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Original Article

Diagnosis of Dengue Virus Infection in Oral Fluid using Immunochromatographic Tests.

Tahmina Akther[1], Biswajit Paul[2], Saif Ullah Munshi[1], Shahina Tabassum[1]

[1]*Department of Virology, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka.* [2]*Department of Biotechnology and Genetic Engineering, Mawlana Bhashani Science and Technology University (MBSTU), Santosh, Tangail*

Abstract

Dengue viral infections are diagnosed by detecting dengue NS1 antigen and dengue specific IgM/IgG antibody by traditional serological tests using patient's blood. Recently saliva is being used employed for diagnosis of different infectious diseases including dengue. In the present study, dengue NS1 antigen and anti-dengue IgM/IgG antibody were detected using rapid immunochromatographic (ICT) kits for diagnosis of dengue and compared with the results of serum ICT results performed on corresponding sera collected from the same individuals. A total of 215 suspected dengue patients were tested and dengue infection was found in 176 (81.9%) sera and 161 (74.9 %) oral fluid samples. Among the 30 dengue NS1 antigen positive sera, 28 were also positive for NS1 in oral fluid indicating high sensitivity (93.3%), specificity (100%), diagnostic accuracy (99.1%), PPV (100%) and NPV (98.9 %) of testing dengue NS1 antigen in oral fluid. Similarly oral fluid assay for anti-dengue IgM showed sensitivity, specificity, diagnostic accuracy, PPV and NPV of 87.3%, 100.0%, 95.8%, 100.0% and 94.1% respectively. Test for anti-dengue IgM/IgG in oral fluid showed sensitivity, specificity, diagnostic accuracy, PPV and NPV of 89.7%, 100.0%, 98.1%, 100.0% and 97.7% respectively. All these parameters for detection of anti-dengue IgG by ICT showed 100%in oral fluid. Thus, results from this study indicates that detection of dengue NS1 antigen or anti-dengue IgM/IgG in oral fluid is an alternative tool for dengue diagnosis. It may benefits dengue diagnosis especially in infants and children since it is easy to collect and require no additional sample processing. It also has the potential to use for epidemiological survey.

Key words: Dengue, Immunochromatographic test, Oral fluid, Serum.

Introduction:

Dengue infection (DF) is a self-limiting infection occasionally causing dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) which are the two major life threatening conditions. One hundred million cases of DF and 250,000 to 500,000 cases of DHF/DSS occur annually.[1] Serology is currently the method of choice mostly applied for routine diagnosis which requires blood as a specimen for testing.[2] Until recently, only blood samples were used for dengue diagnosis but it has been shown in several studies that saliva can also be employed for detection of dengue NS1 antigen or dengue specific IgM and IgG antibody.[3,4] Saliva which produced by the submandibular, sublingual, parotid glandsand salivary glands constitutes a part of oral fluid. Oral fluid is a physiological fluid that can be collected from the oral cavity of the mouth.[5] Oral fluid, being non-invasive, is cost effective, simple and easy to collect, available in sufficient quantity and easy to store and transport; without needing any auxiliary personnel.[6] The use of oral fluid samples for disease detection has also been reported for Human Immunodeficiency Virus (HIV), Hepatitis A virus (HAV) and Hepatitis B virus (HBV), Measles, Mumps, and Rubella.[7] To detect acute dengue infection, dengue NS1 antigen and/or dengue specific anti-dengue IgM and to diagnose previous infection dengue specific anti-dengue IgG are the serological tests performed on patient's serum or

✉ ***Correspondence:***
Dr. Tahmina Akther
Medical officer
Dept. of Virology,
Bangabandhu Sheikh Mujib Medical University
E-mail address: tahminaakther76@yahoo.com
Telephone No: 01712105687

plasma. Sometimes it is difficult to collect blood from suspected dengue patients for testing who have already developed DHF/DSS. Moreover, venous blood sampling from infants and young children for diagnosis or for community based study from remote settings is cumbersome. As the collection of oral fluid specimens is a non-invasive procedure, it is a preferred alternative for the diagnosis of any disease specially infections. Therefore, this study was designed to evaluate oral fluid as an alternative of blood sample to use in diagnosis of acute and past dengue infection. In present study, immunochromatographic (ICT) kits designed for detection of serum / plasma dengue NS1 antigen and anti-dengue IgM&IgG antibody were used to detect dengue NS1antigen and anti-dengue IgM&IgG antibody in oral fluid and compared with the results obtained from the dengue NS1 antigen and anti-dengue IgM&IgG antibody tests performed on serum. conducted in Rajshahi Medical College Hospital & Dhaka Medical College from August 2014 to July 2015. Total 323 blood samples were collected from suspected enteric fever patients and isolation rate of Salmonella was 9.29% [S.typhi (3.41%), and S.paratyphi A (5.88%)]. Among isolated S.typhi, 9.09% were resistant to chloramphenicol, co-trimoxazole and cefixime and there were no S.typhi resistant to azithromycin and cefotaxime. Among the isolated S.paratyphi A, 5.26% were resistant to chloramphenicol, co-trimoxazole, azithromycin, cefotaxime, and cefixime. There were no ceftriaxone resistant Salmonella. Low proportion of resistance to first line antibiotics (chloramphenicol, co-trimoxazole) suggests that these drugs can be used once again.

Methodology:

This study was performed from March' 2016 to September' 2016 at the Department of Virology, BSMMU, Dhaka. A total of 215 oral fluid samples and corresponding sera were collected at the same time points from the individuals who were suspected for dengue infections. After taking written informed consent, oral fluid was collected by dribbling (drooling, spitting) into a 15 ml conical centrifuge tubes and kept untreated and uncentrifuged. Approximately 5 ml of venous blood was collected in a vacutainer tube using aseptic venipuncture techniques and after clot retractionap-proximately 2 ml of serum was collected for testing. All tests for dengue were performed within half to one hour after collecting samples. Both the oral fluid samplesand sera were simultaneously tested for dengue NS1 antigen and anti-dengue IgM&IgG using qDetectTMdengue NS1 antigen test kit (OMC, Healthcare, Bangladesh) and Dengue IgG&IgM Test Device (HangzhouDeangel Biological Engineering Co, Ltd, China; Mainland), respectively according to the manufacturer's instruction. For detection of dengue NS1 antigen in oral fluid and serum, 50µl of oral fluid and serum sample and 3 drops of the chase buffer were used. The result of the test was read after 20 minutes in both kinds of tests. For anti-dengue IgG and IgM rapid test, 15 µl of oral fluid and 10 µl of serum with 2 drops of buffer were used and waited for 15 minutes in case of serum but 20 minutes for oral fluid. All the statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS) version 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA).The results of serum ICT were considered as gold standard and compared with the test results obtained from ICT performed with oral fluid. The diagnostic efficacy was evaluated in terms of sensitivity, specificity, diagnostic accuracy, positive predictive value (PPV), negative predictive value (NPV).The study was approved by Institutional Review Board of BSMMMU (IRB No. BSMMU 2016/3467).

Results

A total of 215 oral fluid samples and sera were collected from patients who were presented with symptoms indicative of dengue infection. The mean age of the study participants included in the study was 37.2 ±13.5 years, where 132 (61.1%) were males and 83 (38.4%) were females. Out of 215 cases, 67.44% samples were collected within 2-5 days and remaining samples were collected between 6-8 days after the onset of fever. Among the collected 215 sera, 81.9% (176/215) were positive for one or multiple dengue infection parameters where dengue NS1 antigen was detected in 30 (13.95%) sera, anti-dengue IgM in 71 (33.02%) sera, both anti-dengue IgM and IgG was detected in 39 (18.13%) sera and anti-dengue IgG alone was detected in 36 (16.74%) sera (Table-I).On the contrary, 74.9 % (161/215) corresponding oral fluid showed positivity for any or multiple dengue diagnostic parameters. Among the 30 (13.95%) dengue NS1 antigen positive sera, dengue NS1 antigen was detected in 28 (13.02%) oral fluid, anti-dengue IgM in 62 oral fluid of 71 anti-dengue IgM positive sera. Among all the patients, 39 (39/215) serum samples 39 (18.13 %) were positive for both anti-dengue IgM and IgG in which 35 oral fluid samples (16.27 %) were also positive for anti-dengue IgM and IgG in oral fluid. ICT performed on both serum and oral fluid obtained the same result for anti-dengue IgG i.e. out of all samples, 36 (16.74%) sera and oral fluid samples were positive for anti-dengue IgG antibody.

The sensitivity, specificity, diagnostic accuracy, PPV and NPV of ICT for dengue NS1 antigen in oral fluid was 93.3%, 100.0%, 99.1% ,100.0% and 98.9%, respectively (Table-II). Among the only anti-dengue IgM cases, oral fluid assay showed sensitivity, specificity, PPV, NPV and diagnostic

accuracy of 87.3%,100.0%, 95.8%, 100.0% and 94.1% respectively. The sensitivity, specificity, diagnostic accuracy, PPV and NPV of anti-dengue IgM/IgG on oral fluid were 89.7%, 100.0%, 98.1%, 100.0% and 97.7%, respectively. Detection of dengue IgG antibodies by ICT in oral fluid showed 100% in sensitivity, specificity, diagnostic accuracy, PPV and NPV.

Table-I: Detection of different dengue parameters in saliva samples are presented corresponding to serum samples (n = 215)

	Serum (215)		Oral fluid (215)	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Dengue NS1 antigen	30 (13.95)	39 (18.13)	28 (13.02)	54 (25.11)
Anti-dengue IgM	71 (33.02)		62 (28.83)	
Anti-dengue IgM&IgG	39 (18.13)		35 (16.27)	
Anti-dengue IgG	36 (16.74)		36 (16.74)	
Total	176 (81.86)	39 (18.13)	161 (74.9)	54 (25.11)

Table-II: Different performance indicators of dengue NS1 and dengue IgM and IgG tests performed in oral fluid in comparison to serum samples (n=215).

Parameters	NS1 antigen	IgM antibody	IgM+IgG	IgG antibody
Sensitivity (%)	93.3	87.3	89.7	100.0
Specificity (%)	100.0	100.0	100.0	100.0
Diagnostic Accuracy (%)	99.1	95.8	98.1	100.0
Positive predictive value (%)	100.0	100.0	100.0	100.0
Negative predictive value (%)	98.9	94.1	97.7	100.0

Discussion

In present study, a total of 215 oral fluid samples were collected from the dengue suspected febrile patients and subjected to dengue NS1 antigen and anti-dengue IgM/IgG tests using two ICT kits with few modifications which were designed to use with plasma or serum or whole blood. Corresponding serum samples were also collected from the same study participants at the same time and tested with the same ICT kits. In comparison to dengue tests performed in serum, it was possible to diagnose correctly almost all acute and past dengue infected patients with oral fluid either using dengue NS1 antigen or IgM and IgG antibody tests. It was observed that among the 30 dengue NS1 antigen positive sera, 28 were also positive for NS1 antigen in oral fluid indicating sensitivity of 93.3% and specificity of 100%. The high diagnostic accuracy (99.1%), PPV (100%) and NPV (98.9%) of testing dengue NS1 antigen in oral fluid indicates that oral fluid is a good alternative of serum. Interestingly, the sensitivity and specificity of the tests performed in oral fluid observed in the present study is better than the previously published studies. In earlier studies, sensitivity of detection of NS1 in oral fluid was comparatively less i.e. 64.7% and 86% though the specificity was almost same.[8,9] After 5 days of fever, detection of dengue specific IgM antibody is the mainstay for diagnosis of acute dengue. In current study, the sensitivity, specificity, PPV & NPV of anti-dengue IgM observed in the present study is 87.3%, 100%, 100% and 94.1 % which shows slight better performance than other study.[10] Presence of anti-dengue IgG is an indicator of past infection. The sensitivity for anti-dengue IgG test in oral fluid observed in current study is better (100%) in comparison of previous study (88%), though the specificity in both the studies was similar (100%).[11]

The concentration of antibody in saliva is less comparing to serum which may cause slight loss of sensitivity in all the dengue tests performed in oral fluid. It should be noted that this discordance may be avoided by adding preservative to the oral fluid specimens or by using a collection device.[12] Even after such limitations, this study proofs that diagnosis of dengue infection can be achieved by detecting dengue NS1 antigen or dengue specific antibodies in oral fluid.

In patients where collection of blood sample is burdensome, the availability of an oral fluid sample would allow a high specific, although less sensitive, diagnostic assay for dengue. Thus, detection of dengue NS1 antigen or anti-dengue IgM/IgG in oral fluid specimens provide an alternative tool that has the benefits of easy sample collection, requiring neither special materials nor expertise in phlebotomy. This is particularly important in dengue-endemic, resource-poor countries such as Bangladesh, where many infants and children become infected every year. In addition, oral fluid is more cost-effective test sample than serum since, it is cheaper to collect and require no additional sample processing. It also shows promise of using in studies related epidemiological survey.

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