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Original Article

Evaluation of Immune Status to Measles in Vaccinated Population in Tehran, by Enzyme-linked Immunosorbent Assay and the Hemagglutination Inhibition Techniques (1386-1387)

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Abstract

Background and Aims: Measles remains one of the leading causes of childhood morbidity and mortality in developing countries and is still a major public health concern in developed countries. Although live attenuated vaccine is used throughout the world, out breaks of disease still occur in many countries including Iran.

Methods: The present study was performed to evaluate the immune status against measles after the mass campaign vaccination in 2003.

Results: In this study, Approximately 172 sera were analysed by ELISA and HI tests. The results indicated, 162 were positive (94.2%) and 10 were negative (5.8%) by HI test and 165 were positive (95.5%) and 7 were negative (4.1%) by the ELISA test.

Conclusion: Understanding measles outbreaks that occur after the initiation of measles elimination efforts will be critical in refining the strategies for measles elimination.

Keywords: Measles; Immune Status; ELISA

Introduction

Measles is of the most contagious diseases which is caused by a virus belonging to the family of paramyxoviridae, genus morbilivirus. The nucleocapsid is surrounded by a viral membrane which contains several viral proteins. Some of these proteins such as viral hemag-glutinin induces viral specific antibody which plays a major role in immunity and protection against the virus (18). This antibody can be measured by several tests such as hemagglutination inhibition and Elisa tests. Measles has caused millions of death since its emergence, thousands of years ago. The disease is characterized by a prodromal illness of fever, cough, coryza and conjunctivitis followed by the appearance of a generalized maculopapular rash (16). Death from measles is mainly due to an increased susceptibility to secondary bacterial and viral infections, which is attributed to a prolonged state of measles virus- induced immune suppression. In most countries of the world, measles vaccination has had considerable impact on the control of the disease (1). But many countries have reported measles epidemics despite high vaccine action coverage. Measles virus infections in Iran has decreased dramatically since the use of live attenuated measles vaccine (1, 3). If administered properly, live attenuated measles vaccine can induce life long immunity greater than 85% with one dose and about 90% with two doses (2). In the present cross sectional study, HI and ELISA tests have currently gained acceptance as the methods of choice in

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the diagnosis of measles virus infection and in the evaluation of the immune status of an individuals (4, 9). However, **HI** test has been shown to be less sensitive than the enzyme-linked immunosorbent assay test (5-7).

Methods

Sera

The study population who were vaccinated against measles in Tehran without history of the disease included 172 serum samples (61 males and 111 females) in different age groups. Sera were obtained by centrifugation of whole blood collected in tubes without anticoagulant and they were stored at -20 C until used.

HI Test

The HI test was performed by a modification of the method of Gershon and Krugman (4). Serum samples were treated with heating at 56 C for 30 minutes to inactivate complement proteins and then were treated with 25% kaolin in phosphate buffered saline to remove nonspecific factors. The supernatant was mixed with 10% green monkey red blood cells to remove nonspecific factors for agglutination. Briefly, serum samples were diluted 1:8 in phosphate-buffered saline (PBS; pH 7.2) containing 0.4% bovine albumin in If-bottom *96*well micro plates. Two-fold serial dilutions of sera were made and then four hemagglutinating units of antigen in a volume of 0.025 ml were added. Each well received 0.025 ml of a 0.5% suspension of African green monkey erythrocytes. Plates were shaken and incubated for 1 h at 36°C. The reciprocal of the dilution which completely inhibited hemagglutination was taken as the HI antibody titer. Complete inhibition of agglutination at a >1:8 dilution of serum was considered indicative of immunity.

ELISA Test

The BElA measles IgG quant Kites a quantitative Enzyme- Linked Immunosorbent assay was used for the detection of specific IgG antibodies to measles virus.

During the first incubation, only anti-measles specific antibodies present in serum or plasma bind to the inner surface of the wells coated with the measles antigen. After the first incubation the wells were washed to remove non-reactive serum components. During the second incubation a monoclonal antibody anti-human IgG conjugated with horseradish peroxides (HRP) was added. After a second washing cycle, a sub strate TMB solution was dispensed in to the wells in order to detect specific antibodies during a subsequent incubation. The enzymatic reaction was then stopped by adding stop solution.

The amount of color was directly proportional to the specific IgG anti-measles concentration in the patient samples. The IgG titer was calculated in international mu/ml by a calibration curve traceable to the international standard. The IgG titer of> 125 mu/rnl was considered to be Positive, The IgG titer of <85 mu/rnl was negative and IgG titer between mu/ml was equivocal 85-125.

Statistical analysis

X^2-Test was used to analyze data obtained by SPSS 11.5 software. Differences or correlation with $p<0.05$ were considered statistically significant.

Results

Determination of antimeasles anti-bodies by the HI test

Sera from 172 vaccinated individuals were tested with the standardized HI test for measles virus. As it is shown in table l, Of these 162 were positive with a titer of > 1:8. In total 94.2% of these sera were positive and 5.8% were negative.

Application of Elisa test for antibody detection

Sera from 172 vaccinated cases were also tested with the standardized test for ELISA measles virus. The results are shown in table 2. OF these sera, 165 had IgG titer of> 125 mu/rnl which were considered to be positive (95.5%) and 7 were negative (4.1 %) (Table 2).

Comparison of ELISA and HI results

Because HI test has been commonly used as a standard method for determining immune status, all sera tested by ELISA were also evaluated for HI antibody. ELISA and HI were performed on a total of 172 sera. Of these sera,

Table 1. HI Results for evaluation 0f immunity to measles virus in vaccinated individuals.

HI Results	Frequency	Percent
Positive	162	94.2
Negative	10	5.8
Total	172	100

159 (96.4%) were positive and 4 (57.1 %) were negative by both tests; 6 sera that were positive by the ELISA (3.6%) were negative by HI; 3 sera that were positive by HI (42.9%) were negative by ELISA (Table 3).

Discussion

Worldwide, it is estimated that measles kills some 880,000 children annually, a toll more than any other vaccine-preventable disease. The global plan, established by the World Health Organization (WHO) and United Nations Children's Fund (UNICEF), was to cut this burden by two-thirds between 2000 and 2005, and thereafter to prevent 600,000 measles fatalities annually (16).

In most countries of the world, measles vaccination has had considerable impact on the control of the disease. But many countries havereported measles epidemics despite high vaccine action coverage. Example of these ismeasles epidemics of 1988 1990 in the United States of America, Canada, Hungary, Taiwan (11, 12, 13). The present study was performed to evaluate the immune status against measles after the mass campaign vaccination in 2003. In this study, 172 sera were analyzed by ELISA and HI tests. Our study showed that 162 cases (94.2%) of total

Table 2. ELISA Results for evaluation of immunity to measles virus in vaccinated individuals.

ELISA Results	Frequency	Percent
Positive	165	95.5
Negative	7	4.1
total	172	100

population (172) were immune against measles and 10 cases (5.8%) were negative by HI test and 165 cases (95.5%) of total population (172) were immune against measles and 7 cases (4.1 %) were negative by the ELISA test. By using chi-square test, there was no significant correlation between the age group and the mean titers of measles antibodies. Also, there was no significant statistical difference between the male and female in the immunity level ($p>0.05$).

In one study in Iranshahr district in 1994, among 411 vaccinated children, only 64.3(271 cases) of the children under the study had antibody against measles virus while 95.6% of these group had been vaccinated (17).

The main purpose of measles vaccine-ation is to prevent the numerous complications that can occur with measles virus infection. If administered properly, live attenuated measles vaccine can induce lifelong immunity. Vaccination failure could be attributed to non-observance of preservation guide lines, use of unsuitable solvents for vaccines, wrong inoculation techniques, low virus efficiency which may be responsible for this lack of responsiveness (10). In addition, it is important to employ sensitive tests to measure immune

Table 3. Comparison of ELISA and HI results in vaccinated individuals.

		+	-	Total
HI Results	+	159 96.4%	3 42.9%	162(94.2%)
	-	6 3.6%	4 57.1%	10(5.8%)
	Total	165(95.5)	7(4.1)	172(100%)

responses induced against a particular vaccine (8, 13, and 14). The results of this study indicated that the level of immune status against measles is acceptable so the immunity is probably lifelong.

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