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COMPARISON OF MICRO NEUTRALIZATION TEST WITH HEMADSORPTION-INHIBITION TEST, AND IMMUNOFLUORESCENCE TECHNIQUE FOR DETECTING MUMPS ANTIBODIES

Roustai , M.H.*

ABSTRACT. **The microneutralization test was successfully adapted to the detection and titration of mumps virus antibodies in human sera. A total of 913 serum samples obtained from the students of south of Tehran were tested . Six hundred and fourteen (67.3%) sera were found to be positive with titers from 1/2 to 1/32. In another attemp 149 sera collected from male student, in year 1986, were tested. The results of this test were compared with those of hemadsorption-inhibition test an indirect immunofluorescent technique. There was not a good correlation between the results of the three techniques.**

Key words: TECHNIQUE/IMMUNOFLUORESCENCE/ANTIBODIES/MUMPS VIRUS/HEMADSORPTION REACTION

INTRODUCTION

The virus of mumps was isolated in 1934 by Johnson

* From the faculty of medical sciences, University of Tarbiat Modarres, Tehran, I.R. of Iran.

and Goodpasture from 4 of 6 specimens of saliva from patients with epidemic parotitis (1). It may be propagated in vitro in several cell cultures. The virus belongs to the paramyxoviruses and agglutinates red blood cells of fowl, man, guineapig and other species.

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 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 are correlated with history of mumps , with individual susceptibility as measured by subsequent infection, and with one another (2).

Microneutralization test for determination and measuring mumps antibodies was developed by Kenny et al.(3) and has been used extensively. Cells infected with mumps virus can adsorb RBC from different species and this reaction may be inhibited by application of anti - mumps serum. (4) .

The indirect immunofluorescence technique was also successfully adapted to the detection and titration of mumps virus antibodies in human Sera (5). The most important function of a test is to distinguish between individuals who are truly susceptible and those who are immune as a result of inapparent or forgotten infections, among persons who give no history of mumps. Previous studies demonstrate that the neutrlization test is the one best correlated with history (2). In this paper the correlation of the results of hemadsorption inhibition test

(HADI) , and indirect immunofluorescence techniques was examined with those of microneutralization test.

MATERIALS AND METHODS

Sera. Serum samples were obtained from 501 and 412 school girls and boys respectively. The students were seven to twenty years old and studied at primay, intermediate,and high schools located in south of Tehran. These sera were used for micro neutralization test (MNT). In addition 149 sera were collected from daughter students, aged 16-20 years, and used for comparison of the tests. All serum samples were inactivated and stored at -20°C until tested.

Cell cultures . Hela and Vero cells were used for microneutralization test and fluorescent antigen preparation respectively.

A 75 cm^2 corning culture flask of Hela cells was obtained from the division of virology, Pasteur institute of Iran.The cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10 per cent 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. The FBS was reduced to 2 per cent in the maintenance medium. The vero cell line of African green monkey kidney cells was maintained by weekly passage, using Dulbecco's medium supplemented with 5 percent inactivated FBS, 100 μg/ml of streptomycin, 100 units/ml of penicillin, 50 μg/ml of kanamycin,and 5 μg/ml of fungizone. Cells were grown in Corning 25 cm^2 cell culture flask. As cell growth approached a nearly confluent

monolayer, the growth medium was replaced with the medium containing 2% FBS.

Virus . Attenuated mumps virus strain, Hoshino, received from Razi Institute was used for MNT, preparation of fluorescent antigen, and mumps specific antiserum.

Preparation of rabbit antiserum against mumps virus. Specific antiserum was prepared by inoculation of two healthy rabbits, using the mumps virus. The test animals as well as a control were first bled. 2.5 ml of the virus suspension, with the titer of $10^{3.6}$/0.025 ml in micro test, was them inoculated via the marginal vein of the ear and an additional dose intramuscularly for each test rabbit.

Second inoculations were given seven days later. The control rabbit was inoculated with growth medium . All animals were bled from the heart on 14th and 21 rst days after first inoculation. Blood samples were allowed to clot at room temperature. After breaking up the clot and freeing it from the vessel wall, the entire specimens were refrigerated overnight at 4°C. The samples were then centrifuged at 2000 r.p.m. for 15 minutes at 4°C., and the supernatant antiserum removed (6).

Each serum was mixed with equal volume of Hela cell suspension containing 3 x 10^5 cells/ml, and left at 4°C overnight. The sera were then separated by centriugation, and stored at -20°C until tested.

Microneutralization test with specific and control sera. This was performed in sterile 96 - well (Costar, Cambridge, Mass) plates with flat bottoms, perfilled with 0.025 ml of the growth medium. For each sample to be tested, 0.025 ml heat-inactivated (56°C.30 min) serum was added to an edge will with a 0.025 ml

diluting the serum from 1/2 to 1/128 .

Thus, with one instrument both addition and dilution of the serum could be accomplished. For each serum, three parallel rows of dilutions were made. The third row was used for serum toxicity control. Consequently 4 sera were tested in one plate (1,2). Each well of the first two serum dilution series then received an equal volume (0.025 ml) of challenge virus diluted in growth medium to give approximately 100 median tissue dulture infective doses in 0.025 ml at 6 days. Each well of the remaining serum dilution series received 0.025 ml of growth medium and served as the serum control .

Another plate was used for the remaining sera, including a commercial antiserum against mumps virus. In the third plate, a virus titration of four paralel rows of dilutions from 1/1 to 1/16384 and two rows of unifected cells were included. After an incubation period of 1 hours at 37°C each well of the test received 0.1 ml of Hela cell suspension in growth medium adjusted to contain 2 x 10^5 viable cells per ml. Each plate was then sealed perfectly and incubated at 37°C. The tests were read at 6 days for CPE by using an inverted microscope, and then 0.025 ml fo a 0.5% guinea pig erythrocyte suspension in PBS was added to each well and allowed to adsorb at room temperature for 5-10 minutes followed by washing with PBS for two times. The plates were then observed microscopically for presence or absence of hemadsorption. The neutralizing antibody titer of each serum was calculated by the Spearman karber method.

Microneutralization test with the serum samples. The above mentioned method was used for measuring the mumps

antibodies in the sera.

Hemadsorption - inhibition test (HADI) . Monolayer cultures of Hela cells in the 96-well plates were infected with 100 TCID50 of mumps virus in 0.025 ml. Five days later, the cultures were used for the test. Uninfected cultures were used as controls. The media were removed from all cultures and the cell sheets were washed once with normal saline. A 0.025 ml portion of the serum to be tested was added to each infected culture. Control cultures received normal serum and rabbit hyperimmune serum with titer of 1/32 respectively. After incubation at room temperature for 30 minutes, 0.025^{32} ml of a 0.5% guinea pig erythrocyte suspension in normal saline was added to each well and allowed to adsorb at room temperature for 5 minutes followed by washing with normal saline. The plates were inspected for hemadsorption microscopically.

Indirect immunofluorescence (IIF). One 25 cm^2 Corning culture flask of the vero cells was inoculated with one ml of virus suspension containing 100 TCID50 per 0.025 ml. One culture was also used for control cells and inoculated with maintenance medium. After 7 days of incu - bation when 70% of inoculated cells showed CPE , the control and infected cells were collected separately. On each slide were made 10 circles in two rows. The upper row was used for the infected cells and the lower row for control ones. Smears of the cells were made on the slides, fixed in acetone and presеved at -20°C until used as antigen. The smears of each slide were covered with the serum dilutions to be tested. After 30 minutes at room remperature, the slides were washed three times with PBS at PH 7.2 and incubated for an additional 30 minutes with

an appropriate dilution of fluorescein-labeled antiserum. The slides were washed again three times, covered with 90% glycerine and examined under fluorescent microscope.

RESULTS

Table 1 showes the composition of study population by sex, and age. The age of students ranged from 7 to 20 years. The geometric mean antibody titers of mumps neutralizing antibodies obtained from MNT are given in Table 2. As are shown in this table among 913 serum samples tested 299 (32.7%) had no neutralizing antibodies against the virus and 614 (67.3%) were found to be positive,with titers from 1/2 to 1/32 . The percentage of samples having neutralizing antibodies varied from 78.5 for age 13 to 59.2 for age 7. However, the presence of mumps neutralizing antibodies, was in general similar in both sexes. The percentage of positive sera obtained from girls and boys was 67.7 and 66.7 respectively.

The highest percentage of positive sera(79%)obtained from females was recorded for age 13, and the lowest percentage of positive ones (50%) was recorded for age group 18-20 . In order to investigate the above result more accurately, 67 serum samples from dughter student aged 16-17 and 58 sera from age group of 18-20, collected two years before starting this study, were tested separately. The results showed taht 69.5% and 75% of the sera were positive respectively. As far as the sera from males were concerned the lowest positive percentage (48.6%) was recorded for age 16 and the highest positive percentage (77.8%) obtained from age 13. The geometic mean antibody

titers (GMT) were lowest among students of 16 years old in both sexes. The highest G.M.T. were 6.3 and 11.7 for 17 years old female and 7 years old male respectively . However, as are indicated in table 2, it was the same in both total populations.

Table 1. Distribution of study population by sex and age.

Sex / No. and % / Age	Female		Male		Total	
	Number	Percent	Number	Percent	Number	Percent
7	34	6.7	15	3.6	49	5.3
8	77	15.3	20	4.8	97	10.6
9	89	17.7	24	5.8	113	12.4
10	61	12.1	38	9.2	99	10.8
11	41	8.1	39	9.4	80	8.7
12	36	7.1	41	9.9	77	8.4
13	38	7.5	27	6.5	65	7.1
14	34	6.7	56	13.5	90	9.8
15	26	5.1	32	7.7	58	6.3
16	25	4.9	35	8.4	60	7
17	22	4.3	37	8.9	59	7
18-20	18	3.6	48	11.7	66	7.2
Total	501	100	412	100	913	100

Table 2. Results of MNT for mumps neutralizing antibodies by sex and age.

Sex / Age (years) / (No.), (%), GMT	Female				Male				Total			
	Tested (No.)	Positive test (No.)	(%)	GMT	Tested (No.)	Positive (No.)	test (%)	GNT	Tested (No.)	Positive test (No.)	(%)	GNT
7	34	18	53	5.0	15	11	73.3	11.7	49	29	59.2	6.94
8	77	47	61.1	5.8	20	13	65	9.0	97	60	61.8	6.4
9	89	58	65.2	5.3	24	17	71	5.4	113	75	66.4	5.6
10	61	40	65.6	4.8	38	24	63	5.7	99	64	65.0	5.0
11	41	29	70.8	5.9	39	25	64	3.5	80	54	67.5	4.6
12	36	26	72.2	3.9	41	30	73	4.189	77	56	73.0	4.0
13	38	30	79	4.0	27	21	78	4.134	65	51	78.5	4.0
14	34	27	79.2	3.8	56	39	70	4.5	90	66	73.3	4.2
15	26	19	73	3.9	32	18	56	5.0	58	37	64.0	4.6
16	25	19	76	3.2	35	17	49	3.4	60	36	60.0	3.3
17	22	17	77.2	6.3	37	25	68	4.8	59	42	71.2	5.3
18-20	18	9	50	4.0	48	35	73	4.5	66	44	66.7	4.4
Total	501	339	67.7	4.8	412	275	66.7	4.8	913	614	67.3	4.8

Comparison of MNT and HAdI for screening of antibodies to mumps virus. Serum samples (149)were tested in MNT and HAdI for antibodies to mumps. The results are shown in Table 3. In 79 cases antibodies were detected by both methods; i,e., neutralizing antibody titers of 2 or more and inhibition of RBC adsorption to inoculated cells were demonstrable. Twenty-six samples were negative in both tests. Seven serum samples inhibited adsorption of RBC to the cells but were negative in MNT. Finally , of 37 serum samples with neutralizing antibody titers of 2 or more, all were negative in HAdI.

Table 3. Results of MNT and HAdI tests for detection of antibodies to mumps virus.

MNT \ HAdI	Positive	Negative	Total
Positive	79	37	116
Negative	7	26	33
Total	86	63	149

Comparison of MNT an IIF for screening of antibodies to mumps virus. Seventy-seven sera were tested in MNT an IIF for antibodies to mumps. The results are shown in Table 4. In 48 cases antibodies were detected by both methods. Five cases were negative in both tests. Four sera were positive in IIF and negative in MNT . Finally, none of 20 serum samples with neutralizing antibody ti- ter of 2 or more was positive in IIF.

Table 4. **Results of MNT and IIF method for detection of antibodies to mumps virus.**

IIF / MNT	Positive	Negative	Total
Positive	48	20	68
Negative	4	5	9
Total	52	25	77

DISCUSSION

The causative agent of mumps is present through out the world , and most people ultimately become infected by the time they are young. Approximately a third of the cases, particularly in infants or children, are asymptomatic (1). Postpubescent children and adults tend to have more overt, clinically apparant multiple organ involve - ment (8). As far as the presence of the disease in Iran is concerned rise in the neutralizing antibodies to mumps was first demonstrated in sera of two patients with pacreatitis in 1973 (9). The virus was isolated form a patient's saliva 5 years later (10).

Following infection with the virus, CF,neutralizing, and HI antibodies are formed. Neutralizing antibodies appear several weeks following infection and persist for long time albeit at low levels. The neutralizing antibodies correlate best with immune status, and the serum neutralization test has been claimed to be one of the most sensitive test for measuring mumps antibodies(11,12,13,14) .

In this study microneutralization test was applied for measuting mumps antibodies in the sera of 913 students from south of Tehran. If titer of $\frac{1}{2}$ or greater confers some definite immunity (7,15) 67.3% of students tested were immune. Because there was no available data on vaccination status of the students against mumps it was not possible to say if the antibodies were created in response to natural infection or immunization. It is necessary to do further clinical, epidemiological, and sero-epidemiological surveys inorder to determine the patterns of susceptibility and immunity within a community.

Since neutralization tests are generally satisfactory for determining the immune status and are not considered to be practical as a routine diagnostic procedure, hemadsorption-inhibition test and the indirect fluorescent antibody technique were used to detect the mumps antibodies in this study. Both tests showed less sensitivity and had lower specificity, comparing with MNT. One reason for the lower susceptibility of the HAdI can be attributed to the presence of share antigenic components between mumps virus and parainfluenza viruses(16). As a result of this antigenic relationship antibodies against para - influenza viruses may react with mumps antigen. As far as IIF is concerned, Brown, et al. used this method for detection of mumps antibodies in human sera at the time of onset. These antibodies persisted unchanged for at least three months and then reduced (5). It is suggested that at the time of taking blood samples for this study the above antibodies had reached to a nondetectable level in some cases. And it might be the reason for the lower susceptibility of the test. On the other hand low specificity

of the test could conceivably represent cross-reactions between parainfluenza virus antibodies and mumps antigens (5).

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