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Original Research Article

To study the seroepidemiology of measles at a tertiary care centre

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Abstract

Back ground: The "gold standard" for diagnosing an MV infection in a lab is the presence of particular immunoglobulin M (IgM), which can be detected using either an ELISA (enzyme-linked immunosorbent assay) or immunofluorescence. A most popular type of testing is serological because it is quick, affordable, and reliable and can be done in a high-throughput setting. According to earlier research comparing IgM tests, with RT-PCR-based assays as a reference, sensitivities ranged from 89.9 to 98.8% and specificities ranged from 92.5 to 97.9%.

With this background, this study was conducted to serologically diagnose clinically suspected cases of measles by IgM Antibody detection in our setting and also to correlate measles infection with vaccination status.

Materials and Methods: We included all patients aged less than 24 years belonging to both genders visiting the Paediatrics/Medicine OPD at Sir Ronald Ross Institute of Tropical and Communicable diseases, Nallakunta, Hyderabad, during March 2019 to March 2020. The sample size considered was 100. ELISA kit with IgM for measles was used to serologically diagnose measles in suspected cases.

Results: Among 100 suspected measles cases, IgM ELISA was positive in 52% of cases and

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majority of the children had OD values between 0.5 to 0.8 (46%). In our study, there were 21 children who did not know about measles vaccination status. We found that there were more unvaccinated children were ELISA IgM positive compared to vaccinated children, however this was not statistically significant. ELISA was associated significantly with age in our study. We found that 23.1% of ELISA positive children were aged less than 1 year compared to 4.2% in ELISA negative group were aged <1 year. This difference was statistically significant. No other clinical parameters had significant association with ELISA positivity

Conclusions: The findings indicated that children under the age of five remain the most vulnerable population with the highest absolute number of confirmed cases, which raises the prospect of novel forms of measles transmission (from adults to unimmunized children).

Keywords: Measles, serology, ELISA, IgM antibody

Introduction

Despite the existence of a secure and reliable vaccination, more than 140 000 people died from the measles in 2018, the majority of whom were children under the age of five [1]. The measles virus is the cause of a severe febrile disease that is highly contagious. Due to the inability to sustain high levels of vaccination coverage, measles is still a frequent sickness in many underdeveloped nations even if it is now rare in developed ones. When it comes to vaccine-preventable deaths, measles is the greatest cause of morbidity and mortality in children. Before the measles vaccine's release in 1963 and widespread immunization, significant outbreaks occurred around every 2-3 years, and measles was thought to be responsible for 2.6 million annual deaths [1].

The WHO advises genotyping representative strains as part of a strategic plan for the eradication of measles because although the virus is monotypic by serotype, diversity within the complete hemagglutinin [H] gene and the C-terminal part of the nucleoprotein [N] gene has allowed classification of the virus into eight clades, A to H, including 22 genotypes.

In nations that have eradicated the measles, several genotypes have been found among the rare instances, therefore ongoing surveillance of virus genotypes in circulation is crucial. Therefore, WHO advises virological surveillance during all measles control phases.

In particular in nations on the verge of MeV elimination, high-quality laboratory testing is essential for a swift and accurate diagnosis and containment of MeV infections [2]. For the laboratory confirmation of MeV infections, the sequential development of virus-specific IgM and IgG antibodies (as a correlate of the host's humoral immune response against the virus) can be employed, in addition to direct molecular assays measuring viral RNA [3, 4].

The "gold standard" for diagnosing an MV infection in a lab is the presence of particular immunoglobulin M (IgM) [5], which can be detected using either an ELISA (enzyme-linked immunosorbent assay) [6] or immunofluorescence [7]. A most popular type of testing is serological because it is quick, affordable, and reliable and can be done in a high-throughput setting [8]. According to earlier research comparing IgM tests, with RT-PCR-based assays as a

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reference, sensitivities ranged from 89.9 to 98.8% and specificities ranged from 92.5 to 97.9% [9-11].

With this background, we conducted this study to serologically diagnose clinically suspected cases of measles by IgM Antibody detection in our setting and also to correlate measles infection with vaccination status.

Methodology

The study was conducted after obtaining institutional ethics committee approval. All patients who met the WHO clinical case definition for measles: "any person with a generalized maculopapular rash (i.e., non-vesicular), and a history of fever of 38 °C or more, and at least one of the following: cough, coryza (i.e., runny nose), or conjunctivitis (i.e., red eyes); or: any person in whom a health professional suspects measles" [12]. The clinical symptoms were always present at the moment of sampling. Samples were collected after having obtained informed consent from the parents. We included all patients aged less than 24 years belonging to both genders visiting the Paediatrics/Medicine OPD at Sir Ronald Ross Institute of Tropical and Communicable diseases, Nallakunta, Hyderabad, during March 2019 to March 2020. The sample size considered was 100 depending on the assumption that at least 50% of the clinically diagnosed patients are serologically positive, therefore p is taken as 50. According to the formula 4pq/l2 the sample size is rounded off to 100.

After getting consent, a thorough history and physical examination were performed and demographic information was collected. Serological assays and a minimal diagnostic workup

were performed on all individuals using ELISA kit with IgM for measles. Blood samples from all suspected cases and serum were separated after centrifugation at 3000 rpm for 10 minutes and then kept in a 2 ml cryovials tube at -20 °C until further processing. Following the manufacturer's instructions, the serum was analysed for anti-MeV-IgM antibodies using an Measles IgM enzyme-linked immunosorbent assay test kit from Immunolab, Eurofins, Germany. Each ELISA plate used a positive and a negative control from the kit. An ELISA reader operating at 450nm and with both positive and negative control readings measured the optical density of the wells. Results were calculated according to the manufacturer's instructions.

All the data was entered in excel spread sheet and analysed using SPSS version 21. Continuous variables were expressed as mean ± standard deviation, and categorical variables as frequencies (%). Quantitative data were compared using the chi-square test. A p-value of less than 0.05 was considered as statistically significant.

Results

A total of 100 consecutive patients of suspected measles cases were interviewed during the study period. The gender distribution of our study participants was almost equal with 51% males and 49% females. Majority of the children were aged between 1 to 5 years (52%) followed by 5 to 10 years (34%) and 14% were aged < 1 year. Majority of children had fever

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since 5 to 10 days (64%) and rash for <5 days (85%). The most common associated symptoms were cough (92%), coryza (72%) and conjunctivitis (63%). Majority of the suspected measles cases were not vaccinated (43%).

Table 1: Demographic and clinical symptoms in suspected measles cases

Parameters		**Frequency**
Age	< 1 year	14
	1 to 5 years	52
	5 to 10 years	34
Gender	Males	51
	Females	49
Fever	< 5 days	25
	5 to 10 days	64
	>10 days	10
	Absent	1
Rash	< 5 days	85
	5 to 10 days	15
Associated symptoms	Conjunctivitis	63
	Coryza	72
	Cough	92
	Diarrhea	22
	Joint pains	15
Vaccination status	Vaccinated	32
	Partially vaccinated	4
	Not vaccinated	43
	Don’t know	21

Among 100 suspected measles cases, IgM ELISA was positive in 52% of cases and majority of the children had OD values between 0.5 to 0.8 (46%). IgM-positive samples rate in our study was 52%.

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Table 2: ELISA in suspected measles cases

ELISA parameters		Frequency
ELISA	Positive	52
	Negative	48

In this study, there were 21 children who did not know about measles vaccination status, so considered 79 for association between ELISA and vaccination status. We found that there were more children who were unvaccinated were ELISA IgM positive compared to vaccinated children, however this was not statistically significant when chi-square test was applied (p>0.05).

Table 3: Association of ELISA with vaccination status

ELISA	Vaccination status	
	Vaccinated	Unvaccinated
ELISA IgM Positive	17	27
ELISA IgM Negative	15	20
Chi-square value	0.1441	
P value	0.704	

ELISA was associated significantly with age in our study. We found that 23.1% of ELISA positive children were aged less than 1 year compared to 4.2% in ELISA negative group were aged <1 year. This difference was statistically significant when chi-square test was applied. No other clinical parameters had significant association with ELISA positivity.

Table 4: Association of clinical and demographic parameters and ELISA

Parameters		IgM Negative	IgM Positive	P value
Age	< 1 year	2 (4.2%)	12 (23.1%)	**0.023**
	1 to 5 years	27 (56.3%)	25 (48.1%)	
	5 to 10 years	19 (39.6%)	15 (28.8%)	

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Gender	Males	25 (52.1%)	26 (50%)	0.835
	Females	23 (47.9%)	26 (50%)	
Fever	< 5 days	15 (31.3%)	10 (19.2%)	0.508
	5 to 10 days	27 (56.3%)	37 (7.2%)	
	>10 days	5 (10.4%)	5 (9.6%)	
	Absent	1 (2.1%)	0	
Rash	< 5 days	39 (81.3%)	46 (88.5%)	0.313
	5 to 10 days	9 (18.7%)	6 (11.5%)	
Associated symptoms	Conjunctivitis	26 (54.2%)	37 (71.2%)	0.078
	Coryza	34 (70.8%)	38 (73.1%)	0.803
	Cough	43 (89.6%)	49 (94.2%)	0.392
	Diarrhea	7 (14.6%)	15 (28.8%)	0.085
	Joint pains	10 (20.8%)	5 (9.6%)	0.116
Vaccination status	Vaccinated	15 (31.3%)	17 (32.7%)	0.513
	Partially vaccinated	2 (4.2%)	2 (3.8%)	
	Not vaccinated	18(37.5%)	25 (48.1%)	
	Don’t know	13 (27.1%)	8 (15.4%)	

Association of age and the clinical parameters was significant according to the ‘p’ value i.e., 0.023.

Discussion

Measles is known as Captain of the killer team, in India. The prevalence of measles infections has decreased, but outbreaks and isolated cases still happen occasionally. Scientific data strongly suggests that immunization can prevent measles.

In our investigation, we found that children aged 1 to 5 were the most often affected age range by suspected measles. In other investigations, it was discovered that children under the age of five are most commonly affected by measles infection. The prevalence of measles infection in children decreased as they aged. Children under the age of five have a significant risk of exposure due to the endemic nature of the measles virus, but children beyond the age of five would have acquired lifetime immunity [13-21]. The variety of age groups impacted by

outbreaks in various geographic locations reveals the variety of measles transmission patterns in India. This finding argues for standardized outbreak investigations that take into account all cases, the scope of the outbreak and all age groups within the study population. Cases among people older than 5 years old were documented by studies in Tamil Nadu and Kashmir [22, 23].

IgM testing is still a crucial component of clinical virology's MeV diagnosis. When performed 2-3 days following the appearance of the rash, the most often used test, the measles-specific IgM antibody assay, is nearly 100% sensitive [24, 25]. ELISA was used in this investigation to measure measles antibody levels. IgM-positive samples rate in our study was 52%.

The highest rates were noted in other studies conducted around the globe. For instance, Wairagkar and Collaborators [26] reported that 62.8% of IgM-positive samples were found in data from an Indian retrospective research conducted between 2005 and 2010. Despite certain outbreaks, such as those in Bulgaria in 2009 [27], Romania in 2010 [28], Germany [29] and more recently in Ireland [30], England in 2012 [31] or the Netherlands in 2013 [32], it is evident that the rates are substantially lower in the WHO Europe region.

Conclusion

The findings indicated that children under the age of five remain the most vulnerable population with the highest absolute number of confirmed cases, which raises the prospect of novel forms of measles transmission (from adults to unimmunized children). The current study has demonstrated the effective use of serological methods to monitor the measles scenario and the changing epidemiology of measles in modern India. This study also indicate that the burden of measles infection is still high among vaccinated and unvaccinated children.

References

1. Measles [Internet]. Who.int, 2022. [cited 14 August 2022]. Available from: https://www.who.int/health-topics/measles#tab=tab_1

2. Hubschen JM, Bork SM, Brown KE, Mankertz A, Santibanez S, Ben Mamou M, *et al*. Challenges of measles and rubella laboratory diagnostic in the era of elimination. Clin Microbiol Infect. 2017;23:511-515. 10.1016/j.cmi.2017.04.009.

3. WHO. Manual for the laboratory diagnosis of measles and rubella virus infection, 3rd ed. World Health Organization (WHO), Geneva, Switzerland, 2018.

4. Riddell MA, Chibo D, Kelly HA, Catton MG, Birch CJ. Investigation of optimal specimen type and sampling time for detection of measles virus RNA during a measles epidemic. J Clin Microbiol. 2001;39:375-376. 10.1128/JCM.39.1.375-376.2001.

5. Grandien M, Osterhaus ADME, Rota PA, Smaron MF, Wild TF. Laboratory diagnosis of measles infection and monitoring of measles immunization: memorandum from a WHO

meeting. Bull WHO. 1994;72:207-211.

6. Erdman DD, Heath JL, Watson JC, Markowitz LE, Bellini WJ. Immunoglobulin M antibody response to measles virus following primary and secondary vaccination and natural virus infection. J Med Virol. 1993;41:44-48.

7. De Swart RL, Vos HW, Uytde Haag FGCM, Osterhaus ADME, Van Binnendijk RS. Measles virus fusion protein-and hemagglutinin-transfected cell lines are a sensitive tool for the detection of specific antibodies in a FACS-measured immunofluorescence assay. J Virol Methods. 1998;71:35-44.

8. Moss WJ. Measles. Lancet. 2017;390:2490-2502. 10.1016/S0140-6736(17)31463-0.

9. Sampedro A, Rodriguez-Granger J, Gomez C, Lara A, Gutierrez J, Otero A. Comparative evaluation of a new chemiluminescent assay and an ELISA for the detection of IgM against measles. J Clin Lab Anal. 2013;27:477-480. 10.1002/jcla.21630.

10. Sanz JC, Mosquera M, Ramos B, Ramirez R, De Ory F, Echevarria JE. Assessment of RNA amplification by multiplex RT-PCR and IgM detection by indirect and capture ELISAs for the diagnosis of measles and rubella. APMIS. 2010;118:203-209. 10.1111/j.1600-0463.2009.02581.x.

11. Gomez-Camarasa C, Lara-Oya A, Cobo F, Sampedro-Martinez A, Rodriguez-Granger J, Gutierrez-Fernandez J, *et al*. Comparison of two chemiluminescent immunoassays in the detection of measles IgM antibodies. J Virol. Methods. 2016;237:38-39. 10.1016/j.jviromet.2016.08.018.

12. WHO. WHO Guidelines for Epidemic Preparedness and Response to Measles Outbreaks. WHO/CDS/CSR/ISR/99.1.Geneva, Switzerland: WHO, 1999.

13. Arunkumar G, Vandana K, Sathiakumar N. Prevalence of measles, mumps, rubella, and varicella susceptibility among health science students in a University in India. American journal of industrial medicine. 2013;56:58-64.

14. Gupta SN, Gupta N, Gupta S. A mixed outbreak of rubeola-rubella in district Kangra of Northern India. Journal of Family Medicine and Primary Care. 2013;2:354.

15. Bhuniya S, Maji D, Mandal D. Measles outbreak among the Dukpa tribe of Buxa hills in West Bengal, India: Epidemiology and vaccine efficacy. Indian Journal of Public Health. 2013;57:272.

16. Chow SC, Wang H, Shao J. Sample size calculations in clinical research. CRC Press, 2007.

17. Cutts F, Clements C, Bennett J. Alternative Routes of measles immunization: A Review. Biologicals. 1997;25:323-338.

18. Low N, Kraemer S, Schneider M. Immunogenicity and safety of aerosolized measles vaccine: systematic review and meta-analysis. Vaccine. 2008;26:383-398.

19. Henao-Restrepo AM, Greco M, Laurie X. Measles aerosol vaccine project. Procedia in Vaccinology. 2010;2:147-150.

20. Yeung LF, Lurie P, Dayan G. A limited measles outbreak in a highly vaccinated US boarding school. Pediatrics. 2005;116:1287-1291.

21. Sheets F. District Level Household and Facility Survey.

22. Parray SH, Gaash B, Ahmad M, Kadri SM. Changing face of measles in Kashmir. Indian J for the Practising Doctor. 2008;5:5-6.

23. Mohan A, Murhekar MV, Wairagkar NS, Hutin YJ, Gupte MD. Measles transmission following tsunami in a population with high one dose vaccination coverage, Tamil Nadu, India 2004-2005. BMC Infect Dis. 2006;6:143.

24. Mayo DR, Brennan T, Cormier DP, Hadler J, Lamb P. Evaluation of a commercial measles virus immunoglobulin M enzyme immunoassay. J Clin Microbiol 1991;29:2865-7.

25. Bellini WJ, Helfand RF. The challenges and strategies for laboratory diagnosis of measles in an international setting. J Infect Dis. 2003;187(1):S283-90.

26. Wairagkar N, Chowdhury D, Vaidya S, Sikchi S, Shaikh N, Hungund L, *et al*. Measles Net India collaborators. Molecular epidemiology of measles in India, 2005-2010. J Infect Dis. 2011 Jul;204(1):S403-13. Doi: 10.1093/infdis/jir150 PMID: 21666192.

27. Marinova L, Muscat M, Mihneva Z, Kojouharova M. An update on an ongoing measles outbreak in Bulgaria, Euro Surveill. 2009 April-Nov:14:19-442.

28. Necula G, Lazar M, Stanescu A, Pistol A, Santibanez S, Mankertz A, *et al*. Transmission and molecular characterisation of wild measles virus in Romania, 2008 to 2012. Euro Surveill. 2013 Dec;18(50):206-58.

29. Mankertz A, Mihneva Z, Gold H, Baumgarte S, Baillot A, Santibanez S, *et al*. Spread of measles virus D4-Hamburg, Europe, 2008-2011. Emerg. Infect Dis. 2011;17(8):1396-401.

30. Fitzpatrick G, Ward M, Ennis O, Johnson H, Cotter S, Carr MJ, *et al*. Use of a geographic information system to map cases of measles in real-time during an outbreak in Dublin, Ireland. Euro Surveill., 2011, 17(49).

31. Ramsay ME. Measles: the legacy of low vaccine coverage. Arch Dis Child. 2013 Oct;98(10):752-4.

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32. Knol MJ, Urbanus AT, Swart EM, Mollema L, Ruijs W, Van Binnendijk R, *et al*. Large ongoing measles outbreak in a religious community in the Netherlands since. Euro Surveill. 2013 May;18(36):205-80.