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RESEARCHARTICLE

A UNIQUE PHENOMENON IN HBsAG SCREENING–THE HOOK EFFECT

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ABSTRACT

Screening for HBsAg is done routinely in patients attending various departments of Hospital. Different methods of conducting HBsAg tests include solid phase assays, flow through, agglutination, and lateral flow. These rapid tests need a backup of standard EIA testing and /or DNA based methods like PCR as gold standard for evaluating their sensitivity and specificity. In our setup we perform rapid tests (J mitra's Hepacard) based on Immunochromatographic method, Elisa and PCR for confirmation and further evaluation. When Screening for HBsAg was done in a 32 year orthopaedic patient showing pink coloured line in test reigion of Hepacard, without developing colour band in the control line. In the absence of control line we could not interpret the test result immediately. So Elisa was performed on this sample which gave reactive result for HBsAg antigen. This prompted us to do serial dilution of serum sample (1:10, 1:20) and on repeating the test, positive results with control line was observed in Hepacard. Hence we assume that inconclusive result observed earlier using Hepacard is due to Hook effect. Hook effect might have occurred due to large quantities of HBsAg antigen in serum, which impairs antigen-antibody binding, resulting in low antigen determination. Further when Quantitative PCR was done in this sample high concentration of HBV DNA was present. HenceHook effect has to be kept in mind when screening of HBsAg is done routinely.

INTRODUCTION

Viral hepatitis is a disease with multiple causes that was first *described in fifth century B.C. When Hippocrates described epidemic jaundice, he was undoubtedly referring to persons infected with acute hepatitis B virus (HBV) as well as other agents capable of infecting the liver (Mahoney, 1999).HBV infection is a global health problem. Two billion people are infected worldwide and approximately 360 million suffer from chronic HBV infection; over 5,20,000 die each year(50,000 from acute hepatitis B and 4,70,000 from cirrhosis or liver cancer).HBV infection management presents us with many challenges (De Franchis et al., 2002). In the South-East Asia region, the estimated burden of chronic HBV infection is 100 million and HBV is the second most common cause of acute viral hepatitis after HEV in India. With 3.7%prevalence, there are, over 40 million HBV carriers, India is considered to have an intermediate level of HBVendemicity. Every year, one million Indians are at risk for contacting HBV and about 100,000 die from HBV infection (NCDC, 2014).In high prevalence regions, the lifetime risk of HBV infection is greater than 60%,as the infections are acquired at birth or early childhood. This is because of the absence of adequate immune response and the rate of development of chronic* infection is greater, as in children most infections are asymptomatic and hence acute disease related to HBV is rare.But in adults the rates of chronic liver disease and liver cancer are very high (Andre, 2000). The envelop protein are expressed on the surface of the virions. The spherical particles of (22nm diameter) and filamentous particles (42nm) in peri pheral blood of patients suffering from HBV Infection constitutes the Hepatitis B surface antigen (Ananthanarayan and Panikers, 2013). HBsAg is highly antigenic and the most common antigenic marker detected routinely (Bernerd *et al*., 1999).

Quatitative Enzyme immunoassays(EIA) are considered to be the most sensitive test with 100% sensitivity and specificity and are widely used at well equipped reference clinical laboratories (HBsAg, 2001; Clement *et al*., 2002; Lau *et al*., 2003 and Verstraeten and Keya, 1997).Rapid tests are intended for qualitative detection of HBsAg in human serum,plasma or whole blood whenever enzyme immunoassays methods are impractical or cannot be sustained (Torlesse *et al*., 1997).The advantage of tests based on immunochromatographic method is that it can be completed in 10-20 mins and can be performed by technicians or nurses with a minimum training (Sato *et al*., 1996). Screening is important in identification of carrier state andto prevent the spread of infection in the community. Despite the high level performance of screening assays, transfusion associated HBV infection is still reported (Hoofnagle, 1990 and Kjima *et al*., 1991).

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There are three possible explanations for false- negative results in commercial assays. In chronic HBV carriers, the HBsAg level may be below the detectable limit. Another explanation is that virus variants yield sequences that are not recognized by the antibodies employed in the assays (Bernerd*et al*., 1999). Third explanation for false negative results is the Hook's effect that is the misdetection of the viral antigen in an immunological assay due to high concentration of HBsAg antigen (Kafil Akthar and Rana K Sherwani *et al*., 2009).

MATERIALS AND METHODS

This case was reported in a tertiary care hospital, Hoskote, Bengaluru. The patient was admitted in orthopaedic ward for evaluation of low backache. Blood is collected byvenipuncture and was sent to Department of Microbiology. It was allowed to clot and serum was taken for examination. Rapid test using Hepacard was performed on the sample. Hepacard- aone-step immunoassay based on antigen capture or "sandwich" principle. The method uses monoclonal antibodies conjugated with colloidal gold and polyclonal antibodies immobilized on a nitrocellulose strip in a thin line (test line-T) and an additional line of anti-mouse antibody (control line-C)at a distance from the test line to serve as procedural control. Two drops of serum was added to the sample well, this was allowed to react for 20 mins and results read thereafter. If the sample contains HBsAg antigen, a pink band (test line-T) along with the control line has to be formed indicating that the sample is reactive for HBsAg. In this case the serial dilutions (1:10,1:20) of the patients sample in normal saline was done and the test was re-run. Hepa Elisa was used to confirm the presence of Hepatitis B surface antigen. The test sample was taken for Hepatitis B virus DNA detection test (Real time PCR assay) to determine the concentration of HBV DNA.

RESULTS

The Hepacard device when read after 20 mins showed only one distinct pink test line and no control line as shown in Fig.1. Diluted sample showed positive control and test line (Fig.1-1:10) & (Fig 2-1:20). Hepa Elisa showed reactive results. Real time PCR assay – HBV DNA levels was 107 IU/ml(1 IU=6 Copies/ml).

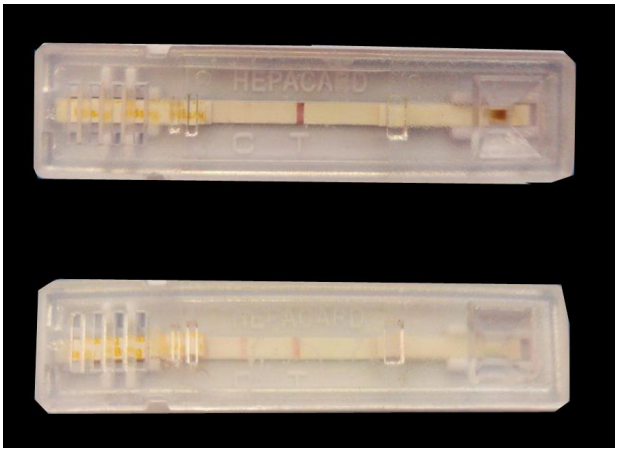

Fig. 1. First hepacard device- showed Appearence of one distinct line in control reigion

Second hepacard device-Sample 1:10 dilution showed both control "c" and faint pink test "t" line.

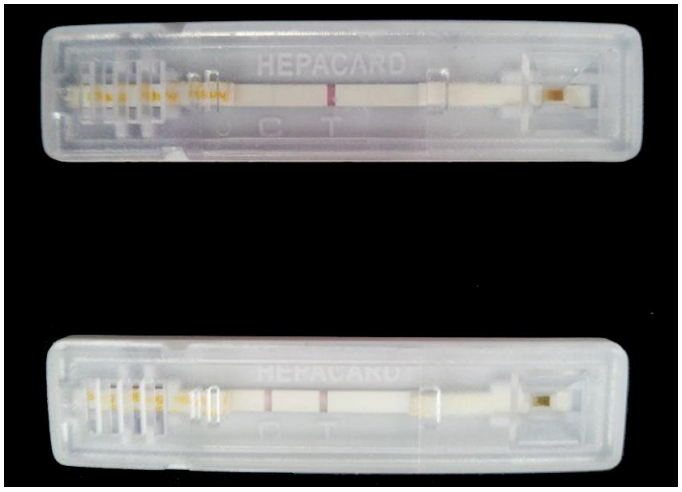

Fig. 2. First hepacard device showed appearence of one distinct line in control reigion

Seccond hepacard device- Sample 1:20 Dilution-Darker bands in both test "T" and control "C" region

The rapid test is a screening method that is able to discriminate an infected individual from an uninfected subject (Sato *et al*., 1996 and Raj *et al*., 2001). Various viral markers, including molecular methods such as DNA: DNA hybridization and PCR, are highly sensitive and quantitative. They are useful to monitor the disease progression closely (Mvere *et al*., 1996).The first description of prozone effect in the literature was made by Miles *et al*. (1974). Large quantities of antigen in serum impair antigen-antibody binding, resulting in low antigen determination. This is called prozone or high dose Hook effect, which describes the inhibition of immune complex formation by excess antigen concentration. The prozone or high dose hook effect, documented to cause false – negative assay results more than 50 years ago still remains a problem in immunometric assay (Brensing, 1989; Haller *et al*., 1992; Landsteiner, 1946). To detect prozone effect,samples are often tested undiluted and after dilution (saryan *et al*., 1989). If the results of diluted sample is higher than for Theundiluted sample, then the undiluted sample most likely exhibited the prozoneeffect. This approach increases labour and reagent cost for assays that encounter extremely high analyte concentration (KafilAkhtar, 2012).

Conclusion

It is important that Immunochromatographic Tests (ICT) should have high sensitivity and specificity levels that meet standards established by Enzyme immune assay(EIA).Many ICT are less sensitive than EIA. Results of ICT should be interpreted with caution. Negative control line does not indicate, the card is invalid. Hook phenomenon has to be considered while interpreting the result. Further tests will be useful to confirm the diagnosis.

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