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

Sebahat Seker, BS, MS, M. Fatih Abasiyanik, BS, MS, Barik A. Salih, MS, PhD.

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.

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
genital abnormalities.21-24 Earlier studies conducted
in other cities in Turkey detected rubella IgG
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 enrolment
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 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
Dagnostica 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 Dagnostica GmbH, Germany). The tests
were employed according to the manufacturer's
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. 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%
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
Rubella immune status in women ... Seker et al

WHO 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). 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%. Prevalence of rubella IgG antibodies in Nigerian women revealed 77% positivity with measured titers in the range of 15-100 IU/ml. 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. 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. 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. 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, in this study we have detected 100% seropositivity for IgG in all women tested, an indicator for widespread 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 within the Izmir city region were IgG seropositive.

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

<table>
<thead>
<tr>
<th>Subject</th>
<th>n tested</th>
<th>IgM + (%)</th>
<th>IgG values &gt;50 IU/ml n (range/average)</th>
<th>IgG values &lt;50 IU/ml n (range/average)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Married women</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant normal delivery</td>
<td>33</td>
<td>0 (0)</td>
<td>30 (54-129/91)</td>
<td>3 (31-39/37)</td>
</tr>
<tr>
<td>Aborted</td>
<td>8</td>
<td>1 (12)</td>
<td>7 (54-143/86)</td>
<td>1 (43-43/43)</td>
</tr>
<tr>
<td>Non pregnant</td>
<td>7</td>
<td>0 (0)</td>
<td>6 (77-115/94)</td>
<td>1 (37-37/37)</td>
</tr>
<tr>
<td><strong>Single women</strong></td>
<td>112</td>
<td>1 (2)</td>
<td>102 (67-135/96)</td>
<td>10 (24-46/35)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>160</td>
<td>2 (2)</td>
<td>145 (54-143/92)</td>
<td>15 (24-46/38)</td>
</tr>
</tbody>
</table>

p<0.05, immunoglobulin (Ig)

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

<table>
<thead>
<tr>
<th>Avidity test</th>
<th>n of samples</th>
<th>Avidity index (%)</th>
<th>High</th>
<th>Intermediate</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>with IgM +</td>
<td>2</td>
<td>1 (50)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with IgM -</td>
<td>158</td>
<td>116 (73)</td>
<td>22 (14)</td>
<td>20 (13)</td>
<td></td>
</tr>
</tbody>
</table>

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.
In another 2 studies from Ankara city, 98% and 82% seropositivity for pregnant women was reported. It appears that virus circulation in the above mentioned cities was not sufficient 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. 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. However, several reports brought the attention to the persistence of such antibodies for periods that range from several months to even years after infection. 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. Similarly, IgM was detected in 16% of vaccinees for up to 3 years. 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 to complement IgM antibody assay in assessment of rubella infection. 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. 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.

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.

Acknowledgment. The authors would like to thank the gynecologists at the Sisli Etfal hospital for providing the necessary information and for their help in collecting the samples.

References

Rubella immune status in women ... Seker et al