In a pilot study, conducted in August-Sept 2005, a correlation of 90% between OD values of newly delivered infant’s OF and cord serum was observed. Therefore, this study was undertaken with an aim to study the concordance of OF and serum values in a larger age group of (seven months to 12 years) asymptomatic children.

Materials and Methods

The study was conducted at an out-patient department (OPD), Department of Paediatrics, of a tertiary care health centre, Sassoon General Hospitals and BJ Medical College, Pune India; in collaboration with Reference Measles Laboratory at National Institute of Virology, Pune, India, on apparently asymptomatic children visiting OPD for age certificate, along with ill siblings or as convalescent follow-up. Any probable history of measles vaccination, infection, contact and clinical features, for the child, were elicited from guardians. Details of child’s age, education, religion, occupation, diet, dental hygiene and the family’s socio-demographic profile were noted. Written informed consent was taken from the respective parents/guardians of all participants. The Institutional Ethics Committee approved the study.

The OF was collected and transported using “Oracol” (Saliva Collection System – Malvern Medical Developments Limited, Barbourne, Worcester, UK) and extracted from the sponge using extracting fluid. The blood was withdrawn (two to three cc) from children by veni-puncture using needle-syringe or butterfly needles and collected in BD Vacutainer SST™ II Advance. It was allowed to clot and serum was separated by centrifugation at 2000rpm for 30-60 secs. Both samples were stored at minus 70° C until assayed. The IgG antibody status was determined using commercially available – Measles IgG Capture ELISA. Sensitivity 89.5%, specificity 90.6% Concordance of 89%, coefficient of correlation r is equal to 0.97 (Karl Pearson’s) and rho is equal to 0.86 (Spearman’s), was found between OD value of OF and serum.

Key words: Measles IgG antibody, Optical density, Oral fluid

Introduction

Measles is a highly infectious endemic disease causing extensive morbidity and mortality in the developing countries. Its elimination and control programs stress the need for monitoring immunization status, post-vaccination sero-conversion, rate of transmission, individual and population susceptibility.[1-3] Oral fluid (OF); the fluid containing gingivo-crevicular exudates and the oral mucosal transudate is a serum derived fluid present in the gingivo-crevicular sulcus. Measles IgM antibody was detected in OF in United Kingdom in 1991-93.[4] Measles IgM and IgA have been used for disease diagnosis and IgG antibody in prevalence surveys.[5-8] Sensitivity, specificity and concordance of 98,73,95%; 90.4,77.8,80.2%; 97,87%;,[4] have been reported between OF and serum for Measles IgG antibody in previous studies[7,9,10] respectively. The age-wise estimation, more so, for infants and under-five has not been done extensively. During community surveys, children and parents prefer collection of painless non-invasive OF over blood. OF is more suitable for non-technical staff and is free from risk to exposure to blood borne pathogens. Being easy to store and transport, OF is clinically and epidemiologically better. We therefore analyzed the potential of OF to replace serum to monitor measles IgG antibody by comparing the OD of antibody in OF and serum detected using the commercially available ELISA kit.

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Capture ELISA (Measles IgG Capture EIA, an enzyme immunoassay, for the detection of human IgG antibodies to measles virus in human oral fluid and serum/plasma sample; Micro immune Limited, Brentford, Middlesex, United Kingdom). It’s antibody status was interpreted from the OD value; the cut-off values to identify measles positivity were calculated separately for each run, according to manufacturer’s instructions. Positive - greater than 1.25x the mean OD of three negative controls [ODnc]; negative - less than 1.1x ODnc and equivocal - 1.1 to 1.25x ODnc. A test of significance and coefficient of correlation (r-Karl Pearson’s and rho-Spearman’s) was calculated using SPSS Statistical Software. Factors thought to affect antibody levels, namely, age, gender, immunization history, history of past infection were analyzed.

**Results**

The 100 (mean age four years, range seven months to 12 years) enrolled subjects showed male dominance. (66:34). Six subjects gave positive history of measles infection in the past (three males and three females). All of them were seen in the first year. History of Measles vaccination coverage was 90.5% (67/74 of children over nine months). The OF and serum OD value pairs were labeled concordant if the interpretation for both of the OF and serum was the same i.e. the pairs are either positive – positive; negative-negative or equivocal-equivocal; else they were labeled discordant (D). Concordance of 89% between OF and serum was observed. Measles-specific IgG antibodies in paired OF and serum samples are presented in (Table 1). As inferred from the table, IgG antibodies were detected in 55% in OF and 57% in serum. Further, considering serum as gold standard and equivocal results as negative, the sensitivity and specificity of sensitivity 89.5%, specificity 90.6%, concordance of 89% between OD values of OF and serum pairs; with coefficient of correlation between OD values of OF and serum pairs being r is equal to 0.97 and rho is equal to 0.86 respectively. We can thus extrapolate value of serum measles IgG from OF values. This significant finding should be confirmed in a larger multi-centric study. The possible cause of discordance like sensitivity of the kit, hypersecretory status of oral mucosa, cross reactivity during test should also be further evaluated.

**Discussion**

Gingival sulcus is flooded with gingivo-crevicular exudates and the oral mucosal transudate owing to its rich blood supply. These fluids constitute the OF and contains antibiotics proportionate to levels circulating in blood for majority of normal individuals. This is supported by our finding of sensitivity 89.5%, specificity 90.6%, concordance of 89% between OD values of OF and serum pairs; with coefficient of correlation between OD values of OF and serum pairs being r is equal to 0.97 and rho is equal to 0.86 respectively. We can thus extrapolate value of serum measles IgG from OF values. This significant finding should be confirmed in a larger multi-centric study. The possible cause of discordance like sensitivity of the kit, hypersecretory status of oral mucosa, cross reactivity during test should also be further evaluated.

The results are important for measles community surveys requiring high community participation. The OF is easy to collect, store and transport ensuring community acceptability. It is non-invasive and free from danger of blood borne pathogens associated with serum. It has, therefore, no ethical issues involved because of its non-invasive nature and is more suitable for non-technical staff at home, rural and field settings. Hence, it becomes the sample of choice for the masses supported by the fact that gender and history of past infection or immunization does not have any statistical bearing on correlation between OF and serum.

**Table 1: Detection of IgG antibodies against measles in paired serum of samples**

<table>
<thead>
<tr>
<th>Serum</th>
<th>Positive</th>
<th>Negative</th>
<th>Equivocal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>51</td>
<td>3</td>
<td>3</td>
<td>57 (%*)</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>35</td>
<td>1</td>
<td>37</td>
</tr>
<tr>
<td>Equivocal</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>55 (%*)</td>
<td>38</td>
<td>7</td>
<td>100</td>
</tr>
</tbody>
</table>

Sensitivity = 51/57 = 89.5, Specificity = 39/43 = 90.6, Serum is considered as gold standard, and equivocal inter-operated as negative for calculations, *Percentage Positivity
At present, the cost of oral swab is the limiting factor in cost-benefit analysis. Despite the psychological and physical trauma associated with needle prick, especially for infants and under-five in blood collection outweighs the cost of the OF collection device; with government support and indigenous manufacturing, even the cost can be markedly reduced.

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References


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