Evaluation of Immune Response to Measles Virus Antigen

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Abstract: Increasing of measles infection cases in last few years is an important issue, which must be dealing with to assess the reasons. Immunizing lab animals with measles vaccine to is a better way understanding of vaccine-induced immune responses against measles vaccine. The current study is done investigated the phagocytosis index and the titer of specific antibody in response to measles vaccine measured by HI test in rabbits who received the vaccination to estimate the immune status after vaccination. Our study revealed that the rabbits subjected to the vaccine demonstrated an increase of 4-fold antibody concentration and increase in phagocytosis index (55.2, 59.8, 53.7, 51.5, 41.7)% after 14 days from immunization with the antigen comparing to the control. These results showed that the live attenuated measles vaccine is efficient. The aim is to evaluate the immune status after vaccination with measles virus, by calculating the phagocytosis index and the titer of specific antibodies produced because of stimulation the immune system to measles vaccine, to evaluate the effectiveness of the vaccine.

Keywords: measles, virus, immune, response, antigen

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I. Introduction

Measles, red measles, is a very infectious viral agent (1). Infected person suffers from fever, more than 40 °C, cough and red eyes (2). After few days the symptoms start as spots start from the patient's mouth, these spots are Koplik's spots. Then a red rash start as facial spots then appeared on all over the patient's body (3). Symptoms appear within two weeks of exposure to an infected one (4). The infected person may suffer from complications occur in a ratio of 30%, including diarrhea, blindness, may be inflammation in brain and pneumonia (5). The virus is an airborne spread infected agent via coughs and sneezes and may spread via contact with salivary and nasal secretions (4). The patients can be infectious to others before and after four days of rash appearance (5). An individual can be immune after infection (4).

The vaccine is very potent. Immunization decrease deaths in a ratio of 75% from 2000 to 2013. There is no treatment, Supportive care can decrease the disease outcomes, rehydration can be beneficial, good nutrition and medications to decrease fever (5). For a secondary bacterial infection, antibiotics can be used (4). About for million cases in one year were occurred in the United States of America before vaccination (5), and those many of children under five years old were died (4).

The genetic material of Measles Viruses a −ssRNA, enveloped, genus Morbillivirus. Paramyxoviridae family. Isolated Nobel L., John E. and Thomas P. isolated the virus for the first time from infected person having Koplik's spots (6). Human is the only natural host, no other animal reservoir. The risk factors for measles infection is immunodeficiency resulted from infection with HIV, immunosuppression after organ plant operations or receipt of a cell, or after corticosteriod therapy. The WHO reports showed that there are more than 158,000 deaths were resulted from the infections with measles virus. The number decreased to 63,000 deaths in 1990 (7). Measles still the most reason of deaths in 2013, also in states where the vaccine has been introduced and could be in high rate. There were 10,000 cases of infection with Measles in thirty European countries, Most cases reported were in unvaccinated individuals (8).

Detection of viral infection can be estimated by fever for three days and may be cough or coryza or conjunctivitis comnined with the formation of spots (9). Also, detection can be through clinical laboratory tests to confirm the presence of anti-measles IgM, or by isolation the viral genetic material from nasal secreemens, people how is difficult to obtain blood samples from them, saliva may be collected to detect measles-specific IgA (10).

The children in developed countries, are Immunized with measles at age of one year. Generally, trivalent Measles-Mumps-Rubella vaccine. Vaccine does not agemtnstrated before one year of age due to inadequate response as a result of the immature immune system. Specific measles antibodies can be transferred from pregnant mothers to their newborn inafents but in low amounts and still for only six months. Children
become susceptible to infection at age of one year (11). Booster dose of the vaccine can be given between ages of 4-5, in order to enhance the immune response. Immunization can cause fever and pain at the injection site. It is preferred to give two doses in developing countries, because of high endemic of measles virus infection (12).

II. Methodology

*Staphylococcus aureus* suspension:
The bacterial strain was provided kindly by biology department laboratories. The suspension was prepared in a concentration of $1 \times 10^8$ cell/ml.

*Haemagglutination Ag*:
The antigen was prepared from measles vaccine which was provided by WHO, kept in 4°C until used.

*Alsever’s solution*:
Alsever’s was prepared by adding 2.05%dextrose, sodium citrate0.8%, citric acid0.055%, and sodium chloride 0.42%.

*Phosphate-buffered saline* PH:7.4:
One tablet of (PBS) in 100 ml distilled water and sterilized in autoclave. Bovine serum albumin was added in concentration of 0.1%, kept in 4°C.

*Kaolin (Aluminum silicate)*, PH= 8.6. Was prepared in two dilutions:
- Suspension of 50%.
- Suspension of 0.75%.

III. Methods

Four rabbits, which with 1kg weight were used in this study. Three of them were immunized with 1 ml measles vaccine and the fourth one didn’t immunize as a control. Blood samples were taken before vaccination, then once after 2 weeks after vaccination. For evaluation of immune responses to measles antigens. Phagocytosis in vitro was done and Measles-specific antibodies were measured by haemagglutination inhibition test.

**Blood Samples Collection:**
Blood was collected by heart puncture using a syringe, then 0.5 ml the blood was distributed into the first container with anticoagulant (heparin). These samples were used to calculate the percentage of the efficiency of phagocytic cells to estimate the immune response. The test was done within (1-2) hours of blood collection.

The remainder of the samples were added to test tubes, incubated at 37°C for a quarter of an hour to complete the clotting process and then placed in the refrigerator for two hours, the tubes were centrifuged 2000 r/min for ten minutes, then the serum was collected and transferred to another sterile tube for hemagglutination inhibition test.

1. **Phagocytosis Test**:
   This test was done to determine phagocytosis index. The test was done according to Furth et al. 1985 (13). The phagocytosis index was determined before and after immunization with measles vaccine for each experimental animal. Phagocytosis index was calculated by counting 200 phagocytic and non-phagocytic cells.

   \[
   \text{Phagocytosis index} = \frac{\text{no of phagocytic cells}}{200 \text{phagocytic and nonphagocytic cells}} \times 100
   \]

2. **Hemagglutination Inhibition Test (HI):**
Red blood cells from human O blood group was collected in Alsever’s solution were used (14).

**Haemagglutination Test (HA):**
A volume of 25µl PBS was added all wells, then 25µl of measles antigen was added to the first well and two fold dilutions were done, the control wells were free of virus. 25µl from 0.5% RBCs suspension was added the wells. The plate was taped to mix the components. The results obtained after 1 hr of incubation. of incubation at room temperature.

The result of HI was 128
4 HA units: $128/4 = 32$
to prepare total volume = 32 ml of the 4HA antigen. 31 ml of buffer was added to 1 ml of the original suspension.

**Heamagglutination-Inhibition Determination (HI):**
Kaolin treatment: A volume of 0.05 ml from PBS was added to 0.05 from each heat inactivated serum (56°C for 30 minutes). A volume of 0.01 ml of kaolin (25% W/V) the mixture was mixed well.
The tubes were incubated in 25°C for 20 minutes. The tubes were centrifuged at 1200 rpm for 5 minutes. The supernatants were collected. RBCs suspension in 10% concentration in volume of 25µl was added. Incubated at 4°C for 1 hr. Centrifuged at 250 rpm for 5 minutes. The supernatants were collected.

The Haemagglutination inhibition test was done as below: 25µl of phosphate buffer was put in each well of the microliters plate. 25µl of each serum was added in the first line of wells. Then two-fold was made along eachrow. 25µl of 4HA antigen dilution of was putin each well except the last wells. Slightly the plates were taped in order to mix the components, the plates were covered by a lid and incubated30 minutes at room temperature.25µl of 1%R.B.Cs was put in well, then incubated f 45 minutes at 25°C. The results of settling patterns were read. The results were recorded to determine the endpoint. The HI titer in International Units/ml calculated according to the equation below:

\[
HI (Ab) = \frac{\text{INVERT of HIGHEST DILUTION} \times \text{HAUNITS}}{256}
\]

256: A factor of conversion to the International units /ml for sera under examination.

IV. Results And Discussion

The innate and the adaptive immunity are crucial to give an effective immune response to the measles vaccine. Moreover, good vaccination have to induce a good stimulation of both the cellular and humoral immune responses of adaptive immune response resultinig production memory and effector cells (15,16). In order to assess the efficiency of the measles vaccine phagocytosis in vitro and HI test were done, the results of this study showed that the vaccine has the potency and effectiveness to induce an adequate immunity to measles virus.

Phagocytosis:

The phagocytosis index results in test groups 1, 2, 3, 4 before immunization were 41.3, 35.7, 39.5 and 40.4 respectively while they were 55.2, 59.8, 53.7, 54.5 respectively for them after immunization. These results demonstrated that there is an elevation in phagocytosis index within the test groups after immunization than before in cooperation to control group. The results of phagocytosis experiment are shown in the table (1). The phagocytosis index was elevated in test groups as compared to the control with mean of 52.55%.

Table 1: Phagocytosis Index in experimental groups and control before and after immunization with measles

<table>
<thead>
<tr>
<th>Samples</th>
<th>Phagocytosis index% before immunization</th>
<th>Phagocytic Index % After immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test 1</td>
<td>41.3</td>
<td>55.2</td>
</tr>
<tr>
<td>Test 2</td>
<td>35.7</td>
<td>59.8</td>
</tr>
<tr>
<td>Test 3</td>
<td>39.5</td>
<td>53.7</td>
</tr>
<tr>
<td>Test 4</td>
<td>40.4</td>
<td>51.5</td>
</tr>
<tr>
<td>Control</td>
<td>42.3</td>
<td>41.7</td>
</tr>
</tbody>
</table>

Innate immune response is a very conserved strategy against a many infectious agents. Induction of innate immune response can cause an inflammatory response to control the infections before it spread. The cell of innate response products can induce adaptive immune responses to develop (17). The Phagocytic cells are important in acute inflammatory response due to its efficiency in engulfment and destruction of many pathogens. Phagocytic cells are neutrophils; macrophages; monocytes; and eosinophils and they are called provisional Phagocytes. Phagocytosis process is important in host defenses against pathogens (18). polymorphonuclear cells (PMN) are the first line of cellular response. The number of PMN phagocytosis increases rapidly from the beginning to prevents any infection. The neutrophils exhibit pseudopods on their surface to increase the surface area, this helps in enhancing phagocytosis by increasing phagosomes formation (19). Our result shows a good elevation in phagocytosis index after vaccination, which means that the vaccine that introduced the Iraqi recipient is effective and can give a good innate immunity response representing the activity of cellular immune response of phagocytic cells. The results revealed that despite the difficulties that face our health employees who are responsible for the transportation of vaccine in Iraq due to country situations and deteriorating economy, the delivery of the vaccine to the health centers and then to the recipient, mostly children. Also, the insurance of the characteristics and effectiveness of vaccine are not easy to be ensured which need a series of cooling, has been provided well.

Experiments of nature case reports, seroepidemiology, outbreak investigations and animal studies all suggest that the cellular response is sufficient to protect against measles (20). Measles vaccines result a mild infection and can inducecellular and humoral immune response. Serologic evidence shows that measles vaccines can induce long lasting immunity (21). These studies are compatible to the results of this study.
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Figure 1: Phagocytic Cells

**Haemagglutination Inhibition:**
The haemagglutination inhibition measles antibodies titer for test groups 1, 2, 3, and 4 were 512, 256, 236, and 512 respectively. These titers represent 8, 4, 4, and 8 HI IU/ml units respectively. The results are in Table (2).

**Table 2: Haemagglutination Inhibition antibody concentrations after immunization with measles vaccine.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>HI titer</th>
<th>HI in IU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test 1</td>
<td>512</td>
<td>8</td>
</tr>
<tr>
<td>Test 2</td>
<td>256</td>
<td>4</td>
</tr>
<tr>
<td>Test 3</td>
<td>256</td>
<td>4</td>
</tr>
<tr>
<td>Test 4</td>
<td>512</td>
<td>8</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The basis of the HI assay is that anti-measles antibodies prevent attachment of the virus to the R.B.Cs. for that reason hemagglutination is inhibited (22). The results of this study showed an increase in HI units of measles antibodies in serum of animals after vaccination with measles vaccine, which revealed a good humoral response to measles antigens. Therefore, we recommended to use the vaccine as its effective and also safe.

About 95% of vaccines examined after immunization had detectable antibodies to measles antigen (23). The high titer relative to the HI titer was most likely due to the presence of the immunoglobulin M antibody which is efficient in agglutination, the initial high ratio of PHA to HI titers is due to the presence of immunoglobulin M (IgM) antibody, which is known to be highly efficient in agglutination because of its multiple valence. It is noteworthy that both of these aspects distinguishing PHA from HI are based on the unique feature of the former that requires very firm binding of antibody to the antigen over the erythrocyte surface (24). The HI titer reached its peak 3 weeks after infection and started to decline after few months. Measles vaccine produces mild infection among 95% of vaccinated individuals can develop measles antibodies (25).

In spite of that vaccination may produces antibodies lower than that of natural infection; the vaccine can induce long-term immunity and may be for lifetime. Vaccinated persons who lose antibody show an increase in antibody titer upon revaccination which indicates that they are still immune (26). The incidence of infections with viruses, which have to be disappeared within populations after vaccine introduction, is increased in few recent years. Studies in the field of efficiency and safety of vaccines are really
needed and the survey for each vaccine is very important to ensure the eradication of these infections which may harm the community health and cause endemic or could be epidemic infections. This study was done to evaluate the efficiency of Measles vaccine due to the serious disease caused by this virus and the rapid spread through the populations to assess if it is efficient or not by evaluation the humoral and cellular immune response results from the immunization with the vaccine. Also this study can assess the safety of the vaccine and its effect on animals after vaccination. Periodical assessment for measles vaccine is important as the live attenuated virus of vaccine can be returned to active infectious one if there is any defect in cooling series or in moisture or other condition during transportation and storage. In Iraq, we have many problems that make the preservation is not easy. This study revealed that the phagocytosis index is elevated after immunization and the antibody titer is increased to sufficient titers that can give a good protection from infection.

The live attenuated vaccine is favorable than killed one because it can give long-term protection, such as mumps and measles vaccines. These vaccines induce the cellular and humoral immunity (1). The permissive to Measles infection can result in upregulation of co-stimulatory molecules (2). When the viral infection occur, the immature dendritic cells will be mature and it will transport the virus to the local lymphatic nodes, the viral antigens will be presented to T-cells (3). Live attenuated measles virus vaccines can induce cellular and humoral immune response. The vaccine protection results from the stimulation of specific antibodies and the efficient memory cellular immunity can protect the vaccines from infection.

In a cohort study which focused on low-income countries in West Africa where there is a high rate of death caused by opportunistic infections, If there is immune suppression in acute measles it can lead to mortality. About 50% of all children deaths were recorded because of measles infection within two months only for 5 years, suggesting that there is no detection for long-term squeal in infected children (5) or there is no evaluation for the vaccine efficiency, which should be done frequently.

Our study shows that the live attenuated vaccine is very effective and safe, even though it is important to check its effectiveness and safety after transportation and before administration to the recipients.

V. Conclusion
The animals under this experiment had elevation in phagocytosis index and excellent antibody responses after measles vaccination. These results indicate that this vaccine is effective.

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