EVALUATION OF SENSITIVITY AND SPECIFICITY OF ORAL FLUID SAMPLE FOR DIAGNOSIS OF HEPATITIS B

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Abstract

Objectives: To assess the sensitivity and specificity of oral sample in detection of Hepatitis B surface antigen (HBsAg), which is the hallmark of the infection as compared to serum sampling using commercially available serologic test kits.

Study design: The study group comprised of 80 patients, 40 of whom were known to be hepatitis B positive (test group) and 40 sero negative (control group). The subjects were tested for HBsAg using serologic ELISA kit without any modifications. Absorbance values for serum and saliva were determined and the data analyzed using paired‘t’ test.

Results: In seropositive patients, the mean absorbance value of serum was 1.9402±0.440319, and that of saliva was 0.5228±0.175297. P value was < 0.001 and thus the mean score between serum and

saliva in seropositive patients was found to be extremely significant. Validation of the oral fluid test gave sensitivity of 95% and specificity of 100% as compared to serum sampling.

Conclusion: Oral fluid can be used as a safe and suitable diagnostic medium, alternative to serum in the diagnosis of hepatitis B infection for epidemiological and diagnostic studies as well

Key words: Oral fluid, saliva, ELISA, Hepatitis B.

Introduction

Hepatitis B is a global public health problem. Although most developed countries are showing trend towards decline of Hepatitis B Virus (HBV) infection, developing countries including India have shown no evidence of any decline.[1] HBV carrier pool in India has increased to 43-45 million, constituting the second largest pool next to China, globally.[2] Nearly 3 to 4% of the population in India are infected by the virus.[3]

Hepatitis B is a serious infectious disease of the liver caused by DNA virus belonging to the family called Hepadna viruses. HBV is a blood borne virus and the infection is transmitted mainly by parenteral and perinatal mode. The non-parenteral way of transmission has also been indicated and hepatitis B surface antigen (HBsAg) has been detected in various body fluids including urine, saliva and stools apart from blood.[2]

Enzyme-linked immunosorbent assay (ELISA) is considered a reliable indicator of detection of this disease. Because as little as 0.0001 mL of infectious blood can transmit the disease,[4,5] serological testing can prove to be hazardous for health professionals and paramedical staff. There is also the potential risk of disease transmission through needlestick injuries.[3] In addition, obtaining blood samples for large-scale detection of HBV is inconvenient because of the extra equipment required. In addition, the patient population may be reluctant to subject themselves to the invasive procedure of collecting blood samples. The use of saliva as

a diagnostic medium alternative to blood, provides substantial advantages in sampling convenience as it is a non-invasive technique. Literature reports indicate that viral hepatitis markers can be detected in saliva as well [6] . This study aims to establish oral fluid as a reliable diagnostic tool for detecting HBsAg using ELISA, simplifying the diagnosis of HBV. This can serve as a boon for health professionals and patients as well as in endemic situations.

MATERIALS AND METHODS

A total number of 80 subjects were selected for the study following approval from the Institutional review board. Informed consent was taken from each patient after explaining the aim and methodology of the study. Of the 80 patients, 40 were known to be HBV seropositive (test group) and 40 HBV sero-negative (control group). The seropositive patients (test group) were selected from the Department of General Medicine, M.S Ramaiah Medical College, Bangalore, India.

Method of Collecting Data:

Of the 40 patients in the seropositive group, 31 were males and 9 females, among the seronegative group 30 were males and 10 females. 2 ml of oral fluid (unstimulated saliva) was collected (dribble method) from all patients, in a sterile plastic container following thorough oral examination. Patients with gingival and mucosal lesions with a tendency to bleed were excluded from the study. The oral fluid samples were checked visually for blood contamination, if occurred were excluded from the study. The whole saliva was centrifuged at 4000 rpm for 15 min and supernatant was collected in cryovials, labelled and subjected to ELISA testing. For serum samples 2ml of blood was collected from anticubital vein in the anticubital fossa under aseptic conditions. This was centrifuged at 2000 rpm for 5 mins. Serum was separated and subjected to HBsAg analysis. Same cutoff value was applicable to

oral samples as well. The samples were then stored at -20˚C until analysis was carried out. The results were compared with paired serum specimens.

Testing for HBsAg was done with the help of commercially available kit 'General Biological's SURASE B-96 TMB, solid-phase enzyme immunoassay, Tetramethylbenzidine kit, Taiwan which meets the requirements for a third generation test as per the Food and Drug Administration (FDA) standards, intended as a laboratory screening test. Same kit was used to test both serum and oral fluid. The 'SURASE B-96 TMB kit adopts the sandwich principle, antibody-antigen-antibody as the basis of assay. Both serum and oral fluid samples were tested independently.

RESULTS:

A wide variation in age was noted, ranging from 7 - 70 years. Among the test group 31 (77.7%) were males and 9 (22.5%) were females with a mean age of 39.35±18.78 years .Among the control group 30 (75%) were males and 10 (25%) were females and the mean age was 34.93±16.477 years.

Validity of the tests were checked by comparing the absorbance value of blank, each negative control, mean negative control and difference between mean positive and negative control with one given by the manufacturer, and tests were found to be valid. Cutoff value was calculated as per the manufacturer's formula. Out of the total 80 patients, (40 seropositive and 40 seronegative), HBsAg was found in the oral fluid of 40 seropositive patients and in none of the seronegative patients.

The reliability of saliva as a diagnostic medium of hepatitis B infection was measured by calculating sensitivity and specificity. Standard formulae was used to calculate the sensitivity and specificity of oral-fluid relative to serum results, and exact 95% confidence limits (95% CL) were calculated. In this study sensitivity of 100 % (95% CL: 91.19-100) and also specificity of 95% (95% CL: 91.19-100) was found.

The sensitivity of using saliva for detection of HBsAg was found to be 95% while the specificity was 100%. The relation between saliva and HBsAg detection was found to be highly significant. The kappa value obtained is 1 which shows that there was perfect agreement between saliva and serum for diagnosis of Hepatitis B.

Paired‘t’- test was used to analyze the statistical significance between serum and saliva samples. The mean scores between serum and saliva absorbance values in seropositive and seronegative patients was calculated and P value obtained.

Among seropositive patients, (test group) the mean absorbance value of serum was 1.9402±0.440319, and that of saliva was 0.5228±0.175297 (Graph 2). The P value was < 0.001 and thus the mean score between serum and saliva in seropositive patients was found to be extremely significant.

Among seronegative patients, the mean absorbance value of serum was 0.0338 ±0.018042 and that of saliva was 0.0284±0.018188. The P value was calculated as 0.135 which was not significant.

DISCUSSION:

HBsAg is considered to be the hallmark of HBV infection. Transmission of HBV in the absence of apparent parenteral, sexual or perinatal exposure is common in highly endemic settings and occur within and between households. HBsAg, has been reported in feces, urine, bile, semen, tears, and saliva.[3,7] . The average volume of blood inoculated during a needle stick injury with a 22 gauge needle is approximately 1 µl[8] , sufficient quantity to contain up to 100 infectious doses of HBV [9] .In studies of health care workers who sustained injuries from needles contaminated with blood from HBsAg positive patients, 6-14% developed clinical hepatitis B and 25-45% developed serological evidence of HBV infection.[10] The

constitutional symptoms of HBV infection include fever, anorexia, nausea, myalgia, jaundice, or icteric hepatitis. These symptoms are reported in only 20% of the positive patients and more than 80% have subclinical or anicteric hepatitis.

In 1976, Peterson and colleagues conducted a study in two Alaskan villages and concluded that saliva and cutaneous exudates containing HBV viruses may play a role in the transmission of HBV infection and hence may be of diagnostic value.[11] Since then many trials have been conducted to prove the diagnostic efficacy of saliva in the detection of HBV. Collection of whole saliva is easy as it requires minimal armamentarium and there is more patient compliance. Whole saliva contains a detectable quantity of HBsAg, which is derived from gingival crevicular fluid. Whole saliva is considered a better saliva sample for HBsAg detection in contrast to stimulated saliva.[12,13]. Therefore, in the present study, whole saliva was used to detect the presence of HBsAg. The presence of HBsAg in saliva can be explained by the admixture of the crevicular fluid with saliva. In the present study, we chose to detect only HBsAg and not the other HBV antigens because, HBsAg is a reliable marker of HBV infection and it is the first antigen to appear after the infection. HBsAg appears in serum as early as 1 to 10 weeks following exposure to HBV and even before the prodromal phase of HBV infection. It is also the last antigen to disappear after remission of the infection.[4,14]

The results of our study conclude that sensitivity and specificity of saliva as a diagnostic tool for detecting HBsAg is 95% and 100% respectively. This is in agreement with the results of Ben-Aryeh and Ben-Porath[15] who established a sensitivity and specificity of 90% and 100% in detecting the salivary HBsAg using ELISA.

The probable reason for absence of HBsAg in the saliva of one sero-positive patient could be that the concentration of HBsAg in saliva was much lower compared to blood because of the sub-clinical state of the infection. Our results are not in agreement with other Indian studies

conducted by Wanjari et al[16] in Amravatti who established a sensitivity of 76% and Gagandep Arora et al[17] who found a sensitivity of 74.29% and specificity of 100% though the same standard method was used. In our study sensitivity is greater because most of the patients were symptomatic, reporting to hospital for the same whereas in other studies the patients were asymptomatic HBV carriers selected from blood banks of the hospitals. Thieme et al established a sensitivity and specificity of 100% for detection of HBsAg in saliva.

CONCLUSION:

Our results suggest that oral fluid could have the potential to replace serum in HBV surveys. The test is economical, convenient and safer compared to serological tests for HBV infection and has wider applications in both patient and outbreak management

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