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DETERMINATION OF NEUTRALIZING ANTIBODIES AGAINST THE MUMPS VIRUS IN SELECTED GROUPS IN TEHRAN AND TWO IRANIAN VILLAGES

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ABSTRACT

A microneutralization test was used for the detection and evaluation of mumps neutralizing antibodies. A total of 1037 blood samples from selected groups of students aged 7 to 22 years in south and east of Tehran, 139 blood samples from the umbilical cords of pregnant women at two Tehran hospitals at delivery time, and 190 samples from children under five years of age who were living in two villages located in Zandjan province, were tested. The results of this study indicated that the percentage of the above mentioned sera having mumps virus neutralizing antibodies with a titer of 1:2 or greater was 69.6%, 92.8%, and 29.5%, respectively.

Vaccination against mumps is optional in the Islamic Republic of Iran and therefore it is believed that the virus is circulating among susceptible subjects. These data also suggest that children could be a target for mumps vaccination.
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INTRODUCTION

Mumps is an acute communicable disease of children and young adults caused by a single strain of virus. An outbreak of what was probably mumps or epidemic parotitis was described by Hippocrates in the 5th century B.C. The virus is a member of the genus paramyxovirus in the family *Paramyxoviridae*. The virus is able to propagate in different tissues of the host and cause various signs and complications. The most common signs are fever and swelling of the parotid glands, either unilaterally or bilaterally. The sublingual and submaxillary glands may also be involved. Central nervous system complications, mumps orchitis, pancreatitis, hearing loss, thyroiditis, arthritis, renal complications, oophoritis and myocarditis are observed less frequently. In 1934 the viral etiology of mumps was documented by transmission studies.[1] The mumps virus was isolated in 1945, and in 1948 a killed mumps virus vaccine was licensed. After the virus was successfully attenuated in chick embryo tissue culture in 1963, a live attenuated virus vaccine—the Jeryl Lynn strain—was licensed in December 1967.

Various tests are available for the detection of previous mumps infection, through which the attempt is made to determine an individual's state of immunity to mumps. This assessment of immunity is desirable because of the relative frequency of adult mumps, and the occurrence of such complications as meningoencephalitis, pancreatitis, and orchitis. Tests have been evaluated by the extent to which they correlate with a history of mumps, with individual susceptibility as measured by subsequent infection, and with one another.[2]

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A microneutralization test (MNT) for determination and measuring of mumps antibodies was developed by Kenny et al.[3] and has been used extensively. Cells infected with the mumps virus can adsorb RBCs from different species, a reaction which may be inhibited by application of anti-mumps serum.[4] In this study, attempts were made to apply MNT with some modifications based on available reagents and facilities.

MATERIAL AND METHODS

Source of specimens

We attempted to test serum samples collected from different age groups who were living in different socioeconomic areas. Serum samples were obtained from the following sources: (a) 1037 sera from students aged between 7 and 22 years who were living in south and east of Tehran, (b) 139 blood samples from the umbilical cords of pregnant women at Akbarabadi and Shariati hospitals at the time of delivery, and (c) 190 sera from children aged between 1 and 5 years from Aghkand and Torkmanchai villages located in Zandjan province. All samples were transferred to the virology laboratory aseptically and stored at -20°C until testing.

Cell culture

MRC-5 cells were used for primary passages of the mumps viruses. These viruses were then adapted to HeLa cells, which were used for all serological tests during this study.

Medium

Eagle's minimum essential medium (MEM) supplemented with 10% inactivated fetal bovine serum (FBS), 100 µg/ml of streptomycin, 100 units/ml of penicillin, 50 µg/ml of kanamycin, and 5 µg/ml of fungizone was used during the tests. The FBS was reduced to 2% in the maintenance medium.

Viruses and antiserum

An attenuated mumps virus strain, named Hoshino, was obtained from Razi Research Institute. This virus was first propagated in MRC-5 cells and then adapted to HeLa cells. A local strain of the virus designated S#12 was also used in this study. This virus yields better titers than the Hoshino strains.[5]

Antiserum against the virus was prepared in rabbit by a conventional method described previously.[6]

Neutralization test

Mumps antiserum prepared in rabbits was used to run a macroneutralization test. Two-fold dilutions in MEM of the inactivated mumps antiserum, as well as the control rabbit serum, were mixed with an equal volume of virus suspension containing 100 TCID50. The mixture was kept at 37°C for 1 hour, and then 0.2 ml of each mixture was inoculated into each of four HeLa cell culture tubes. After an adsorption period of one hour, 1.5 ml of the growth medium was added to each tube and all tubes were left at 37°C. For virus control, the test virus dilution was mixed with an equal volume of diluent. Final readings were made when the cells containing virus control showed CPE. Serum titers were expressed as the reciprocal of the serum dilution that inhibited CPE completely in test tubes.

Table I. Distribution of neutralizing antibody titers to mumps virus among selected students from south and east of Tehran.

Titer	$\leq\frac{1}{2}$		$\frac{1}{2}$		$\frac{1}{4}$		$\frac{1}{8}$		$\frac{1}{16}$		$\frac{1}{32}$		Total	
Sera tested / Age (year)	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
7	20	40.8	8	16.3	2	4	7	14.3	12	24.5	-	-	49	100
8	37	38.1	14	14.5	13	13.4	12	12.4	21	21.7	-	-	97	100
9	38	33.6	16	14.2	26	23	16	14.2	15	13.3	2	1.8	113	100
10	35	35.3	17	17.2	21	21.2	14	14.2	11	11.1	1	1	99	100
11	26	33	17	21.5	15	19	13	16.5	8	10	-	-	79	100
12	21	27.3	26	33.8	11	14.3	11	14.3	8	10.4	-	-	77	100
13	14	21.5	16	24.6	21	32.3	11	17	3	4.6	-	-	65	100
14	24	26.7	23	25.5	24	26.7	10	11.1	9	10	-	-	90	100
15	21	36.2	10	17.2	13	22.5	10	17.2	4	6.9	-	-	58	100
16	28	38.9	22	30.5	14	19.5	6	8.3	2	2.8	-	-	72	100
17	32	28	19	16.7	23	20.2	29	25.5	11	9.7	-	-	114	100
18-22	34	27.4	39	31.5	25	20.2	18	14.5	8	6.5	-	-	124	100
Total	330	31.8	227	21.9	208	20	157	15.1	112	10.8	3	0.3	1037	100

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Table II. Distribution of neutralizing antibody titers to mumps virus among parturient women from south and north of Tehran.

Titer	<1/2		1/2		1/4		1/8		1/16		Total	
Sera tested / Hospital location	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
South of Tehran	7	6.3	67	62.2	23	21.3	11	10.5	0	0	108	100
North of Tehran	3	9.7	22	70.9	4	13	2	6.5	0	0	31	100
Total	10	7.2	89	64	27	19.5	13	9.3	0	0	139	100

Table III. Distribution of neutralizing antibody titers to mumps virus among children 1-5 years of age from two villages of Zandjan.

Titer	<1/2		1/2		1/4		1/8		1/16		Total	
Sera tested / Age (year)	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1-5	132	69.5	4	2.1	5	2.6	20	10.5	29	15.3	190	100

Microneutralization testing

All test sera were investigated for the presence of mumps neutralizing antibodies using a conventional method described previously.[6] Minor modifications were made whenever necessary.

Hemadsorption

Because CPE produced by the mumps virus cannot be recognized easily, the hemadsorption test was also performed in order to obtain more reliable results. The following procedures were undertaken:

a. The medium of each well was removed from the culture and the cell sheet was washed with PBS.

b. A sterile suspension of 0.5% guinea pig RBC in PBS was prepared and added in 0.025 ml volumes to the wells. Uninoculated cultures of the same batch were treated in the same manner for control purposes.

c. The plates were incubated at room temperature for 5-10 minutes.

d. All cells were washed with PBS thoroughly and then 0.025 ml of PBS was added to each well.

e. The infected cell sheets were examined microscopically at low magnification.

RESULTS

It was shown that macro- and microneutralization tests can both be used for detecting and measuring mumps virus antibodies. The reproducibility of both systems for titrations of the antibodies was investigated by performing a number of replicate titrations and similar results were obtained. Direct observation of CPEs produced by the virus was not a reliable method of reading the results of the tests. The sensitivity and specificity of the above mentioned method in relation to the hemadsorption test were 68.1% and 78.3%, respectively.

The results of the tests are shown in Tables I to III. Table I shows the composition of the study population of the students and their antibody status against mumps. As indicated, out of 1037 sera tested, 330 (31.8%) had antibody titers less than 1:2 and the antibody titers of the remaining sera were distributed between 1:2 and 1:32 of which 21.9%, 20%, 15.1%, 10.8%, and 0.3% had mumps antibody titers of 1:2, 1:4, 1:8, 1:16 and 1:32, respectively.

Table II shows the levels of mumps antibody titers in sera collected from the umbilical cords of parturient women. As shown, only 7.2% of total sera lacked neutralizing antibodies at dilutions of 1:2 or greater. The percentage of the sera having a reciprocal titer of 2 was 64%. By comparing Tables I and II, it is obvious that the percentage of sera from pregnant women having an antibody titer of 1:2 is greater than those of students, but the percentage of students having antibody titers of 1:4 or higher is greater than women. It was shown that the results were significantly different in vaccinated individuals.

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Table III shows the results obtained from sera belonging to some rural children between 1-5 years old. None of these children were vaccinated against mumps and all were living in two villages in Zandjan province and did not show signs or symptoms of any disease at the time of sampling. As indicated in Table III, 132 (69.5%) serum samples were free from neutralizing antibodies to mumps virus. This figure is much greater than those obtained from other study groups. While the highest percentage of the student's and parturient women's sera having mumps antibodies to mumps virus. This figure is much greater than those obtained from other study groups. While the highest percentage of the student's and parturient women's sera having mumps antibodies showed a titer of 1:2, this was equal to 1:16 in children. The significance of these findings are discussed below.

DISCUSSION

The causative agent of mumps is present throughout the world, and most people ultimately become infected when they are young. Approximately a third of the cases, particularly in infants and children, are asymptomatic.[1] Postpubescent individuals and adults tend to have more overt, clinically apparent multiple organ involvement.[7] Concerning the presence of the disease in Iran a rise in the titer of neutralizing antibodies against mumps was first demonstrated in the sera of two patients with pancreatitis in 1973,[8] and the virus was isolated from a patient's saliva 5 years later.[9]

Following infection with the virus, complement fixing, neutralizing, and hemagglutination inhibiting antibodies are formed. Neutralizing antibodies appear several weeks following infection and persist for a long time, albeit at low levels. The neutralizing antibodies correlate best with the subject's immune status, and the serum neutralization test has been claimed to be one of the most sensitive tests for measuring mumps antibodies.[10-14]

Immunity to clinically apparent mumps infection correlates best with a neutralizing antibody titer of 1:8 or greater.[14] Most individuals develop clinically apparent infection only once during their lifetime and maintain low but protective levels of neutralizing antibody thereafter. However, it has been shown repeatedly that at least 90% of individuals having a neutralizing antibody titer of 1:2 are immune against mumps.[15,16]

As shown in Tables I and II, 68.2% of the students and 92.8% of the parturient women tested in this study showed neutralizing antibody titers of 1:2 or greater. In other words, the majority of the individuals tested were immune against mumps. The natural tendency of neutralizing antibody levels to decline gradually to nonprotective levels overtime may be offset by booster effects from repeated exposure to and subclinical infection by the mumps virus during an individual's lifetime, and this might be the reason why a range of neutralizing antibody titers is seen among tested sera.

Regarding the results of our study, it is deduced that less 1-5 year old children have been infected with the virus compared with other age groups and these findings correlate very well with the results of epidemiologic studies done in a number of other countries.[14]

None of the conventional tests, such as complement fixation and hemagglutination inhibition, which are used for measuring mumps antibodies in Iran[5] can be relied on for determining whether an individual is susceptible to the disease. The neutralization test is very specific and highly reliable for determining immunity in this regard.[14]

As far as vaccination of susceptible individuals is concerned, it is recommended that attenuated mumps virus vaccine be given to children 12 months of age or older. However, the trivalent measles, mumps, rubella vaccine should not ordinarily be given to children under 15 months of age.

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