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Serodiagnosis of Measles in Patients Attending a Tertiary Care Hospital in Visakhapatnam: A Retrospective Study

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Abstract: Background: Measles remains a major public health concern in India, particularly among infants and young children, despite the existence of an established vaccination program. The disease continues to contribute significantly to morbidity and mortality, especially in resource-limited settings. Aim: This study aimed to assess the prevalence of measles-specific IgM antibodies in clinically suspected cases and to evaluate the demographic and clinical characteristics associated with seropositive results. Methods: A retrospective study was conducted over one year (January to December 2017) at King George Hospital, Visakhapatnam. Blood samples from 86 clinically suspected measles patients were tested for measles-specific IgM using an ELISA method. Patient data, including age, sex, vaccination status, and presenting symptoms, were recorded and analysed. Results: Out of 86 patients, 6 (6.9%) tested positive for measlesspecific IgM antibodies. Among them, 4 were males and 2 were females, with a male-tofemale ratio of 2:1. Two of the positive cases were infants aged below 6 months and were unvaccinated; the remaining four were adults aged 30-40 years with unknown immunization status. Fever was a universal symptom, and all positive cases belonged to low socioeconomic backgrounds. Conclusion: The findings indicate a significant burden of measles among infants too young to be vaccinated, highlighting the potential need for earlier immunization strategies. Strengthening routine immunization and targeted public health campaigns is essential to reduce measles incidence and associated complications.

Research Paper

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Introduction

Measles is not only one of the most contagious viral diseases but also one of the most preventable. It remains a significant cause of vaccine-preventable deaths globally, particularly affecting children under five years of age [1]. The measles virus, which belongs to the *Morbillivirus* genus of the *Paramyxoviridae* family, is transmitted through direct contact with infectious droplets or airborne spread [2]. Due to its high transmissibility, the virus can rapidly spread in populations with low immunization coverage, resulting in periodic outbreaks and clusters, particularly in densely populated areas and regions with weak health infrastructure [3].

Globally, the disease continues to challenge public health systems despite the availability of effective and affordable vaccines [4]. According to WHO estimates, in 2022, measles caused significant mortality, particularly in low- and middle-income countries [5]. Even with substantial global progress in measles control,

many regions—especially in Africa and Southeast Asia—continue to report sporadic outbreaks and high incidence rates [6].

In the Indian context, the Universal Immunization Programme (UIP) has been a cornerstone of child health strategies since 1985. The addition of the second dose of the measles-containing vaccine and the introduction of the Measles-Rubella (MR) campaign have been pivotal in increasing coverage [7]. Nevertheless, inequities in access, vaccine hesitancy, and inconsistent recordkeeping continue to pose challenges to universal immunization [8]. Urban-rural disparities, gender bias in healthcare access, and vaccine misinformation further exacerbate these issues. Moreover, mobile populations and internally displaced groups remain difficult to reach consistently [9].

Measles typically presents with an initial prodromal phase of fever, malaise, cough, coryza, and conjunctivitis. The appearance of Koplik's spots,

followed by a generalized maculopapular rash, is a characteristic feature [1]. However, the clinical course may be complicated by serious consequences such as pneumonia, otitis media, diarrhoea, encephalitis, and, in rare cases, SSPE [10]. The severity of illness is amplified in malnourished children and those with compromised immune systems [11].

Infection in infants younger than 9 months is particularly concerning because they are not yet eligible for routine vaccination under India's standard schedule. These infants depend primarily on maternal antibodies, the levels of which may be insufficient if the mother acquired immunity through vaccination rather than natural infection [12]. This vulnerability emphasizes the importance of considering modified vaccination schedules or targeted strategies during outbreaks [5].

Laboratory diagnosis using serological tests like enzyme-linked immunosorbent assay (ELISA) for IgM antibodies plays a crucial role in confirming suspected cases and guiding public health interventions [13]. Confirmatory testing becomes especially important in cases of atypical presentation or during periods when multiple exanthematous illnesses (such as dengue, rubella, or parvovirus B19) are co-circulating [14].

The present study was conducted at King George Hospital in a tertiary care hospital setting in Visakhapatnam, Andhra Pradesh, a region that has witnessed fluctuating measles incidence despite efforts to improve immunization coverage. By evaluating seropositivity using IgM ELISA and analysing clinical and demographic factors associated with confirmed measles cases, this study aims to contribute valuable insights for strengthening regional measles control and elimination strategies in alignment with national goals.

AIM

- 1. Determine the proportion of clinically suspected measles cases confirmed serologically by IgM ELISA over one year.
- Describe the demographic (age, sex) and clinical characteristics of laboratory-confirmed cases.
- 3. Explore factors associated with infection, particularly vaccination status.
- 4. Contribute local epidemiological data to inform measles control and elimination efforts in the region.

MATERIALS AND METHODS

Study Design and Period:

Retrospective observational study analysing data from patients investigated for measles between January 2017 and December 2017.

Study Setting:

Department of Microbiology, Andhra Medical College, affiliated with King George Hospital (KGH), Visakhapatnam. KGH is a government tertiary care hospital serving a large population from the Visakhapatnam district and neighbouring regions.

Study Population:

Patients of all ages presenting to outpatient departments, inpatient wards, or the emergency department of KGH during the study period with a clinical presentation suggestive of measles, as per the attending physician's assessment. The core case definition included fever and maculopapular rash. Additional supportive features, such as cough, coryza, or conjunctivitis, were often present but not mandatory for inclusion as a "clinically suspected" case in this retrospective analysis based on laboratory requisitions.

Sample Size:

A total of 86 serum samples received by the Department of Microbiology for measles IgM testing during the study period met the inclusion criteria (clinically suspected case) and were included in the analysis. This represents a convenience sample based on clinical suspicion and physician request for testing.

Sample Collection and Processing:

- Approximately 3-5 mL of venous blood was collected from each patient under aseptic precautions using standard venipuncture techniques.
- Blood was allowed to clot at room temperature (approximately 30 minutes).
- Serum was separated by centrifugation at 2500-3000 rpm for 10-15 minutes.
- Separated serum was aliquoted into sterile cryovials.
- Serum samples were stored at 2°C-8°C if testing was planned within 48 hours. Samples not tested immediately were stored at -20°C until analysis.

Laboratory Method:

 Detection of measles-specific IgM antibodies was performed using the commercially available EUROIMMUN Anti-Measles Virus ELISA (IgM) kit (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany), following the manufacturer's instructions precisely.

• Principle:

The assay is based on the indirect ELISA technique. Microplate wells are coated with purified, inactivated measles virus antigen. Diluted patient serum is added. If measles-specific IgM antibodies are present, they bind to the immobilized antigen. After washing, an enzyme-labelled (peroxidase) anti-human IgM antibody

is added, which binds to the captured human IgM. Following another wash step, a chromogenic substrate (TMB) is added. The enzyme catalyses the conversion of the substrate, producing a blue colour. The reaction is stopped with sulfuric acid, turning the solution yellow. The intensity of the yellow colour, measured as optical density (OD) at 450 nm (reference filter 620 nm), is proportional to the amount of measles-specific IgM antibody present in the sample.

- Interpretation: Results were interpreted according to the kit protocol using the calculated ratio of the sample's OD to the OD of the calibrator (cut-off control) provided in the kit:
 - o Ratio < 0.8: Negative for measles-specific IgM
 - o Ratio ≥ 0.8 to < 1.1: Equivocal/Borderline
 - o Ratio \geq 1.1: Positive for measles-specific IgM
 - Internal quality controls (positive and negative controls provided in the kit) were run with each batch of samples. External quality assurance participation was maintained as per the laboratory's protocol.

Ethical Considerations:

The study protocol was reviewed and approved by the Institutional Ethics Committee of Andhra Medical College, Visakhapatnam. As a retrospective study utilizing anonymized laboratory and clinical data collected as part of routine care, the requirement for individual informed consent was waived by the IEC. Patient confidentiality was strictly maintained throughout the study; data were analysed using unique identifiers, not patient names.

Data Collection:

Relevant demographic data (age, sex, residence), clinical presentation (symptoms, duration, complications), vaccination history (documented status where available or caregiver recall), and outcome (survived, deceased) were extracted from the laboratory requisition forms, patient case records, and hospital information system for the IgM positive cases and the overall cohort of 86 suspected cases where possible. Socioeconomic status was inferred based on the type of hospital admission (general ward vs. private) and residence address.

Statistical Analysis

Data management and analysis were carried out using Microsoft Excel 2019. The analysis was primarily descriptive. Categorical variables such as sex, IgM result, vaccination status, clinical symptoms, and patient outcomes were summarized using frequencies and percentages. Continuous variables like age were grouped into categories and reported in percentage terms. Due to

the small number of IgM-positive cases (n=6) and the retrospective design of the study, especially the limited availability of complete vaccination data, no inferential statistical tests were applied. Comparative analyses between groups (e.g., IgM-positive vs. IgM-negative) were not undertaken, as the sample size was insufficient for meaningful statistical inference. Instead, the focus remained on describing and highlighting the patterns observed in confirmed measles cases within the overall suspected cohort.

RESULTS

Out of the 86 clinically suspected measles cases tested over the one year, six were confirmed positive for measles-specific IgM antibodies using ELISA, accounting for a seropositivity rate of 6.9%. The clinical and demographic profiles of these IgM-positive individuals are summarized in Table 1. Two of the cases were infants under 6 months of age who had not received any measles vaccination and, unfortunately, succumbed to the illness. The remaining four cases were adults between 30 to 40 years of age with unknown vaccination status; all recovered. Fever and rash were universal among all six confirmed cases.

Table 2 depicts the age and sex distribution of IgM-positive individuals. Among the six confirmed cases, four were male and two were female, giving a male-to-female ratio of 2:1. Age-wise, two cases were infants below 1 year and four cases were adults aged 30–40 years, indicating susceptibility at both extremes of the age spectrum.

The clinical symptom profile is detailed in Table 3. While all six patients presented with the hallmark symptoms of fever and rash (100%), additional classical symptoms such as cough, coryza, and conjunctivitis was variably present. Cough was observed in 66.7% of cases, coryza in 50%, and conjunctivitis in 33.3%, reflecting typical but not uniformly present features of clinical measles.

A broader overview of the entire study population is provided in Table 4. Among the 86 suspected cases, only six were confirmed measles cases, with infants and adults accounting for all positives. The two infant fatalities (33.3% of IgM-positive cases) emphasize the vulnerability of unvaccinated infants. The unknown vaccination status of the four adult cases highlights the need for improved documentation and perhaps adult immunization strategies. These findings collectively support the need for targeted public health interventions, especially focused on infants under 9 months and adults potentially missed by historical immunization efforts.

Table 1: Demographic and Clinical Characteristics of IgM-Positive Cases

Case ID	Age (months/years)	Sex	Fever	Rash	Vaccination Status	Outcome
1	4 months	M	Yes	Yes	Not vaccinated	Deceased
2	5 months	F	Yes	Yes	Not vaccinated	Deceased
3	32 years	M	Yes	Yes	Unknown	Recovered
4	35 years	F	Yes	Yes	Unknown	Recovered
5	30 years	M	Yes	Yes	Unknown	Recovered
6	36 years	M	Yes	Yes	Unknown	Recovered

Table 2: Age and Sex Distribution of IgM Positive Cases

Age Group	Male	Female	Total
<1 year	1	1	2
30–40 years	3	1	4
Total	4	2	6

Table 3: Summary of Symptoms among IgM-Positive Cases

Symptom	Number of Cases (n=6)	Percentage (%)
Fever	6	100
Rash	6	100
Cough	4	66.7
Coryza	3	50
Conjunctivitis	2	33.3

Table 4: Summary of Study Population and IgM Results

Parameter	N
Total suspected measles cases	86
IgM-positive cases	6 (6.9%)
Male: Female ratio (IgM+)	2:1
Infant cases (age <6 months)	2
Adult cases (age 30–40 years)	4
Unvaccinated (infant cases)	2
Unknown vaccination status	4
Fatalities among IgM+ cases	2 (33.3%)

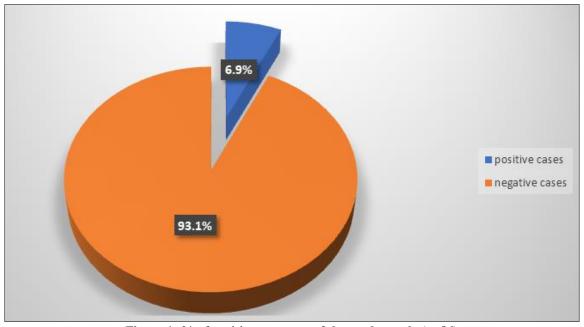


Figure 1: % of positive cases out of the total sample (n=86)

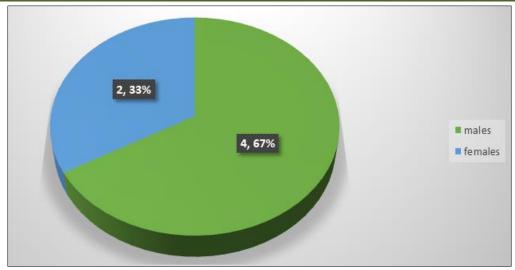


Figure 2: Sex wise distribution of positive gender

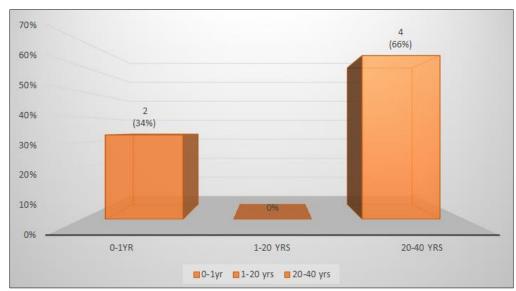


Figure 3: Age wise distribution

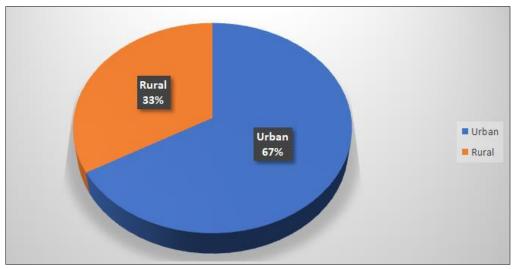


Figure 4: Geographic distribution

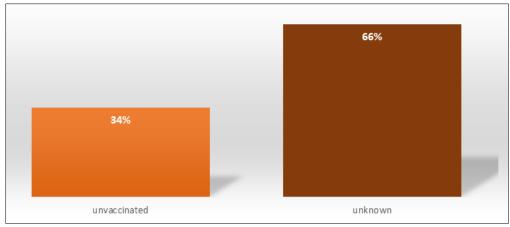


Figure 5: Immunization status (n=6)

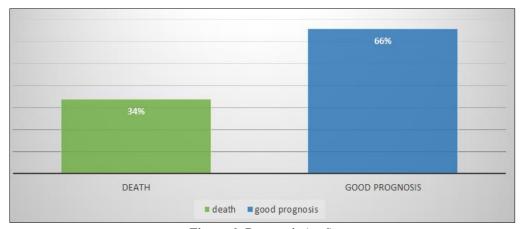


Figure 6: Prognosis (n=6)

DISCUSSION

This study's findings were broadly consistent with those reported in previous Indian studies. The 6.9% IgM seropositivity rate observed aligns with that reported by Kairi *et al.*, (2017), who identified similar positivity rates in a tertiary care setting using ELISA [15]. Indwar *et al.*, (2016) [16], also noted a concerning proportion of measles cases occurring in infants younger than 6 months, echoing our observation of fatal outcomes in this vulnerable, unvaccinated age group.

This retrospective analysis presents critical data on the serodiagnosis of measles in a tertiary healthcare setting in Visakhapatnam. A seropositivity rate of 6.9% (6/86) using IgM ELISA aligns with rates observed in inter-epidemic periods in similar Indian hospital settings [15]. Although seemingly modest, the detection of active infections, including fatal outcomes in infants, emphasizes the continuing circulation of measles and the need for intensified surveillance [11].

One of the study's most significant observations was the vulnerability of infants younger than 9 months. Two unvaccinated infants, aged 4 and 5 months, succumbed to measles—a tragic yet avoidable outcome. The Indian schedule administers the first dose

of the MR vaccine at 9–12 months, leaving younger infants reliant on maternal antibodies for protection [12]. However, maternal antibody levels, especially in vaccinated mothers, wane earlier than those from natural infection, sometimes rendering infants susceptible as early as 4–6 months [1]. This immunological gap could be bridged through early MR doses during outbreaks or in high-incidence areas, as recommended by WHO [5]. Localized studies from India and Southeast Asia consistently report increased measles mortality among unvaccinated infants [16].

Adult measles, as reported in this study, also demands attention. Four of six confirmed cases were aged between 30 and 40 years, highlighting the risk of measles resurgence among adults due to secondary vaccine failure, waning immunity, or never having been vaccinated during childhood [4]. As India only achieved improved measles vaccine coverage in the late 1990s, many adults may still be susceptible. This warrants targeted MR catch-up campaigns for high-risk adult groups, including healthcare workers, teachers, and students [3].

Gender-based disparity in measles incidence was observed in this study, with a male-to-female ratio of 2:1 among seropositive patients. While the biological

basis remains under-researched, sociocultural factors such as greater healthcare access for males and preferential treatment in healthcare-seeking behaviour may explain this trend [9]. This underscores the importance of promoting gender equity in immunization access and awareness campaigns [8].

Complications mortality and were predominantly seen among those from low socioeconomic strata. The two infant fatalities belonged to impoverished families. Socioeconomic determinants such as malnutrition, overcrowding, and limited healthcare access have been associated with severe measles outcomes [11]. Nutrition-based interventions, maternal education, and community-based monitoring could mitigate such disparities [6].

The unknown vaccination status in four adult cases reflects the widespread problem of inadequate immunization documentation. Many individuals lack verifiable vaccine records, leading to data gaps that impede epidemiological assessments. Digital immunization tracking systems and community surveys could bridge this information void [7].

Lastly, the measured 6.9% seropositivity must be contextualized. Factors such as physician selection criteria for testing, timing of sample collection (since IgM detection is time-sensitive), and test kit sensitivity can all influence observed positivity rates [13]. Therefore, enhanced surveillance protocols—including those for rubella and other differential diagnoses—are essential for accurate estimation of disease burden [14].

In conclusion, this study underlines the persistent threat posed by measles in both paediatric and adult populations. Revising immunization policies, expanding adult vaccination, enhancing awareness, and improving surveillance infrastructure are key to achieving measles elimination goals.

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