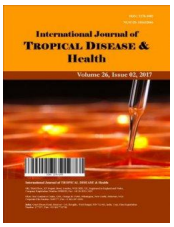

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Diagnostic Relevance and Clinical Interpretation of Hepatitis B Virus Serological Profile Testing

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Authors' contributions

This work was carried out in collaboration between all authors. Author CU designed and wrote the first draft of the manuscript. Authors ISY, HKM and AS reviewed and made substantial contributions to the intellectual content of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Hepatitis B virus (HBV) infection constitutes a public health menace in many regions of the world. The dynamic nature of the infection and the characteristic variations in the serological markers associated with the various phases of the infection makes its classification, treatment and management a serious challenge to health professionals. Hepatitis B serologic profile is generated from the measurement of several hepatitis B virus specific antigens and antibodies. Immunological responses from the constellation of the assay report can be used to differentiate acute from chronic infection; classify the different phases of the infection; recognise past, resolved and active infection;

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identify occult and mutant form of the infection. Accurate interpretation of the profile report will abet the diagnosis of hepatitis B virus infection and its associated disease, hence, identifying and linking infected individuals, to appropriate care and treatment services, thereby reducing hepatitis B related mortality and unnecessary expenses on unwarranted medications. This review presents a comprehensive overview of the various forms of HBV infection, the natural history of chronic hepatitis B infection, the serological markers and a guide to the interpretation of the various serological patterns associated with hepatitis B virus infection with the sole purpose of providing myriad of relevant information to guide healthcare providers in the diagnosis, treatment and management of those infected with HBV.

Keywords: Chronic hepatitis B; hepatitis B envelope antigen negative; hepatitis B surface antigen; hepatitis B virus; hepatitis B virus DNA; immunological response; occult Hepatitis B; serological markers.

1. INTRODUCTION

Hepatitis B virus (HBV) infection is a major cause of acute and chronic liver disease. It is an infectious disease that leads to a wide spectrum of liver complications, ranging from acute hepatitis, including fulminant hepatic failure to chronic hepatitis, decompensated cirrhosis, hepatocellular carcinoma (HCC) and end-stage liver disease [1]. Despite the existence of an effective vaccine since 1982, the burden of viral hepatitis B continues to constitute a public health threat in many regions of the world, particularly in developing countries [2]. Current reports by the world Health Organization (WHO) estimates that 60% of the world's population lives in areas where HBV infection is highly endemic, particularly in Asia and Africa with about 248 million people living with chronic HBV infection (CHB) and an estimated 1.4 million deaths recorded annually resulting from cirrhosis and HCC [3]. Hepatitis B virus is an enveloped DNA virus that infects the liver and causes hepatocellular necrosis and inflammation [4]. HBV is transmitted predominantly by percutaneous or mucosal exposure to infected blood and body fluids, which includes saliva, menstrual, vaginal and seminal fluids [5]. Perinatal transmission has been identified as the major route of HBV transmission in many parts of the world and a notable factor in sustaining the reservoir of the infection in some regions, particularly in South-East Asia [6,7]. The diagnosis of HBV infection and its associated disease is based on the accurate interpretation of clinical, biochemical, histological, and serologic findings [8]. The World Health Organization (WHO) guideline on Hepatitis B testing described testing and diagnosis of HBV infection as the entry point to gaining access to prevention, care and treatment services, as well as a crucial component of an effective response to the hepatitis epidemic [3]. HBV testing is done with the following specific objectives; identifying and linking infected individuals to appropriate care and treatment services; reducing hepatitis B related mortality by providing treatment to those affected; providing link to preventive interventions such as hepatitis B vaccination to reduce transmission; monitoring response to antiviral treatment; surveillance to detect outbreaks; monitor trends in incidence and identifying risk factor; surveillance to estimate the prevalence of chronic infection, monitor trends in sentinel groups and screening in blood transfusion services to prevent transmission via infected blood and its products [3]. Serological assays and nucleic acid testing (NAT) are the screening and diagnostic tool utilised to detect HBV infections in many regions of the world. Serological assays are employed as the first line testing strategy due to their relatively low cost compared to NAT. They are based on immunoassay principle and can detect hepatitis B surface antigen (HBsAg) in infected body fluids. NAT technologies are deployed to detect the viraemic state of the infection. They are instrumental in differentiating active from inactive infection, priotizing those eligible for treatment and signaling when antiviral treatment should be discontinued due to non-response, resistance or to effective suppression of the virus [3]. Complete eradication of HBV is difficult because of its tendency to integrate into the host genome. Treatment is however, targeted at sustaining suppression of the HBV DNA levels; normalizing alanine aminotransferase (ALT) levels; delaying or arresting the progression of liver injury and development of cirrhosis and HCC [9]. HBV infection is an intractable pestilence, however, WHO recommends the use of antiviral agents tenofovir and entecavir for treatment of HBV infection. These agents have been shown to effectively suppress HBV replication, prevent

progression to cirrhosis, and reduce the risk of HCC and liver-related morbidity and mortality [4,10].

2. VARIOUS FORMS OF HEPATITIS B VIRUS INFECTION

2.1 Acute Hepatitis B Virus Infection

Acute HBV infection is characterized by the presence of HBsAg and immunoglobulin M (IgM) antibody to Hepatitis B core antigen (HBcAg) [5]. The acute form of the infection is usually self-limiting, consequent to the spontaneous clearance of the virus in 90–95% patients within 6 months of infection [8]. Acute infection can however, progress to acute inflammation and hepatocellular necrosis, with a case fatality rate of 0.5–1% [1].The clinical incubation period of acute hepatitis B infection averages between 2–3 months and can range from 1–6 months after exposure [8].

2.2 Chronic Hepatitis B Virus Infection

CHB infection has a variable and dynamic course and can be defined as the persistent of HBsAg in the blood or serum for longer than six months after an acute infection, with or without associated active viral replication, evidence of hepatocellular injury and inflammation [1]. Worldwide, only 5–10% of patients with acute HBV infection progresses to the chronic state, which is associated with considerable mortality and morbidity [11]. Acquisition of HBV infection at birth or early childhood has been shown to result to chronic condition in about 90% of infected individuals [12].

2.3 Hepatitis B Envelope Antigen Negative Chronic Hepatitis B Virus Infection

CHB infection can be broadly categorised into hepatitis B envelope antigen (HBeAg) positive and HBeAg negative chronic HBV infection based on the expression of HBeAg [9]. HBeAg is a biomarker of high levels of HBV replication and infectivity. It is expressed in the active state of acute and chronic HBV infection and it is associated with an increased risk of HCC [13,14]. Chronic HBeAg negative infection is the predominant form of CHB infection worldwide [15]. HBeAg-negative CHB infection consists of individuals with varying levels of viral replication and liver disease status [16]. It can be broadly sub-categorized into inactive and active forms. The former can be characterized with a normal ALT levels and low HBV DNA levels without significant necroinflammatory disease. Whereas, the latter group can comprise individuals with chronic necroinflammatory disease of the liver and a naturally occurring mutant form of HBV that does not produce HBeAg because of a mutation in the precore or core promoter region of the HBV genome [17,18]. The predominant form of precore mutation involves a point mutation from G to A at nucleotide 1896 (G1896A), which creates a stop codon that results in loss of HBeAg synthesis. Similarly, the most commonly observed core promoter mutation involves a two-nucleotide substitution at nucleotides 1762 and 1764 [19,20]. Persons with the active form of HBeAg-negative infection have the propensity of developing marked viral reactivation and a tendency of being refractory to antiviral treatment [21]. It is pertinent to identify and closely monitored this group of patients as treatment may be indicated to prevent progression to fibrosis and cirrhosis [16]. HBeAg-negative form of chronic HBV was considered in the context of the various forms of HBV infection in this write up for two obvious reasons. Firstly, a larger proportion of HBV infected individuals are asymptomatic and predominantly HBeAg negative, thus, portending the need for further assessment to differentiate those who are inactive carriers from those harboring the precore or basal core promoter mutants of the virus. Differentiating between these groups of patients will require the use of additional investigations like measuring aminotransferase levels to help determine liver inflammation, quantification of HBV DNA levels to differentiate active from inactive HBeAg-negative infection and the use of non-invasive diagnostic tests (Transient elastography, aspartate aminotransferase (AST)-to-platelet ratio index (APRI), and fibrosis-4 (FIB-4)) to assess the stages of liver disease (3). However, the accessibility and cost implication of the additional investigations can become a significant impediment to the effective care and management of people with CHB. Secondly, CHB is a heterogenous disease with a dynamic natural history. Serological markers fluctuate as the infection transcends through the various phases of the natural course of the disease. Continuous monitoring of HBV DNA and other markers in both the active and inactive form of the disease is of paramount necessity in identifying disease progression and when to initiate therapy. However, proper classification of the disease, the precise correlation of laboratory results with clinical findings and the cost

implication of the repeated assays might also be a hindrance to the proper care and management of people with CHB.

2.4 Occult or Latent Hepatitis B Virus Infection

Occult or latent HBV infection (OBI) is a persistent form of HBV infection, characterized by the absence or undetected HBsAg in the serum [22-24]. It can be categorised as either seropositive or seronegative OBI. Seropositive OBI occurs when serum HBV DNA is detectable and both hepatitis B core antibody (anti-HBc)/hepatitis B surface antibody (anti-HBs)IgGs antibodies are positive or only anti-HBc IgG is positive. Seronegative OBI on the other hand is evident by the detection of only HBV DNA in serum/or liver tissue without the corresponding detection of anti-HBc IgG and or anti-HBs IgG antibodies [25]. The reduced expression of HBV surface proteins and the low binding affinity of monoclonal antibody against HBsAg in OBI cases have been attributed to various forms of mutations in the HBsAg gene [26,27]. HBV Genotypes A, C, G, E and D have been found among patients with OBI in different regions of the world. The predominant form is usually region specific [25]. OBI is of clinic relevance and a public health concern due to the risk of reactivation it poses to individuals with previous history of HBV infection along with patients co-infected with hepatitis C virus/human immunodeficiency virus and patients undergoing chemotherapy. These patients are at risk of developing liver cirrhosis and HCC [22-24]. Furthermore, recipients of blood donation and organ transplant are also at risk of acquiring OBI, thus, necessitating the advocacy for a highly sensitive molecular means in screening of HBV DNA in blood and organ donors [28,29].

3. NATURAL HISTORY OF CHRONIC HEPATITIS B

The various liver pathologies associated with CHB are the cumulative effect of the interplay between the host defense system and HBV activities. The clinical presentations manifest as the infection progresses through the natural course of the disease. The natural history of CHB can be divided into 4 phases: The natural course of CHB begins with immune tolerant phase. This phase is observed primarily in perinatally acquired infection and individuals in this phase are positive for HBeAg with elevated levels of HBV DNA>20,000 IU/ml but have a normal serum ALT level. Liver biopsy usually shows no or minimal histological changes [16,30]. The increased viral activity during this period can be attributed to the role played by HBeAg, acting as an immune tolerant protein, thus, aiding the virus to evade the immune system [31]. Immune clearance phase, also known as immune active phase is the next phase in the natural history of CHB infection. It is the phase where the host's immune system recognizes and responds to the invading HBV pathogen thus leading to the damage of hepatocyte [32]. This phase is characterized with an elevated ALT level and decreased HBV DNA level. HBeAg may be cleared with seroconversion to anti-HBeAg but may still persist in some individuals [33]. Protracted immune clearance phase may increase the risk of liver fibrosis and therefore disease progression [34]. Immune response to HBV leads to the suppression of HBV DNA, normalization of ALT and subsequent seroconversion of HBeAg to anti-HBe antibody. This low replicative phase is known as the inactive carrier state or the immune control state of the disease [35,36]. Inactive phase of the disease depicts a state with low risk of liver disease progression and has a good prognosis [37]. Patients in the inactive carrier state constitute the largest group of persons with chronic HBV infection. Although, they are usually asymptomatic with low or no virological activities occurring at the stage, however, spontaneous reactivation might be observed in approximately 20%-30% of the inactive HBsAg carriers and some may revert to a phase of significant viraemia with fluctuating ALT. Multiple episodes of reactivation or a sustained reactivation can cause progressive liver damage [38]. In addition to the risk of reactivation and the emergence of mutant viruses that fail to express HBeAg, inactive HBsAg carrier state can also be associated with a number of other potential outcomes, which includes; indefinite persistence of the inactive carrier status, resolution of chronic infection (evident by HBsAg clearance, appearance of anti-HBsAg antibody and reactivation of the disease due to recrudescence of the original infection [39-41]. The reactivated phase is the fourth phase in the natural history of CHB infection, those arising as a result of mutations in the precore or core promoter region of the HBV give rise to HBeAg negative CHB disease ($HBeAg^-$CHB). Individuals in this phase react negative to HBeAg, with or without seroconversion to anti-HBeAg. HBV DNA can be detected, serum ALT level fluctuates and liver biopsy usually shows active inflammation [39,42].

Suffice to say that, not all patients go through each of the four phases [30]. Serological markers that aid in differentiating these phases are depicted in Table 1.

4. SEROLOGICAL MARKERS OF HEPATITIS B VIRUS INFECTIONS

4.1 Hepatitis B Surface Antigen (HBsAg)

HBsAg also known as the Australian antigen is the first serological marker of HBV infection to appear. The window period between HBV infection and detection of HBsAg is estimated to be around 38 days; however, this depends on other factors such as the analytical sensitivity of assay used, the immunocompetence of the host, individual virus kinetics and occult HBV type of infection where HBsAg is undetected [3]. HBsAg level varies throughout the different phases of CHB infection and across different HBV genotypes [43,44]. Quantification and monitoring of HBsAg level has been identified as a potential alternative marker for assessing HBV viraemia and response to antiviral treatment [3]. Quantification of HBsAg can also be utilised to differentiate active from inactive disease among HBeAg-negative individuals and to predict disease reactivation in asymptomatic HBeAg-negative patient [45,46].

4.2 Hepatitis B Surface Antibody (Anti-HBs)

Anti-HBs is a neutralizing antibody that confers protective immunity against HBV infection. It is produced immediately after the window period of HBV infection, after the spontaneous clearance of HBsAg or following immunization with HBsAg vaccine preparations [3]. Anti-HBs can be used to monitor post-immunization responses. In this case, anti-HBs appears in the absence of anti-HBc. However, anti-HBs can coexist with anti-HBc in patients with resolved HBV infection. Anti-HBs can also be detected in patients with reactive HBsAg. Its presence in this scenario cannot be used to exclude current infection [47].

4.3 Hepatitis B envelope Antigen (HBeAg)

HBeAg is a secreted, immunologically soluble antigen that is associated with high levels of HBV replication and progressive liver disease. The presence of HBeAg is a marker of high infectivity and it's useful in deciding when to commence and stop antiviral therapy [48].

4.4 Hepatitis B envelope Antibody (Anti-HBe)

Anti-HBe is a neutralizing antibody that emerges during HBeAg seroconversion. The detection of Anti-HBe in the serum is associated with clinical improvement of hepatitis which includes; reduced HBV DNA, indicating decreased viral replication and infectivity, normalized serum aminotransferase levels, and quiescence of inflammation in the liver usually indicating decreased viral replication and infectivity [49]. Anti-HBe is usually detected in the immune-control and immune-escape phases of the natural course of chronic HBV infection. It may also be detected at the end of immune-tolerance phase when it coexist with HBeAg during the period of seroconversion from envelop antigen to envelop antibody [50,51].

4.5 Hepatitis B core Antibody (Anti-HBc)

Anti-HBc are non neutralizing antibodies directed against HBV core (capsid) protein. Anti-HBc IgM antibodies are expressed during acute HBV infection but may remain detectable for up to 6 months. Detecting Anti-HBc IgM is diagnostic, as it can be used to differentiate between acute and chronic HBV infection. However, its reappearance during "flares" in chronic HBV infection makes it an unreliable indicator of recent primary HBV infection [3].Commercially diagnostic assays detect anti-HBc total antibodies (Anti-HBc (total)) which comprises both IgM and IgG antibodies. Anti-HBc (total) is the most constant marker of HBV infection and when it appears together with anti-HBs, it indicates resolved infection. However, the presence of anti-HBc in the absence of HBsAg and anti-HBs is defined as "isolated anti-HBc" or "anti-HBc alone" and the serological response is compatible with acute, resolved, or chronic HBV infection but might also represent occult HBV infection or infection with atypical variant strains of HBV [52,53,54]. The routine screening of blood donors for anti-HBc has been implemented in many countries [52,55]. This is attributed to the recognized fact that anti-HBc may be the only detectable serological marker of HBV infection in blood donors with resolved infection, low grade chronic infection or infection with atypical variant strains of HBV [56-58].

4.6 Hepatitis B Virus DNA (HBV DNA)

HBV is an envelope virus with a partially double stranded, circular DNA. The full length DNA

Table 1. Differentiating chronic hepatitis B phases using hepatitis B virus serological markers

CHB Phase	ALT	HBsAg	Anti-HBs	HBeAg	Anti-HBe	Anti-HBc	HBV DNA (UI/ml)
Immune tolerance	Persistently normal	Reactive	Non-Reactive	Reactive	Non-Reactive	Reactive	**>**20,000 IU/mL
Immune clearance	Persistently elevated	Reactive	Non-Reactive	Reactive	Non-Reactive[a]	Reactive	**>**20,000 IU/mLor**<**20,000 IU/mL
Inactive or carrier state	Usually Normal; Characterized occasionally with periodic flares	Reactive	Non-Reactive	Non-Reactive	Reactive	Reactive	**<**20,000 IU/mL
Reactivation (HBeAg$^-$ CHB)	Characterized with Periodic flares (Persistently or intermittently Abnormal)	Reactive	Non-Reactive	Non-Reactive	Reactive	Reactive	**>**20,000 IU/mLor**<**20,000 IU/mL

CHB: Chronic Hepatitis B; ALT: Alanine aminotransferase; HBsAg: Hepatis B surface antigen; Anti-HBs: Hepatitis B surface antibody; HBeAg: Hepatitis B envelope Antigen; Anti-HBe:Hepatitis B envelope antibody; Anti-HBc: Hepatitis B core antibody; HBV DNA: Hepatitis B virus DNA; HBeAg$^-$ CHB; Hepatitis B e antigen negative chronic hepatitis B. [a]Anti-HBe may coexist with HBeAg at the end of immune-tolerance phase during the period of seroconversion from e antigen to e antibody

Table 2. A guide to the interpretation of various hepatitis B virus serological patterns

HBsAg	Anti-HBs	HBeAg	Anti-HBe	Anti-HBc	Interpretation
Reactive	Non-Reactive	Reactive	Non-Reactive	Reactive	This is compatible with an acute or chronic active HBV infection, with ongoing viral replication. Anti-HBc IgM antibodies can be used to differentiate active from chronic infection. More so, persistence of HBsAg in the serum for more than six months is diagnostic of HBV chronic infection.
Reactive	Non-Reactive	Non-Reactive	Reactive	Reactive	These serological responses are compatible with HBV carrier
Reactive	Non-Reactive	Non-Reactive	Non-Reactive	Reactive	status / inactive state, or HBeAg- CHB disease arising as a result of mutation in the precore or core promoter region of HBV. Both presentations can be differentiated using additional HBV markers such as those that assess the stages of liver disease such as ALT, liver histology, APRI score, transient elastography (Fibroscan). More so, HBV DNA is a more direct and accurate measurement of active HBV viral replication, which correlates with disease progression.
Non-Reactive	Non-Reactive	Non-Reactive	Non-Reactive	Reactive	This immunological response is referred to as "isolated anti-HBc" or “anti-HBc alone.” It is compatible with any of the

HBsAg	Anti-HBs	HBeAg	Anti-HBe	Anti-HBc	Interpretation
					following; acute HBV infection during the window period, resolved HBV infection, CHB infection and occult HBV infection. It can also be attributed to a false positive reaction. Unraveling the clinical status of individuals with isolated anti-HBc requires further investigations such as HBV DNA and anti-HBs measurement. Hepatitis B vaccination of individuals with isolated anti-HBc to measure anti-HBc response has been employed to distinguish the various outcomes associated with isolated anti-HBc. This is based on the premise that those with previous exposure to HBsAg will show anamnestic anti-HBs response after a single dose of vaccine. The identification of isolated anti-HBc is significant in organ transplant donors and in candidate patients for chemotherapy and Immunosuppressive therapy due to the risk of HBV reactivation.
Non-Reactive	Reactive	Non-Reactive	Non-Reactive	Reactive	These serological responses are compatible with resolved or past HBV infection. The presence of Anti-HBc in both episodes indicates past exposure to the virus.
Non-Reactive	Reactive	Non-Reactive	Reactive	Reactive	
Non-Reactive	Reactive	Non-Reactive	Non-Reactive	Non-Reactive	This immunological pattern is compatible with serological response associated with hepatitis B vaccination. The absence of anti-HBc indicates the non existence of ongoing HBV infection or previous exposure to HBV.

HBsAg: Hepatis B surface antigen; Anti-HBs: Hepatitis B surface antibody; HBeAg: Hepatitis B envelope Antigen; Anti-HBe:Hepatitis B envelope antibody; Anti-HBc: Hepatitis B core antibody; HBV DNA: Hepatitis B virus DNA; HBeAg- CHB; Hepatitis B e antigen negative, chronic hepatitis B; CHB: Chronic Hepatitis B; ALT: Alanine aminotransferase; HBV: Hepatitis B virus; APRI: Aspartase aminotransferase-to-platelet ratio index; TE: Transient elastography

strand is about 3020–3320 nucleotides long, while the short length strand is about 1700–2800 nucleotides long [59]. HBV viral genomes can be detected and quantified in serum using nucleic acid testing technologies (NAT). The measured HBV DNA which is also called HBV viral load is expressed in international units per milliliter (IU/ml) as the recognized international standard. It can also be expressed in copies/ml. conversion between IU/ml and copies/ml is possible using the formular; 1 IU/ml = 5.3 copies/ml [3]. High level of HBV DNA, can range from tens of thousands to billions, and it is a direct and accurate measure of active HBV viral replication, which correlates with disease progression and a marker of infectivity [57]. Moderate amount of HBV DNA however, drifts around 10,000 IU/ml while low or undetectable levels are less than 2,000 IU/ml which indicate "inactive" infection [60]. Measurement of HBV DNA can be used to diagnose early HBV infection before the appearance of HBsAg. Similarly, HBV DNA estimation can be used to differentiate active from inactive HBeAg-negative infection. In conjunction with ALT levels and degree of liver fibrosis, it can be used to determine the need for antiviral therapy [61]. HBV viral load is pivotal in deciding and selecting HBV infected candidates eligible for antivral therapy, consequently, it can be used in monitoring treatment response. Elevated values while on treatment may indicate inadequate adherence or the emergence of resistant variants [62,63,64]. HBV DNA detection is also a useful marker in diagnosing HBV occult infection [65,66].

5. CONCLUSION

Considering the dynamic nature of HBV infection and the variations associated with the immunological responses in the various phases of the infection, proper classification of the disease and the precise correlation of laboratory results with clinical findings is vital in identifying and linking infected individuals, to appropriate care and treatment services.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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