Clinical Medicine and Health Research Journal (CMHRJ)
Volume 02, Issue 06, November - December, 2022
Page No. 245-254

Research Article

Occult Hepatitis B Virus: Implications in Endemic Region like Nigeria.

[1]Akinbobola Ayokunle Adeniyi, [2]Olubunmi Aanu Adeniyi, [3]Olalekan Ademola Kemiki, [4]Stephen Oluwasegun Adetunji, [5]Priscilla OgoOluwa Olamiju, [6]Damilola Christianah Aiyegbokiki, [7]Akinjinmi Akintunde Akintayo, [8]Oludare Joseph Akinola, [9]Cosmas Tochukwu Eze, [10]John Faith OYERINDE,

[1]Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan.

[2]Department of Medical Laboratory Services, Association For Reproductive and Family Health (ARFH), Ibadan.

[3]Department of Medical Laboratory Science, Babcock University Ilishan-remo.

[4]Department of Microbiology and Infection Services, University Hospital Sussex, United Kingdom

[5]411 Jefferson's Avenue, Gaylord, MN 55334

[6]Department of Medical Laboratory Science, Babcock University Ilishan-remo.

[7]Department of Medical Laboratory Sciences, Ajayi Crowther University Oyo, Oyo State, Nigeria.

[8]Alliance for International Medical Action (ALIMA) Owo Mission Nigeria.

[9]Department of Medical Microbiology and Parasitology, University College Hospital Ibadan.

[10]54gene Ltd. Ikate-Lekki, Lagos

Received: 28 September, 2022 Accepted: 28 October, 2022 Published: 01 November 2022

Abstract:

The situation whereby there is appearance of HBV DNA in the absence of observable hepatitis B surface antigen (HBsAg) has been described as occult hepatitis B virus infection. The above situation was first mentioned in late 1970s and its prevalence depends on the differences in the distribution of HBV in each region or community, the study population, sensitivity and specificity of the adopted screening method. The sero-prevalence of HBsAg in Nigeria is estimated to range from 10-40% and this qualifies Nigeria as hyper- endemic area (Odemuyiwa *et al*., 2001; Fasola *et al*., 2008). There is also observable increase in detected HBV DNA among patients who were previously screened as negative for HBsAg (Adeniyi *et al*., 2022). Furthermore, high prevalence of HBsAg has been reported in the population of screened blood donors (Ejele and Ojule, 2004) and also in patients attending clinics (Nwokedi *et al*, 2011; Adeniyi *et al*., 2022).
Occult HBV is clinically important as it can be contacted by means of blood transfusion and organ transplant among others (Kwak *et al*., 2014). In Nigeria, there has being increase in prevalence of occult HBV despite ongoing immunization program (Opaleye *et al*., 2015; Adeniyi *et al*., 2002). When compared to similar viruses that could be transmitted during transfusion, occult HBV is more common (Comanor *et al*., 2006).

Keywords: **Occult HBV, NAT, ELISA, PCR, Molecular method**

Introduction

In the decades after World War II, various studies begin to differentiate the differences in critical hepatitis. Krugman *et al.* (1967) documented two types of hepatitis: serum hepatitis (SH) and infectious hepatitis. Hepatitis B, which was formally referred to as serum hepatitis, is transmitted through body fluids. Study to identify this virus was conducted by Prince *et al.* (1964) and also by Blumberg *et al.* (1965).
Identification of variance in genetic makeup in various community or region was conducted using polyclonal antibodies to test for presence of proteins in human serum (Blumberg *et al*., 1965). The human serum contains antibodies that are capable of coagulating proteins different in genetic makeup from the blood recipients. In a study conducted, an indigenous Australia was tested to have a new antigen which was then named after Australia (Au) (Blumberg *et al.*, 1965). One of the study conducted by Prince *et al.* (1964) identified an antigen denoted by SH similar to above Australia antigen (Au) and both are identical to antigen found on the surface of hepatitis B (HBsAg) (Prince *et al*., 1964; Krugman *et al*., 1967). The above studies have helped in serological detection of HBV and in different epidemiological studies. It is not new that HBV infection has affected over 300 million individual globally. The infection is presented in various clinical ways from asymptomatic status to people with various forms of liver problem resulted from HBV infection (Lee, 1997). One of the means of detecting HBV infection is by the presence of its surface antigen (HBsAg) in the circulation. There is now more sensitive molecular biology technique which has made it possible to identify presence of HBV in people without HBV

surface antigen, with presence or absence of antibody to the surface antigen (anti-HBs) and also HBV core antigen (anti-HBc) (Bréchot *et al*., 1981; Loriot *et al*., 1992).
There is no doubt that many people around the world are now been exposed to HBV (Lavanchy *et al*., 2004) and of which majority of this population are ignorant of their HBV status. One could conclude that the course of HBV infection is complex, considering environmental factors, virus and the infected individual which all play important roles in determining the outcome of disease.

Classification of HBV

Hepatitis B virus belongs to Hepadnaviridae family (Mason *et al*., 2005) with two genera namely, orthohepadnaviruses (infecting mammals) and those common among birds, Avihepadnaviruses (Fauquet *et al*., 2005). All the viruses classified under this family have a significant similarity in genome organisation and replication. Asides from viruses in the family Spumaviridae, Hepadnaviruses is a DNA virus that infects animals and replicates using reverse transcription from viral RNA. Studies have shown more than 35% divergence in genetic makeup of orthohepadnaviruses when compared to more than 19% found in avihepadnaviruses (Guo *et al*., 2005). HBV has small genomic size of about 3-3.3kbp with unique arrangement which makes the virus stand out and these are criteria for designation of Hepadnaviridae into a distinct family (Howard, 1986).
The understanding of the potent DNA sequence similarity and almost a complete lack of homology exhibited by members of this family formed the basis for assigning the viruses into two different genera: orthohepadnaviruses and avihepadnaviruses. On the other hand, the allocation of individual members of this group into above genera depends largely on the viral host preference. Human HBV are assigned into different eight groups of genotypes from A to H depending on their genetic variance which is put at 8% but less than 17%. The supposed number ninth group would have been group I but has less than 8% divergence (Olinger *et al*., 2008).

Structure of HBV

There are three viral particles recognised with the use of electron microscopy and these are two spherical shaped structures of around 20nm in diameter and also the filaments shaped structures of about 22nm in width (Fig. 1). The two above are not infectious though they have viral surface antigen (HBsAg) but lack nucleic acids. The third one is HBV virion also known as Dane particles. The diameter is around 42 nm, has envelope that contains lipid embedded with HBsAg which surround the HBV core antigen (HBcAg) (Gerlich *et al*., 1980).

Genomic Organisation of HBV

The genome of HBV is described as partially double stranded DNA which is circle and is 3.2 kb pairs (Figure 2). At the 5-prime end is attached, with covalent bond, the viral polymerase to the negative strand (Gerlich *et al*., 1980). According to work done earlier on HBV, the genome has four open reading frames (ORF) namely: S, C, P and X. They are in the same direction (which defined the plus and minus strands of HBV DNA), and these ORF help in translation of seven proteins. This means that a small genome contains a large amount of coding information. The P ORF runs almost the entire length of the genome thereby overlaps other segments like C and X and covers S portion of the genome. The genome also has some other characters like promoters, enhancer and polyadenylation signals.
HBV transcripts are encoded by minus strand, according to Elders et al. (1985), and are capped and polyadenylated. The lengths of important transcripts are pre-C/C (3.5 kb), pre-S (2.4 kb), S (2.1 kb) and X (0.7kb) (Enders *et al*., 1985).

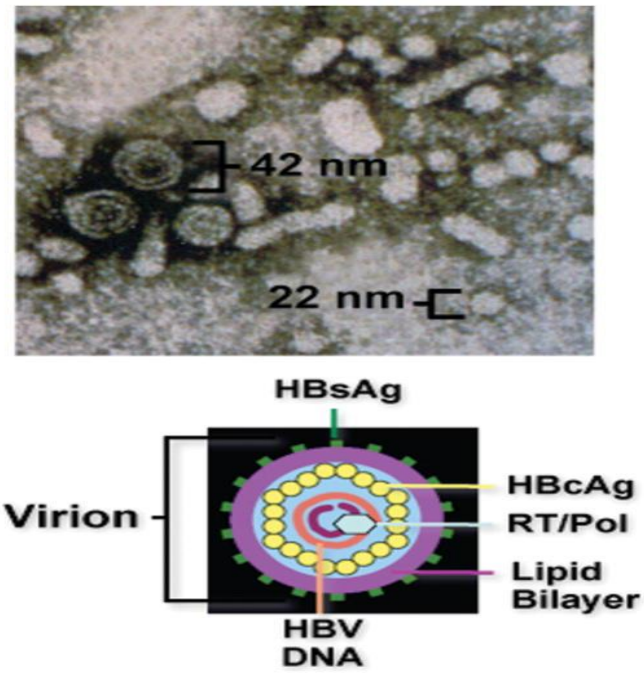

Figure 1: Electron micrograph of circulating forms of HBV particles in the blood is shown at the top and a schematic drawing of Dane particle, the infectious HBV particle, is shown at the bottom with various structural features (Liang, 2009).

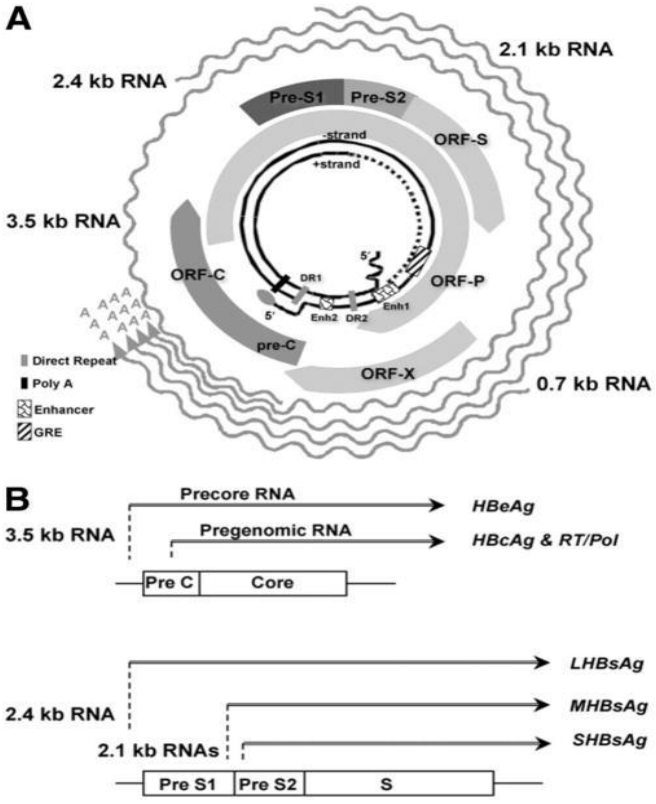

Figure 2: The HBV genome. (A) The genomic organization, RNA transcripts and gene products are shown with several key regulatory elements. (B) The transcription start sites of various HBV transcripts and the proteins they encode (see text for details).
Source: (Liang, 2009)

Considering the 3.5kb transcript from preC/C mRNAs, it has two longer RNAs referred to as pre-core (pre-C) mRNAs and also a short one referred to as pregenome RNA (PgRNA) which is necessary for DNA synthesis (Ganem and Schneider, 2001). pre-C protein also referred to as HBeAg is important in regulating immune response to HBV infection (Chen *et al.*, 2004). HBeAg is also important in predicting current infection.

Pol gene is important in the production of DNA polymerase of the virus and has N-terminus necessary for terminal protein production which functions as primer for negative sense DNA production synthesis (Lin and Anderson, 2000). The section of C-terminal is important in production of reverse transcriptase while PreS/S genes are important in the production of viral envelope glycoproteins. S protein is usually the common envelope protein in the viral structures (Bruss and Ganem, 1991).

Genes and Proteins of HBV Virus

HBV surface envelope proteins (HBsAg) is encoded by *S* ORF, it is structurally and functionally segmented into pre-S1, pre-S2, and S portionsas seen in (Figure 2). Robinson et al., 1976 make us to understand that the pre S – S known in full term as 'presurface surface' region of viral genetic makeup is responsible for production of surface antigen of the virus. HBsAg also known as 24-KD S protein is said to be the common type of surface antigen protein. Pre S2 or M protein is another HBV protein though the function is unknown. Pre S1 or L protein is important in attachment of HBV to the host cell and also helps in the release of the virus from the cell after virion assembly (Bruss and Ganem, 1991). HBcAg is produced by C ORF from translation of core region while HBeAg is produced from precore region. HBeAg as being described by Milich *et al*., 2003 as antigen yet to understand its function but it is believed that its presence indicate ongoing infection.

The pol known as polymerase is produce in P ORF of the virus genome and is said to be large protein with different functions: production of negative sense strand and encapsidation took place in terminal protein segment; synthesis of genome is enhanced in reverse transcriptase domain denoted by RT; replication is facilitated by ribonuclease H domain which also involves in clearing of pregenomic RNA. The (HBxAg) a is 16.5-kd protein that is encoded by HBV *X* ORF and its function includes DNA repair, signal transduction, inhibition of protein degradation and transcriptional activation (Cross *et al*., 1993; Zhang et al., 2001; Bouchard *et al*., 2004; Hu *et al*., 2006). The HBxAg mechanism of action and biologic function in viral life-cycle remain largely unknown but it is believe to contribute to the oncogenic potential of HBV. Literature has also revealed the importance of HBxAg in productive HBV infection in vivo.

Epidemiology of HBV

Death recorded as a result of HBV infection are put around 500,000 to over 1 million annually (Lee, 1997; Mahoney, 1999) and it accounts for 10th most causes of mortality around the world. Depends on varying geographical area and different population subgroups, the prevalence of HBV infection are different (Yu *et al.*, 2000; WHO, 2015). Many areas like Angola, Benin, Bostwana, North Korea South Korea, Mongolia and Alaskan have high endemicity with HBsAg prevalence >8% while part of Bulgaria, Czech Republic, Germany, Poland are considered as having intermediate HBV endemicity with HBsAg prevalence of 2 - 7% and France, Belgium, Sweden, Iceland, US, Mexico and Australia are considered as having low HBV endemicity with HBsAg prevalence of < 2% (Lavanchy, 2004). It is not surprising that regions with a high prevalence of HBV infection are also implicated with high rates of HCC. HBV is responsible for about 60%-80% of the World's primary liver cancer and this is common cause of mortality in Asia and Africa (Lemon *et al.*, 2000; McGlynn *et al.*, 2001).

Literatures revealed that the risk of developing chronic HBV infection ranges from less than 5% in adult to 25% - 30% in infants and children under 5 and to more than 85% in children born to HBeAg-positive mothers after acute exposure (Lok and McMahon, 2001). Chronic HBV infection is likely to develop in immunosuppressed patients after acute infection. Data have shown that more than half of the global population are living in highly endemic areas, while about 2 billion are estimated to be infected (WHO, 2015). Of the 2 billion people who have been infected globally, there are more than 3 million acute cases yearly and more than 350 million are chronic carriers of HBV (WHO, 2015; Goldstein *et al.*, 2005) and approximately 15-40% of the infected patients will develop cirrhosis, hepatocellular carcinoma (HCC) or liver failure (Lok, 2001).

Genetic Diversity of HBV

Lack of proof reading activity by the viral polymerase (pol) and the use of RNA intermediate during its replication has resulted in HBV high genetic variability (Seeger and Mason, 2000; Ganem and Schneider, 2001). The classical serotype based classification of HBV strains has being replaced by HBV genotypes and subgenotypes (Datta, 2008). HBV has 10 genotypes and its genotypes/subgenotypes have been linked to different geographical locations (Okamoto *et al.*, 1998). HBV/A is usually found in North America, Northern Europe, and Central Africa and is pandemic while HBV/B and HBV/C are found in the far East and Southeast Asia. HBV/D is prevalent in India, Mediterranean region and Middle East. Likewise, HBV/E and HBV/ F have been reported in Nigeria and other neighbouring countries. HBV/G has been found in the USA, Germany and France, HBV/H in Mexico and South America, HBV/ I in Vietnam and Laos and HBV/ J in the Ryukyu Islands in Japan (Norder *et al.*, 1994; Magnius and Norder, 1995; Stuyver *et al.*, 2000; Croagh *et al.*, 2015).

Transmission of HBV

HBV infection spreads easily through contact with infected body fluids and thus said to be highly contagious. Human served as the only natural host of the virus and the possible means of transmission are through vehicles such as body fluids like blood, semen and saliva (Scott *et al.*, 1980). Hepatitis B virus could be as high as 10^{10} Dane particles per ml in body fluid of the carrier and it could be transmitted through sexual contact, sharing of sharp object with infected person and also during birth from infected mother to the child.

Depend on the geographical regions; the risk of vertical transmission varies. Some of the routes of HBV transmission like mothers to child as stated above includes: transmission through placental in pregnancy, delivery, during care or breastfeeding (Hou *et al.*, 2005). Many studies have shown rare cases of uterus transmission and this account for < 3% of perinatal infections (Tang *et al.*, 1998). The HBeAg status of the infected mother determines vertical HBV transmission (Xu *et al.*, 1985). More than 35% of HBV infections in US are traced to act of practising multiple sexual partners and more than 20% are found in people with gay relationship. This

statistics demonstrated that sexual contact is most likely route of HBV transmission in such a low endemic areas (Wasley *et al.,* 2008).

There are other likely means of HBV transmission such as the practise of injecting drug common among gangsters, circumcision, use of infected blood products or whole blood and working in health care facility (Margolis *et al.,* 1991).

Pathogenesis of HBV Viral Infection

HBV of about 10^{10} to 10^{12} genome copies per ml found in body fluid is enough for possible transmission (Schildgen *et al.,* 2006). On entering the host, the Dane particle locates the hepatocyte cells, its main target, for replication and persistence. There has been no evidence of HBV causing cytotoxic effect on the affected hepatocytes and no evidence of cytopathic change under normal conditions of infection (Thimme *et al.,* 2003; Wieland *et al.,* 2004).

There are evidences that many people infected with HBV are usually asymptomatic and may have little injury of the liver, even when the virus is actively replicating (deFranchis *et al.,* 1993). There are different schools of thought that believe the response of the host cells to viral antigen is responsible for liver injury. This observation is same with the fact that patient with low immunity tend to have minimal liver injury despite the high burden of the viral infection (Stevens *et al.,* 1975). Consequently, confirmed by experimental data (Ando *et al.,* 1994; Guidotti *et al.,* 2000; Kakimi *et al.,* 2001), generally, massive cytotoxic T Lymphocytes (CTL) and Natural Killer cells (NK) action result in the killing of infected hepatocytes and is essential for the elimination of the infection.

The Liver and Its Response to HBV Infection

Liver has remains primary site of HBV replication in humans. There has not been evidence that other cells in the body can be use to replicate the virus. In term of structure, blood enters from the hepatic artery, through miscroscopic lobules of the liver, and portal vein which is located in a structure known as portal triad then comes out through hepatic vein. The miscroscopic lobules has parenchymal cell where larger percentage of the liver mass are situated and is referred to as hepatocyte. Clearance of hepadnavirus infections leads to destruction of large numbers of hepatocytes causing jaundice (icterus) in some patients as a result of build up of bilirubin in the blood thereby giving a yellow colouration of the eyes and skin (Thorgeirsson and Grisham, 2003).

Immune Response to Hepatitis B Virus Infection

The process of HBV pathogenesis is not injurious to liver cell even after all the liver cells have been infected. The immune response to infection is usually slow as a result of minimal observable cells injury (Jilbert *et al.,* 1992; Kajino *et al.,* 1994; Thimme *et al.,* 2003; Wieland *et al.,* 2004). Due to much number of infected hepatocytes, there is massive attack by the immune response on the hepatocytes during the clearance stage of the infection (Summers *et al.,* 2003). More than 90% population of immunocompetent adults are able to develop enough immune response during clearance stage to resolve the infection but that is not the case in less than 10% of adult and children below one year of age whom may not be able to clear the infection.

Chronic hepatitis B is seen as a result continuous injury to liver cells which is a result from recruitment of immune mediated T-cell against hepatocytes with viral antigens (Ganem and Prince, 2004). It is usually lifelong and is believed to be as a result of dysfunction HBV-specific T-cell from exhausted T-cell thereby affecting the normal function of the cells to fight infection (Das *et al.,* 2008). There is also observable reduction in viral load during the infection which could be due to production of antiviral cytokines which hinder viral replication (Meuleman *et al.,* 2006).

Clinical Features of HBV Infection

There is dynamics in HBV infection and it clinical manifestation is determine by patients' age when infection occur (young children generally have milder disease than adults), the immune status, the infecting virus dose (higher doss appeared to result in shorter incubation period and generally more severe hepatitis) and also at what point the disease was observed (Robinson, 1995). At the onset of infection, the patient may develop fever like symptoms like feeling unwell, vomiting and headache. Jaundice may be noticed but more sometimes HBV infection produces no symptoms or jaundice (Hollinger and Liang, 2001). It has been stated earlier that most adult recovered after the episode of infection while few others may not recover but develop fulminant hepatitis and die (Robinson, 1995; WHO, 2015).

Patterns of HBV Infection

During HBV infection (primary infection), those who are susceptible (non-immune) can be either symptomatic or asymptomatic. While the asymptomatic is more common, symptomatic is less common, especially in young children. Both are usually self-limited in adult with clearance of virus from blood and liver and the development of lasting immunity to re-infection (Hoofnagle, 1981; Wright and Lau, 1985). It should be noted that some primary infections in healthy adults do not resolve as would be expected but develop into persistent infections. Persistent HBV infection can be presented as symptomatic or asymptomatic. Asymptomatic chronic HBV carriers are usually people with subclinical persistent infection, normal or nearly normal findings on liver biopsy and normal serum aminotransferase levels; chronic hepatitis B explain those with abnormal liver function and histologic; Liver cirrhosis explains is seen when new nodules and fibrosis develop acute liver injury. More than 15% of people with chronic hepatitis B develops cirrhosis (Ganem and Prince, 2004).

Primary Infection in HBV

Primary infection is characterised with production of IgM isotope against HBV core antigen (anti-HBc antibody) and at this time HBsAg is seen in circulation (Hoofnagle, 1981). The titre of virus in the blood will be at high level with detectable number of HBeAg (Ribeiro *et al.,* 2002).

After liver injury, the level of alanine aminotransferase (ALT) is only increase following established viral infection. This is an indication of time needed for the immune response mediated by T-cell to initiate liver injury. The clearance of hepatocyte of viral infection without necessarily causing liver injury shows the potency of non-cytolytic mechanisms' ability to achieve clearance (Ganem and Prince, 2004). Clearance of HBV infection is characterised by absence of HBsAg and HBeAg antigens with observable level of anti-HBs antibodies in circulation. There is also persistence of HBV DNA in the blood for long period or life (Prince *et al.,* 2001).

Occult Hepatitis B Infection

This is use to describe the detection of HBV DNA in the absence of observable HBsAg and with presence or absence of anti-HBC or anti-HBs after incubation period (Allain, 2004).

There are still many things unclear about occult HBV infection especially its role in clinical and biological spectrum of infection caused by HBV. There is persistence of HBV DNA even after the clearance of HBsAg in the liver and serum (Feitelson *et al.,* 1994; Mosley *et al.,* 1995; Yuki *et al.,* 2003). There is report of occult HBV infection among population of asymptomatic patience like blood donors, patience with good result of liver test and patients coming for general medical check up (Hennig *et al.,* 2002; Adeniyi et al., 2022).
Occult HBV can be found in different conditions including (1) people who was once infected with HBV with evidence of anti-HBs (2) people infected with escape mutant (3) apparently healthy HBV infected individual at nonproductive stage with presence of anti-HBc and absence of detectable anti- HBe (4) people who has chronic hepatitis or apparently healthy individual with no indication of HBV except HBV DNA (Allain, 2004).

Occult HBV in Recovered Infection

Replication of HBV DNA, at very low level with persistence detection over the years in the liver, peripheral blood, mononuclear cells or serum, continues in an individual who have recovered from HBV infection and produced neutralizing anti-HBs (Norborg *et al.,* 2000; Rehermann *et al.,* 1996). In such cases, the virus is either complexed with anti-HBs, or free in the circulation, or both forms might be present (Yotsuyanagi *et al.,* 1998). Many authors have shown level of HBV DNA in OBI and put the viral load at range between 5 and 1000 IU/ml. An explanation to this might be as a result of cytotoxic T-lymphocyte (CTL) inadequate efficiency response resulted in clearing most, but not the entire virus produced (Rehermann *et al.,* 1996).

HBsAg Mutants and Occult HBV

Mutation in genetic makeup of HBV results in variant different in phenotype from the initial virus (de Franchis *et al*., 2003). In some occasion mutation is conferred by pressure from the host immune response to infection given rise to change in sequence at S ORF as seen in vaccine-escape mutants. Likewise, changes can occur at P ORF as a result of pressure from taken some antiviral drugs. Over time there will be some stability in the HBV mutants and could be transmitted either by horizontal or vertical means (Weber, 2005). On the other hands, Carman *et al*. (1996) observed that immune-escape mutants result from pressure imposed as a result of humoral and cellular immune response to invading virus thereby making HBV invisible to detect. Someone who has been successfully vaccinated could still come down with infection by S-immune escape mutants like G145R and D145R (Dindoost *et al*., 2012).

Occult HBV and Anti-HBC

In time past presence of anti-HBc is important in determining non-B hepatitis but with the introduction of anti-HCV, anti-HBc test is no more important except in prevention of occult HBV transmission (Kleimman and Busch, 2001). The distribution of HBV in an area is an important factor in determining the HBV markers that would be found in anti-HBc positive individual (Allain, 2004). The presence of anti-HBc without observable anti-HBs is called anti-HBc- only and it could be explain in either two ways (Grob *et al.,* 2000). The first case is seen in long time chronic HBV with no observable HBeAg and the level of HBsAg become undetectable as a result of its low level in the system (Allain *et al.,* 2003). The second case is seen in a situation whereby an individual have recovered from HBV infection with short persistence of anti-HBs. The anti-HBs become undetectable but only anti-HBc is observed (Almeida-Neto *et al*., 2001).

Occult HBV without Serological Markers

Screening for anti-HBc in blood donors are important in avoiding transmission of HBV infection in the two circumstances mentioned above. It is now important to design blood safety strategy in lieu of rise in detection of HBV DNA in the absence of observable HBV blood markers (Allain, 2004). There have been situation of reported HBV DNA alone in hepatocellular carcinoma (HCC) or hepatitis (Berasain *et al.,* 2000; Shiota *et al.,* 2000). Some vaccinated children were reported to have no detectable anti-HBs with HBV DNA below 10^3 copies per ml. (Feitelson *et al.,* 1994). In another study, a case was reported of a retrospective study of population who earlier received blood transfusion (Baginski *et al.,* 1992).

Occult HBV Infection and Clinical Implication

The clinical implication of OBI has remained unknown over the years. People with Hepatocellular carcinoma or unclassified chronic liver disease were first to be detected of OBI (Brechot *et al.,* 2001). This shows association between occult HBV (OBI) and liver disease, other things been equal like absence of HCV and abnormal alcohol intake. It is noteworthy to mention that people with OBI are usually asymptomatic and screening of large population is a prerequisite for its detection. Occult OBI has potential for reactivation and eventually causes hepatocyte inflammation in immunosupressed individual or people who are undergoing chemotherapy. OBI could also result in hepatocellular carcinoma in individual by its ability to influence hepatic inflammation and fibrosis (Giovanni *et al*., 2013).
Blood ALT is usually normal in people with OBI and anti-HBs (Michalak *et al.,* 1994). There are scanty report of ALT level and liver disease in blood donors with anti-HBc only. Studies have shown no observable change in ALT level or liver disease in OBI patients with either anti-HBs or ani-HBc only (Yotsuyanagi *et al.,* 1998). In another study, more than 1.9% of patients with chronically elevated ALT levels were detected of HBV DNA and one of these patients was a previous blood donor (Berasain *et al.,* 2000). This shows the importance of screening for OBI before blood transfusion.

Prevalence of Occult HBV Infection

Distribution of OBI in a population varies and depends on the level of endemicity of HBV infection in the study area, the type of assay adopted for the studies, and the population being studied. Many of the studies above have shown that the prevalence of OBI is higher in people who are at more risk of HBV infection and with liver disease than people with less risk of having HBV infection and without liver disease (Maria *et al*., 2015). OBI is predominant in regions with high prevalence of HBV infection (Chemin and Trepo, 2005; Adeniyi *et al*., 2022). HBV infection in endemic area is predominantly gotten perinatally or while growing up as a child. Prevalence of OBI is also depending on the sample being tested with higher detection seen using liver compared to serum sample (Chemin and Trepo, 2005).

Diagnosis of Hepatitis B Virus

The use of different serological markers of HBV and by inclusion of other screening method to exclude other viral agent like hepatitis A and C viruses helps in diagnosing HBV.

Vaccine-induced immunity could be detected using serological tests and to differentiate acute, self-limited infections from chronic HBV infections. Measurement of HBV viral load and antiviral agents' potency can be accomplished by Nucleic acid testing for HBV-DNA (Mel *et al*., 2005). Blood is usually preferable sample for the detection of HBV infection. Assay for viral DNA, though rigorous, can be used to determine the presence of circulating virus. A viral load around 2,000IU/ml is considered enough to cause episode of hepatitis and also damage to liver cells (Chu *et al.,* 2002). In occult HBV, viral load is usually low and may not be detected even with PCR diagnostic technique (Raimondo *et al.*, 2008).

Serologic Diagnosis of HBV

The use of enzymes immunoassays (EIAs) is good in accomplishing detection of HBV using their antigen and corresponding antibodies (Table 1) like HBeAg and anti-HBe, HBsAg and anti-HBs, anti-HBc and HBcAg, though not freely found in circulation (Servoss *et al.,* 2005). HBsAg are found in excess in serum (Martin *et al*., 1993), this first marker indicate presence of HBV infection and seen within 7 days from first exposure. Presence of anti-HBs in the circulation indicate HBV infection resolution and gives lasting immunity to the individual (Servoss and Friedman, 2006).

Presence of IgM anti-HBc is important in diagnosing acute HBV infection during window period. IgG anti-HBc soon replaces IgM anti-HBc whose presence indicate resolved HBV infection (with observable HBsAg) (Servoss and Friedman, 2006). When the virus is finally cleared from the system, HBeAg level decreased and then replaced with anti-HBe. The level of HBV DNA is markedly reduced during this period. Chronic HBV infection is indicated by the presence of HBeAg in serum (Servoss and Friedman, 2006). There is also presence of HBsAg exceeding 6 months with more than 10^5 HBV DNA copies/ml and elevated serum ALT level (Lok *et al.,* 2001). There is varying appearance of HBV serological markers during HBV infection depends on either the infection is an acute or chronic HBV infection (Table 2.) (Gitlin N, 1997; Mahoney, 1999; Cabrerizo *et al.*, 2000).

Table 1: Antigens and antibodies markers of HBV infection

Antigens	Antibodies
HBsAg Hepatitis B surface antigen is the earliest indicator of acute infection and is also indicative of chronic infection if its presence persists for more than 6 months. It is useful for the diagnosis of HBV infection and for screening of blood. Its specific antibody is anti-HBs	*Anti-HBs* This is the specific antibody to hepatitis B surface antigen. Its appearance 1–4 months after onset of symptoms indicates clinical recovery and subsequent immunity to HBV. Anti-HBs can neutralize HBV and provide protection against HBV infection
HBcAg Hepatitis B core antigen is derived from the protein envelope that encloses the viral DNA, and it is not detectable in the bloodstream. When HBcAg peptides are expressed on the surface of hepatocytes, they induce an immune response that is crucial for killing infected cells. The HBcAg is a marker of the infectious viral material and it is the most accurate index of viral replication. Its specific antibody is anti-HBc	*Anti-HBc* This is the specific antibody to hepatitis B core antigen. Antibodies to HBc are of class IgM and IgG. They do not neutralize the virus. The presence of IgM identifies an early acute infection. In the absence of HBsAg and anti-HBs, it shows recent infection. IgG with no IgM may be present in chronic and resolved infections. Anti-HBc testing identifies all previously infected persons, including HBV carriers, but does not differentiate carriers and non-carriers

Table 1: Antigens and antibodies markers of HBV infection (continuation)

Antigens	Antibodies
HBeAg Hepatitis B e antigen appearing during weeks 3–6 indicates an acute active infection at its most infectious period, and means that the patient is infectious. Persistence of this virological marker beyond 10 weeks shows progression to chronic infection and infectiousness. Continuous presence of anti-HBe indicates chronic or chronic active liver disease. HBeAg is not incorporated into virions, but is instead secreted into the serum. Mutant strains of HBV exist that replicate without producing HBeAg. HBeAg's function is uncertain. Its specific antibody is anti-HBe	*Anti-HBe* This is the specific antibody to hepatitis B e antigen. During the acute stage of infection the seroconversion from e antigen to e antibody is prognostic for resolution of infection. Its presence in the patient's blood along with anti-HBc and in the absence of HBsAg and anti-HBs indicates low contagiousness and convalescence [31]
HBXAg Hepatitis BX antigen is detected in HBeAg positive blood in patients with both acute and chronic hepatitis. HBX Ag is a transcriptional activator. It does not bind to DNA. Its specific antibody is anti-HBX	*Anti-HBX* This is the specific antibody to hepatitis BX antigen. It appears when other virological markers are becoming undetectable
HBV DNA HBV DNA is detectable by PCR as soon as 1 week after initial infection, but the test is generally only performed for research purposes or to detect mutants that escape detection by current methods	
HBV DNA polymerase Tests for the presence of HBV DNA polymerase, detectable within 1 week of initial infection, are only performed for research purposes	

Source: (Cabrerizo *et al.,* 2000)

Table2: Stages of HBV infection with serological markers

Stage of infection	HBsAg	Anti-HBs	Anti-HBc IgG	Anti-HBc IgM	HBeAg	Anti-HBe
Late incubation period	+	-	-	-	+/-	-
Acute hepatitis B or persistent carrier state	+	-	+	+	+	-
HBsAg-negative acute hepatitis B infection	-	-	-	+	-	-
Recovery with loss of detectable anti-HBs	-	-	+	-	-	-
Healthy HBsAg carrier	+	-	+++	+/-	-	+
Chronic hepatitis B, persistent carrier state	+	-	+++	+/-	+	-
HBV infection in recent past, convalescence	-	++	++	+/-	-	+
HBV infection in distant past, recovery	-	+/-	+/-	-	-	-
Recent HBV vaccination, repeated exposure to antigen without infection, or recovery from infection with loss of detectable anti-HBc	-	++	-	-	-	-

Source: (Cabrerizo *et al.,* 2000)

Molecular Diagnosis of HBV

Molecular detection of HBV infection has greatly improved diagnosis. It could be use to achieve both qualitative and quantitative diagnosis of HBV. The quantitative diagnosis of HBV has achieved much success including: the detection of acute HBV infection, differentiation of ongoing from resolving HBV infection and also monitor patience response to antiviral treatment (Servoss and Friedman, 2006).

HBV DNA could be assayed both qualitatively and quantitatively. The use of signal or target amplification method could help to quantify HBV DNA in the serum. Signal amplification, like branched DNA technique, are very specific but not as sensitive to detect low level of HBV DNA. On the other hands, target amplification, like PCR technique, are very sensitive and could detect HBV DNA as low as 10 copies per ml (Loeb *et al.*, 1991). Recent advance in PCR technique is real-time PCR which could detect and quantify HBV DNA simultaneously in real time. It is very sensitive and could detect viral genome as low as 10 copies per ml (Sum *et al.*, 2005).

Prevention and Control

We have basically three main strategies put in place to prevent HBV infection and this includes passive immunoprophylaxis, active immunization and behaviour modification. An individual could gain immunity as a result of exposure to antibodies (monoclonal and polyclonal) thereby prevents infection (i.e passive immunoprophylaxis). An active immunization involves inoculation of an individual with all or part of live or killed microorganism to activate the host cells to produce immune response to infection. Behavioural modification involves changes in sexual activities, adequate screening of blood and blood products and many more. Even though neonates and children are more at risk of infection in early stage of their growing up, active and passive immunisation would be of great benefit in preventing infection (Hou *et al.*, 2005).

Conclusion

Occult HBV poses much concern especially in HBV endemic region, like Nigeria, where its spread as a result of lack of appropriate molecular diagnostic techniques in routine laboratory blood screening before transfusion or even among general population is being increasingly observed. It is important to screen for HBV DNA, HBcAg, anti-HBsAg, HbeAg, anti-HbeAg in individual. In addition to the use of ELIZA diagnostic technique, blood donors and the general population should be screened for the presence of occult HBV. This will assist in preventing circulation of Occult HBV in the population in addition to effort being put in place by immunization program.

References

1. Adeniyi AA, Olamiju PO, Adeniyi OA, Adetunji SO, Odiabo GN and Kemiki OA. 2022. Detection of HBV DNA among HBsAg Negative Patients. *Virol Immunol j;DOI:*10.23880/vij-16000294.
2. Allain JP, Candotti D, Soldan K, Sarkodie F, Phelps B, Giachetti C, Shyamala V, Yeboah F, Anokwa M, Owusu-Ofori S, Oparesem O. 2003. The risk of hepatitis B virus infection by transfusion in Kumasi, Ghana. *Blood* 101: 2419-2425.
3. Allain JP. 2004. Occult hepatitis B virus infection. *Transfus Clin Biol* 11:18–25.
4. Almeida-Neto C, Strauss E, Sabino EC, Sucupira MC, Chamone DA. 2001. Significance of isolated hepatitis B core antibody in blood donors from Sao Paulo. *Rev Inst Med Trop Sao Paulo* 43: 203-208.
5. Ando K, Guidotti LG, Wirth S, Ishikawa T, Missale G, Moriyama T. 1994. Class-1 restricted cytotoxic T-lymphocytes are directed cytopathic for their target cells in-vivo. *J Immuno* 152(7): 3245-3253.
6. Baginski I, Chemin I, Hantz O, Pichoud C, Jullien AM, Chevre JC. 1992. Transmission of serologically silent hepatitis B virus along with hepatitis C virus in two cases of post-transfussion hepatitis. *Transfussion* 32: 215-20.
7. Berasain C, Betes M, Panizo A, Ruiz J, Herrero JI, Civeira MP. 2000. Pathological and virological findings in patients with persistent hypertransaminasaemia of unknown aetiology. *Gut* 47: 429-35.
8. Blumberg BS, Alter HJ, Visnich S. 1965. A "New" Antigen in Leukemia Sera. *The Journal of the American Medical Association* 191: 541–546.
9. Bouchard MJ, Schneider RJ. 2004. The enigmatic X gene of hepatitis B virus. *J Virol* 78:12725–12734.
10. Brechot C, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Brechot P . 2001. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: Clinically significant or purely "occult"? *Hepatology* 34: 194–203.
11. Bruss V, Ganem D. 1991. The role of envelope proteins in hepatitis B virus assembly. *Proc Natl Acad Sci* 88: 1059-63.
12. Cabrerizo M, Bartolome J, Caramelo C, Barril G, Carreno V. 2000. Molecular analysis of hepatitis B virus DNA in serum and peripheral blood mononuclear cells from hepatitis B surface antigen-negative cases. *Hepatology* 32: 116–123.
13. Carman W.F., Molecular varients of hepatitis B virus. *Clin Lab Med* 1996; 16: 407-428.
14. Chemin I, Trepo C. 2005. Clinical Impact of occult HBV infection. *J Clinical Virol* 34:
15. Chen MT, Billaud JN, Sallberg M, Guidotti LG, Chisari FV, Jones J, Hughes J, Milich DR. 2004. A function of the hepatitis B virus precore protein is to regulate the immune response to the core antigen. *Proceedings of the National Academy of Sciences of the United States of America* 101:14913–14918.
16. Chu CJ, Hussain M, Lok AS. 2002. Quantitative serum HBV DNA levels during different stages of chronic hepatitis B infection. *Hepatology* 36: 1408–1415.
17. Comanor L, Holland P. 2006. Hepatitis B virus blood screening: unfinished agendas. *Vox Sang* 91: 1-12.
18. Croagh CM, Desmond PV, Bell SJ. 2015. Genotypes and viral varients in chronic hepatitis B: A review of epidemiology and clinical relevance. *World J. Hepatol* 7(3): 289-303.
19. Cross JC, Wen P, Rutter W. 1993. Transactivation by hepatitis B virus X protein is promiscuous and dependent

on mitogen-activated cellular serine/threonine kinases. *Proc Natl Acad Sci U S A* 90:8078–8082.
20. Das A, Hoare M, Davies N. 2008. Functional skewing of the global CD8 T cell population in chronic hepatitis B virus infection. *Journal of Experimental Medicine* 205(9): 2111–2124.
21. Datta S. 2008. An overview of moleculer epidemiology of Hepatitis B virus (HBV) in India. *Virology Journal* 5: 156.
22. de Franchis R, Hadengue A, Lau G, Lavanchy D, Lok A. 2003. EASL International Consensus Conference on Hepatitis B. 13-14 September, 2002 Geneva, Switzerland. Consensus statement (long version). *J Hepatol* 39 Suppl 1: S3-25.
23. de Franchis R., Meucci G, Vecchi M. 1993. The natural history of asymptomatic hepatitis B surface antigen carriers. *Ann Intern Med* 118: 191-4.
24. Dindoost P, Jazayeri SM, Karimzadeh H, Saberfar E, Miri SM. (2012) HBsAg variants: common escape issues. *Jundishapur J Microbiol* 5(4): 521-527.
25. Ejele OA and Ojule AC. 2004. The prevalence of hepatitis B surface antigen (HBsAg) among prospective blood donors in Port-Harcourt, Nigeria. *Niger J Med* 13(4): 336-8.
26. Enders GH, Ganem D, Varmus H. 1985. Mapping the major transcripts of ground squirrel hepatitis virus: the presumptive template for reversetranscriptase is terminally redundant. *Cell* 42:297–308.
27. Fasola FA, Kotila TR, Akinyemi JO. 2008. Trends in transfusion-transmitted viral infections from 2001 - 2006 in Ibadan, Nigeria. *Intervirology* 51: 427-431.
28. Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA. 2005. Virus Taxonomy: Eighth Report of the International Committee of Taxonomy of Viruses. Hepadnaviridae. San Diego, CA: *Elsevier Academic Press* 2005:373–384.
29. Feitelson M, Lega L, Guo J, Resti M, Rossi ME, Azzari C. 1994. Pathogenesis of post tranfussion viral hepatitis in children with betathalassemia. *Hepatology* 19: 558-68.
30. Ganem D, Prince AM. 2004. Hepatitis B virus infection Natural history and clinical consequences. *New England Journal Medicine* 350: 1118–1129.
31. Ganem D, Schneider R.J. 2001. Hepadnaviridae: the viruses and their replication. In: Fields virology 4th edition. Edited by: Knipe DM; Howley PM. *Philadelphia: Lippincott Williams & Wilkins* 2923 - 2969.
32. Gerlich W, Robinson WS. 1980. Hepatitis B virus contains protein attached to the 5′ end of its complete strand. *Cell* 21:801–811.
33. Giovanni Raimondo, Gaia Caccamo, Roberto Filomia, and Teresa Pollicino. Occult HBV infection. Semin Immunopathol. 2013 Jan; 35(1): 39–52.
34. Gitlin N. 1997. Hepatitis B: diagnosis, prevention, and treatment. *Clin Chem* 43(8B):1500–1506.
35. Goldstein ST, Zhou F, Hadler SC, Bell BP, Mast EE, Margolis HS. 2005. A mathematical model to estimate global hepatitis B disease burden and vaccination impact. *Int J Epidemiol* 34(6): 1329.
36. Grob P, Jilg W, Bornhak H, Gerken G, Gerlich W, Günther S, Hess G, Hüdig H, Kitchen A, Margolis H. 2000. Serological pattern “anti-HBc alone”: report on a workshop. *J Med Virol*. 62:450–455
37. Guidotti LG, McClary H, Loudis JM, Chisari FV. 2000. Nitric oxide inhibits hepatitis B virus replication in the livers of transgenic mice. *J Exp Med* 191(7): 1247-1252.
38. Guo H, Mason WS, Aldrich CE, Saputelli JR, Miller DS, Jilbert AR and Newbold JE. 2005. Identification and classification of avihepadnaviruses isolated from exotic anseriformes maintained in captivity. *Journal of Virology* 79:2729–2742.
39. Hennig H, Puchta I, Luhm J, Schlenke P, Goerg S, Kirchner H. 2002. Frequency and load of hepatitis B virus DNA in first-time blood donors with antibodies to hepatitis B core antigen. *Blood* 100: 2637-41.
40. Hollinger FB, Liang TJ. 2001. Hepatitis B virus. In: Fields Virology 4th edition. Edited by: Knipe D.M., Howley P.M. Philadelphia: *Lippincott Williams & Wilkins* 2001: 2971-3036.
41. Hoofnagle JH. 1981. Serologic markers of hepatitis B virus infection. *Annu Rev Med* 32:111.
42. Hou J, Liu Z, Gu F. 2005. Epidemiology and Prevention of Hepatitis B Virus Infection. International Journal of Medical Sciences 2.1: 50–57.
43. Howard CR. 1986. "The biology of hepadnaviruses". *J. Gen. Virol.* 67 (7): 1215–35.
44. Hu Z, Zhang Z, Kim JW, Huang Y, Liang TJ. 2006. Altered proteolysis and global gene expression in hepatitis B virus X transgenic mouse liver. *J Virol* 80:1405–1413.
45. Jilbert AR Wu TT, England JM. 1992. Rapid resolution of duck hepatitis B virus infections occurs after massive hepatocellular involvement. *Journal of Virology* 66: 1377–1388.
46. Kajino K, Jilbert AR, Saputelli J. 1994. Woodchuck hepatitis virus infections: very rapid recovery after a prolonged viremia and infection of virtually every hepatocyte. *Journal of Virology* 68: 5792–5803.
47. Kakimi K, Guidotti LG, Lane TE, Chisari FV. 2001. Cutting edge: Inhibition of hepatitis B virus replication by activated NK cells does not require inflammatory cell recruitment to the liver. *J Immonol* 167(12):6701-6705.
48. Kleimman SH; Busch MP. 2001. HBV amplified and back in the blood safety sportlight. *Transfusion* 41: 1081-5.
49. Krugman S, Giles JP, Hammond J. Infectious hepatitis: evidence for two distinctive clinical, epidemiological, and immunological types of infection. JAMA 1967;200: 365-73.
50. Kwak MS, Kim YJ. 2014. Occult hepatitis B virus infection. World J Hepatol. 6(12): 860–869.
51. Lavenchy D. 2004. Hepatitis B virus epidermiology, disease burden, treatment and current and emerging

prevention and control measures: a review. *J Viral Hepat* 11: 97-107.
52. Lee WM. 1997. Hepatitis B virus infection. *N Engl J Med* 337:1733–1745.
53. Lemon SM, Layden TJ, Seeff L. 2000. The 20th United States- Japan Joint Hepatitis Panel Meeting. *Hepatology* 31(3): 800–806.
54. Liang TJ. 2009. Hepatitis B: The Virus and Disease. *Hepatology* 49(5 Suppl): S13–S21.
55. Lin B, Anderson DA. 2000. "A vestigial X open reading frame in duck hepatitis B virus". *Intervirology*. 43 (3): 185–90.
56. Loeb DL, Hirsch RC, Ganem D. 1991. Sequence independent RNA cleavages generate the primers for plus strand DNA synthesis in hepatitis B viruses: implications for other reverse transcribing elements. *The EMBO Journal* 10: 3533–3540.
57. Lok AS, McMahon BJ. 2001. Chronic hepatitis B. *Hepatology* 34:1225-41.
58. Loriot MA, Marcellin P, Bismuth E, Martinot-Peignoux M, Boyer N, Degott C, Erlinger S, Benhamou JP. 1992. Demonstration of hepatitis B virus DNA by polymerase chain reaction in the serum and the liver after spontaneous or therapeutically induced HBeAg to anti-HBe or HBsAg to anti-HBs seroconversion in patients with chronic hepatitis B. *Hepatology* 15:32–36.
59. Magnius LO, Norder H. 1995. Subtypes, genotypes and molecular epidemiology of the hepatitis B virus as reflected by sequence variability of the S-gene. *Intervirology*; 38(1-2): 24-34.
60. Mahoney FJ. 1999. Update on diagnosis, management, and prevention of hepatitis B virus infection. *Clin Microbiol Rev* 12(2): 351–366.
61. Margolis HS, Alter MJ, Hadler SC. 1991. Hepatitis B: evolving epidemiology and implications for control. *Semin Liver Dis* 11: 84-92.
62. Maria LG, Conrado MF, Jose LL, Ingrid B. 2015. Prevalence of occult hepatitis B virus infection. *World J Gastroenterol* 16:12.
63. Martin P, Friedman LS, Dienstag JL. 1993. Diagnostic approach in viral hepatitis: scientific basis and clinical management. *Edinburgh: Churchill Living-stone P*. 393-408.
64. Mason WS, Jilbert AR Summers J. 2005. Clonal expansion of hepatocytes during chronic woodchuck hepatitis virus infection. *Proceedings of the National Academy of Sciences of the United States of America* 102: 1139–1144.
65. McGlynn K A, Tsao L, Hsing AW. 2001. International trends and patterns of primary liver cancer. *Int J Cancer* 94(2): 290–296.
66. Mel K, Gail M, and Martin P. 2005. The Laboratory Diagnosis of Hepatitis B Virus. *Canadian J inf. Disease and Med Mic* 16(2):65-72.
67. Meuleman, P., Libbrecht, L. and Wieland, S. (2006). Immune suppression uncovers endogenous cytopathic effects of the hepatitis B virus. *Journal of Virology* 80: 2797–2807.
68. Michalak TI, Pasquinelli C, Guilhot S, Chisari FV. 1994. Hepatitis B virus persistence after recovery from acute viral hepatitis. *J Clin Invest* 93: 230-239.
69. Milich D, Liang TJ. 2003. Exploring the biological basis of hepatitis B e antigen in hepatitis B virus infection. *Hepatology* 38:1075–1086.
70. Mosley JW, Stevens CE, Aach RD, Hollinger FB, Mimms L, Solomon LR. 1995. Donor screening for antibody to hepatitis B core antigen and hepatitis B virus infection in transfusion recipients. *Transfussion* 35: 5-12.
71. Norborg U, Gusdal A, Horal P, Lindh M. 2000. Levels of viremia in subjects with serological markers of past or chronic hepatitis B virus infection. *Scand J Infect Dis* 32: 249-252.
72. Norder H, Courouce´ A-M, Magnius LO. 1994. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. *Virology* 198: 489–503.
73. Nwokedi EO, Odimayo MS, Emokpae AM, Yahaya IA, Sadiq MN, Okwori EE. 2011. Seroprevalence of hepatitis B surface antigen among patients attending Aminu Kano Teaching Hospital, Kano. *Niger J Med* 20(2): 213-5.
74. Odemuyiwa SO, Mulders MN, Oyedele OI, Ola SO, Odaibo GN. 2001. Phylogenetic analysis of new hepatitis B virus isolates from Nigeria supports endemicity of genotype E in West Africa. *J Med Virol* 65: 463-469.
75. Okamoto H, Tsuda F, Sakugawa H, Sastrosoewignjo RI, Imai M, Miyakawa Y, Mayumi M. 1998. Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. *J Gen Virol* 69: 2575 - 2583.
76. Olinger CM, Jutavijittum P, Hübschen JM, Yousukh A, Samountry B, Thammavong T, Toriyama K, Muller CP. 2008. "Possible new hepatitis B virus genotype, southeast Asia". *Emerging Infectious Diseases*. 14 (11): 1777–80
77. Opaleye OO, Tong HV, Bui Tien S, Fagbami AH, Adekanle O, Ojurongbe O. 2015. Occult Hepatitis B Virus Infection in Nigerian Blood Donors and Hepatitis B Virus Transmission Risks. *PLoS ONE* 10(7): e0131912.
78. Prince AM, Fuji H, Gershon RK. 1964. Immunohistochemical studies on the etiology of anicteric hepatitis in Korea. *Am J Hyg* 79:365-81.
79. Prince AM, Lee DH, Brotman B. 2001. Infectivity of blood from PCR-positive, HBsAg-negative, anti-HBs-positive casesof resolved hepatitis B infection. *Transfussion* 41: 329-32.
80. Raimondo G, Allain JP, Brunetto MR, Buendia MA, Chen DS, Colombo M. (2008). Statements from the Taormina expert meeting on occult hepatitis B virus infection. *J Hepatol* 49: 652–657.
81. Rehermann B, Ferrari C, Pasquinelli C, Chisari FV. 1996. The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. *Nat Med* 2: 1104-1108.

82. Ribeiro RM, Lo A, Perelson AS. 2002. Dynamics of hepatitis B virus infection. *Microbes Infect* 4: 829-35.
83. Robinson WS. 1995. Hepatitis B virus and Hepatitis D virus. In: Mandell G.L., Bennett J.E., Dolin R., eds. *Principles and Practice of Infectious Disease*, 4th Ed. New York, *Churchill Livingstone* 1406-1439.
84. Schaefer S. Hepatitis B virus: Significance of genotypes. *J Viral Hepat* 2005. 12: 111- 124.
85. Schildgen O, Helm M, Gerlich W. 2006. Non-response to adefovir: host or virus dependent? *J Clin Virol* 37: 327-8.
86. Scott R.M, Snitbhan R, Bancroft W.H, Alter H.J, Tingpalapong M. 1980. Experimental transmission of hepatitis B virus by semen and saliva. *J Infect Dis* 142: 67-71.
87. Seager C and Mason W. 2000. Hepatitis B virus Biology. Microbiol. *Mol biol rev* Vol 64: No 1. p.51- 68.
88. Seeger C., Zoulim F and Mason W. HEPADNAVIRIDAE. In*: Fields Virology* 5th edition. Edited by: Knipe D.M, Howley P.M. Philadelphia: Lippincott Williams & Wilkins; 2007: 2979-3013
89. Servoss J.C., Freidman L.S., Dienstag J.L. Diagnostic approach to viral hepatitis. In:
90. Servoss JC, Freidman LS. 2006. Serologic and Molecular diagnosis of hepatitis B virus. *Infect Dis Clin N Am* 20: 47-61.
91. Shiota G, Oyama K, Udagawa A, Tanaka K, Nomi T, Kitamuri A. 2000. Occult hepatitis B virus infection in HBs antigen-negative hepatocellular carcinoma in a Japanese population. *J Med Virol* 62: 151-8.
92. Stevens CE, Beasley RP, Tsui J, Lee WC. 1975. Vertical transmission of hepatitis B antigen in Taiwan. *N Engl J Med* 292: 771-4.
93. Stuyver L, De Gendt S, Van Geyt C, Zoulim F, Fried M, Schinazi RF, Rossau R. 2000. A new genotype of hepatitis B virus: Complete genome and phylogenetic relatedness. *J. Gen Virol* 81: 67- 74.
94. Sum SS, Wong DK, Yuen JC. 2005. Comparison of the COBAS Taq Man HBV test with the COBAS Amplicor monitor test for measurement of hepatitis B virus DNA in serum. *J Med Virol* 77: 486-90.
95. Summers J, Jilbert AR., Yang W. 2003. Hepatocyte turnover during resolution of a transient hepadnaviral infection. *Proceedings of the National Academy of Sciences of the United States of America* 100: 11652–11659.
96. Tang JR, Hsu HY, Lin HH, Ni YH, Chang MH. 1998. Hepatitis B surface antigenemia at birth: a long-term follow-up study. J Pediatr. 133: 374-7.
97. Thimme R., Wieland S, Steiger C. 2003. CD8 T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. *Journal of Virology* 77: 68–76. A. Thomas H.G, Lemon B, Zuchman A.J, editors. *Viral Hepatitis*: 2005. P. 50-64.
98. Thorgeirsson SS, Grisham JW. 2003. Overview of recent experimental studies on liver stem cells. *Seminars in Liver Disease* 23: 303–312.
99. Weber B. 2005. Genetic variability of the S gene of hepatitis B virus: clinical and diagnostic impact. *J Clin Virol* 32(2): 102-112.
100. WHO (2015) Hepatitis B. Fact sheet N° 204.
101. Wieland SF, Spangenber HC, Thimme R. 2004. Expansion and contraction of the hepatitis B virus transcriptional template in infected chimpanzees. *Proceedings of the National Academy of Sciences of the United States of America* 101: 2129–2134.
102. Wright TL, Lau JY. 1985. Clinical aspects of hepatitis B virus infection. *Lancet* 342:1340-4.
103. Xu ZY, Liu CB, Francis DP. 1985. Prevention of perinatal acquisition of hepatitis B virus carriage using vaccine: preliminary report of a randomized, double-blind placebo-controlled and comparative trial. *Pediatrics* 76:713-718.
104. Yotsuyanagi H, Yasuda K, Iino S, Moriya K, Shintani Y, Fujie H, Tsutsumi T, Kimura S, Koike K. 1998. Persistent viremia after recovery from self-limited acute hepatitis B. *Hepatology* 27: 1377- 1382.
105. Yu MW, Chang HC, Liaw YF. 2000. Familial risk of hepatocellular carcinoma among chronic hepatitis B carriers and their relatives. *J Natl Cancer Inst* 92: 1159-1164.
106. Yuki N, Nagaoka T, Yamashiro M, Mochizuki K, Kaneko A, Yamamoto K. 2003. Long-term histologic and virologic outcomes of acute self-limited hepatitis B. *Hepatology* 37: 1172 – 9.
107. Zhang Z, Torii N, Hu Z, Jacob J, Liang TJ. 2001. X-deficient woodchuck hepatitis virus mutants behave like attenuated viruses and induce protective immunity in vivo. *J Clin Invest.*108:1523–1531.