Yonsei Medical Journal
Vol. 22, No. 1, 1981

A Measurement of Rubella Antibodies among Korean Children by Enzyme-linked Immunosorbent Assay*

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We measured the degree of immunity of 326 Korean children to rubella virus by enzyme-linked immunosorbent assy (ELISA). They were admitted to Pediatrics Unit of Yonsei Medical Center between April and July, 1980 with various illnesses excluding rubella disease. Among the 326 tested, 172 cases gave a positive titer of antibodies (mainly, IgG and IgM antibodies) and 127 had IgG antibody against rubella virus antigen. These represented 52.8% and 39.0%, respectively, of the total number of children tested. There was no significant difference in the rate of positivity between sex, but the positive rate increased as the age increased. The antobody titers of positive individuals to rubella virus were higher among the older children. Results and a brief outline of the ELISA method for serodiagnosis of rubella in clinical use will be discussed.

Infection by rubella virus, one of self-limited communicable diseases, is often characterised by mild constitutional symptoms, such as fever, malaise, rash and enlargement of superficial lymphnodes (Park and Good, 1974). But intrauterine infection, especially during the first trimester of pregnancy may cause not only intrauterine growth retardation, but also multiple congenital birth defects such as cataracts, microcephalus, deafness, and venticular septal defects. Rubella infection is considered as one of diseases yielding discomfort to an individual and family as well as the community and nation. Thus, the major concern of rubella research and immunization programs is to protect females of child-bearing age from its infection in order to minimize incidence of congenitally handicapped people.

Rubella infection, probably transmitted by respiratory droplets, is more prevalent in late winter and spring. Recovery from the infection yields a long-lasting immunity due to immunoglobulin M and G antibodies, so an apparent infection or sporadic exposure to the causative agent in childhood or during the early adolescent perio will provide an adequate protection to an individual. Also, susceptible individuals

Received June 27, 1981
*This study was supported by a CMB research grant (1979-1981).

may obtain immunity to a certain extent against rubella infection by vaccination with a preparation of live virus, but this is not recommended for female individuals who are currently pregnant or suspected of pregnancy.

As one of the assured ways to prevent or to reduce the incidence of congenital birth defects due to intrauterine infection of rubella virus, proper vaccination to the vulnerable female population prior to be pregnancy should be done.

In Korea, there are a great number of congenital anormalies, but there is lack of survey on the etiology. Therefore, need of a systemic survey on the causes of congenital birth defects is great. The Evaluation of the immune status of the Korean population, particulary of young female adults to rubella virus will meet such a need to a certain extent. This is surely providing pieces of information in setting up a proper vaccination program for the mother-to-be females as a preventive measure of intrauterine infection of rubella virus.

Since isolation of rubella virus in 1962 (Parkman, *et al,* 1962), a number of tests for detecting antibodies to rubella virus, such as hemagglutination inhibition (HAI) test (Stewart, *et al,* 1967, Liebhaber, 1970), virus neutralization (Nt) test (Rawls, *et al,* 1967, Sato, *et al,* 1979), indirect immunoflourescent (IF) technique (Brown, *et al,* 1964), complement fixation (CF) assay (Sever, *et al,* 1965) and radioimmunoassay (RIA) (Kalimo, *et al,* 1976) were developed, but each test has some disavantages or limitations in routine clinical use. Recently, a simple and sensitive method of enzyme-linked immunosorbent assay (ELISA) for quantitation of rubella antibody was devised (Engvall and Perlmann, 1972, Voller and Bidwell, 1975), which offers some advantages over the HAI, Nt, IF, CF and RIA tests (Gravell, *et al,* 1977).

The purpose of the present study is to introduce the ELISA method and to evaluate immune status of different age groups of Korean children by this method with an aim to assess the applicability of ELISA method in routine clinical use and in estimating herd-immunity of population.

METERIAL AND METHOD

Sera

Serum specimens were obtained from the patients (new born to 15 year-old) admitted to the Pediatrics unit of Yonsei Medical Center, Seoul, Korea between April and July, 1980. None of them were considered as rubella patients.

Rubella virus antigen and reference antisera

Rubella virus antigen, rubella human high titered, low titered and negative reference antisera were purchased from M.A. Bioproducts, Walkersville, MD, USA. The high titered and low titered reference sera were equivalent to 1:128 or greater and 1:16 to 1:32 by hemagglutination inhibition (HAI) test, respectively. The negative reference antiserum is less than 1:8 by HAI test. We followed the same criteria for positiveness, high and low titers of rubella antibodies for serum specimens (Bidwell *et al.*, 1977).

Enzyme-linked antiglobulin conjugates and substrate

Alkaline phosphate conjugated sheep anti-human immunoglobulin and alkaline phosphates conjugated rabbit anti-human IgG were also purchased from M.A. Bioproducts. Substrate solution of enzyme was prepared by dissolving five mg of one tablet of para-nitrophenyl phosphate (= Sigma 104 Substrate, Sigma Chemical Co., St. Louis. Mo. USA) in 5 ml of 10% diethanolamine buffer (pH, 9.8) (Lee, 1978)

ELISA Procedures

The method described by Voller *et al.*, (Voller, *et al.*, 1976) and Forghani and Schmidt (Forghani & Schmidt, 1979) was employed in the experiments. The optimal dilutions of rubella virus antigen and enzyme-conjugated antisera were determined by preliminary block titration against corresponding antisera or virus antigen. The optimal dilution of virus antigen was prepared in 0.006M bicarbonate coating buffer, pH 9.6 and added to 0.2 ml of antigen solution to a well of disposable polystylene microplate in duplicate (Micro ELISA® 96-well U-plate, Cooke Laboratory Products, Division of Dynatech, Alenxandria, Va, USA). The plates were kept overnight at 4°C and the unabsorbed antigen was washed out by empting and filling the plate wells three times with 0.01 M phosphate buffered-saline, pH 7.4, containing 0.05% Tween-20 as well as 0.02% sodium azide (PBS-Tween).

For the test proper, positive and negative reference sera were diluted in PBS-Tween buffer (1:400 and 1:800 for the high titered serum, 1:200 and 1:400 for the low titered serum and 1:100 and 1:200 for the negative control serum). Patient sera were also diluted in PBS-Tween to 1:100 for the primary screening test for rubella antibodies and for the quantitation of rubella antibodies in patient sera, 1:200, 1:400 and 1:800 dilutions of testing sera were made. Two-hundred ul of each of the diluted sera was added to the antigen-coated wells and the plates were incubated for 2 hours at room temperature in a humid chamber and then, the plates were washed with PBS-Tween same as before. The optimal dilution of freshly diluted enzyme-conjugated anti-human sera in a volume of 200-μl was added to wells *in lieu* of antigen-test sera and incubated overnight at 4°C in a humid chamber. The following day the wells of plates were washed three times with PBS-Tween as before. Two-hundred-μl of the enzyme substrate solution (1 mg of para-nitrophenyl phosphate/ml) prepared in 10% diethanolamine-10^{-3} M $MgCl_2$ buffer, pH. 9.8 was added to the well and incubation of plate was carried out at room temperature for 30 minutes. The enzyme reaction was stopped by adding 50 μl of 3N NaOH. The intensity of color of the reaction was read at 405nm in a spectrophotometer (ELISA reader, microtiter,® Dynatech Laboratories, LTD, Sussex, UK). The sole substrate solution was used as the blank of spectrophotometry.

RESULTS

Sera from 326 Korean children who were admitted to Pediatrics Unit of Yonsei Medical Center with various illnesses were subjected to the measurement of rubella antibody titer by ELISA method. Table 1 represents the distribution of postitive cases of total rubella immunoglobulins, both of IgG and IgM, of testees by different age groups, whereas Table 2 is more specifically for only rubella IgG. On the basis of the data in Table 1 and 2, one would admit that the positive rate of antibody titer to rubella virus gradually increased with increased age, regardless of sex. For instance, there is only 25% of positiveness of rubella IgM and IgM antibodies for the under one month group, but 84% for the age group of 10-15 year-old (Table 1). In the case of rubella IgG, there is about 20% and 76% of positiveness for the under one month and the 10-15 year-old groups, respectively (Table 2). It is more clearly depicted in Figure 1 and 2.

Not only the positive rate of rubella antibody in patients gradually increased by age, but also the incidence of a higher titer was more frequent among the older groups as observed in Table 3 and 4. For instance, 8 among 55 positive cases for rubella antibodies up to one year-old showed

Table 1. Distribution of Rubella Antibodies (Ig M + Ig G) by Age Group

Age group	Male			Female			Total		
	Total no.	Positive no.	+ %	Total no.	Positive no.	+ %	Total no.	Positive no.	+ %
under 1 month	29	7	24.1	27	7	25.6	56	14	25.0
1 month-1 yr	68	29	42.6	29	12	41.4	97	41	42.3
1yr-6yrs	58	38	65.5	34	18	52.9	92	56	60.9
6yrs-10yrs	37	28	75.7	19	12	63.2	56	40	71.4
10yrs-15yrs	13	10	76.9	12	11	91.7	25	21	84.0
Total No.	205	112	54.6	112	60	49.6	326	172	52.8

$p < 0.01$

Table 2. Distribution of Rubella Ig G Antibody by Age Group

Age group	Male			Female			Total		
	Total no.	Positive no.	+ %	Total no.	Positive no.	+ %	Total no.	Positive no.	+ %
under 1 month	29	5	17.2	27	6	22.2	56	11	19.6
1 month-1yr	68	15	22.1	29	8	27.6	97	23	23.8
1yr-6yrs	58	27	46.6	34	15	44.1	92	42	45.7
6yrs-10yrs	37	21	56.8	19	11	57.9	56	32	57.1
10yrs-15yrs	13	8	61.5	12	11	91.7	25	19	76.0
Total No.	205	76	37.1	121	51	42.1	326	127	39.0

$p < 0.01$

Table 3. Distribution of High-and low-titers of Rubella Antibody (IgG + IgM) by Age Group

Age Group	Male			Female		
	Total no.	No. of high titer (⩾ 1:32)*	No. of low titer (1:16 ⩽)*	Total no.	No. of high titer (⩾ 1:32)*	No. of low titer (1:16 ⩽)*
under 1 month	7	1	6	7	2	5
1 month-1 yr	29	1	28	12	4	8
1 yr-6 yrs	38	11	27	18	8	10
6 yrs-10 yrs	28	14	14	12	6	6
10 yrs-15 yrs	10	6	4	11	7	4
Total No.	112	33	79	60	27	33

* Titers of rubella antibody expressed which are equivalent to HAI titers.
We followed the same criteria made by Bidwell *et al.* (Bidwell *et al.*, 1977) for High-and low-titers of rubella antibody in the specimens.

Table 4. Distribution of High-and low-titers of Rubella IgG by Age Group

Age Group	Male			Female		
	Total no.	No. of high titers (≥ 1:32)*	No. of low titers (1:16 ≤)*	Total no.	No. of high titers (≥ 1:32)*	No. of low titers (1:16 ≤)*
under 1 month	5	1	4	6	1	5
1 month-1 yr	15	1	14	8	3	5
1 yr-6 yrs	27	5	22	15	6	9
6 yrs-10 yrs	21	13	8	11	6	5
10 yrs-15 yrs	8	6	2	11	7	4
Total No.	76	26	50	51	23	28

*Titers of rubella antibody expressed which are equivalent to HAI titers.
We followed the same criteria made by Bidwell *et al.* (Bidwell *et al.*, 1977) for high-and low-titers or rubella antibody in the specimens.

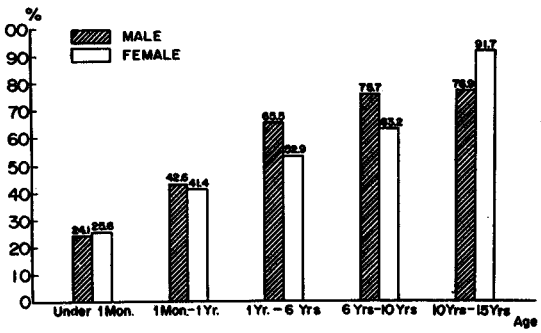

Fig. 1. Positive rates of rubella antibodies (IgG + IgM) by age groups.

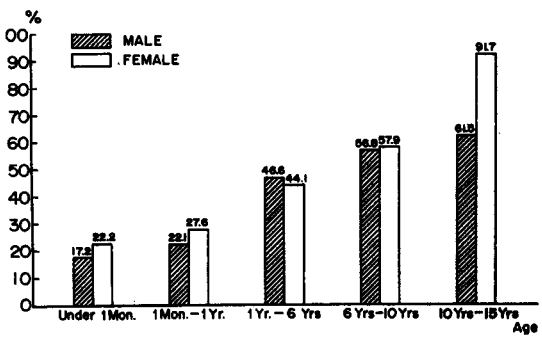

Fig. 2. Positive rates of rubella IgG antibody by age groups.

titers above 1:32, whereas 33 out of 61 positive cases between 6 and 15 year-old groups showed

Table 5. Distribution of Rubella IgM Antibody

Age group	Male	Female
under 1 month	1	1
1 month - 1 yr	5	2
1 yr - 6 yrs	11	3
6 yrs - 10 yrs	4	1
10 yrs - 15 yrs	2	0
Total	23	7
% *	20.5	11.7

* (No. of positive case of rubella IgM antibody/No. of positive case of total rubella antibodies (IgG + IgM)) x 100

the titers above 1:32 (Table 3). The same trend was also seen for rubella IgG antibody in Table 4. The contents of Table 5 are the positive cases of rubella IgM and these contents were derived from an assumption, that is, the difference in titers of each positive individuals for rubella antibodies (IgM and IgG) and only for rubella IgG may be theoretically considered as titers of rubella IgM antibody, although there is some experimental deviation. According to Table 5, only a minor portion of the population subjected

Table 6. Distribution of Rubella Antibody Titers (total immunoglobulins) by Age Group

Age group	Male						Female					
	High titers			Low titers			High titers			Low titers		
	1:128	1:64	1:32	1:16	1:12	1:10	1:128	1:64	1:32	1:16	1:12	1:10
⩽ 1 month	0	0	1	1	3	2	1	0	1	0	5	0
1 month-1 yr	1	0	0	4	8	16	1	1	2	2	2	4
1 yr-6 yrs	3	4	4	11	4	12	3	3	2	3	4	3
6 yrs-10 yrs	4	8	2	2	4	8	1	5	0	3	2	1
10 yrs-15 yrs	1	4	1	1	1	2	2	4	1	4	0	0
Total No.	9	16	8	19	20	40	8	13	6	12	13	8
		33			79			27			33	

Table 7. Distribution of Rubella Antibody Titers (IgG) by Age Group

Age group	Male						Female					
	High titers			Low titers			High titers			Low titers		
	1:128	1:64	1:32	1:16	1:12	1:10	1:128	1:64	1:32	1:16	1:12	1:10
⩽ 1 month	0	0	1	1	2	1	1	0	0	1	3	1
1 month-1 yr	0	1	0	4	5	5	0	2	1	3	2	0
1 yr-6 yrs	2	2	1	10	4	8	2	3	1	3	4	2
6 yrs-10 yrs	4	7	2	2	4	2	1	5	0	1	4	0
10 yrs-15 yrs	1	4	1	1	1	0	1	5	1	2	2	0
Total No.	7	14	5	18	16	16	5	15	3	10	15	3
		26			50			23			28	

to this test turned out to be positive for rubella IgM antibody. Table 6 and 7 are the distributing pattern of positive titers of rubella antobody (IgM and IgG) and that of IgG only of positive cases, respectively.

DISCUSSION

Since rubella virus infection is one of the self-limited diseases with mild constitutional symptoms of fever, malaise, rash, etc., the disease itself needs only symptomatic care and treatment, but intrauterine infection of the fetus especially during the first trimester, may yield multiple congenital defects in about 20% (Sever, *et al.*, 1969, Wasseman and Slobody, 1974). Rubella is considered as one of the air-borne diseases. It may occur widely in the world and often becomes epidemic, regardless of geographic environment.

In Korea, there is yet no official report about rubella, although it is generally admitted that the local epidemics of rubella occured, sporadically presumably with conceivable cases throughout the nation of intrauterine infection by rubella virus. One may understand the reasons for the lack of a report on rubella infection in Korea because the disease itself is very obscure and too easily confused to give a clear-cut diagnosis with only clinical symptoms

and the lack of a laboratory facility to identify the causative virus. Thus reports on rubella are lacking as well as the incidence of handicapped people due to congenital rubella. In this regard, one faces difficulties in planning preventive measures against congenital defects due to rubella virus.

The diagnosis of rubella based on the clinical symptoms is erroneous and not reliable comarped with the laboratory diagnosis. The laboratory diagnosis of rubella infection is possibly made by either isolation of rubella virus from the patient or a variety of serological tests for increase rubella antibody titer in the serum during or after infection. Isolation of the causative virus from patients is, of course, more direct and of firmative, but it requires a suitably equipped laboratory for virus culture. Also the isolation and identification of the virus is rather expensive and time consuming. The serological tests for rubella antibody are less accurate than the isolation of the causative virus for the diagnosis of active infection, but it is more common as a tool of laboratory diagnosis with many advantages over the virus isolation. Also the serological tests are useful in detecting the non-immune individuals as well as estimating general herd-immunity of the population. A number of methods for detecting rubella antibody are now available, but each method has some advantages as well as some limitations. Among the variety of the bewildering serologic tests for viral antibidies, the recently devised ELISA method (Engvall and Perlman, 1971, Voller *et al.*, 1976), is rather simple, sensitive and rapid compared with other methods. Thus it might be applicable for routine clinical use or measuring general herd-immunity against rubella virus. Like the others, the ELISA method also has a limitation in that the antibody titers measured by the method are expressed with correlation to the comparative titers to those of the positive reference sera measured by other methods, i.e. hemagglutination inhibition test or complement fixation test (Forghani, *et al.*, 1978). The positive serum should be obtained from a very reliable source.

In the present study, we measured the immune state of Korean children to rubella virus by the ELISA method prior to a systematic estimation of immunity in the child-bearing age group of female population. This is a report on the first attempts of employing the ELISA method in measuring viral antibodies in Korea. Eventhough the subjects of this study were the children who were admitted to be in bed in the Pediatric Unit of Yonsei Medical Center, none of them were suspected of having rubella, therefore, we assume that data obtained from the study would not be biased in projecting a whole picture. As mentioned earlier, there is an increase in the positive rate of rubella immunity and higher titers among the older children. This is somewhat compatible with the report on hemagglutination inhibition antibodies of rubella virus among Koreans made by Kim in 1980. On the basis of such trends one can easily assume that there are occasional local epidemics of rubella in the child population and children get immunity during such occasions. There is no doubt that susceptible woman in early pregnancy have the same chance of being exposed to the virus during rubella epidemics thus, causing congenital defects in the fetus as well as in postnatal life.

Through the study, we assessed the applicability of the ELISA method to measure the rubella antibody titer of routine specimens with respect to the possibility of handling large numbers of specimens, reagent supply, equipment, cost, etc., and we came to the conclusion that serodiagnosis of rubella infection as well as the estimation of the immune status of the population to rubella virus by the ELISA method

could be easily done with the facilities we possess.

Thus, we are able to do laboratory serodiagnosis to determine if the pregnant women with mild constitutional symptoms are truly prevent the mishaps of congenital anormalies. Moreover, we can measure the immune status of the population in the child-bearing ages and on the basis of such a survey, a proper vaccination program could be recommended to one without adquate immunity to rubella infection.

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