Saliva as a Diagnostic Tool for Hepatitis B Infection- A Comparative ELISA Study

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Abstract: Background: Hepatitis B viral infection is a major public health problem with a worldwide prevalence. Due to their increasing incidence, early detection and improved diagnoses of severe cases are of primary importance. Currently viral antigens and antibodies are detected by traditional serological tests. However, the introduction of oral fluid as an alternative has led to many researches. Its advantages over venepuncture are many. Hence this prompted us to carry out a pilot study to evaluate the diagnostic efficacy of saliva in detecting hepatitis B surface antigen using Enzyme Linked Immunosorbent Assay (ELISA).

Aim and objectives: To evaluate the presence of hepatitis B surface antigen in saliva and its sensitivity and specificity through ELISA

Methodology and Results: 20 seropositive patients and 20 seronegative patients of hepatitis B viral infection were considered individually. Saliva samples collected from these patients were subjected to ELISA test for hepatitis B surface antigen. A sensitivity of 45% and specificity of 100% was obtained for the diagnosis of hepatitis B infection.

Conclusion: Many studies have been conducted utilizing saliva as a diagnostic tool especially in western population. Its advantages over venepuncture are, being less invasive, less painful, safe (prevention of needle stick injuries), less expensive, allows large numbers of samples to be collected easily for screening and for epidemiological purposes. In a developing country like India, an intermediate endemic zone where the estimated prevalence rate of HBV in the healthy general population is around 4.7%, a recent study showed 5% HBsAg positivity in Health Care Workers, but a highest seropositivity of around 40% among laboratory technicians.

The World Health Organization has estimated that there are more than 2 billion HBV infected people and about 378million chronic carriers worldwide. The toll of approximately 1 million deaths from chronic liver disease and hepatocellular carcinoma per year demonstrates the scale of the global health problem it poses.

It was estimated that more than 50% of liver cancers were attributable to HBV infection. Although most carriers will not develop hepatic complications from chronic hepatitis B, 15% to 40% will develop serious sequelae during their lifetime. In India, an intermediate endemic zone where the estimated prevalence rate of HBV in the healthy general population is around 4.7%, a recent study showed 5% HBsAg positivity in Health Care Workers, but a highest seropositivity of around 40% among laboratory technicians.

The major concern at this prime hour is the need to curb the disease due to their increasing incidence, which can be achieved by early detection and improved diagnosis. Currently viral antigens and antibodies are detected by traditional serological tests. However, the introduction of oral fluid as an alternative to venepuncture has led to many researches. A variety of studies have indicated the potential use of oral fluid for clinical diagnosis of infectious diseases and to evaluate immunity levels of important vaccine-preventable virus infections. The enormous advantages saliva holds, makes it a valuable tool for screening purposes.

Hence this prompted us to evaluate the presence of hepatitis antigen, its sensitivity and specificity by ELISA using saliva as diagnostic tool.
II. MATERIALS AND METHODS

20 seropositive patients of hepatitis B infection and 20 seronegative cases who were admitted to the M S Ramaiah hospital and college were considered. Consent for the study was obtained from every individual participating in the study. Ethical committee approval was also obtained.

Unstimulated saliva samples were collected from both the groups at convenience in a 20ml wide mouthed bottle. The saliva collection was about 3ml- 5ml which were stored immediately at -20 C until analysis. Before commencing with the procedure, samples were thawed to room temperature. For hepatitis B virus surface antigen detection, SD HBs Ag ELISA 3.0 Bio-standards diagnostics was employed. The procedure was standardized for detection of the antigen in saliva by running seropositive and seronegative cases, where the serum samples were used as controls. As instructed by the manufacturer the ELISA procedure was carried out with positive and negative controls provided in the kit. On completion of the method, the readings were obtained from an ELISA reader adjusted to the wavelength of 450nm and the results were tabulated (Table 1).

With a cut off value of 0.50, a sensitivity of 45% and specificity of 100 % was obtained. Out of the 20 seropositive cases of hepatitis B infection only 9 were positive with saliva samples while the remaining 11 cases were negative. On the other hand all the 20 seronegative cases of hepatitis infection were negative with saliva samples too. The inference noted here is that saliva when used as a diagnostic tool in our study; we obtained a sensitivity of 45% and specificity of 100%

III. DISCUSSION

A number of chronic infections contribute to the burden of cancer in humans. Chronic HBV infection alone has been estimated by the WHO to be the tenth leading cause of death globally, and HBV as the second most important known human carcinogen, after tobacco. [9] HBV seems to provide one of the examples whose genetic variability is observed both as the evolution of genotypes and as the emergence of mutations in each infected subject. [10] Volumes of research & literature exist on hepatitis B, its mode of transmission and infection.

Hepatitis B virus is present in the blood, saliva, semen, vaginal secretions, menstrual blood, and to a lesser extent, perspiration, breast milk, tears, and urine of infected individuals. A highly resilient virus, HBV is resistant to breakdown, can survive outside the body, and is easily transmitted through contact with infected body fluids.

The routine method of investigation being the blood has its own disadvantages. The relative inconvenience of obtaining blood samples, risk of disease transmission associated with needlestick injuries, requirement of consent and cooperation of the patient, the need for a trained venipuncturist, the need to separate serum before testing and the difficulty and added risk of venipuncture in children make serologic testing unattractive. Due to invasive nature of blood collection, it is also stated that contaminated needles cause 8–16 million HBV infections each year, compared with 2.3–4.7 million hepatitis C virus infections, and 80 000–160 000 human immunodeficiency virus infections. [5]

Saliva considered as the mirror of the body is an emerging biofluid for early detection of diseases. Ease, safety, and the minimally invasive nature of oral fluid collection are the major advantages of this technique. Studies have shown that saliva sample collection have a significant comfort and convenience level when compared to urine and blood. Hence our pilot study aimed to detect hepatitis B surface antigen (HBsAg), which is the hallmark of the infection; using ELISA as our screening tool and replacing serum with saliva.

Out of our 20 seropositive patients, 10 patients weren’t aware of their seropositivity of hepatitis B on admission. They were admitted for other medical reasons. As most of the serology analyses do consider screening for hepatitis B as a routine, we were able to identify such patients. Our study received good response from the patients when requested for the saliva sample collection and participation when explained about the study.

The sensitivity and specificity of detection of hepatitis B surface antigen in saliva was 45% and 100% respectively. The explanation that can be derived from these results is that all the seronegative patients did not show the presence of the antigen in their saliva and results were compatible with the serum samples. Hence 100% specificity was obtained. Only in 9 seropositive patients was the antigen detected in saliva while the remaining 11 were negative presenting with only 45% sensitivity.

Literature reviews we encountered have shown studies in agreement between the results for the serum and saliva specimens with an excellent reproducibility for detection of HBsAg.

Hutse, V., et al carried out a study on forty-three HBsAg positive and seventy-three HBsAg negative paired serum/oral fluid samples. The detection of HBsAg in oral fluid was carried out using a modified ETI-MAK-4 ELISA. The validation of this oral fluid test gave a sensitivity and specificity of 90.7% and 100%, respectively. They concluded that the modified ETI-MAK-4...
ELISA is a suitable test for oral fluid samples for epidemiological purposes. [11]

Cruz, H. M. et al carried out a study where HBsAg was detected by the ELISA in oral fluids and whole saliva samples with paired serum samples. Sensitivities of whole saliva and oral fluid were 93.6 and 85.1%, respectively, whereas specificities of whole saliva and oral fluid were 92.6 and 94.1%, respectively. They suggested the use of whole saliva and oral fluid together with the modified commercial EIA for Hepatitis B virus infection surveillance. [12]

Arora G et al conducted a study on 35 hepatitis B seropositive and seronegative subjects. The sensitivity and specificity of saliva as a diagnostic tool for detecting HBsAg antigen using ELISA was 74.29% and 100%, respectively. They concluded that because of its noninvasive nature, saliva can be effectively used for large-scale Hepatitis B virus detection. [13]

Table 1. Tabulated results of saliva samples for detection of Hepatitis B surface Ag

<table>
<thead>
<tr>
<th>Serum samples</th>
<th>Positive</th>
<th>Negative</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (n=20)</td>
<td>9</td>
<td>11</td>
<td>45%</td>
<td>100%</td>
</tr>
<tr>
<td>Negative (n=20)</td>
<td>--</td>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The above studies showed an appreciable sensitivity and specificity for detection of the antigen in saliva. The sensitivity in our study was comparatively low. This made us to investigate the protocol that we followed. We obtained evidence that the diminutive size of the antigen particles could be the reason. These antigens have greater chances to get entrapped within saliva, especially mucous which makes them unavailable for reaction and therefore detection. Hence pretreatment of saliva samples for digestion of mucin for studies especially related to detection of antigen and antibody is required to accomplish better results.

Investigators have detected a large number of viruses in oral samples by using an antigen, an antibody or nucleic acid targets. The literature regarding salivary-based antibody tests for detection of viral infections is extensive. Clinicians can use a number of oral samples to diagnose viruses, but the use of these tests by dental professionals has been limited. Salivary tests, although rapidly increasing in use, still constitute a minority of all diagnostic tests performed. Thus, by conducting salivary tests in a dental setting, practitioners would be able to identify infections in a cohort that might not otherwise be detected, at least in suspected individuals. [31]

IV. CONCLUSION

In our endeavor to serve the community we made an attempt to evaluate the detection of hepatitis viral infection using saliva-an emerging biofluid. We did succeed to an extent and ascertained the pretreatment of saliva samples as a cardinal step prior to analysis.

Today, a growing number of proof-of-principle assays have been established using saliva to monitor diseases or bodily conditions such as HIV infection, immune responses to viral infections and the detection of illicit drug use. [14]

Although a number of very sensitive and specific serologic tests for viral hepatitis are commercially available, the use of oral fluid samples makes it a satisfactory alternative, since the possibility to detect immunity using body fluids that can be easily and self-collected will facilitate the investigation, the follow-up of the outbreak, and the surveys of immunity in representative samples of the general population. However, despite the much progress in understanding the natural history of HBV infection, we still have a long way to go before we can conquer hepatitis B infection.

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