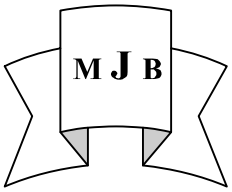
Immune Status Study of Dentistry Student and Children Naturally Infected with Measles Virus for Detection of Primary and Secondary Immune Failures

Younis Abdulredha K. Al-Khafaji
Branch of Medical Microbiology, College of Dentistry, University of Babylon, Iraq.



Abstract

This study was carried out on 52 subject of Babylon dentistry student and children who were naturally infected with measles virus and laboratory confirmed as measles during 2009 by detection of specific anti-measles IgM .They were tested for IgG avidity to estimate their immune status and to distinguish between primary measles infection and reinfection due to secondary vaccine failure. The study reflected that all unvaccinated subjects (19.2%) showed a primary immune response whereas secondary immune responses were detected in(46.09%). Of 34 subjects sourly vaccinated (42.9 %) of them reflected a primary immune response, whereas (57.1%) of them reflected secondary immune response ,thereby indicating a secondary vaccine failure that was seen at very high proportion in subjects > 11 years old, so that revaccination of 11 years old children is recommended for reactivation of their immune status to restrict secondary reinfection.

دراسة الحالة المناعية لطلبة كلية طب الأسنان ولأطفال المصابين طبيعيا بفيروس الحصبة لتحديد فشل الاستجابة المناعية الأولية والثانوية.

الخلاصة

تم دراسة الحالة المناعية لطلبة كلية طب الأسنان والأطفال المصابين طبيعيا بفيروس الحصبة في بابل خلال العام الدراسي 2009وقد أجريت الدراسة على 52 إصابة تم تشخيصها مختبريا من خلال التحري عن الغلوبيولين المناعي M كما تم التحري عن الغلوبيولين المناعي G بغية التفريق بين الإصابة الأولية بفيروس الحصبة وبين عودة الإصابة بسبب الفشل الثانوي للقاح . وعكست الدراسة بان جميع المرضى غير المطعمين (19.2%) أعطوا استجابة مناعية أولية ، بينما أبدى (57.1%)منهم استجابة مناعية ثانوية كإشارة إلى فشل ثانوي للقاح،والذي لوحظ بنسبة عالية جدا في الأعمار أكثر من احد عشر عاما ،وعليه أرى ضرورة إعادة تطعيم الأطفال بهذا العمر لتنشيط الحالة المناعة لديهم للحد من العدوى الثانوية بالفيروس.

Introduction

Measles is a highly communicable infections disease , it remains the leading cause of vaccine preventable childhood mortality in developing countries, and is still a major public health concern in the developed countries [14]

Measles virus (MV) is a member of the family paramyxoviridae, genus Morbillivirus. It is transmitting via the respiratory rout and has an incubation phase of 9 to 19 days [5].

Measles outbreaks are known to occur even in highly vaccinated population despite the availability of an effective live attenuated measles virus vaccine [19]. Mild or asymptomatic measles infection are probably very common among measles- immune persons exposed to measles cases and may be the most common manifestation of measles

during outbreaks in highly immune populations [9] .

Measles control has a high priority in many countries, and it is important that questions surrounding possible vaccine failures eliminate measles may be evaluated and strengthened [20].

A study from the united states of 80 blood donors of whom 8 developed measles during an outbreak in a university college , reported that individual with low measles antibody titers (≤ 120 m IU) were susceptible to infection [2] .

Observation from immunized populations suggest that undetectable antibodies may not necessarily imply that the individual is fully susceptible to disease [1] ,[13].

Measles virus-specific high-avidity antibodies are associated with pre-existing B-cell memory, whereas low avidity IgG is an indication of the primary immune response [18], [7] ; [21] .Thus avidity measurement can be used to assess the success of measles vaccination [3],[7] and offers a way of assessing the type of vaccine failure without knowledge of prior antibody status [16]. In this study, we used IgG avidity assay to analyze sera from conformed measles patient in order to determine how many cases of reinfection (high- avidity antibodies) and how many were cases of primary infection (low- avidity antibodies) in adult subject and study of there immune response.

It is important that highly sensitive andspecific laboratory test Enzyme Immune Assay (EIA) used to accurately determine the antibody level resulting from vaccination or naturally infection with measles virus. Several enzyme immune assay (EIA) kits for anti-measles virus antibodies are available commercially, among which the Dade Bering EIA kit was previously found to perform better in comparison with other commercial EIA kits [10]. Therefore this kit was used in evaluation of the immune response in this work.

The objectives of present study were:

1. To evaluate measles-specific humeral immune response IgM in teenager (Dentistry students), as well as children naturally infected with measles virus.
2. To differentiate between primary and secondary immune failure by detection the avidity of immunoglobulin G.

Materials and Methods

During March to June 2009 several cases of measles were detected in Babylongovernorate including teenager university student, and childrens. The first case came from Wasit governorate who live in student resident house, in Hilla city. Most students in contact with him became ill and seafaring from fever, cough and coryza most of them after 3 days later developed a generalized maculo-papuler rash and was subsequently confirmed by serological study to have measles. Information about students' illness, vaccination status and their age were reported. Blood samples by vein puncture at acute and convalescent period were collected to test for the presence of measles- specific antibodies .

Serological analysis

Serum were separated from collected blood samples , they were tested for the presence of measles specific IgM and IgG antibodies by using previously described indirect enzyme immunoassay that have been shown to be highly sensitive (99.6%)and highly specific (100%){Enzygot, Dade Behring, Marburg, Germany} (Hamkar R. et al., 2006).

Study group samples

Nine sera sample were collected from laboratory confirmed measles patients aged ≤ 2 years old

whom were received single dose of measles virus (except two of them of unknown vaccination status) experienced primary infection, and were not re infected by measles virus, therefore their sera were regarded as low avidity anti- measles IgG.

Another 7 sera sample were collected from subjects (3 male and 4 female) age 12 years old who had been vaccinated twice for measles at 9 months with measles vaccine and 15 months of age with MMR vaccine during vaccination program in Hilla city Babylon governorate (one of them of unknown vaccination status).

The third group of sera sample were colleted from 36 university student aging from 19 – 25 years whom were suffering from acute measles infection regarded as having high avidity IgG against measles virus.

At the time of sera sample collection, a question about personal data, vaccination status was completed. Cases were confirmed as measles when testing for anti-measles IgM gave positive IgM results when using Enzygost anti-measles IgM and IgG (EIA) kits (Dade Behring, Marburg, Germany) .

Avidity measurement

All sera samples were subjected to anti-measles IgG avidity assay according to [2],[7];[8]. The avidity of IgG for measles virus was measured by a protein- denaturing enzyme immune assay where the antibodies were first allowed to bind to the virus antigen followed by elution with or without, six molar urea. Each sample was tested at dilution of 1:21 and each sample at 4 replicates. For each replicate a single serum dilution (1:21) of the kit serum diluents was applied to each of 4 wells on one row of ELISA plates. (Two wells of measles antigen positive - coated and 2 wells of measles antigen negative - coated).

After incubation for 1 hour, test plates were washed 4 times according to ELISA kit procedure. Then 2 wells (one antigen – positive and one antigen – negative were soaked for 5 minutes in wash buffer containing 6 mol / L. urea. Fresh buffer were applied and the soaking was carried out twice more.

The plates was then washed 4 times with wash buffer. Then the test was continued according to the kit restriction manual. The remaining specific antibody was then detected according to the EIA kit procedure. An avidity index (AI) was calculated from the optical density (OD) of the wells according to the following formula :

$AI = (\Delta OD \text{ with urea} / \Delta OD \text{ with wash buffer}) \times 100$

Three control were used for testing EIA plates serum sample containing strong high- avidity, weak high avidity and low- avidity anti-measles IgG- antibody, in addition to kit positive and negative control were also applied.

Results

Serum samples included in this study was grouped by age and measles vaccination status and their distribution was shown in table (1).

All samples were tested and confirmed as measles case in laboratory, (anti-measles- IgM positive). Nine ≤ 2 year old children and seven (12) years old subjects samples were used as a control source of low and high avidity from all age groups, 10 (19.2%) had unknown vaccination status, 8 (15.4%) had not been vaccination, and 34(65.4%) had received one (21 subjects) or two (13subjects) doses of measles vaccine.

Overall, 24 (46.2%) measles cases confirmed by a positive IgM test exhibit high-avidity IgG representing secondary immune response to measles (that representing secondary

vaccine failure),while remaining other 28 subjects (53.8%)of them exhibiting a primary immune response (low avidity) indicating primary measles infection see table (2). The table reflected also anti-measles virus low and high- avidity IgG which was distributed as following: subjects less than 2 years old show 100% low avidity and this proportion decreased gradually with increasing age and reach 28.5% of those age 23 years.

High avidity anti - measles virus IgG in higher proportion at subjects age 23 years (71%) followed by 22 years (70%) then 21 years(60%). Whereas 2 cases (28.6%) was found in 12 years age group and non in ≤ 2 years age group .

The distribution of anti-measles virus high and low-avidity IgG among the study group by vaccination status was reflected in table (3) below. Non of the 8 unvaccinated patients (0 %) reflect high- avidity anti-measles IgG so their infection was regarded as a primary measles infection. Of the remaining 35 patients which were sourly vaccinated 15 (42.9%) showed low avidity (i.e., a primary immune response) and 20 (57.1%) showed a secondary immune response, which indirectly reflecting a secondary vaccine failure .

There was no relationship between vaccination status and age($P > 0.05$), but there was a clear relationship between anti-measles virus IgG avidity with age ($P < 0.05$). Also there was no relationship between vaccination and avidity ($P > 0.05$) but there was a direct relationship between number of vaccine dose and avidity. Table (3) also reflected that subjects who received two dose of measles virus (infected and vaccinated) showed high-avidity anti-measles virus IgG proportion (55%) , where as subjects who received single dose vaccine showed lower proportion (45%) .

Discussion

Although vaccination program with measles virus live attenuated virus vaccine in Iraq started since 1980s, by a routine immunization program, under which the vaccine is given in one dose scheduled at 9 months of age, with a recommendation for vaccination at 18 month age with MMR trivalent vaccine. yet measles cases were reported every year anywhere in Iraq and neighboring countries, however during March 2009 a large episodes of measles infection was seen and teenager subject were included most of them had been vaccinated, so this study was directed mainly toward teenager patients (dentistry student). Several research published that vaccine- induce protection with less duration less robust than naturally acquired immunity against measles virus [7],[15], also high occurrence of mild symptoms measles due to secondary vaccine failure [4], [7]; [9]; [11]; [17]; [18] has been found among measles patients vaccinated over decade age especially among those who were revaccinated [7],[17]. Hence the evolution of measles control programmes in Iraq and neighboring countries require well understand of the resions for primary and secondary vaccine failure. The presence of high proportion of primary vaccine failures in vaccinated patients with measles, give an indication of improper vaccine handling, for example, improper cold chain or misvaccination, in addition to suppressing factors influencing Iraqi population since more them 3 decades, besides persistent exposure to naturally circulating measles virus. All this gave reasons to follow up the immune status of Iraqi population, to improve measles control in Iraq. Therefore introduction of good diagnostic test for detection of measles such as IgM- capture EIA for detection

of measles IgM is not sufficient to differentiate between primary infection and re infection due to second vaccine failure [7],[17]. Detection of specific measles IgM detected only in primary measles infection or vaccination but also inducible by overcame end.

Few researcher used IgG avidity test as a useful procedure for identifying primary and secondary immune responses [7], yet few reports upon this test used during measles outbreaks [7],[17]. However in this study 46.2 % of 52 of measles cases confirmed by a positive IgM EIA test mounted a secondary immune response, giving an indication that presence of IgM can not be used as a reliable indicator of a primary immune response [7],[17]. The present study also reflected that all unvaccinated subjects showed a primary immune response supporting the information giving by the IgG avidity test. This finding inconsistent with other researchers, and I had concluded that:

1. measles virus can infect previously immune responded persons, producing classic symptoms of measles in some, and mild or no symptoms in others.
2. The protective immunity induced by vaccination may not be life long without being boosted by an exposure, mostly sub clinically, to a naturally circulating virus.
3. Due partly if not entirely, to the secondary vaccine failure, the numbers of measles cases among adults in Iraq increase in recent over the previous year.
4. Measles is still endemic in Iraq, and person has the potential for repeated exposure to wild type measles virus.

1- Large scale serological estimation of immune status of Iraqi population is required in all governorates to find the population requiring vaccination to be included in rotten vaccination program

2-Implementation of better vaccination programs is urgently required especially for those aging 15-20years as a boaster dose.

3-IgG immunoglobulin avidity test is a good parameter for detection of primary and secondary immune response failure.

Table1 Distribution of anti-measles IgM-positive cases by age and measles vaccination status

Age (years)	Total subject Tested	vaccination status				No. of vaccine doses(Known vaccination status)		
		unknown		Known				
		No.	%	No	%	0 No.	1 No.	2 No.
≤2	9	2	22.2	7	77.8	2	2	3
12	7	1	14.3	6	85.7	0	0	6
19	6	2	33.3	4	66.7	1	2	1
20	8	1	12.5	7	87.5	2	4	1
21	5	0	0	5	100	0	4	1
22	10	2	20	8	80	2	5	1
23	7	2	28.6	5	71.4	1	4	0
Total	52	10	19.2	42	80.8	8	21	13

Table 2 Distribution of low and high- avidity anti-measles virus IgG in the study group by age.

Age (years)	Total subject Tested	anti-measles virus IgG Low- avidity		high- avidity	
		No.	%	No.	%
≤2	9	9	100	0	0
12	7	5	71.4	2	28.6
19	6	3	50	3	50
20	8	4	50	4	50
21	5	2	40	3	60
22	10	3	30	7	70
23	7	2	28.5	5	71
Total	52	28	53.8	24	46.2

Table 3 Distribution of low and high- avidity anti-measles virus IgG in the study group by vaccination status.

anti-measles virus IgG	Total subject Tested No.	vaccination status unknown		known		known vaccination status (No. of doses of vaccine) 0		1		2	
		No.	%	No	%	No.	%	No.	%	No.	%
High-avidity	24	4	44.4	20	46.5	0	0	9	60	11	55
Low-avidity	28	5	55.6	23	53.5	8	100.0	6	40	9	45
Total	52	9	100.0	43	100.0	8	100.0	15	100.0	20	100.0

References

1- Bin D., Zhihui C.; Qichang L.;et al.1991. Duration of immunity following immunization with live measles vaccine: 15 yearsof observation in Zhejiang Province, China. Bull WHO.69:415-23.

2- Chen, R. T., L. E. Markowitz, P. Albrecht, J. A. Stewart, L. M. Mofenson, S. R. Preblud, and W. A. Orenstein. 1990. Measles antibody: reevaluation of protective titers. J. Infect. Dis. 1621036-1042.

3- De Souza V. U.A, Pannuti C.S.; Masami S.; and De Andrade H.F. 1997. Enzyme-linked immunosorbent assay-IgG antibody avidity test for single sample serologic evaluation of measles vaccines. Journal of medical virology. 52:275–9.

4- Erdman, D. D., J. L. Heath, J. C. Watson, L. E. Markowitz, and W. J. Bellini. 1993. Immunoglobulin M antibody response to measles virus following primary and secondary vaccination and natural virus infection. J. Med. Virol. 1:44-48.

5- Griffin, D. 2001. Measles virus, p. 1401-1441. In D. M. Knipe and P. M. Howley (ed.), Fields virology, 4th ed. Lippincott Williams and Wilkins, Philadelphia, Pa.

6- Hamkar, R., M. Mahmoodi, R. Nategh, K.N. Jelyani, M.B. Eslami and T. Mohktari -Azad. 2006 Distinguishing between primary measles infection and vaccine failure reinfection by IgG avidity assay; Health journal. 12 (6):775-782.

7- -Hamkar, R., M. Mahmoodi, R. Nategh, K.N. Jelyani, M.B. Eslami and T. Mohktari-Azad. 2006. Distinguishing between primary measles infection and vaccine failure reinfection by IgG avidity assay; Health journal . 12 (6):775-782.

8- Hedman, K., and S. A. Rousseau. 1989. Measurement of avidity of specific IgG for verification of recent primary rubella. J. Med. Virol. 27:288-292.

9- Helfand, R. F., J. L. Heath, L. J. Anderson, E. F. Maes, D. Guris, and W. J. Bellini. 1997. Diagnosis of measles with an IgM captures EIA: the optimal timing of specimen collection after rash onset. J. Infect. Dis. 175:195-199.

10- Hesketh, L., A. Charlett, P. Farrington, E. Miller, T. Forsey, and P. Morgan Capner. 1997. An evaluation of nine commercial EIA kits for the detection of measles specific IgG. J. Virol. Methods 6651-59.

11- Hidaka, Y., T. Aoki, M. Akeda, C. Miyazaki, and K. Ueda. 1994. Serological and clinical characteristics of measles vaccine failure in Japan. Scand. J. Infect. Dis. 26:725-730.

13- Krugman S1983. Further-attenuated measles vaccine: characteristic and use. Rev. Infect.Dis.5:477-81

14- Moss, W. J., and D. E. Griffin. 2006. Global measles elimination. Nat. Rev. Microbiol. 4900-908.

15- Mossong J, Muller CP. 2003. Modelling measles re-emergence as a result of waning of immunity in vaccinated populations. Vaccine. 21:4597–603.

16- Narita, M., S. Yamada, Y. Matsuzono, O. Itakura, T. Togashi, and H. Kikuta.1996 Immunoglobulin G avidity testing in serum and cerebrospinal fluid for analysis of measles virus infection. Clinical and diagnostic laboratory immunology. 3:211–5.

17- Pannuti, C. S., R. J. Morello, J. C. de Moraes, S. P. Curti, A. M. S. Afonso, M. C. C. Camargo, and V. A. U. F. de Souza. 2004. Identification of primary and secondary measles vaccine failures by measurement of immunoglobulin G avidity in measles cases during the 1997 São Paulo epidemic. Clin. Diagn. Lab. Immunol. 11:119-122.44

18- Paunio, M., K. Hedman, I. Davidkin, M. Valle, O. P. Heinonen, P. Leinikki, A. Salmi, and H. Peltola. 2000. Secondary measles vaccine failures identified by measurement of IgG avidity: high occurrence among teenagers vaccinated at a young age. Epidemiol. Infect. 124:263-271.

19- Poland, G. A., and R. M. Jacobson. 1994. Failure to reach the goal of measles elimination. Apparent paradox of measles infections in immunized persons. Arch. Intern. Med. 1541815-1820.

20- Ratnam, S., V. Gadag, R. West, J. Burris, E. Oates, F. Stead, and N.Boullianne. 1995. Comparison of commercial enzyme immunoassay kits with plaque reduction neutralization test for detection of measles virus antibody. J. Clin. Microbiol. 33:811-815

21- Tuokko H. 1995.Detection of acute measles infections by indirect and mu-capture enzyme immunoassays for immunoglobulin M antibodies and measles immunoglobulin G antibody avidity enzyme immunoassay. Journal of medical virology. 45:306–11.