Rubella immune status of pregnant and non-pregnant women in Istanbul, Turkey

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ABSTRACT

Objective: Rubella immunization rates are not optimal and infections during pregnancy still occur since many countries incorporate no rubella vaccine in their national immunization program. The evaluation of immunity to rubella virus relies on the presence of specific antibodies. This study was undertaken to determine in a cross-sectional survey whether rubella virus circulation in the Istanbul city, induces detectable immunoglobulin G (IgG) antibodies with a protective level, in a random group of pregnant and non-pregnant women.

Methods: One hundred and sixty women of 20-41-years of age (average 24-years) were grouped as follows: 1. Forty-eight married women. Among these were 41 pregnant women (33 delivered normally, 8 aborted). 2. One hundred and twelve single women. Samples were collected during the periods from October 2000 through to March 2001 and from November 2001 through to May 2002. Rubella specific IgG antibodies were detected (by the ELISA test) in all women tested.

Results: Quantitative analysis of the IgG levels showed noticeable variability that ranged between 24-143 IU/ml (average 94). One hundred and forty-five (91%) out of 160 women had rubella IgG levels of above 50 IU/ml with a range of 54-143 IU/ml (average 92) while 15 (9%) had a level between 24-46 IU/ml (average 38). Rubella IgG-avidity test revealed that 116 (73%) of women had high IgG avidity, 22 (14%) had intermediate avidity and 20 (13%) showed low avidity. Two women who were IgM positive, each had either high or intermediate IgG avidity.

Conclusion: All women tested were seropositive for rubella specific IgG antibodies suggestive of natural virus circulation within the community. Although the majority appeared to possess protective level of such antibodies, screening for protective immunity appears always to be a necessity for future protection against reinfection.

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Rubella virus infection usually causes a mild disease in humans, but infection during early pregnancy often leads to severe congenital abnormalities.[1-3] Although the incidence of such abnormalities has declined considerably as a consequence of rubella immunization, the immunization rates are not optimal and infections during pregnancy still occur.[4] According to the world health organization (WHO) report,[5] only 105 (49%) of 214 countries had introduced rubella vaccine in their national immunization program. Protection against rubella virus infection and the determination of the immune status relies on the development of specific immunoglobulin G (IgG) antibodies following immunization or natural exposure.[6,7] However, reinfection is still common and could occur in both vaccinated and naturally exposed individuals with most cases being subclinical.[8-19] The detection of IgM on the other hand although being an indicator of acute infection, the interpretation of results requires further confirmation.[1-3] Thus, serology remains the method of choice for the diagnosis of rubella infections and for determination of the immune status and susceptibility.[1,2,20] In Turkey, routine rubella immunization of infants has not been adopted as of

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some concern that it might interrupt the circulation of the virus in the community, shift infection to the childbearing age and as a result, increase the risk of congenital abnormalities.[21] Earlier studies conducted in other cities in Turkey detected rubella IgG seropositivity, but with variable rates and did not determine the protective level.[21-24] Whether rubella virus circulation in the Istanbul metropolitan area (a city of >10 million population) induces detectable antibodies with a protective level, in a random group of pregnant and non-pregnant women, was investigated in this study. Rubella specific IgM antibodies were also examined and tested for non-specific cross reactivity with cytomegalovirus and toxoplasma.

Methods. A representative group of 160 women, with an average of 16 women from 10 different districts that covers wide residential areas in Istanbul, were enrolled in this study. Their age range was 18-41-years (average 24). The enrollment of women was based on being in continuous contact with children either according to their marital status (with children) or to the occupational status (teachers at elementary schools, nurses at children hospitals). Forty-eight were married women (23 housewives, 17 teachers, 8 nurses) of 20-41 years of age (average 26), among these 41 who attended the Sisli Etfal hospital in Istanbul for delivery were selected on residency bases. Thirty-three women had normal delivery while 8 had spontaneous abortion. The other 112 were single women (74 students, 32 teachers, 6 nurses) of 18-30-years of age (average 23) were selected from several schools located at different districts. Serum sample was obtained from each woman and stored at -20°C until used. Those from married women were collected during the period from October 2000 through to March 2001 and those from single women from November 2001 through to May 2002.

Enzyme-linked immunosorbent assay. The quantitative measurement of rubella specific IgG antibodies and the detection of rubella specific IgM was carried out using the ELISA kit (NOVUM Diagnostica GmbH, Germany). Rubella IgG titers of >25 IU/ml were considered positive, 10-25 IU/ml intermediate and <10 IU/ml as negative. Intermediate samples were retested. Sera were also examined for IgM and IgG antibodies to cytomegalovirus and toxoplasma by the ELISA kit (NOVUM Diagnostica GmbH, Germany). The tests were employed according to the manufacturers instructions. The washing steps were carried out using the ELISA washer EL x 50 (BIOTEK, United States of America (USA)) and the results were read using the ELISA reader EL x 800 (BIOTEK, USA). Sample dilutions were carried out according to the instruction manual and all sera were tested at the same time.

Rubella immunoglobulin G-avidity test. Immunoglobulin G avidity was determined by ELISA as described previously.[1] Briefly, the ELISA plates were first incubated with serum samples; then parallel wells were either washed with the usual washing buffer or with 5 M/l urea washing buffer 3 times for 5 minutes each. Enzyme-conjugates were added and the rest of the steps were completed as described by the instruction manual. The avidity index (AI) was calculated as the ratio between the optical-density (OD) of serum samples washed with urea-washing buffer and OD of serum samples washed with washing buffer without urea. Avidity indices <0.3 indicates low IgG avidity, intermediate for those of >0.3-<0.6 and high IgG avidity for those of >0.6.

Statistical analysis. Analysis of data was carried out using statistical package for social science 10.1. The difference between the groups was analyzed by the Chi-square. Statistical significance was set at a p value of <0.05.

Results. Quantitative analysis of the rubella specific IgG antibody levels revealed variable concentrations. Even though all women tested were seropositive for IgG, a wide range of IgG levels between 24-143 IU/ml was detected (**Table 1**). It appeared that 145 out of 160 women (representing 91% of the total women tested) had an IgG level of >50 IU/ml with a range of 54-143 IU/ml (average 92) that was statistically significant. The other 9% women were with <50 IU/ml (range 24-46 IU/ml/ average 38). The detection of rubella virus specific IgM antibody was shown in **Table 1**. Two out of 160 women were IgM-positive (one aborted, one single). Among the 8 women who aborted at 5 or 6 months of their pregnancies, only one who aborted at 2 months of pregnancy was IgM positive. She had an IgG level of above 50 IU/ml, high IgG avidity and no history of fever or contact with an infected child. Rubella avidity test carried out on sera positive or negative for IgM showed that one of the 2 IgM positive women (aborted) had high avidity IgG antibody and one single woman with intermediate avidity. The 158 IgM negative sera showed 116 (73%) with high IgG avidity, 22 (14%) with intermediate avidity and 20 (13%) with low avidity (**Table 2**). Sera tested for non-specific cross reactivity to cytomegalovirus or toxoplasma were all negative for IgM, but had detectable levels of IgG specific antibodies to cytomegalovirus in 148 samples and to toxoplasma in 120 samples (data not shown).

Discussion. The selection of women in this study was based on the fact that rubella reinfection is being asymptomatic in most cases and women might get reinfected through contact with their own

Table 1 - Detection of rubella specific immunoglobulin M and immunoglobulin G antibodies in sera of married and single women.

Subject	n tested	IgM + (%)	IgG values >50 IU/ml n (range/average)	IgG values <50 IU/ml n (range/average)
Married women				
Pregnant normal delivery	33	0 (0)	30 (54-129/91)	3 (31-39/37)
Aborted	8	1 (12)	7 (54-143/86)	1 (43-43/43)
Non pregnant	7	0 (0)	6 (77-115/94)	1 (37-37/37)
Single women	112	1 (2)	102 (67-135/96)	10 (24-46/35)
Total	**160**	**2 (2)**	**145 (54-143/92)**	**15 (24-46/38)**
$p<0.05$, immunoglobulin (Ig)				

Table 2 - Rubella immunoglobulin G-avidity test of women positive or negative for immunoglobulin M reactivity.

Avidity test	n of samples	Avidity index (%)[a] High	Intermediate	Low
with IgM +	2	1 (50)	1 (50)	-
with Ig M -	158	116 (73)	22 (14)	20 (13)
Total	**160**	**117 (73)**	**23 (14)**	**20 (13)**

Ig - immunoglobulin, [a]Avidity index: ratio of optical density of serum samples washed with or without urea. Index values of <0.3 indicate low IgG avidity > 0.3 and <0.6 are intermediate and >0.6 of high IgG avidity

children or with other children based on their occupation. Most rubella infections were found to occur in children of 5-15-years of age in countries where no routine vaccination program is applied.[21] Over 70% of the population of Izmir, Turkey was reported to acquire infection before 10-years of age (average 6), which is similar to averages reported for Brazil, Mexico, Poland and Scotland, while it was estimated to be around 8% for individuals between 15-29-years of age.[21] Thus, women in those countries are at continuous risk of contracting the infection. Serologic testing for rubella provides a useful basis for differentiating recent from previous infection and for determining immunity or susceptibility to infection.[2] Interpretation of our findings was based on the evaluations of results obtained from a previous study[1] and on the WHO reference preparations.[5] Immunity to rubella virus infection was thought to be represented when IgG values were greater than 25 IU/ml, usually above 50 - >200 IU/ml and negative sera reveal less than 10 IU/ml. Sera with titers between 10-25 IU/ml are rated as intermediate reactive.[1] According to the WHO[5] reference preparations of anti-rubella antiserum, IgG determination using 5 different commercial tests was found to be in the range of 91-126 IU/ml (average 107 IU/ml).[1] Our findings that 91% of all women tested had specific IgG levels above 50 IU/ml indicate that the majority of women were endowed with protective immunity against the virus and those with <50 IU/ml representing 9% were considered intermediate reactive.

In several serologic surveys conducted in 13 countries of the Americas from 1962 through to 1991, it was reported that individuals of both sexes who possessed anti-rubella antibodies showed a wide range of seropositivity between 20-100% and in pregnant women between 42-91%.[2] Prevalence of rubella IgG antibodies in Nigerian women revealed 77% positivity with measured titers in the range of 15-100 IU/ml.[25] Immunoglobulin G anti-rubella antibodies were found positive in 86% of serum samples obtained from 1802 pregnant women, 10% were intermediate while 4% had no antibodies.[1] In Hong Kong, 8-11% of women of childbearing age were found to be susceptible to rubella infection and that 14% of those women were negative for rubella IgG antibodies.[26] In another study, 6115 (87.7%) of hospital employees were screened for evidence of rubella immunity and the absence of immunity was identified in 325 (5.3%) employees.[27] So the potential still exists for reinfection in many countries and thus, screening for rubella antibodies appears to be an important practice. Unlike previous reports where susceptibility rates of 10-24% were reported in countries with no policy for rubella immunization being adopted,[5] in this study we have detected 100% seropositivity for IgG in all women tested, an indicator for wide spread of the virus within the Istanbul communities. Previous studies conducted in Turkey showed that 86.5% of women 15-29 years of age 21 and 90% of pregnant women[22] within the Izmir city region were IgG seropositive.

In another 2 studies from Ankara city, 98% and 82% seropositivity for pregnant women was reported.[23,24] It appears that virus circulation in the above mentioned cities was not efficient enough in the induction of detectable antibodies in certain percentages of the population and the authors advice vaccination of women who were negative. Recently Kanbur et al,[29] reported that based on past medical history, at least 34% of the seropositive study population of Turkey had sub-clinical rubella infection and they recommended a nationwide seroepidemiologic survey to determine age-specific rubella immunity. The diagnosis of rubella infections generally depends on the detection of IgM antibodies, usually within one week that disappears 6-8 weeks following infection.[1] However, several reports brought the attention to the persistence of such antibodies for periods that range from several months to even years after infection.[28,30,31] In a previous study, serum samples obtained from 1802 pregnant women showed specific IgM in 18% of them and the investigators recommended confirmation by a second independent test such as immunoblotting or avidity testing.[1] Similarly, IgM was detected in 16% of vaccinees for up to 3 years.[25] In our study, the detection of IgM in 2 of the women examined might suggest that these women either being reinfected with rubella virus or had persisted IgM reactivity. These women could not recall any previous fever episodes or contact with an infected child. The application of the differential assay of high avidity and low avidity IgG antibodies is considered an important alternative tool or to complement IgM antibody assay in assessment of rubella infection.[31,32] The detection of low avidity specific IgG is of value in the diagnosis of recent primary rubella infection or immunization. Immunity was thought to be present when high avidity IgG antibodies are present in sera of women following immunization or reinfection.[1] This is also true for both of our IgM-positive women who had high and intermediate IgG levels. Sera were also found negative for IgM non-specific reactivity against cytomegalovirus and toxoplasma in our study, which is in agreement with previous findings.[28]

In conclusion, all women tested were seropositive for rubella specific IgG antibodies suggestive of proper virus circulation within the community. The majority had an IgG level of above 50 IU/ml, which is as indicated earlier a predictor for protective immunity. Screening for protective maternal immunity appears always to be a necessity for future protection against reinfection.

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