



THE SIGNIFICANCE OF ISOLATED HEPATITIS B SURFACE ANTIGEN: A CASE REPORT AND REVIEW OF LITERATURE

Majd Alsayb<sup>1\*</sup>, MD, Farah Al Gamdi<sup>1</sup> and May A. Alsayb<sup>2</sup>

<sup>1</sup>Department of Gastroenterology and Hepatology, King Fahd Armed Forces Hospital, Jeddah, Saudi Arabia.

<sup>2</sup>Medical Laboratory Technology Department, College of Applied Medical Sciences, Taibah University, Al Madinah, Saudi Arabia.

\*Corresponding Author: Majd Alsayb

Department of Gastroenterology and Hepatology, King Fahd Armed Forces Hospital, Jeddah, Saudi Arabia.

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ABSTRACT

**Background:** One of the common cause of viral hepatitis is Hepatitis B virus. Acute infection is characterized by the appearance of HbsAg, HBcIgM and detectable HBV DNA. Isolated HbsAg positivity is unusual serological marker with no clear interpretation neither its significance. **Case presentation:** A 38year old Saudi male patient who had a screening test that showed an isolated HbsAg positivity with undetectable HBV DNA repeatedly. We considered his isolated HbsAg as a false positive test. He received 3 doses of recombinant hepatitis B vaccines. His follow up serology showed negative HbsAg, HbsAB was 51.4 mIU/mL and HbcAB remained negative. **Conclusion:** Weakly isolated HBsAg positivity often do not reflect actual HBV infection. Different possible mechanisms could lead to this unusual serological profile and further investigations and confirmations should be considered in the interpretation guideline for hepatitis B infection.

**KEYWORDS:** Hepatitis B surface antigen (HBsAg), Hepatitis B surface Antibodies (HBsAB), Hepatitis B core Antibodies (HBcAB), hepatitis B core antigen (HBcAg), hepatitis B envelope antigen (HBeAg), hepatitis B envelope antibodies (HBeAb), chemiluminescent microparticle immunoassay (CMIA), polymerase chain reaction (PCR), signal-to-cutoff (S/CO).

INTRODUCTION

Hepatitis B is associated with significant complications involving cirrhosis and hepatocellular carcinoma. During HBV infection, 4 key markers are used to diagnose the hepatitis B infection and monitor the course of the disease. HBsAg is a protein encoded by the virus genome which forms part of the outer shell of the virus. If antibodies are produced to this protein (HBsAb), then the patient usually clears the virus and becomes immune.<sup>[1]</sup> Hepatitis B core antigen (HBcAg) and its antibodies (HBcAb) along with Hepatitis B e- antigen (HBeAg) and its antibody (HBeAb) are used along to monitor the course of the infection as well. Acute hepatitis B infection is characterized by the appearance of serum HBsAg and HBcIgM, which then disappear during recovery and confirmed with HBV DNA. Chronic hepatitis B infection is defined by positive HBsAg for more than six months with a positive HBcAb with or without a detectable HBV DNA.<sup>[2]</sup> Interpretation of hepatitis B serology markers are important. Unusual isolated HBsAg test result can occur sometimes in laboratory examination and it's not clear how this profile should be interpreted neither its significance.<sup>[3]</sup> In some cases, vaccination for HBV can cause transient HBsAg seropositivity. Most commonly this occurs a few weeks after first dose of Hepatitis B vaccine, due to

synthetic HBsAg in vaccine.<sup>[4]</sup> Here we present a case of atypical serological markers, including positive (HBsAg) in the absence of HBcAb and HBV DNA. We present this case to emphasize the importance of confirmatory testing.

Case Presentation

A 34-year-old Saudi male not known to have any medical problem. He had a routine laboratory test in August 2015 that showed a weakly positive HBsAg, Hepatitis B surface Antibodies (HBsAB) 0.05mIU/ml, Hepatitis B core Antibodies (HBcAB) negative, Hepatitis B e Antigen (HBeAg) negative, Hepatitis B e-Antibodies (HBeAB) negative (see table 1). His prior Hepatitis B serology were all negative in January 2015. His HIV and HCV Ab tests were negative. The patient exhibited no gastrointestinal complaints or any viral hepatitis risk factors. He had no history of recent vaccine or flu like symptoms. On physical examination there were no stigmata of chronic liver disease. His liver function tests were normal, including an alanine transaminase (ALT) of 17. His viral hepatitis A, and D were negative. HBV polymerase chain reaction (PCR) assay was not detected. His abdominal ultrasound was also normal His hepatitis B serology were repeated, and the results came back with low titers of HBsAg and negative for HBcAB, HBeAg

and HBeAB. Initially, false positive HBsAg was suspected given the patient's unusual serological test results. He received 3 doses of recombinant hepatitis B vaccines and his serological response was tested 1 month

after the third vaccination and showed negative HBsAg, HBsAB was 51.4 mIU/mL and HBeAB remained negative.

**Table 1: Serological markers.**

| Test | HBsAg    | HBeAb    | HBeAg    | HBeAb    | HBsAb      | HBV DNA      |
|------|----------|----------|----------|----------|------------|--------------|
| 1st  | positive | negative | negative | negative | 0.05ml/mL  | undetectable |
| 2nd  | positive | negative | negative | negative | 0          | undetectable |
| 3rd  | negative | negative | negative | negative | 51.48ml/mL | undetectable |

## DISCUSSION

HBsAg is the first serological marker after infection with hepatitis B virus, appearing between 6 weeks and 6 months after exposure. During the acute phase, HBsAg will remain positive and clears late in the recovery period. Failure to clear HBsAg within six months indicates a chronic HBsAg carrier state (1, 2). The importance of a positive HBsAg is determined by assessing it in correlation to the other hepatitis B serology markers and clinical presentation.

In our laboratory, as a screening test for HBV infection we used Abbott ARCHITECT hepatitis B virus surface antigen which is a qualitative assay using chemiluminescent microparticle immunoassay (CMIA). A reactive specimen must be retested to determine whether it is repeatedly reactive. Then the specimen should be confirmed using the ARCHITECT HBsAg Qualitative Confirmatory assay, a neutralization procedure utilizing human anti-HBs. A positive HBsAg sample is determined by comparing the chemiluminescent signal in the reaction to the calibration signal cutoff (S/CO). Signals greater than or equal to the cutoff signal is considered reactive for HBsAg. In our laboratory the S/CO is  $\geq 1$ . Our patient HBsAg was weakly positive with a S/CO 1.2. Given the repeated weak positive test result, a false positive HBsAg was initially suspected. He received 3 doses of recombinant hepatitis B vaccines and his serological response was tested 1 month after the third vaccination and showed negative HBsAg, HBsAB was 51.4 mIU/mL and HBeAB remained negative.

The natural history of Hepatitis B infection determined by the interaction between viral replication and host immune response. Clearance of HBV mediated by both cellular and humoral immune response. The antibody-mediated immune response aim to clear the circulating viral proteins, thus, absence of antibody response could indicate host atypical immune reaction to HBV.<sup>[5]</sup> Such unusual serological profile in hepatitis B virus infection may indicate the lack of host immune response or variant to the HBV.

The accuracy of HBV is very essential especially in patient for dialysis, organ transplant and chemotherapy. HBsAg is detected using an immunoassays that utilize HBsAb to capture HBsAg in the sample. As with all immunoassays, a false positive results can generate by

non-specific binding.<sup>[6]</sup> DNA test is the most sensitive methods to detect HBV and used as a confirmatory test for hepatitis infection, negative HBV DNA indicates absence of HBV. The presented case show that HBV DNA is not detected while HBsAg is low. Mutations in the genetic sequence of the HBV DNA in such condition (positive for HBsAg and negative for anti-HBe) were found rarely. Hepatitis B virus 2 (HBV2) describe possible variants of HBV that do not stimulate a proper immune response to the anti-HBe in immune-competent patients.<sup>[7]</sup> HBV2 showed to be prompted by mild-grade hepatitis, yet the patients in this case did not present any symptoms of mild-grade hepatitis.

An interfering substance with the testing platform could be a possible explanation of HBsAg result in the presented case. Heterophilic antibody has been associated with false positive HBsAg and shown to persist for several months.<sup>[8,9]</sup> Presence of heterophilic antibody for this long is unknown, but a possible cause for heterophile antibodies, such as infection, tumor, systemic disease or transfusion. In previous case report of isolated HBsAg in a patient with parathyroid adenoma, heterophilic interference from the paraneoplastic syndrome was associated with the HBsAg seropositivity.<sup>[9]</sup> A positive HBsAg can be detected in patients after HBV vaccination for up to 2 weeks<sup>[4]</sup>, yet, in our case the patient had not been vaccinated and HBsAg was repeatedly positive for more than a year. Unfortunately, one of the limitations here is the inability to test patient serum for HBsAg with heterophilic antibody blocking reagent. We suggest it is worthwhile to evaluate the heterophilic antibody in subsequent cases for a prolonged length of time if possible.

Molecular mimicry of amino acid sequence shared between viral protein and self-antigen has been widely investigated. Known epitopic regions on HBsAg share similar sequence in self antigen protein such as myelin basic protein (MBP) and myelin oligodendrocyte protein (MOG) known to associate with autoimmune disease multiple sclerosis (MS).<sup>[10]</sup> It would be interesting if such similarity could induce cross reactivity and interfere with hepatitis B routine serological test.

Therefore, multiple causes can possible be associated with isolated HBsAg positivity. The original hypothesis of HBV acute infection seems unlikely, based on the

PCR sensitivity and the negative HBcAb result in our patient.

#### IN CONCLUSION

Our case reveals that weakly isolated HBsAg positivity often do not indicate actual Hepatitis B infection, highlighting the importance of confirmatory testing. This unusual serology profile could be a result of altered host immune response or as a result of HBV variants.<sup>[11]</sup> To the best of our knowledge, this is the first reported case in the literature of a false-positive HBsAg that became negative after 3 doses of hepatitis B vaccinations in a medically free patient.

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