Acta Pharmaceutica Turcica
XXXX (3) 141-144 (1998)

APPLICATION OF ELISA AND HAI TEST FOR THE DETECTION OF MUMPS VIRUS ANTIBODIES

KABAKULAK VİRUSU ANTİKORLARININ SAPTANMASINDA ELİSA VE HAİ TESTİNİN UYGULANMASI

NURTEN ALTANLAR, AHMET AKIN

Department of Pharmaceutical Microbiology, Faculty of Pharmacy,
Ankara University, 06100, Ankara- Turkey

In this study, Mumps specific antibodies were investigated with ELISA and HAI test in 405 sera obtained from different age groups. Specific viral antigen was prepared with Mumps virus which was produced in vero cell. The control antigen was prepared in the same way.

In ELISA, indirect technique was applied by using polystyrene microtitre plates. Antigen, control antigen and serum dilutions were determined by using block titrations.

405 sera samples belonging to different age groups, specific antibodies first were tested by HAI test. In this test 347 out of 405 sera (85.7%) were proven seropositive, 58 (14.3%) sera were not found positive.

In total, using ELISA, out of 405 sera samples, 349 (86.2%) IgG were found positive and 16 (4%) of those were found to be IgM seropositive, 56 (13.8%) sera were found seronegative.

Bu çalışmada, farklı yaş gruplarından alınan 405 serumda ELISA ve Hemaglütinasyon İnhibisyon testi (HAI) ile Mumps spesifik antikorları araştırılmıştır. Spesifik viral antijen, vero hücresindeüretilen Mumps virusundan hazırlanmıştır. Kontrol antijen de aynı şekilde hazırlanmıştır.

ELISA'da polistiren mikropleytler kullanılarak indirekt teknik uygulanmıştır. Antijen ve kontrol antijen ve serum dilüsyonları blok titrasyon yapılarak saptanmıştır.

405 serumda spesifik antikorlar önce HAI testiyle belirlenmiştir. 347(%85.7) serumda seropozitiflik belirlenirken 58 (%14.3) serumda ise pozitiflik saptanmamıştır.

ELISA'da ise 349 (%86.2) serumde IgG ve bu 349 serum içinde 16(%4) serumda aynı zamanda IgM pozitifligi belirlenirken, 56 (%13.8) serumda ise pozitiflik bulunmamıştır.

Keywords : *Mumps Virus; ELISA; Hemagglutination Inhibition Test*

Anahtar kelimeler : *Kabakulak virusu; ELISA; Hemaglütinasyon Önlenim Testi*

Introduction

Mumps virus is a member of the paramyx oviridae family from the paramyxovirus genus (3,5,6,8). It is synthesized in the cytoplasm, but assembly occurs the cell surface (6). Mumps virus has single-stranded RNA and a diameter of 150-200 nm. Nucleocapsid is enclosed by an envelope and the envelope is composed of a matrix protein and two surface glycoproteins, the hemagglutinin-neuraminidase and the fusion factor (2,3,9,17,18). Only one serotype of Mumps virus is known (6,17).

Mumps is an acute generalized viral infection that occurs at childhood. Bilateral and unilateral parotitis is the most common clinical feature. Mumps virus has important complications as menengitis and epididimo-orchitis. Orchitis complication is important especially for adult men because patients with bilateral orchitis have shown 10% sterility. Mumps virus may produce 30% subclinical infection (9).

For determination of Mumps, different immunity tests have been described. As the technique is highly sensitive and requires only one serum sample, ELISA is the preferred method for determination of specific IgM and IgG antibodies (4,10,12,14,15).

In this study, ELISA and HAI methods were compared for measuring Mumps virus antibodies.

Vero cell infected by Mumps virus were used as antigen in ELISA and HAI. The assays were carried out in microtiter plates. 405 sera samples belonging to different age groups, specific to IgG and IgM antibodies were investigated with ELISA which was prepared in our laboratory.

Materials and Methods

Serum Specimens

405 serum samples were collected from the diagnostic laboratories of GATA Haydarpaşa Education Hospital Microbiology Department. The sera were kept frozen at 40°C.

Prepation of Antigens

The Enders strain of Mumps virus was propagated in vero cell line. The cells were incubated at 37°C and harvested when 75-100% cytophatic effect was observed, the infected vero cell line were frozen and thawed three times and then centrifuged at 5000 r.p.m. for 10 min. at 4°C. Non infected vero cells prepared in the same way were used as antigen control.

Determination of Mumps antigens optimum dilution for ELISA

The optimal concentrations of the Mumps antigen were determined by chequerboard titrations using known positive and negative sera and they were tested at 1/100, 1/200, 1/400, 1/800 dilutions. The Mumps antigen were diluted to 1/4, 1/8, 1/16.

Antigen Coating

The Mumps antigen were diluted to 1/16 liter in 0.1 M bicarbonate buffer solution at pH 9.6. Polystyrene flat bottomed microtiter plates were coated with Mumps antigen (100 µl/well). After overnigt incubation at 4°C in a wet chamber, antigen solutions were aspirated and washed three times with washing buffer (containing PBS pH 7.2-0.05%Tween 20). After this, blocked unbound sites on the plates by incubating 1% milk powder (100 µl/well) for 1h at 37°C and were washed three times with washing buffer. The plates were sealed with tape and kept at room temperature for 18 h and then stored at 4°C until used.

Application of ELISA

Because of false positive results, serum samples were adsorbed to vero cell line. In the ELISA test, one-high-titer positive, one low-titer positive two negative serum samples by HAI were included. The negative samples were from two children with no history of Mumps infection or Mumps vaccination.

In the ELISA assay, serum samples were diluted to 1/100 with PBS containing 1% bovine serum albumin (BSA) and 0.05% Tween 20 for the detection of Mumps IgG and IgM antibodies. Serum samples were incubated for 1h at 37°C, washed three times with PBS- Tween 20 and 100 µl purified peroxidase conjugated goat anti-human IgG and IgM at 1/1000 dilutions were added to each well. They were incubated for 1h at 37°C and washed three times with PBS- Tween 20. The substrate solution (OPD) was added and the mixture was allowed to react for 30 min at 37°C. After 30 min 50 µl 1N H_2SO_4 were added to the wells. OD values were measured with a Bio Tek Instrument Micropleyt Reader at 492 nm (11,14).

The IgM assay was performed after all serum was absorbed with anti-human IgG to eliminate interference of rheumatoid factor and diminish competition of specific IgG. Positive and negative controls were included in the assay. Titrations and incubation times were as for the IgG assay.

Hemagglutination inhibition test (HAI)

Before HAI test nonspecific inhibitors and heterofil antibodies of sera were removed by heat inactivation, guinea pig red blood cells (RBCs) and sodium periodate. Serial twofold dilutions of treated sera, from 1/10 to 1/10240, were tested in a standart HAI test with 4HA units of virus and 0.5% suspension of guinea pig RBCs in phosphate-buffered saline. The HAI was the reciprocal of the highest dilution of serum that completely inhibited agglutination of RBCs.

Results and Discussion

Results by the ELISA assay

Seropositivity and seronegativity of Mumps IgG and IgM antibodies according to age are shown in Table 1.

Table 1. Distribution of Mumps antibodies in different age groups with ELISA

Age Groups	Negative (%)		Positive (%)		Total
	IgM	IgG	IgM	IgG	
0 - 15	47 (88.7)	12 (22.6)	6 (11.3)	41 (77.4)	53
16 - 30	150 (97.4)	20 (13.0)	4 (2.6)	134 (87.0)	154
31 - 45	48 (100.0)	5 (10.4)	0 (0.0)	43 (89.6)	48
46 - 60	31 (100.0)	4 (12.9)	0 (0.0)	27 (87.1)	31
>60	17 (100.0)	4 (23.5)	0 (0.0)	13 (76.5)	17
Pregnant	96 (94.1)	11 (10.8)	6 (5.9)	91 (89.2)	102
Total	389 (96.0)	56 (13.8)	16 (4.0)	349 (86.2)	405

As seen in Table 1, 405 sera samples were tested for Mumps IgG and IgM antibodies by ELISA test and 349 (86.2%) of those were found IgG positive and 56 (13.8%) sera were IgM seronegative. Seropositivity was also observed as 16 (4.0%) for IgM in 349 sera samples. In pregnant women, seropositivity was observed as 91 (89.2%) for IgG, 6(5.9%) for IgM in 102 sera samples. The percentage of positivity was 77.4% among 0-15 year old and over 80% in the group aged 16 years and older. The rate of seropositivity increased

with age. The highest positivity was found at the age group 31-45 years (89.6%), with older ages, positivity became less (at the ages over 60).

The ratio of seropositivity and seronegativity are shown in Table 2.

Table 2. Numerical and percentage distribution of Mumps antibodies seropositive and seronegative in different age groups

Age Groups	SEROPOSITIVE		SERONEGATIVE		Total Sera
	Number	%	Number	%	
0-15	45	84.9	8	15.1	53
16-30	133	86.4	21	13.6	154
31-45	42	87.5	6	12.5	48
46-60	26	83.9	5	16.1	31
>60	12	70.6	5	29.4	17
Pregnant	89	87.3	13	12.7	102
Total	347	85.7	58	14.3	405

The ratios of seropositivity and seronegativity were found as 85.7% (347/405), and 14.3% (58/405) respectively in different age groups by HAI test. The percentage of positivity was 84.9% between the ages 0-15 years. These results demonstrate that Mumps infections are widespread during childhood.

ELISA appeared to be more sensitive than HAI test. HAI and ELISA are both easy to perform.

Although we found the Mumps HAI to be less sensitive than Mumps ELISA with HAI as a standart, ELISA showed a sensitivity and specificity of 99.0% and 83.0% respectively.

ELISA has proved to be superior as it needs a single serum for determining IgG and IgM antibodies separately. While ELISA is more sensitive than the other methods, it can also produce nonspecific reactions. These nonspecific reactions were prevented with Tween 20, nonionic detergent, BSA, gelatin and milk powder or non reactive protein dilution. In our study, Tween 20, BSA and milk powder were used (13,19).

Reference positive and negative sera samples were used for ELISA. In order to obtain confident results, the control antigen must also be tested. Non infected cell line was used as control antigen, to prevent this type of nonspecific reactions, noninfected vero cell were added at 1/2 dilution to the sera.

Scholten et al. (1980) proved that ELISA was a specific test for determining the IgG and IgM antibodies against Mumps virus at routine laboratory conditions (15).

In our study it was determined that ELISA gives the results in a short time and was more sensitive than the other test.

Shebab (1984) and Fedova et al. (1987) used ELISA and proved it's sensitivity against Mumps virus (10,16).

According to our results, the Mumps sensitivity in children was found to be higher than the adults.

The results of our study were compared with the results of Abuherfeil (1989), Benito (1987), Capner (1988) and were found to be in agreement (1,4,7).

Among 405 sera tested, 48 (11.9%) were negative and 342 (84.4%) positive in both test, while 10 (2.5%) sera were positive in ELISA but negative by the HAI.5 (1.2%) sera were negative in ELISA but positive by HAI test (Table 3).

Table 3. Correlation between ELISA and HAI determination of Mumps virus antibodies

	HAI negative	HAI positive
ELISA negative	48	5
ELISA positive	10	342

A correlation of 96.3% was found between the ELISA and HAI tests. The specificity and rapidity of ELISA makes it suitable for routine use in the determination of Mumps virus antibodies in serum samples. Using Mumps antigen, produced in vero cells by us, the sensitivity and specificity of HAI and ELISA tests were proven consistent, but the results have shown ELISA as more reliable. ELISA test prepared in our laboratory costs less and gives similar results.

References

1. Abuherfeil, N., Shehade, A., Sukhon, E.L., Atmeh, R.: Int.J . Epidemiology 18, 690 (1989)
2. Akan, E.: Genel ve Özel Viroloji pp. 365-371 Desen Matbaacılık, Ankara 1990

3. Baum, G.S., Litman, N.: Mumps virus.In: Mandell, G., Douglas, R.G., Bennet, J.E. (Eds) Principles and Practice of Infectious Diseases, pp. 1260-1265 New York 1990
4. Benito, R.J.: J. Infectious Diseases 155, 156 (1987)
5. Black, F.: Measles and Mumps. In: N.R. Fahey, G.Friedman (Eds.)Manual of Clinical Laboratory Immunology, 4, pp. 596-599. Washington D.C. 1992
6. Burnett, W.G., Schuster, S.G.: Review of Pathogenic Microbiology 1, pp. 228-233, 1974
7. Capner, P.M., Wright, J., Miller, C., Miller, E.: British Medical J. 297, 770 (1988)
8. Çetin, E.T.: İnfeksiyon Hastalıkları 3, pp. 27-29 Çeliker Matbaası, İstanbul, 1979
9. Emond, R.T.D., Roland, H.A.K.: Infectious Diseases pp. 268-270 London 1990
10. Fedova, D., Bruckova, M.M., Plesnik, V., Slonim, D., Sejda, J., Svandova, E., Kubinova, I.: J. Hygiene Epidemiology 31, 409 (1987)
11. Juto, P., Settergen, B., Nilsson, M.L.: J. Infectious Diseases 5, 998 (1989)
12. Leinikki, P., Passila, S.:J. Clinical Pathology 29, 1116 (1976)
13. Liddell, J.E., Cryer, A.: A Practical Guide to Monoclonal Antibodies 156 pp. 53-63 New York, Toronto, 1991
14. Sakata, H., Hishiyama, M., Sugiura, A.: J. Clinical Microbiology 19 (1) 21 (1984)
15. Scholten, M.E.N., Ziegelmaier, R., Behrens, F., Höpken, W.: Medical Microbiology and Immunology 168, 81 (1980)
16. Shebab, Z.M., Brunell, A.P., Cobb, E.: J. Infectious Diseases 149 (5) 810 (1984)
17. Swierkosz, E.M.:Mumps virus. In: H. Isenberg, A. Balows, H. Shadomy, J.W. Hausler, L.K. Heirmann. (Eds)American Society for Microbiology, 5 pp. 912-917 Washington D.C. 1991
18. Örvell, C.: J. General Virology 41, 517 (1978)
19. Voller, A., Bartlett, A., Bidwell, A.: Bull. World Health Organization 53, 55(1976)

Accepted: 04.02.1998