Rubella seroprevalence among Turkish adolescent girls living in Edirne, Turkey

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SUMMARY: Öner N, Vatansever Ü, Karasalihoğlu S, Tatman-Otkun M, Ekuklu G, Küçükuğurluoğlu Y. Rubella seroprevalence among Turkish adolescent girls living in Edirne, Turkey. Turk J Pediatr 2006; 48: 288-293.

This study was designed to estimate the rubella seroprevalence in unvaccinated Turkish adolescent girls in urban and rural areas of Edirne, and to create preventive strategies for congenital rubella syndrome (CRS).

The sample, representing 12- to 17-year-old adolescent girls, consisted of 1,600 subjects selected from school lists by systematic and random sampling, which was matched by age and urban-rural residency strata proportional to the corresponding distributions in the Edirne population. For each participant, a questionnaire was completed and rubella-specific IgG antibodies were measured.

After analysis of samples, seropositivity prevalence, equivocal and seronegative samples of adolescent girls in Edirne were determined as 93.1%, 0.6% and 6.3%, respectively.

Data from the present study may indicate that 6.9% of adolescent girls have considerable risk for rubella infection during pregnancy. Eliminating rubella and CRS in Turkey will require national health service efforts, including vaccination of all adolescents and all susceptible women of childbearing age.

Key words: *rubella, seroprevalence, adolescent girls.*

Rubella is a mild viral infection, caused by *Rubivirus* in the family Togaviridae and characterized by maculopapular rash, postauricular, occipital lymphadenopathy, conjunctivitis and coryza. It is highly contagious and can be epidemic and pandemic[1-3]. Before introduction of its vaccine in 1969, pandemics of rubella occurred every 6-9 years[3]. The most dramatic complication of rubella is congenital rubella syndrome (CRS), which affects infants whose mothers are non-immune for this infection. In CRS, the placenta and fetus are affected after maternal viremia and this results in abortion, stillbirth and some permanent manifestations in newborns such as deafness, heart disease, cataract, glaucoma, retinopathy, bone abnormalities, hepatitis, hemolytic anemia, thrombocytopenia, neuromotor retardation, diabetes mellitus, and progressive rubella encephalitis. Moreover, infants with CRS can spread viruses to the environment for many years[1-3].

Rubella immunity in adolescent girls should be a particular area of interest[4,5] because they are at a critical age of childbearing and, even in Turkey, some adolescent girls become pregnant during this period[6]. The aim of this study was to estimate the seroprevalence of rubella in a group of unvaccinated Turkish adolescent girls living in urban and rural areas of Edirne, and to create preventive strategies for CRS.

Material and Methods

Study Design and Subjects

Rubella IgG antibody titers were measured in sera collected from healthy Turkish adolescent girls, aged between 12-17 years and living in

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rural and urban areas of Edirne, between May and July 2001. At the end of 2000, the total population living in Edirne was 380,000, 3.6% of whom were adolescent girls aged between 12-17 years, the majority of whom (91.4%) attended secondary and high schools[7]. In order to estimate precisely the prevalence of rubella seropositivity in adolescent girls, the sample size was calculated by considering reported prevalence values using the Epi Info Program[8]. In the previous research studies, the prevalence of rubella seropositivity among adolescent girls varied from 75% to 95%[4,5,9-12]. In this study, the prevalence of rubella seropositivity, difference and alpha were accepted as 85%, ±2 and 5%, respectively. As a sum, a sample size of 1,225 subjects was required for the calculation of rubella seroprevalence. However, 1600 subjects were chosen to prevent any possible error.

The sample size we obtained for the study was determined by multistage sampling method that includes stratification according to ages (12 to 17 years) and living area and with systematic randomization from the school lists. The "rural areas" represented the villages and countryside, whereas "urban areas" represented towns and the Edirne city center. According to the population census of 2000, 34% of adolescent girls lived in rural areas, whereas 66% lived in urban areas[7]. All secondary and high schools of each area were included in the study and the number of students that were selected from each school was determined according to the total student population of each school by the following formula: [(Number of students of school/total number of students in Edirne) x the sample size)]. The next stage was the weighing of the adolescent girls who were chosen from the schools according to their ages using the following formula: [(Number of students in particular ages/total number of adolescent girls) x the sample size chosen from particular school]. Finally, the classrooms were chosen in systematic random basis, and each adolescent girl was determined randomly from the selected classrooms using a random number table. In each classroom, we selected substitutes (using random number table) (half the number of the selected adolescents) and those individuals were recruited in case of any non-participation of those on the main list (Fig. 1).

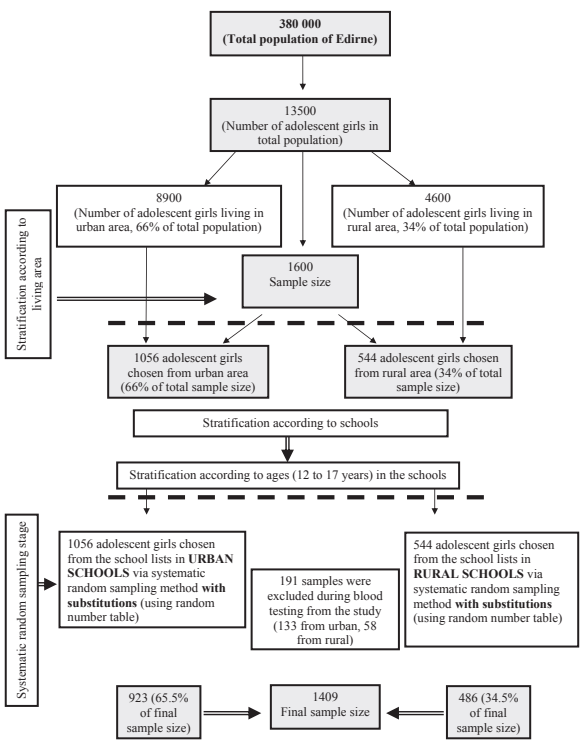

Fig. 1. Sampling methods of the study.

Screening Procedure and Questionnaire

After receiving the approval of the faculty ethics committee for the study procedure, the head teachers of the schools, local public health and education authority, adolescent girls and parents were informed about the study. Informed consent and questionnaire were obtained from both adolescent girls and their parents. The questionnaire included name, birth date and some demographic data such as medical history (any chronic illness), previous history of any exanthematous infection or rubella diagnosis by physician, and immunization status.

Laboratory Measurements

Blood samples were obtained from adolescent girls at school while in a fasting state in the morning. For the rubella IgG antibody titer determination, blood samples were centrifuged at 3500 rpm for 10 minutes and the supernatants were stored at –70°C until assayed. Serum rubella IgG antibody titers were quantitatively determined using Rubella IgG 2.0 kit (Abbott Laboratories, IL, USA), which is based on micro ELISA method. When rubella IgG antibody titers were determined as lower than 4.99 IU/ml, between 5 and 9.99 IU/ml, and higher than 10 IU/ml, they were accepted as seronegative (susceptible), equivocal and seropositive (immune) for rubella, respectively.

Statistics

Rubella seroprevalence was defined as the percentage of subjects whose serum rubella IgG antibody titers were higher than 10 IU/ml. The non-parametric ANOVA (Kruskal- Wallis) analysis was used to determine the significance of the difference for various ages. Rubella seropositivity was compared between rural and urban areas using the chi-square test. All calculations were performed using the Minitab (Release 13) statistical package (Reference number: wcp 133100197). A value of $p<0.05$ was considered as statistically significant.

Results

This study was conducted among 1,600 adolescent girls attending 79 different secondary and high schools in Edirne, Turkey. We took blood samples for rubella IgG antibody titers from 122 of selected substitutes [96 subjects from main list did not want to participate in the study, 14 suffered from chronic illness (renal failure, 2; malignancy, 3; type 1 diabetes mellitus, 2; severe asthma, 5; pulmonary tuberculosis, 1; Bruton agammaglobulinemia, 1) and 12 had rubella immunization]. One hundred and ninety-one blood samples in 1,600 were discarded and could not be analyzed due to inappropriate serum sample, such as hemolysis, insufficient sample, or sample lost during transportation. The final sample of this study included 1,409 adolescent girls, representing 88.1% of the original sample. After analysis of the samples, rubella seropositivity prevalence was determined as 93.1% (n=1312) Turkish adolescent girls between 12-17 years of age. Serum rubella IgG antibody titers were determined to be equivocal levels for 0.6% (n=8) and negative for 7.3% (n=89) (Fig. 2). Rubella seropositivity was found to be 93.7% and 92.8% in rural and urban areas, respectively. There was no statistical difference between adolescent girls living in urban and rural areas for rubella seroprevalence ($p>0.05$).

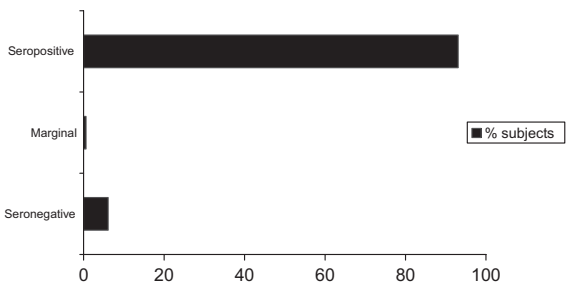

Fig. 2. The percentage of seropositive, equivocal and seronegative subjects.

Among our subjects, rubella seroprevalence rate increased with age. Rubella seroprevalences were 90.3%, 91.7%, 93.9%, 95.0%, 94.1% and 95.4% in 12, 13, 14, 15, 16 and 17 years of age adolescent girls, respectively. Rubella seroprevalence according to age is shown in Figure 3 and Table I.

Of the 1312 rubella seropositive adolescents, 157 (12%) had a past medical history of exanthematous disease, and the remaining 1155 subjects (88%) had no history. Only 34 (2.4%) of the participants had a history of rubella diagnosed by a physician.

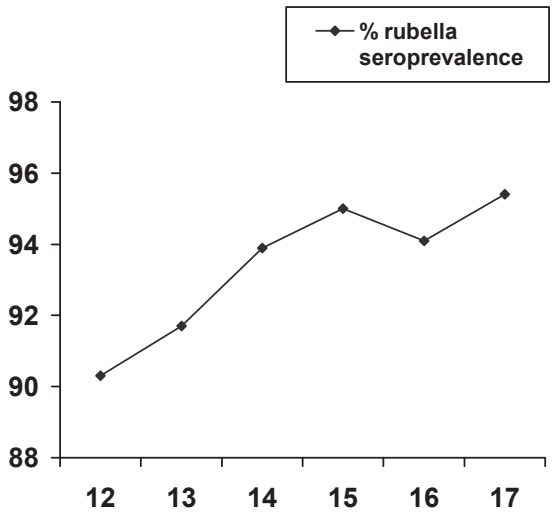

Fig. 3. The percentage of rubella seroprevalence according to age.

Researches associated with rubella seroprevalence have generally included pregnant women or those of childbearing age, and these were generally hospital-based researches[12,17]. In our country, there were two epidemiologic researches from the mid-west (Izmir) and south (Adana) of Turkey. Aksit et al.[9] reported seronegativity rates as 8% and 5% in adolescent girls between the ages 10-14 years and 15-19 years, respectively, in the Turkish population living in Izmir. Karakoç et al.[4] from Adana determined that 7.1% of adolescent girls were susceptible to rubella infection. Kanbur et al.[5] from the capital city of Ankara reported that the proportion of adolescent girls susceptible to rubella infection was 6.6% in 11-13 years old and 1.3% in 14-16 years old. Although their study was hospital-based and its representation was lower, given that the results of these four studies of Turkish adolescent girls do not remarkably differ, it can be said that the susceptibility of adolescent girls in Turkey confirms the high risk of infection during the childbearing period.

Table I. Seropositive, Equivocal and Seronegative Subjects

Age(years)	Seropositive	Equivocal	Seronegative
12	243 (90.3%)	1 (0.4%)	25 (9.3%)
13	278 (91.7%)	–	25 (8.3%)
14	247 (93.9%)	5 (1.9%)	11 (4.2%)
15	209 (95.0%)	–	11 (5.0%)
16	191 (94.1%)	1 (0.5%)	11 (5.4%)
17	144 (95.4%)	1 (0.7%)	6 (3.9%)

Discussion

The most important result of this study is that 6.9% of adolescent girls are still susceptible to rubella infection in our region. These girls are candidates for CRS if they are not immunized. Rubella outbreaks can cause common infection among the non-immunes, and during rubella outbreaks, CRS rate has been determined as between 0.7 to 2.2 in 1000 live births in different epidemiological studies[13-15]. CRS can cause social and healthcare problems. According to the European Advisory Group on the Expanded Program on Immunization of WHO, CRS should be well controlled or eliminated in all European countries by 2010 or earlier[16]. However, rubella vaccine has not yet been incorporated into the Turkish national immunization program. It has been available in the private sector for 15 years in combination with measles and mumps vaccine (MMR), and is used on a private physician's recommendation or upon parental request.

Edirne is located in northwest Turkey, on the border of northern Greece and southern Bulgaria. Gioula et al. (10) from northern Greece reported 22.9% of 11-to-15-year-old adolescents and 8.7% of 16-to-20-year-old adolescents were susceptible to rubella infection. However, 8.6% of 11-to-15-year-old adolescents and 15% of 16-to-20-year-old adolescents had been immunized with rubella vaccine. The main limitation of our study is underestimation of previous rubella infection in our subjects since rubella is a mild viral infection and its diagnosis is mainly clinical. Physicians do not feel the necessity for rubella serologic tests in search of certain conditions except in pregnancy.

Aksit et al.[9] reported that rubella seropositivity rate among rural dwellers was significantly lower than in those living in urban areas. They suggested that circulation of the rubella virus in the crowded urban population is easier than in the rural population. However, Kanbur et al.[5] reported that rubella seropositivity rate was not statistically different between rural vs. urban adolescent girls, and they attributed this result to the limited number of rural participants in their study. We also did not find any difference in rubella seroprevalence rate between rural vs. urban adolescent girls. This may be explained by the similarity of the crowdedness of each class in rural and urban areas as standardized by local education authorities.

Rubella vaccination is mostly performed in the period of 1 to 12 years of age[1-3,12-14]. However, protection time of the vaccine is not exactly known. Clinical efficacy and challenge studies indicate that >90% of vaccinees are protected against both clinical rubella and viremia for at least 15 years, and vaccine-induced protection is generally assumed to be lifelong[13,14,18]. In developed countries, initial MMR vaccinations are routinely performed at 12-15 months while second MMR vaccinations are given at 4-6 years of age. However, the second dose of MMR is performed primarily to boost measles immunization. Moreover, rubella and mumps vaccines do not have any additional side effects in those already-immunized children. In these countries, children who have not previously received the second rubella vaccine dose should be immunized by 11-12 years of age. Most rubella cases are observed in 5 to 15 years of age children in countries where rubella vaccination has not yet been included in the national immunization program[9,11,14,19-22]. The administration time of rubella vaccine in these countries, including Turkey, is still controversial. Like our research, many serologic studies with children in the last 20 years in Jordan[23], Nigeria[24], Yemen[25], Saudi Arabia[26], Libya[27] and Taiwan[28] have all shown an increase in rubella seropositivity with age. This situation may be explained by exposure risk also increasing with age. However, 6.9% of our subjects are still at risk for CRS.

The primary purpose of the rubella vaccine is to prevent CRS, and national policy for rubella vaccination should be based on seroprevalence of rubella infection and CRS. However, these data are still insufficient in Turkey. Moreover, national immunization schedules do not include routine rubella vaccination in our country. Preventive vaccination strategies for CRS would include childhood vaccination alone, selective vaccination of school girls and all women of childbearing age, and a mass campaign for adolescents with routine MMR[13]. These strategies carry many advantages and disadvantages. Protection from rubella infection with vaccine during childhood may not cover women in childbearing age, and this strategy may lead to an increase in CRS cases. Therefore, childhood vaccination alone is not acceptable. Furthermore, in developing countries where rubella is a disease of early childhood, vaccination could lead to an increase in the average age of rubella infection and increased risk of transmission to the pregnants[9]. Selective vaccination of school girls and all women of childbearing age will have very little effect on overall rubella transmission. Furthermore, reaching women of childbearing age is very difficult and pregnancy counseling must be performed in this group before vaccination. According to Plotkin et al.[29], vaccination of all infants will probably eradicate CRS in 30-40 years; vaccination of all schoolgirls will presumably eradicate CRS in 10-20 years; and vaccination of adult women will eradicate CRS immediately, but only if 100% of them are immunized. Performing an obligatory serological test against rubella before legal marriage may be the other preventive method of CRS. However, illegitimacy is also steadily increasing in many developing countries like Turkey.

We think that a mass campaign for rubella vaccination without routine serologic tests for adolescents is the best preventive method for CRS because it not only covers women of childbearing ages for the prevention of rubella, but also decreases overall rubella transmission. However, this strategy alone without vaccination of all women in childbearing ages will not prevent CRS for many years, until the cohorts that are vaccinated as adolescents reach childbearing age[20]. In addition, further cost effectiveness studies should be performed whether or not routine serological tests before rubella vaccination are required.

We present adolescent girls susceptible to rubella and at risk for CRS from a representative Edirne population. Further longitudinal studies

may be a useful adjunct to this seroprevalence study, including follow-up of susceptible subjects until the end of their childbearing ages and surveillance of CRS cases.

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