Sero-Epidemiological Study of Outbreak of Measles among Children in Diyala – 2009

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Abstract

Background: Measles is a serious infectious disease in children. Despite reaching global measles vaccination coverage of 80% of individuals, measles virus (MV) remains the fifth leading cause of death and the most common cause of vaccine-preventable death in children under 5 years of age.

Objectives: to determine the sero-epidemiological characters of the outbreak of measles among children in Diyala province in 2009.

Subjects and methods: This study was done during the outbreak of measles in Diyala provinc (spring and summer of 2009) in Al-Batool hospital of Pediatrics and Gynecology at Baquba city during a 2-month period from 1 April 2009 to 1 June 2009. A sample of 103 child patients presented with clinically suspected measles was studied by thorough history and physical examination with a determination of immunoglobulin M (IgM) antibodies in serum by enzyme- linked immunosorbent assay (ELISA) testing for measles for each patient.

Results: There was 58.3% (66 out of 103) positive blood samples for IgM of measles in children with clinically evident measles. The study showed that there was no significant difference in the distribution of children with measles positive by IgM according to their age and sex, according to residency, according to mothers previous vaccination status or previous infection with measles. On the other hand, the study revealed that the distribution of IgM positive measles was significantly more ($p<0.05$) in children who did not receive previous measles vaccine than those who received vaccination, and in children with low & medium economic status families than those with good status, respectively.

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Conclusion: It was concluded that the single serum assay of IgM antibodies by ELISA testing has medium sensitivity in the diagnosis of measles in children, there is an increasing susceptibility of infection with measles for infant less than one year of age and for children with poor family economic status.

Key words: Measles, IgM, serology, Children, Diyala

Introduction

Mesles (rubeola) is caused by a single-stranded RNA paramyxovirus with one antigenic type[1]. It is a serious infection characterized by high fever, an enanthem, cough, coryza, conjunctivitis, and a prominent exanthem. After an incubation period of 8–12 days, the prodromal phase begins with a mild fever followed by the onset of conjunctivitis with photophobia, coryza, a prominent cough and increasing fever [2]. Humans are the only natural host[1].

Despite reaching global measles vaccination coverage of 80% of individuals, MV remains the fifth leading cause of death and the most common cause of vaccine-preventable death in children under 5 years of age[3].

Countries in the Eastern Mediterranean Region (EMR) reduced the number of measles-related deaths by approximately 75% from 2000 to 2007. However, large measles outbreaks continue to occur throughout the region, suggesting that much work remains to eliminate measles in the EMR[4-7].

> Despite almost universal use of measles vaccines in recent decades, epidemics of the disease continue to occur. Understanding the role of primary vaccine failure (failure to seroconvert after vaccination) and secondary vaccine failures (waning immunity after seroconversion) in measles epidemics is important for the evaluation of measles control programs in developing countries[8].

MV is one of the most contagious pathogens known to humans, and large measles outbreaks, facilitated by overcrowding in poor communities, continue to occur even in countries that have achieved high vaccine coverage.

The pathogenicity of MV is intimately linked to the immune status of the infected individual. Measles is typically a self-limiting disease; however, individuals who are immunocompromised[9,10], malnourished[11-13], or at the extremes of age[14] are at increased risk for severe measles.

During 1997-1998 in EMR, the number of cases reported increased by 58% from previous outbreak; outbreaks were reported in Iran, Syria, Morocco, and Saudi Arabia (MMWR 1999). In our country an outbreak of measles had occurred at the same period[15].

This article aimed to assess the sero-epidemiological characters of the outbreak of measles among children in Diyala province in 2009. We then discuss the clinical consequences of MV infection in individuals.

Subjects and method

This study was done during the outbreak of measles in Diyala province (spring and summer of 2009) in Al-Batool hospital of Pediatrics and Gynecology at Baquba city during a 2-month period from 1 April 2009 to 1 June 2009. A sample of 103 child patients presented with clinically suspected measles was studied by thorough history (including sex, age, weight, address, previous vaccination status, mothers previous vaccination status and history of previous measles infection, history of contact, family size and economic status of the family) and careful physical examination for signs, symptoms, and complications of measles. A determination of IgM antibodies by ELISA testing for measles for 103 patient was done in the central laboratory of health in Baquba to prove recent infection. Data were statistically analyzed by chi square test.

Serological analysis

Samples 2.5 ml of blood were obtained from the 103 children. The samples were left to coagulate at room temperature and serum was obtained by centrifugation. An aliquot of the serum obtained was frozen at –20°C until serological analysis. Measles IgM antibodies were determined by ELISA (bioactive diagnostica. Product number (103 determinations).

Enzyme Immunoassay for the Determination of IgM Antibodies.

Antibodies against MV were detected in children sera using ELISA-test, which done according to the manufacturer instruction and as follows:

Test procedure:The sera were diluted 1/101 and mixed well, then 100ul of undiluted control sera and diluted samples pipetted in duplicate into respective wells of the microtiter strips (except the well for the blank), the plate was covered and incubated for one hour at room temperature. Wells then emptied by aspiration and unbound sera were removed by three cycle of washing, then 100ul of anti-IgG-HRP conjugate was added into each well, then plate was covered and incubated for 30 minutes at room temperature, then unreacted HRP-Abs were washed by 3 cycles of washing by ready to use washing solution. Then, 100ul of ready to use substrate (TMB) was added into each well, then plate was covered and incubated for 15 minutes at room temperature in the dark, then 100ul of 1M H_2So_4

(stopping reagents) to stop substrate reaction and after thoroughly mixing the color was stable for 30 minutes and the absorbance was measured at 450nm using an ELISA reader.

The low positive control served as the cut-off value and when the absorbance of the subject sample was more than 10% above the cut-off value, the result regarded as positive and the absorbance more than 10% below the cut-off value, the result regarded as negative, results in between that could not clearly be defined and they were regarded as questionable. The higher optical density (OD), the higher levels of anti- immunoglobulins are present. The mean cutoff value was calculated through the OD which was 0.638. Any OD reading higher than this OD reading by 10% was considered as positive, any OD reading below by 10% was considered as negative (according to the manufacturer instruction).

Results

The study revealed that from the total number of 103 blood samples obtained from children with clinically evident measles, 66 samples were positive for IgM of measles, which represents about 58.3 % of the total. The study showed that there was no significant difference in the distribution of children with measles positive by IgM assay according to their age and sex as shown in table (1).

Table (1) : Distribution of children with measles positive by Ig M assay according to their age and sex.

Age by year	Female	Male	Total	
			No.	%
<1	16	13	29	43.4
1-2	10	5	15	22.7
3-4	7	6	13	19.7
≥ 5	2	7	9	13.7
Total	35	31	66	100

df = 3, calculated $X^2 = 4.8$, tabulated $X^2 = 7.85$, $p > 0.05$ [NS]

There was statistically insignificant difference in the distribution of measles positive patients by IgM assay according to residency, table (2).

Table (2) : Distribution of children with measles positive by IgM assay according to residency

Residency	Positve measles		Negative measles		Total
	No.	%	No.	%	No.
Rural	36	54.5	17	45.9	53
Urban	30	45.5	20	54.1	50
Total	66	100	37	100	103

df = 1, calculated $X^2 = 0.519$, tabulated $X^2 = 3.841$, $p > 0.05$ [NS]

The study revealed that the main clinical presentations of measles positive by IgM were skin rash (95%), fever (94), bronchitis (74%), conjunctivitis (68%), and diarrhea (46.9) respectively, as in table (3).

Table (3) : The clinical presentations of measles positive by IgM assay among the studied group.

Clinical presentation	No.	%
Skin rash	63	95
Fever	62	94
Bronchitis	49	74
Conjunctivitis	45	68
Diarrhea	31	46.5
Pneumonia	15	22.7
Koplik spot	12	18.2
Vomiting	4	6
Meningitis	1	1.5

The study revealed that measles positive by IgM assay was significantly more among children who did not receive previous vaccination than those who received vaccination ($p < 0.05$).Table (4).

Table (4) : The distribution of patients with measles positive by IgM according to their previous vaccination against the disease in the studied group.

Vaccination	Positive measles		Negative measles		Total
	No.	%	No.	%	
Given	18	27	10	27	28
not given	36	55	27	73	63
Unknown	12	18	0	0	12
Total	66	100	37	100	103

df = 1, calculated X^2 = 50.2, tabulated X^2 = 3.84, p < 0.05 [S], unknown is neglected.

There were no statistically significant difference between patients (≤9 months of age) whose mothers were previously vaccinated and those whose mothers were not vaccinated to be measles positive by Ig M, table (5).

Table (5) : The distribution of patients (≤ 9 months of age) with measles positivity by IgM assay according to their mothers previous vaccination against measles.

Previous mothers vaccination	Positive measles		Negative measles		Total	
	No.	%	No.	%	No.	%
Yes	14	73.7	5	26.3	19	100
No	5	71.4	2	28.6	7	100
Total	19	73.1	7	26.9	26	100

df = 1, p > 0.05 [NS], unknown is neglected

The study showed that there were no statistically significant difference between patients (≤9 months of age) whose mothers were previously infected with measles and those whose mothers were not infected to be measles positive by Ig M, p> 0,05, table (6).

Table (6) : The distribution of patients(≤ 9 months of age) with measles positivity by IgM assay according to their mothers history of previous infection with the disease.

Mothers previous infection	Positive measles		Negative measles		Total	
	No.	%	No.	%	No.	%
Yes	6	60	4	40	10	100

No	16	16.5	10	38.5	26	100
Total	22	61.1	14	38.9	36	100

df = 1, p > 0.05 [NS], unknown is neglected

The study revealed that there was no statistically significant difference between patients with measles positive by IgM who had positive history of family contact with measles and those who had no such history, table (7).

Table (7) : The distribution of patients with measles positive by IgM assay in relation to the presence of positive family contact.

Family contact	Positive measles		Negative measles		Total
	No.	%	No.	%	No.
Yes	34	51.5	21	56.7	55
No	32	48.5	16	43.3	48
Total	66	100	37	100	103

df = 1, calculated $X^2 = 0.441$, tabulated $X^2 = 3.841$, p > 0.05 [NS]

Finaly, the study showed that the distribution of measles positive by IgM was significantly more ($p<0.05$) in patients with low economic status than those with moderate or good economic status as shown in table (8).

Table (8) : The distribution of patients with measles positive by IgM assay in relation to the economic status in the studied group.

Economic status	Positive measles		Negative measles		Total
	No.	%	No.	%	No.
Low	46	65	25	35	71
Moderate + good	20	62.5	12	37.5	32
Total	66	64.1	37	35.9	103

df = 1, calculated $X^2 = 3.859$, tabulated $X^2 = 3.841$, p < 0.05 [S]

Discussion

This study was conducted during the last outbreak of measles in Diyala province which represents an important medical event in this area, and possibly in other areas of Iraq, that may reflect the different aspects of general child's health including primary health care services, vaccination programs, and other social services.

This study revealed that about 58.3% of blood samples of children with clinically evident measles was measles IgM positive. Other studies revel variable higher rate[16-18]. The apparently significant negative or questionable levels of measles IgM can be attributed to either early sampling of blood, children malnutrition and decreased immunity, or to a less extent due to wrong diagnosis or laboratory errors. However, taking these factors in mind, assays of IgM of measles with careful clinical history and physical examination largely improve the accuracy of diagnosis especially in sporadic cases. This accuracy can be further improved by further blood sampling in questionable levels and possibly by other investigations.

This study find no significant differences in measles distribution according to the sex and the different age groups of patients, which can be explained by the general shortage of the medical services and vaccination programs; and the general malnutrition and overcrowding which affect both sexes and multiple age groups. Most importantly, the increasing incidence of measles attacks below one year of age may reflect lacking immunity against measles in infants as a consequence of absence or failure of vaccination[8] in their mothers.

Measles attacks confirmed by IgM assay were found to be significantly more in previously not vaccinated children than those received vaccination. This reflects the vital importance of vaccination in disease prevention the disease as a known medical fact[19-21] especially in our society which require further vaccination coverage and further social education about the great benefits of vaccination.

The study revealed that there was no statistically significant difference ,regarding measles positive IgM cases, between children (≤9 month of age) whose mothers were previously vaccinated against or infected with measles and those whose mothers were not vaccinated or infected with measles. These findings can be explained by a possible weaning infants' passive immunity[22], maternal vaccination failure[8], maternal malnutrition or immunodeficiency, and a possible defects in information taking by history only without medical records.

The study showed no significant relationship in the distribution of children with measeles positive by IgM according and the presence or absence of family contact with measles. This possibly

due to that many patients might not yet develop high positive titers of IgM, or they had questionable titers, with a possible improper history given by the followers.

Finaly, the study showed that measles positive by IgM was significantly more ($p<0.05$) in patients with low economic status (according to income by ID and family size) than those with moderate or good economic status. This finding may be attributed to the better care, nutrition, vaccination coverage, and less crowding in the second group.

The study conclude the following 1. The single assay of IgM antibodies by ELISA testing has medium sensitivity in the diagnosis of measles. 2. There is increasing susceptibility of infant less than one year of age for infection with measles. 3. The incidence of measles infection in children is inversely related to the economic status of the family.

The study recommend doing second IgM antibody testing by ELISA with negative or questionable result or using other method for diagnosis as polymeras chain reaction (PCR), improvement of maternal vaccination, and improvement of the economic status of the poor families.

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