**Evaluation of Measles Virus Vaccine in Protection against Measles**

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**Abstract :**

 Measles is one of the diseases targeted for global eradication by vaccination. Most strategies for elimination and reducing global measles morbidity and mortality measles, are based on the ability to enhance immune response to measles virus (MV). Immunological response had been studied in seropositive individuals, after the administration of the available measles virus vaccine as a preparation for an anticipated measles elimination programme in Iraq.

 This study aimed to estimate the immunological status for 20 healthy infantswho received a single dose of the measles vaccine (Schwarz strain) and 152 healthy adult who received a second dose of the measles vaccine (Schwarz strain). In addition to 30sera samples collected from normal adults non-vaccinated as well as 10 sera samples from non-vaccinated infants.

 Serological tests had been carried for estimating the levels of IgM, IgG, IgG1, IgG2, IgG3 and IgG4 in the sera of seropositive volunteers after measles vaccination with live attenuated measles vaccine.

 The anti-measles antibody levels were measured by ELISA test. Sero-prevalence for specific measles IgM-Abs among infants and adults after measles vaccination were 75%and23.7% respectively. Measles IgG-Abs among infants and adults after measles vaccinations were 55% and 94.1% respectively. During the follow-up study, the mean of IgM level increased post vaccination and reached its highest level after 21 day and started to decrease gradually. Moreover, the mean of IgG level increased in vaccinated individuals within the first week after booster vaccination, and reached its maximum at 14day and started to decrease gradually but remained higher than starting prevaccination level.

 On the other hand, the predominant subclasses IgG1 and IgG3 were observed in vaccinated individual which was contributed (77.053±25.033) ng/ml and (42.399±21.09) ng/ml respectively, of the total IgG responses. The contributions of specific IgG2 and IgG4 to the total IgG anti-measles response were lower (17.263±11.394) ng/ml and (5.853±4.867) ng/ml respectively. The mean of IgG1 seropositivity gradually increased and reached its peak at 21 day after vaccination while the mean of IgG3 reached its higher level at 14 days and is believed to prevent subsequent reinfection.

**Introduction :**

 Measles is an acute highly infectious respiratory viral disease. It remains a leading cause of death among young children especially in developing countries, despite the existence of an effective vaccine (Zilliox *et* *al*., 2007 ; Okonko *et* *al*., 2009).

 The reason of measles outbreaks depends on vaccination coverage , high levels of a live attenuated vaccine coverage are required to prevent virus circulation in population. Primary failure may be seen in anyone with no antibody production following vaccination (Youwang *et* *al*., 2001).

 Secondary failure of measles vaccine is a reason of measles outbreaks in young and adult population that is caused by decreasing anti measles antibody in the course of time(Erdman *et* *al*., 1993; Ghorbani *et* *al*., 2007).

 Recent studies about incidence of measles infection in Iraqi population indicated that there was marked increase in measles cases during the period of 2008- 2009 and according to the Center of Disease Control reports, the total number of measles cases were 8,134 during the year 2008 and there was marked increase in the cases during first 18 weeks of 2009 and the total number of reported cases were 23,336 (MOH and WHO, 2009).Therefore, Iraqi Ministry of Health decided to perform mass vaccination in (17-10-2011) among populations between (18-24) years of age and this mass vaccination was not a part of routine immunization program. For this reason, this study was designed to evaluate immunity status after recent mass measles vaccination.

**Objectives of the Study:**

**1**- To study the efficiency of available measles virus vaccine.

**2**-To follow-up the vaccinated individual by estimation:

IgM , IgG measles antibodies titer.

**3**- Investigate the immunoglobulin (IgG) subclass profile in vaccinated individuals.

**Materials and Methods:**

 Two handard twelve (212) individuals were included in present study. They were divided into three groups: vaccinated adult, vaccinated infants, and non vaccinated individuals as control group.

* **First group:** includes 152 young adult subjects. They were vaccinated with measles vaccine at 17 - 10 - 2011, their ages ranged from (18-24) years, both males and females .
* **Second group:** includes 20 infants. They were vaccinated with measles vaccine , their ages ranged from (9 -12 month), both males and females.

 **Third group(control):** includes 40 normal non-vaccinated individuals; (30 subjects) ranging in their age from (18-24years) and 10 infants ranging in their age from (9 -12 month), both males and females.

 Enzyme Linked Immuno Sorbent Assay (ELISA) test for the evaluation of serum level of immunoglobulin (IgM , IgG, IgG1, IgG2, IgG3and IgG4).

**Calculation of results:**

 The concentration of the samples can be read directly from standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted.

 Statistical analysis was done by using SPSS (statistical package for social sciences) version 17, in which we use analysis of variance (ANOVA) and independent sample T-test for measurement data and chi square (X2) for categorical data.

We set p value<0.05 as significant.

**Result :**

 A total number of 212 apparently healthy individuals were selected randomly from general population, 152 vaccinated universal students of (18-24 years old), 20 vaccinated infants (9-12month) and 40 serum sample (30 adult & 10 infants) were collected from apparently healthy subjects as a control group (non-vaccinated).

 To test the efficacy of vaccination program against measles virus, serological tests had been carried for estimating the levels of IgM, IgG, IgG1, IgG2, IgG3 and IgG4.

Sero-prevalence for specific measles IgM-Abs among infants and adult after measles vaccinations were 75% (15 of 20)and23.7% (36 of 152) respectively. While measles IgG-Abs among infants and adult after measles vaccinations were 55% (11 of 20) and 94.1% (143 of 152) respectively.

Table (1) showed that there was a significant difference in IgM levels in adult vaccinees compared with adult control group (*p* <0.001) . The table also reflected a significant differences in IgG levels in adult vaccinees compared with control group (*p* <0.006), with a mean titer of IgG (97.879±176.957) U/ml developed in vaccinated adult after measles vaccination. Moreover, there was a significant difference in IgM levels in vaccinated infants compared with control group (*p* <0.001). Although IgG levels in vaccinated infants were slightly higher than control group, no statistically significant difference was observed between vaccinated and control infants (*p*> 0.138).

|  |  |  |
| --- | --- | --- |
| Study groups | IgM U/ml (mean±SD\*) | IgG U/ml (mean±SD\*) |
| Vaccinated adult (N=152) | **9.126±3.709** | **97.879±176.957** |
| Control (N=30) | **6.193±2.397** | **10.547±3.704** |
| P value | **<0.001\*\*** | **0.006\*\*** |
| Vaccinated infants (N=20) | **11.87±3.679** | **23.54 ±20.245** |
| Control (N=10) | **6.022±2.02** | **11.501±5.734** |
| P value | **<0.001\*\*** | **0.138** |

|  |  |  |
| --- | --- | --- |
| **Study groups** | **IgM U/ml** | **IgG U/ml** |
| **POSITIVE** | **NEGATIVE** | **POSITIVE** | **NEGATIVE** |
| **Vaccinated adult (N=152)** | **36(23.7%)** | **116(76.3%)** | **143(94.1%)** | **9(5.9%)** |
| **Non vaccinated adult (Control) (N=30)** | **0(0%)** | **30(100%)** | **1(3.3%)** | **29(96.7%)** |
| **P value** | **0.002** | **0.01** |
| **X****2** | **8.857** | **124.8** |
| **Vaccinated infants (N=20)** | **15(75%)** | **5(25%)** | **11(55%)** | **9(45%)** |
| **Non vaccinated infants (Control) (N=10)** | **0(0%)** | **10(100%)** | **2(20%)** | **8(80%)** |
| **P value** | **0.0001** | **0.068** |
| **X****2** | **15** | **3.326** |

SD = standard deviation⃰ significant =\*\*

Table (1): Immune response of vaccinated and non vaccinated individuals represented by IgM and IgG means ±SD

The study has also shown that the incidence of IgM positivity was very little among vaccinated adult group in comparison with vaccinated infants. Sero-prevalence for specific measles IgM- Abs was 75% (15 of 20) among infants, while the sero-conversion rate was 23.7% (36 of 152) among adult. On other hand, measles IgG-Abs was detected in 55% (11 of 20) among infants, whereas adult seroconversion rate of IgG was 94.1% (143 of 152). However, 100 percent of both infants and adult non vaccinated individuals have shown negative IgM titer, while one non vaccinated adult (3.3%) and two (20%) non vaccinated infants gave positive IgG titer table (2).

# Table (2): Immune response status represented by IgM and IgG percent in both vaccinated and non- vaccinated adult and infants

 It is noteworthy that IgG immunoglobulin titer showed marked high increase in its level which was detected at 7 days after vaccination (77.02±45.60) U/ml and reached its maximum titer (96.815±50.9) U/ml at 14 days post vaccination and remained higher than day zero level even after four months post vaccination (Table 3, figure 1).

|  |  |  |
| --- | --- | --- |
| Immunoglobulin class | Mean ± SD of Immunoglobulin in Pre and post vaccination intervals |  |
| Prevaccination | Post vaccination | P value |
| Day 0 | Day 7 | Day 14 | Day 21 | Day 28 | Day 60 | Day 120 |
| IgMU/ml | 6.53±2.19 | 7.34±2.82 | 8.77±4.13 | 10.10±4.39 | 9.157±3 | 7.973±2.33 | 6.44±1.84 | <0.001 |
| IgGU/ml | 12.80±4.16 | 77.02±45.60 | 96.815±50.9 | 92.03±40.94 | 81.97±37 | 70.86±28.79 | 56.63±26.31 | <0.001 |

Table (3): IgM and IgG immunoglobulin titer in Pre and post vaccination intervals for 25 adult vaccinees.

 detection of IgM and IgG immunoglobulin after 7days post vaccination , had shown a significant difference between vaccinated adult and infants group with respect to IgM levels (*p*< 0.002). Reversely, there was no significant difference with respect of IgG levels between them (*p*>0.063) (Table-4). IgG levels percent was higher (94.1%) in adults than in infants vaccinees (55% ) with significant differences in percent of positive IgM and IgG between both vaccinees (Table -5).

|  |  |  |
| --- | --- | --- |
| **Study group** | **IgM U/ml** | **IgG U/ml** |
| **Vaccinated adult(N=152)** | **9.126±3.709** | **97.879±176.957** |
| **Vaccinated infants (N=20)** | **11.87±3.679** | **23.54 ±20.245** |
| **P value** | **0.002** | **0.063** |

Table (4): Comparison of IgM and IgG between adult and infants vaccinated group .

|  |  |  |
| --- | --- | --- |
| **Study groups** | **IgM U/ml** | **IgG U/ml** |
| **POSITIVE** | **NEGATIVE** | **POSITIVE** | **NEGATIVE** |
| **Vaccinated adult (N=152)** | **36(23.7%)** | **116(76.3%)** | **143(94.1%)** | **9(5.9%)** |
| **Vaccinated**  **infants (N=20)** | **15(75%)** | **5(25%)** | **11(55%)** | **9(45%)** |
| **P value** | **<0.001** | **<0.001** |

Table (5): Percentages of positive and negative IgM and IgG among vaccinated adult and infants .

The study reflected significant differences in IgG1and IgG3 subclass between vaccinated and non vaccinated adult group. Contrariwise, no significant differences was observed about IgG2 and IgG4 between vaccinated and non vaccinated adult group (Table -6, figure -2).

|  |  |
| --- | --- |
| **Study groups** | **IgG isotype (mean ±SD)** |
| **IgG1 ng/ml** | **IgG2 ng/ml** | **IgG3 ng/ml** | **IgG4 ng/ml** |
| **Vaccinated adult (N=152)** | **77.053±25.033** | **17.263±11.394** | **42.399±21.09** | **5.853±4.867** |
| **Control****(N=30)**  | **29.971±5.695** | **18.168 ±5.289** | **7.490±3.933** | **4.879±2.794** |
| **P value** | **<0.001** | **0.354** | **<0.001** | **0.352** |

Table (6): Comparison of different IgG isotypes in relation to vaccination status of adult group

**Discussion :**

 In the present study, vaccinated adult subjects reflected positive (23.7%) and (94.1%) for IgM and IgG respectively in comparison with infants who revealed (75%) and (55%) positive for IgM and IgG respectively (Table-2). This result was consistent with other studies.

  [Chiu](http://www.ncbi.nlm.nih.gov/pubmed?term=El%20Mubarak%20HS%5BAuthor%5D&cauthor=true&cauthor_uid=14695672) *et al*. (1997) found that tested sera of vaccinated young adults (21-30 years old) showed 88% IgG positive immune response to measles vaccine , whereas Lee *et al* . (1999) found that adults over 16 years old showed 92% - 98% measles IgG antibodies in their sera. On the other hand, [Lee](http://www.ncbi.nlm.nih.gov/pubmed?term=El%20Mubarak%20HS%5BAuthor%5D&cauthor=true&cauthor_uid=14695672) *et al*. (2001) found that sera of vaccinated young adults

 (19-25 years old ) showed 95 % positive IgG to measles vaccine . The result also in agreement with Ghorbani *et al*. (2007) finding which reported that 0.7% of military cadets in Iran had IgM antibodies in their sera whereas 97.8% of them reflected IgG anti measles- antibodies in their sera.

 The present study includes the detection of IgM immunoglobulin in sera of primary vaccinated children 9-12 months old. It was found that 75% of them were seropositive. This finding was in agreement with what found by Fang (1992) who observed that 83% IgM sera positivity in children vaccinated sera after primary vaccination.

 Helfand *et al*. (1999) estimated that IgM positivity was 2% at 1 week post vaccination of 209 children whereas 61% at 2 weeks and 79% were positive after 3weeks and dropped to 60% at 4weeks after vaccination. However, IgG was positively detected in 81% of vaccinated 3 week later , and 85% at 4 weeks post vaccination.

 In this study , The predominant subclass was IgG1and IgG3 which contributed on average of (77.053±25.033) and (42.399±21.09) respectively, of the total IgG response . The contributions of specific IgG2 and IgG4 to the total IgG anti-measles response were lower (17.263±11.394) and (5.853±4.867) respectively, (table - 6) .



Figure (1): The mean of IgM and IgG immunoglobulin at different post vaccination interval.



Figure (2): Distribution of immunoglobulin -G (IgG) subclass left for adult vaccinated & right for adult non vaccinated control group



Figure (3): Mean value of different IgG - subclass at pre and post vaccination intervals .

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