COMPARATIVE DETECTION OF MEASLES SPECIFIC IGM ANTIBODY IN SERUM AND SALIVA BY AN ANTIBODY-CAPTURE IGM ENZYME IMMUNOASSAY (EIA)

Talat Mokhtari Azad*, Anahid Ehteda, Parvin Yavari, R. Hamkar, Zahra Safar Pour, M. Essalat and Rakhshandeh Nategh

Dept. Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

ABSTRACT

Laboratory diagnosis of acute measles is usually achieved by serology assays for measle-specific IgM antibody. For comparison of measle-specific IgM antibody in saliva and serum, 95 paired blood and saliva samples were collected 1-14 days after the onset of rash. The specimens were tested for specific IgM antibody by an IgM antibody-capture Enzyme Immunoassay (EIA). Measles IgM antibody was detected in 89 (93.7%) of serum samples and in 85 (89.5%) of saliva specimens. Of the 6 (6.3%) serum samples that were IgM antibody-negative, 2 (2.1%) of the paired saliva samples were IgM antibody-positive. The sensitivity and specificity of saliva testing compared with serum was 95.5% and 66.7% respectively. Positive predictive value (PPV) and negative predictive value (NPV) of saliva testing were 97.7% and 50.0% respectively and the accuracy of saliva testing was 93.7%. Our results indicate that saliva samples provided Enzyme Immunoassay results that were in good agreement with results from serum samples. Salivary IgM antibody detection is a suitable non-invasive method for diagnosing recent measles infections and epidemiological studies, especially in children.

Keywords: Measle, Saliva, IgM antibody, Enzyme Immunoassay.

INTRODUCTION

Measles virus is classified in the family of Paramyxoviridae, and is a member of the genus Morbillivirus.[1] Measles virus is an enveloped, pleomorphic virus ranging from 100-250 nm in diameter with a single stranded linear RNA.[2]

This virus is responsible for an acute childhood disease that each year infects over 40 million individuals and causes the death of more than 1 million.[3] The 45th World Health Assembly established a goal of achieving a 90% reduction in the number of measles cases by the year 1995 as a step toward global eradication of this disease.[4] Since expanded program on immunization in Iran in 1984, coverage has improved,[5] but Iran is currently in the measles elimination stage and has not yet conducted the initial catch-up campaign.[6] Historically, in most countries, measles has been diagnosed based on clinical criteria. However, with high immunization rates, the numbers of mild or asymptomatic infections increased, and medical personnel have less experience diagnosing measles. Inaccurate diagnosis can lead to inappropriate vaccination campaigns and waisted resources or missed opportunities to prevent transmission. Therefore, laboratory confirmation has become important to measles control programs.[7] Existing laboratory methods rely largely on the detection of significant rises in measles antibody titer or the

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* Head of National Measles Center
Address: School of Public Health, Tehran University of Medical Sciences, Department of Virology, P.O. Box 6446-14155, Tehran, Iran.
Fax: 021 6462267, Telephone: 021 6112813, E-mail: mokhtari@sphtums.com, mokhtari@hotmail.com

detection of measles specific IgM antibody and are impractical for widespread use in children as blood samples are required. Blood collection requires specially trained staff and sterile equipment, which restricts its widespread use. So, non-invasive methods for confirming measles infection could have an important role in surveillance in communities with limited measles transmissions.[8]

The use of saliva as a noninvasive alternative to serum for detecting virus-specific antibodies was first described by Parry et al..[9] Subsequently, detection of viral immunoglobulin in saliva was reported for patients with HIV,[10] Hepatitis A or B,[11] measles, mumps, and rubella,[12] and parvovirus B19.[13] In addition, detection of virus-specific IgM antibody in saliva after measles vaccination was also reported by Helfand et al..[7]

Up till now, detection of IgM antibody in saliva samples for virus diagnosis has mostly been performed by radioimmunoassay (RIA).[11,12] However, laboratories in many countries may find that EIA is easier to do and read than RIA. The use of radioactive labelled nucleotides in some settings may be almost impossible due to problems in obtaining, storing, using and disposing of radioactive materials. Moreover, expensive equipment is required to monitor and to measure radioactivity.[7]

This study was designed to validate the salivary assay under conditions of routine community use and to investigate the diagnostic accuracy of notified cases of measles.

MATERIALS AND METHODS
Study group
Our study group consisted of 95 suspected measles cases who were referred to the Center of virology of Tehran University of Medical Sciences in Iran between 1999 and 2001. We collected information about age, sex, prior immunization, date of rash onset and date of specimen collection. Blood and whole saliva were collected as described below.

Specimen collection
Blood (2-3 mL) was collected by using universal precautions. Serum was separated and stored at -20°C. Whole saliva specimens were collected at the same time by using plastic pipet and stored at -20° C .[13]

Specimen testing and interpretation
Sera and saliva samples were tested for measles-specific IgM antibody using Denka-Seiken Capture EIA kit as previously described.[14,15] Briefly, anti-human IgM mouse monoclonal antibody were coated into microtiter plates with preservative solution containing bovine se-

rum albumin and 0.1% sodium azide. The first well of EIA kit was left empty as blank and 100 microlitre of each three standards and specimens were added to their designated wells (two wells were used for each standard and standard 2 was used as cut off value). Standard 1 contained no IgM-type antibody to measles virus, second contained low-titer measles-specific IgM antibody and the third standard contained high-titer IgM-type measles virus. Plates were then incubated for 1 hour at room temperature. After the plates were washed, 100 microlitre of antigen solution containing virus antigen and serum albumin was added to each well, except for the blank and plates were incubated for 1 hour at room temperature. Plates were then washed and 100 microlitre of enzyme-labeled antibody solution containing 1.0 w/v% bovine serum albumin and peroxidase-labeled mouse monoclonal antibody was added to each well, except for blank. Plates were then incubated for 1 hour at room temperature. After a final wash, tetramethyl- benzidine substrate solution was added to each well, including the blank, and the plates were incubated for 30 minutes at room temperature. The reaction was stopped by acidification, and within 30 minutes of adding stop reagent, optical density (OD) of the wells was measured at a filter wave length of 450 nm. Undiluted saliva samples were tested using the same technique.

We calculated the average of the two values of standard 2 and used as "b" and OD value for each specimen was expressed as "a". We assessed the IgM antibody index (X) as follows: $X=a/b$. If $X<0.8$, the result was negative.

Data analysis
We calculated the concordance between saliva and serum samples for detecting the presence and absence of IgM antibodies. We also compared the antibody index (X) values of serum compared with saliva by using the Pearson partial correlation coefficient.

The accuracy of antibody-capture EIA in saliva samples was assessed by the proportion of individuals with anti-measles IgM antibodies among those seropositive (sensitivity) and by the proportion of individuals without detectable anti-measles IgM anibody among those seronegative (specificity). We also determined positive predictive value (PPV) by the proportion of true positive saliva samples and false positive saliva specimens. The negative predictive value (NPV) in saliva was assessed by the proportion of false negative samples and true negative specimens.

RESULTS
Demographic information
The patients age ranged from 14 to 38 years, with

Table I. Detection of measles specific IgM antibody against Measles virus in paired salivas specimens.

	Serum IgM antibody		
	Positive	**Negative**	**Total**
Saliva IgM Ab			
Positive	85	2	87
Negative	4	4	8
Total	89	6	95

the majority (34 cases, 35.8%) belonging to the age group of 21-22 years. The mean age of all enrolled cases was 20.1± 3.7; 87 (91.6%) were male. A documentary history of measles vaccine was obtained from 41 (43.2%) patients, 29 of whom had received two doses of the vaccine.

Laboratory samples

Paired serum and saliva samples taken within 14 days of onset of rash were obtained from 95 suspected measles cases notified between 1999 to 2001. No patient refused permission for donating saliva or blood sample.

Results of serum and saliva samples

Measles specific IgM antibody was detected in 89 (93.7%) serum samples. Overall, out of 89 IgM-positive serum samples, 85 (95.5%) of the corresponding saliva samples were IgM-positive, and out of 6 IgM-negative serum samples, 4 (66.7%) of the corresponding saliva samples were IgM-negative (Table I).

The detection rate increased with increasing time between onset of rash and sampling ($p<0.05$). For instance, all of the 8 (8.4%) specimens that had negative results for measles specific IgM antibody, had been collected 5 days prior to the onset of rash. High rate of positivity (100%) was observed for samples collected after 5 days of rash onset (Fig. 1).

Six serum-saliva pairs gave discordant results. Four patients were IgM positive in serum and IgM-negative in saliva. These patients presumably had been infected with measles virus, and the IgM serum results represented true-positive results. Three of these saliva samples, however, had IgM antibody index (X) values close to the positive cutoff value. Two patients had sera that were IgM-negative but had saliva samples that were IgM-positive (presumably false-positive results).

We next determined the concentration of IgM antibody in serum and saliva specimens by day after rash
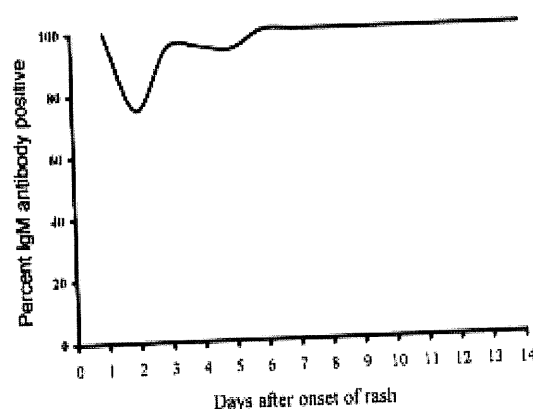

Fig. 1. Correlation between salivary IgM results and the days after onset of rash.

onset by optical density value (OD). Patients had elevated levels of IgM antibody depending on the day following onset of the rash. Furthermore, serum samples had higher concentrations of IgM antibody compared with saliva specimens (r=0.453 and 0.378 for serum and saliva samples respectively) (Fig. 2).

DISCUSSION

Several studies regarding detection of antibodies in saliva specimens are described in the literature.[11,17,18,19] Helfand et al. (1996) using antibody-capture-enzyme-linked immunosorbent assay, showed that virus-specific IgM antibody was detected in 91% of saliva samples collected 2 weeks after measles vaccination.[7] In 1998, Oliveira et al., using antibody-capture radioimmunoassay, also showed that virus specific IgM antibody was detected in 90% of measles saliva samples collected during 1-5 days after rash onset. Between 5 days and 3 weeks after onset, virus-specific IgM antibody was detected in 100% of saliva samples.[20] Our results were very similar to those reported by Helfand
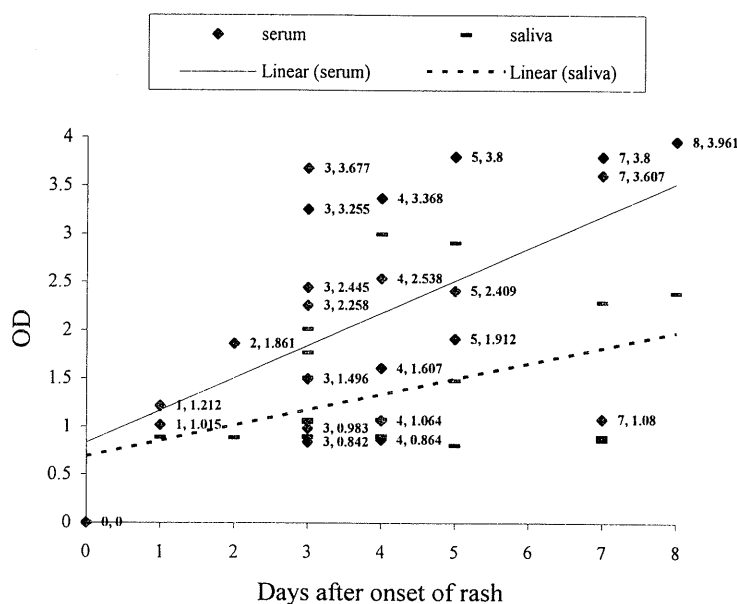
Fig. 2. Comparison of IgM antibody concentration in serum and saliva according to the days after rash onset.

et al., and Oliveira et al. In the first 5 days following onset of rash, infection was verified by salivary test in 88.7% measles cases. However, between 6 and 14 days after rash onset, virus-specific IgM was detected in 100% of saliva specimens, demonstrating the sensitivity of salivary IgM antibody detection.

For diagnosis of recent measles infections by detection of virus-specific IgM antibody in saliva and serum, Perry and colleagues[12] and Helfand et al.[21,22] found that the optimal time for collection of samples was 1 to 5 weeks after onset of rash. IgM capture tests for measles are often positive on the first day of rash onset. However, in the first 72 hours after rash onset, up to 30% of tests for IgM antibody may give false-negative results.[23] As 75% of our saliva samples were collected in the first 5 days after onset of rash and 46% of them were collected in the first 72 hours, we might have missed some positive results.

We used undiluted whole saliva for diagnosis of measles-specific IgM antibody . Detection of IgM antibody in saliva samples for virus diagnosis has mostly been performed by oral mucosal transudate (OMT).[7,16,1,20] George et al.[24] indicated that using whole saliva as a diagnostic medium had shown poor sensitivity and specifity. Our results have demonstrated that whole saliva contains IgM antibody at concentrations high enough to be diagnostically useful.

The sensitivity, specificity, and predictive values of antibody-capture EIA for detection of measles-specific IgM antibody in saliva, have important implications for

their use. The specificity and negative predictive value (NPV) of saliva assay were 66.7% and 50% respectively. The reason for the low specificity and NPV may be due to the number of negative samples. Of 95 specimens, only 8 samples had negative results (4 true-negative and 4 false-negative). So, more specimens should be investigated to determine the specificity and NPV. The two cases with positive results in saliva and negative results in serum may represent a low rate of false positive salivary results. The sensitivity, positive predictive value (PPV) and the accuracy of the saliva assay was 95.5%, 97.7% and 93.7% respectively.

Saliva-based assay for diagnosis offers several advantages over blood such as: acceptability to patients, applicability in children, and ease and safety of specimen collection.[25] According to Holm-Hansen et al., the use of alternative body fluids has other advantages: reluctance to submit to venepuncture is circumvented, reuse of disposable equipment is avoided, and the occupational risk from needle stick injuries is eliminated. Additionally, refusal in collecting blood samples due to cultural or religious traditions and vein problems after venepuncture may increase the difficulty to obtain specimens for testing.[26]

Although our results support the use of saliva specimens for diagnosing measles, they also suggest the need to optimize sampling time to detect IgM antibody after rash onset. The discordant pairs of serum and saliva samples data in our study may have resulted from samples being collected too early in the course of

the IgM antibody response.

In addition to optimizing time for specimen collection, ingestion of water may affect saliva results as well. For example, one of our cases who had a positive result for serum sample, drank water about 2 minutes before collection of saliva specimen. It is possible that her saliva was diluted with water and represented a false-negative result. Further study will be needed to test this hypothesis.

Furthermore, it would be helpful to complement the IgM assay with IgG assay that are optimized with saliva. One group[25] reported good results detecting measles-specific IgG antibodies in oral fluids by using indirect EIA, while another group[26] reported a sensitivity of only 54% by using an IgG antibody-capture RIA.[7]

In summary, our results demonstrated that saliva specimens may be a convenient alternative to serum for diagnosis of recent measles infection. The widespread acceptability of saliva collection should facilitate the investigation of measles outbreaks and have an important role in controlling the disease in regional and national public health laboratories worldwide.

ACKNOWLEDGEMENTS

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