979)***. Before HBV vaccine was integrated into the routine immunization program, the proportion of babies that become HBV carriers is about 10-30% for mothers who are HBsAg-positive but HBeAg-negative. However, the incidence of perinatal infection is even greater, around 70-90%, when the mother is both HBsAg-positive and

HBeAg-positive ***(Xu et al., 1985)***. There are three possible routes of transmission of HBV from infected mothers to infants: transplacental transmission of HBV in utero; natal transmission during delivery; or postnatal transmission during care or through breast milk. Since transplacental transmission occurs antenatally, hepatitis B vaccine and HBIG cannot block this route. Epidemiological studies on HBV intrauterine infection in China showed that intrauterine infection occurs in 3.7-9.9% pregnancy women with positive HBsAg and in 9.8-17.39% with positive HBsAg/HBeAg ***(Xu et al., 1999)*** and it was suggested that a mother with positive HBeAg and a history of threatened premature labor are the main risk factors for intrauterine infection. The studies on transplacental transmission of HBV suggested two possible mechanisms (1) hemagenous route: a certain of factors, such as threaten abortion, can make the placental microvascular broken, thus the high-titer HBV maternal blood leak into fetus' circulation ***(Lin et al., 1987)*** (2) cellular transfer: the placental tissue is infected by high-titer of HBV in maternal blood from mother's side to fetus' step by step, and finally, HBV reach fetus' circulation through the villous capillary endothelial cells (***Xu et al., 1998)***.

For neonates and children younger than 1 year who acquire HBV infection perinatally, the risk of the infection becoming chronic is 90%, presumably because neonates have an immature immune system. One of the possible reasons for the high rate of chronicity is that transplacental passage of

HBeAg may induce immunological tolerance to HBV in fetus ***(Hyams,1995)***.

Sexual Transmission

Sexual transmission of hepatitis B is a major source of infection in all areas of the world, especially in the low endemic areas, such as North America. Hepatitis B is considered to be a sexually transmitted disease (STD). For a long time, homosexual men have been considered to be at the highest risk of infection due to sexual contact (70% of homosexual men were infected after 5 years of sexual activity) ***(Alter, 2003)***. However, heterosexual transmission accounts for an increasing proportion of HBV infections. In heterosexuals, factors associated with increased risk of HBV infection include duration of sexual activity, number of sexual partners, history of sexual transmitted disease, and positive serology for syphilis. Sexual partners of injection drug users, prostitutes, and clients of prostitutes are at particularly high risk for infection ***(Alter and Mast, 1994)***.

Parenteral/percutaneous Transmission

The parenteral transmission includes injection drug use, transfusions and dialysis, acupuncture, working in a health-care setting, tattooing and household contact. In the United States and Western Europe, injection drug use remains a very important mode of HBV transmission (23% of all patients) ***(Margolis et al., 1991)***. Risk of acquiring infection increases

with duration of injection drug use. Although the risk for transfusion-associate HBV infection has been greatly reduced since the screening of blood for HBV markers and the exclusion of donors who engage in high-risk activities, the transmission is still possible when the blood donors are asymptomatic carrier with HBsAg negative ***(Luo et al., 1993)***. Obvious sources of infection include HBV-contaminated blood and blood products, with contaminated surgical instruments and utensils being other possible hazards. Parenteral/percutaneous transmission can occur during surgery, after needle-stick injuries, intravenous drug use, and following procedures such as ear piercing, tattooing, acupuncture, circumcision and scarification. The nosocomial spread of HBV infection in the hospital, particularly in dialysis units, as well as in dental units, has been well described ***(Margolis et al., 1991)***, even when infection control practices are followed. As with other modes of transmission, high vial titers have been related to an increased risk of transmission. People at high-risk of infection include those requiring frequent transfusions or hemodialysis, physicians, dentists, nurses and other healthcare workers, laboratory technicians, intravenous drug users, police, firemen, laundry workers and others who are likely to come into contact with potentially infected blood and blood products ***(Alter, 2006)***.