A Comparitive Study to Detect Hepatitis B Surface Antigen in Saliva and Serum by Enzyme Linked Immunosorbent Assay (Elisa Test) An Invivo Study

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Abstract

Background and Objectives: Hepatitis B virus (HBV) has been well known for its parenteral and sexual dissemination. Applying ELISA it has also been detected in saliva. The high prevalence of HBV among dentist personnel, children in institutions and family members suggests that HBV can be spread by saliva. The present study is done to assess the efficacy of saliva and serum in detection of hepatitis B surface antigen in suspected hepatitis B patients. **method:** This study include 40 pateints with suspected Hepatitis B patients. 2 ml of Saliva and Blood samples were collected from 40 suspected hepatitis B patients. All saliva and serum samples were tested for Hepatitis B surface antigen applying ETI-MAK 4 ELISA KIT (DiaSorin S.p.A., ITALY. **results:** In our study, out of 40 patients, 37 serum samples were positive and 3 samples were negative for HBsAg. Whereas, 31 saliva samples were positive and 9 were negative in saliva samples for HBsAg. Mean value for serum samples was 3.61 and saliva samples 1.69. The mean difference was 1.921. Higher mean hepatitis B antigen is found in serum samples compared to saliva samples. The difference in hepatitis B antigen between serum and saliva samples is found to be statistically significant ($P<0.001$). The data illustrate the diagnostic value of saliva and point to the possible role of saliva as a source of hepatitis B virus infection. **conclusion:** The data revealed that testing of saliva samples for hepatitis B virus markers provided a useful alternative to serum-based assays. The convenience, reliability, and minimal non-invasive nature of this method make it an attractive tool for the selection of non-immune candidates for vaccination against hepatitis B.

Key words: *Hepatitis B, ELISA, HBV antigen, saliva,*

Introduction

Hepatitis is an inflammatory disease of the liver that can severely damage the organ. The disease can result from non-infectious or infectious viral and bacterial agents. There are five main hepatitis viruses, referred to as types A, B, C, D and E.[12]Hepatitis A and E are typically caused by ingestion of contaminated food or water. Hepatitis B, C and D usually occur as a result of parenteral contact with infected body fluids (e.g. from blood transfusions or invasive medical procedures using contaminated equipment).[5,11] Hepatitis B is also transmitted by sexual contact. Viral hepatitis B is major public health problem with over 360 million chronically infected people worldwide.[10] On global scale, chronic hepatitis B virus represents the major cause of chronic hepatitis, cirrhosis and hepatocelluar carcinoma. Therefore, particularly in developing countries with highest prevalence of chronic hepatitis B virus infection, the virus incurs a high incidence of morbidity and mortality.[7] The incubation period for hepatitis B average 12 weeks (range of 6 to 24 weeks). Common symptoms

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include malaise, fever, gastroenteritis and icterus.[13]Over 90% of adult patients with hepatitis B completely recovers from acute illness, approximately 1% die of fulminant hepatitis, and approximately 6 to 10% become chronic active or persistant carriers. HBsAg is the first serological marker to appear in the circulation, well before clinical symptoms, and is the viral component usually found in the highest concentration in the serum of HBV-infected patients. The significance of HBsAg in serum is determined by evaluating it in relationship to the presence or absence of the other HBV markers and the clinical presentation and history of the patient. Up to now,the serologic assay for HBsAg has been routinely applied in clinical practice to diagnose HBV infection,but a non-invasive method for testing pregnant women would be infinitely preferable, especially in developing countries where many patients are reluctant to provide blood samples.Based on the observation that HBV can be transmitted by parenteral exposure to human body fluids such as saliva, breast milk, and semen. Various groups have undertaken to design a detection assay for HBsAg in human saliva. In the search for possible modes of cross-infection, HBsAg has been detected in nearly all body fluids of HBsAg carriers.[1,2]

However, epidemiological and serological evidence implicates saliva as the most probable vehicle for spreading HBV among contacts without apparent percutaneous exposures to blood products.[5]Accordingly, attention has focused on the risks of infection by dental care, either as a result of patient-patient exposures by inadequately sterilized instruments or of dentist-patient exposures by intimate contacts with HBsAg carriers.[3,4] The test thus devised can provide a simplified alternative, at least as far as obtaining the samples, and more significantly, potentially infectious individuals can easily be identified, especially as HBV transmission in saliva has been shown to occur through breaks in the skin.[5,6]

Hence this study being done to detect (diagnose) hepatitis B virus infected person by salivary sample. Hence this study being aimed to detect (diagnose) hepatitis B virus infected person by salivary sample in order to assess the efficacy of saliva in detection of Hepatitis B surface antigen (HBsAg) in suspected Hepatitis B patients, to assessthe efficacy of serum in detection of Hepatitis B surface antigen (HBsAg) in suspected Hepatitis B patients and to compare the efficacy of saliva with serum in detection of hepatitis B surface antigen (HBsAg) in suspected Hepatitis B patients.

Materials and Method

Sources of Data: 40 patients suspected with Hepatits B virus infection will be selected from Out-Patient Dept of General Medicine, KIMS Hospital, Bangalore.

Study material: Commercially available ELISA kit will be used on the selected patients.

Study design: Comparitive non-randomized study.

inclusion criteria: Only individuals with suspected Hepatitis B virus infection will be included in the study. Clinical criteria for suspecting Hepatitis B virus infection: Fever, Loss of appetite**,** Jaundice**,** Liver tenderness / Hepatomegaly**,** Cirrhosis**. exclusion criteria:** Patients who are undergoing treatment for Hepatitis B virus infection and vaccinated against Hepatitis B Virus infection will not be included in the study.

Study method: saliva collection method: Unstimulated whole Saliva sample of about 2 ml will be collected by asking the patient to dribble into sterile wide mouth plastic containers. Using disposable plastic. Collected saliva samples will be stored at -20° c until use. **blood collection method:** Aseptic measures will be used and tourniquet is applied 2 inches above the elbow of the upper arm. The site of puncture is cleaned using sterile gauze dipped in surgical spirit. Using 5 ml syringe with a 22 gauge 1 ½ inch needle, 2 ml of blood is drawn from the antecubital vein. The blood is allowed to clot and the serum separated by centrifugation. Care should be taken to ensure that the serum sample are clear and not contaminated. The collected serum will be stored at -20°c until use.

Materials used :The method of HBsAg determination is based on commercially available ELISA kit.

Composition and Preparation of Reagents

All serum and saliva used to produce the components provided in this kit have been tested for the presence of HBsAg, anti-HCV and anti-HIV-1/2 and found to be nonreactive, except for the positive control, which is reactive for HBsAg.Serum and saliva samples with absorbance value above the Grey zone should be considered as positive or reactive for HBsAg. Serum and saliva samples with the absorbance value below Grey zone should be considered as negative or non-reactive

for HBsAg. The results will be compared and statistically analysed by Descriptive analysis and Chi-square test.

Results

Statistical analysis In this study, out of 40 patients, 37 serum samples were positive and 3 samples were negative for HBsAg. Whereas, 31 saliva samples were positive and 9 were negative in saliva samples for HBsAg. **alternate hypothesis:** There is a significant difference between the values recorded in saliva and serum with respect to the hepatitis B surface antigen i.e. $\eta_1 \neq \eta_2$**. level of significance:** α=0.05**.null hypothesis:** There is no significant difference between the values recorded in saliva and serum with respect to the hepatitis B surface antigen i.e. $\eta_1 = \eta_2$**. statistical technique used:** Mann-Whitney test, Chi-square test(Z)**. decision criterion:** The decision criterion is to reject the null hypothesis if the p-value is less than 0.05. Otherwise we accept the null hypothesis.

Table 1: The computations carried out are given below:

Sample	n	Mean	std dev	mean difference	Z	p-value
Serum	40	3.61	1.06	1.921	-3.893	<0.001
Saliva	40	1.69	1.73			

Higher mean hepatitis B antigen is found in serum samples compared to saliva samples. The difference in hepatitis B antigen between serum and saliva samples is found to be statistically significant (P<0.001). (Graph 1)

Table 2: Results recorded in serum and saliva samples

Saliva	Serum		Total	χ^2	P-Value
	Positive	Negative			
Positive	28 (90.32%)	3 (9.68%)	31	0.009	0.332
Negative	9 (100%)	0 (0.00%)	9		
Total	37 (92.50%)	3 (7.50%)	40		

No statistically significant association is observed between saliva and serum results (P>0.05).

Table 3: Sensitivity, Specificity, PPV & NPV of serum values.

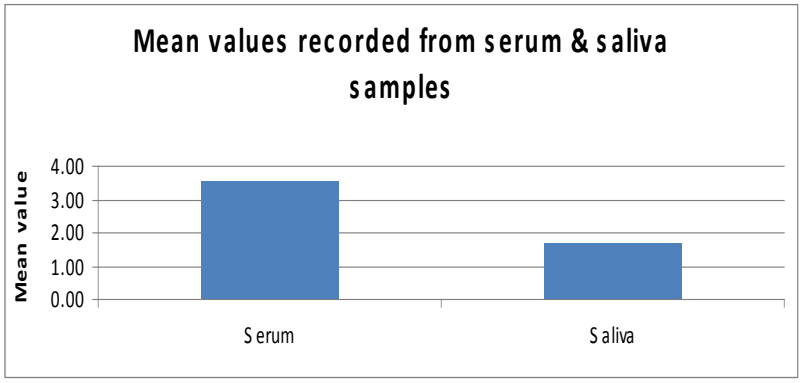
Saliva	Serum		Total	Sensitivity	Specificity	PPV	NPV
	Positive	Negative					
Positive	28	3	31	0.7568	0.0000	0.9032	0.0000
Negative	9	0	9				
Total	37	3	40				

Sensitivity of a test is the probability of a positive test result given the presence of the disease. In other words, it is the probability of identifying true positives. The saliva method is capabale of identifying true positives upto 75.68%.

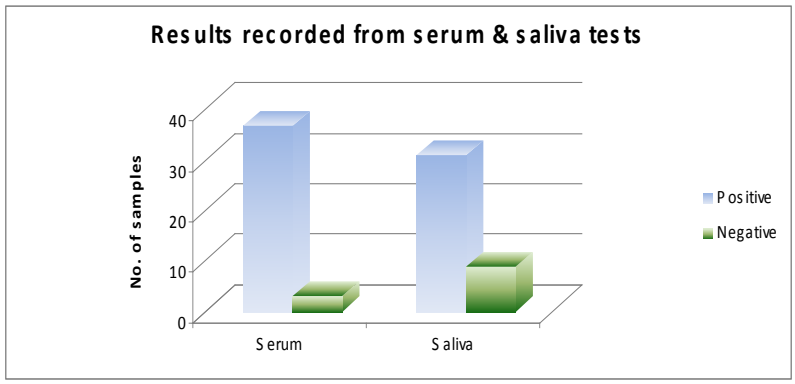
Specificity of a test is the probability of a negative test result given the absence of the disease. In other words, it is the probability of identifying true negatives. The saliva method is not capable of identifying true negatives as the specificity is zero.

The positive predictive value (PPV) also called as precision rate, or post-test probability of disease, is the proportion of patients with positive test results who are correctly diagnosed. Saliva method yielded a PPV of 0.9032 which shows that it is capable of identifying patients diagnosed as positive for Hepatitis B, upto 90.32%.

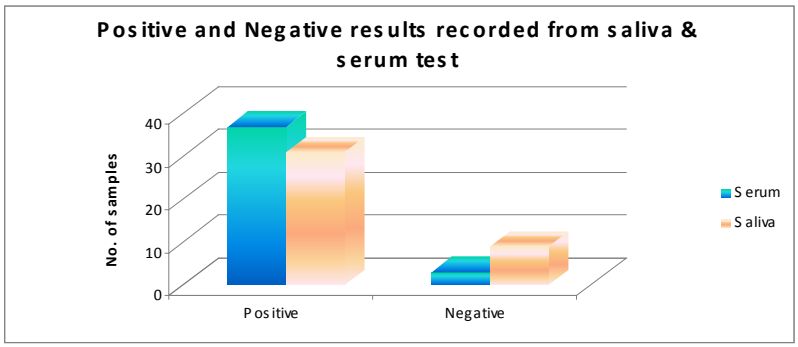
The negative predictive value is the proportion of patients with negative test results who are correctly diagnosed. Saliva method recorded a NPV of 0 implying that it is not capable of identifying patients who were diagnosed as negative for Hepatitis B.



Graph 1: Mean values recorded from serum and saliva samples



Graph 2: Results recorded from serum and saliva tests



Graph 3: showing positive and negative results recorded from saliva and serum samples.

Discussion

High prevalence of hepatitis B virus among dental personnel, children in institution and family member suggest that hepatitis B virus can be transmitted by parenteral exposure to human fluids such as saliva , breast milk and semen.[9]

In this study, out of 40 patients, 37 serum samples were positive and 3 samples were negative for HBsAg. Whereas, 31 saliva samples were positive and 9 were negative in saliva samples for HBsAg.

In this study an ELISA Kit was used, in which the saliva sample incubation step was directly followed by a conjugate incubation step. As a consequence of the limited analytical sensitivity of this ELISA and the low antigen concentrations present in saliva, optical densities of some patient samples were close to the optical densities of negative control saliva. Moreover, evaluation of positivity of the saliva samples in ELISA was based on the absolute value of the corrected optical density (OD), compared with a cutoff. This reduces the reproducibility of the test, because the OD can be influenced by external factors such as temperature, incubation time and the batch of reagents used to perform the test.

Mean value for serum samples was 3.61 and saliva samples 1.69. The mean difference was 1.921. Higher mean hepatitis B antigen is found in serum samples compared to saliva samples. The difference in hepatitis B antigen between serum and saliva samples is found to be statistically significant ($P<0.001$).

Sensitivity of a test is the probability of a positive test result given the presence of the disease. In other words, it is the probability of identifying true positives. The saliva samples were capabale of identifying true positives upto 75.68%.

Specificity of a test is the probability of a negative test result given the absence of the disease. In other words, it is the probability of identifying true negatives. The saliva samples are not capable of identifying true negatives as the specificity is zero.

The positive predictive value (PPV) also called as precision rate, or post-test probability of disease, is the proportion of patients with positive test results who are correctly diagnosed. Saliva method yielded a PPV of 0.9032 which shows that it is capable of identifying patients diagnosed as positive for Hepatitis B, upto 90.32%.

The negative predictive value is the proportion of patients with negative test results who are correctly diagnosed. Saliva method recorded a negative predictive value of 0 implying that it is not capable of identifying patients who were diagnosed as negative for Hepatitis B. The data illustrate the diagnostic value of saliva and point to the possible role of saliva as a source of hepatitis B virus infection.

Based on the high sensitivity of ELISA in saliva, this method appears appropriate for screening. Our results demonstrated that the commercially ELISA kits can be successfully adapted to the use of oral samples in epidemiological studies for hepatitis B.[8]

Conclusion

From the observation gathered comparing detection of saliva to serum samples using a commercially available ELISA kit without modification, the sensitivity was 75.68% and Saliva method yielded a positive predictive value of 0.9032 which shows that it is capable of identifying patients diagnosed as positive for Hepatitis B, upto 90.32% which achieved in the diagnosis of hepatitisB using oral samples suggest that saliva could be used as a diagnostic tool for hepatitis B virus infection.

Testing of saliva samples for hepatitis B virus markers provided a useful alternative to serum-based assays. The convenience, reliability, and minimal non-invasive nature of this method make it an attractive tool for the selection of non-immune candidates for vaccination against hepatitis B. Based on the observation that hepatitis B virus can be transmitted by parental exposure to human body fluids such as saliva, breast milk, and semen. The test thus devised can provide a simple alternative, at least as far as obtaining samples ,and more significantly potientially infectious individuals can be easily identified, especially as hepatitis B virus in saliva has been shown to occur through breaks in skin.

Conflict of Interest: None

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