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IgM CAPTURE ELISA IN LABORATORY DIAGNOSIS OF MEASLES AT SIR RONALD ROSS INSTITUTE OF TROPICAL AND COMMUNICABLE DISEASES

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ABSTRACT: Measles is a vaccine preventable disease, causes extensive morbidity and mortality in tropical countries. Although the diagnosis of measles can be based on clinical findings, laboratory confirmation is extremely important to prevent complications. During November and December 2013, 50 patients aged between 13 months to 17 years were admitted at SRRIT & CD with clinical symptoms and signs of measles. Serum samples were collected from these patients during acute and convalescent phases and tested for Measles specific IgM antibodies by IgM capture ELISA. Out of 50, 39 were paired sera and 11 were single serum samples, 39 were positive, in both acute and convalescent phases, 8 were positive in acute phase while 3 were negative. Since clinical diagnosis of measles can be difficult even for experienced practitioners, Measles virus specific IgM Serology is the standard test for rapid laboratory diagnosis of measles. Apart from this the present study also shows changing epidemiology of the disease with a shift in the age group affected towards higher side.

KEYWORDS: Measles IgM antibodies, Sero diagnosis, Capture ELISA.

INTRODUCTION: Measles is a severe, vaccine preventable disease that causes extensive morbidity and mortality in large parts of the world.^[1] Measles virus is a member of the Morbilli virus genus of the paramyxoviridae family. The disease is highly transmissible and usually affects children. It is also known to cause transient immune suppression and lead to complications as mild as bronchitis to severe like encephalitis, which are especially observed in malnourished children.^[2,3] Despite the wide spread use of measles vaccine, either as a single antigen vaccine or as a component of the triple vaccine against measles, mumps and rubella (MMR) 2,78,358 measles cases were reported and an estimated 1,64,000 deaths from measles occurred worldwide in 2008.^[1] Although the diagnosis of measles can be based on clinical findings, laboratory confirmation is extremely important due to possible confusion with other rash causing illnesses.^[4] This helps in early limitation of specific measures and prevent complications which include diarrhoea, pneumonia, malnutrition, sub-acute sclerosing pan encephalitis (SSPE), blindness, deafness, mental retardation or death.^[5] Measles specific immunoglobulin M (IgM) serology is the standard test for the laboratory diagnosis of measles, and IgM testing is now almost exclusively performed with commercial enzyme immune assay (EIA) kits, an IgM capture assay developed by the centres for Disease control and prevention (CDC) which does not require removal of IgG antibodies and is considered to be more specific than the indirect EIAs for detection of measles IgM antibodies.^[6,7] The present study was done to detect IgM antibodies in clinically diagnosed measles cases admitted at SRRIT & CD during Nov & Dec 2013 by IgM capture ELISA.

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SUBJECTS AND METHODS: 50 patients aged between 13 months to 17 years admitted at SRRIT & CD with clinical symptoms and signs of measles constitute the study group. From these patients serum samples were collected during acute (1st day of rash onset to 5 days afterwards) and convalescent phases (14 days after rash onset). Out of these thirty nine were paired serum samples and eleven were single serum samples. IgM antibodies were detected by IgM capture ELISA by Euroimmun diagnostika.

FUNCTION PRINCIPLE: The sample buffer (Green coloured) contains an anti-human antibody preparation from goat, IgG from a serum sample is bound with high specificity by these antibodies and precipitated. If the sample also contains Rheumatoid factors, these will be absorbed by the IgG antihuman IgG complex.

ANTIGEN: The antigen source is provided by inactivated cell lysates of Vero cells infected with Edmonston strain of measles viruses.

CALCULATION OF RESULTS: The extinction value of the calibrator defines the upper limit of the reference range of non-infected persons (Cut off) recommended by Euroimmun. Values above the indicated cut off are to be considered as positive, those below as negative.

SEMI QUANTITATIVE: Results can be evaluated semi-quantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction of calibrator. The following formula is used to calculate the ratio.

Extinction of the control or patient sample = Ratio Extinction of Calibrator.

Euroimmun recommends interpreting results as follows:

Ratio < 0.8 – negative.

Ratio > 0.8 to 1.1 – borderline.

Ratio > 1.1 – positive.

CROSS REACTIVITY: The quality of the antigen used ensures a high specificity of the ELISA. Sera from patients with infections caused by various agents were investigated with EUROIMMUN antimeasles virus ELISA IgM.

Antibodies against *Borrelia burgdorferi*, CMV (cytomegalovirus) EBV (Epstein barr virus), Mumps virus, Parvovirus B19, Rubella virus, *Toxoplasma gondii*, VZV (Varicella zoster) were all negative.

RESULTS: Blood samples were collected within 1-14 days after onset of the rash from 50 clinically diagnosed measles patients aged between 13 months to 17 yrs. 24 (48%) were below 5 yrs. of age, 14 (28%) were between 5-10 yrs. 12 (24%) were above 10 yrs. of age [Table:1], out of these 24 were males and 26 were female patients [Table: 2]. Of 50 serum samples 39 were paired serum samples and 11 were single serum samples, (As these patients left against medical advice before 1 week of admission). All 39 paired samples were positive for anti-measles IgM antibody tested by IgM capture ELISA. Out of 11 single samples 8 showed positive OD values. The duplicate determination of the paired serum samples taken at an interval of at least 7 days showed that the OD values were the same or increased.

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All the positive OD values were more than 2 [Figure 1].

Age group	0 – 5 yrs.	5-10 yrs.	>10 yrs.
Number	24	14	12
Percentage	48	28	24

Table 1: Showing age distribution of Measles cases

Males		Females	
Positive	Negative	Positive	Negative
24	0	23	3
Total: 24		Total: 26	

Table 2: Showing sex distribution of Measles cases

DISCUSSION: Measles is one of the most infectious diseases known to man and remains the leading cause of vaccine preventable deaths in children worldwide. In most developing countries measles is still a formidable public health concern.^[8,9,10] The ultimate goal of measles control program is to stop the indigenous circulation of measles virus. Monitoring the success of such programs requires a sensitive surveillance system.^[11,12] Misdiagnosis of measles is more common as it may resemble infections with rubella, dengue, Echo virus, coxsackie virus, parvovirus B₁₉ and herpes virus 6, as well as some bacterial and rickettsial diseases.^[13] Laboratory confirmation is essential for all outbreaks and all sporadic measles cases. Detection of measles – specific IgM antibody and measles RNA by real-time RT-PCR are the most common methods for confirmation of measles infection.^[14] In our study serum samples were collected from 50 clinically diagnosed measles cases for detection of IgM anti measles antibodies by IgM capture ELISA.

Out of 50 cases, 39 were paired serum samples and 11 were single serum samples. All 39 paired serum samples were positive for anti-measles IgM antibodies. Out of 11 single serum samples 8 were positive and 3 were negative, showing 73% positivity and 7% negativity. IgM positivity is based on the timing of sample collection. In one study from Tamil nadu it was noticed that samples collected between days 4 and 22 after the onset of rash showed 100% positivity whereas samples collected between days 1 and 3 after the onset of rash showed 71% positivity. In the present study 39 acute phase blood samples were collected 5 days after onset of rash and 3 were collected on 1st day of rash. All these 3 samples were negative. Second serum sample (convalescent) was not collected for these 3 cases and in 8 cases convalescent samples could not be collected as these patients left hospital after one week. 39 convalescent serum samples were collected 14 days after onset of rash.

Timing of sample collection is probably responsible for negativity in epidemics. The optimum time for blood sample collection for IgM detection is 4-28 days post onset of rash.^[15] A Sudan study also observed that 26% of clinically diagnosed measles cases were negative.^[4] Classically, measles behaves as an acute infection of infancy and young childhood between 6 months and 3 years of age. However with effective vaccination programmes in developing countries an upward shift in mean age of infection appeared which can be attributed to less chances of natural childhood infection.^[16] In the present study out of 50 cases 24 [48%] were below 5 years, 14 [28%] were between 5 -10 years and 12 [24%] were more than 10 years old, showing shift in the age group towards higher side.

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CONCLUSIONS: Immunity in the immunized individuals is waning with passing years, leaving aside the unimmunized and under – immunized children, making them susceptible for infection.

One dose of measles vaccine during infancy fails to develop immunity in a large percentage of children therefore to prevent adult measles a repeat dose of vaccine might be necessary at a higher age.

Fig.1

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