International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 9; Issue 02 (C); February 2020; Page No.21298-21301
DOI: http://dx.doi.org/10.24327/ijcar.2020.21301.4181

Research Article

SEROPREVALENCE OF RUBELLA ANTIBODIES AMONG SCHOOL GOING ADOLESCENT GIRLS IN THE AGE GROUP OF 10 TO 18YRS OF PUNE

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A R T I C L E I N F O

Article History:
Received 14th November, 2019
Received in revised form 29th December, 2019
Accepted 05th January, 2020
Published online 28th February, 2020

Key words:

Adolescent girl, Rubella Antibody, Pune

A B S T R A C T

Infection with rubella virus can be disastrous in early gestation. The classical triad for congenital rubella syndrome is: Sensorineural deafness, Eye abnormalities and congenital heart disease especially pulmonary artery stenosis and patent ductus arteriosus.

Material and Method: This cross sectional analytical study was conducted on 269 adolescent girls in Kendriya Vidyalaya (Central School) of Camp area in Pune.

Results: 55.01% girls mentioned their family background as Urban, while remaining 44.98% as Rural. In our study, 81.78% gave history of complete immunization and 18.21% girls gave a negative history i.e. either none or incomplete immunization. 71.37% were found positive of Rubella antibodies.

Conclusion: There are still substantial number of high risk population uncovered with immunization. Therefore it is requirement to increase the coverage of universal immunization program and also to intensify the 2 dosage of MR vaccine though ongoing campaign.

INTRODUCTION

Rubella, also called German measles, is a viral infection of children and adults, and most often occurs in late winter and early spring. Before rubella vaccine was used, children 5 to 9 years old accounted for most of the cases. Two German physicians first described rubella, in the mid-18th century.[1,2] Rubella virus was first isolated in 1962 by Parkman and Weller. This was a major historical development in the understanding of rubella, including isolation of virus, vaccine development, and WHO's recommendations for prevention of Congenital Rubella Syndrome (CRS). [3, 5, 6]

Infection with rubella virus can be disastrous in early gestation. The virus can virtually affect all organs and cause a variety of congenital defects. The classical triad for congenital rubella syndrome is: Sensorineural deafness, Eye abnormalities especially Cataract, retinopathy and microphthalmia and congenital heart disease especially pulmonary artery stenosis and patent ductus arteriosus. Serious Infection may even lead to fetal death, spontaneous abortion, or premature delivery. The severity of the effects of rubella virus on the fetus depends largely on the time of gestation at which infection occurs. Up to 85% of infants infected in the first trimester of pregnancy were found to be affected, if followed up after birth.

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[4] Infants with congenital rubella syndrome can transmit virus and can infect susceptible children till one year of age. [4-6].

The 1964 rubella pandemic in the U.S. caused over 12 million infections, 11,000 foetal losses, and 20,000 cases of CRS in infants [3]. After the introduction of Rubella vaccine in 1969, Rubella vaccination emerged as the most effective public health measure against congenital rubella infection. It was launched in universal immunization program which the incidence reported cases to a0.1/100,000 population and no indigenous CRS case by 1993-94 [2]. Maternal rubella is now rare in many developed countries due to rubella vaccination programmes. However, in many developing countries congenital rubella syndrome (CRS) remains a major cause of developmental anomalies. [7].

The endemicity of rubella has been well established in India. Prevalence of Rubella infection and CRS data in India is scanty due to poor notification and technical difficulties in isolating the organisms [10].

In India, 50% of children acquire rubella antibodies by the age of 5 years and 80 to 90% become immune by the age of 15. Studies from India and abroad have found that 10-20% women in childbearing age are susceptible to rubella and between 6-12% babies born with congenital malformations or with serological evidence of rubella [11].

The present study was carried out to find the seroprevalence of rubella in adolescent girls of a school with heterogeneous

population. School selected for study was Kendriya Vidyala, situated in camp area where school children of entire country were studying being wards of soldiers of different states. The main objective of this study was to study the seroprevalence and also to study the socio-demographic determinants on seroprevalence of Rubella infection in adolescent girls.

MATERIAL AND METHODS

This cross sectional analytical study was conducted on adolescent girls in Kendriya Vidyalaya (Central School) of Camp area in Pune. The girl student population in Kendriya Vidyalaya provides a heterogeneous population from different parts of the country belonging to both rural and urban backgrounds, with exposure to a vast range of environmental factors. This diversity makes for an ideal population for the study on the prevalence of any disease, particularly in the population of that age group. Working definition of Adolescent age group for this study was selected as 10-18 years as defined by WHO and ICDS. Anticipated prevalence of rubella antibody was assumed on the basis of previous studies was taken as 80%. With CI of 95% and precision level of 6%, required sample size was 171 however all 310 adolescent girls of the school were selected in the study. Girls with previous history of Rubella vaccination, either as single vaccine or as combination vaccine and girl with precious history of diagnosed Rubella infection were excluded from the study. Total **41**(14%) girls were excluded in which **36** girls with positive history of Rubella vaccination, **1** girl with history of Rubella infection and **4** girls did were not willing. This study was conducted from 01st Aug 2004 to 31st Jul 2005. Data was collected based on a pre-tested structured questionnaire. Questionnaires were distributed to girls, one week prior to the commencement of study, to obtain the written consent of parent/guardians to include them into the study. Questionnaire included data on demographic profile like age, birth order, parent's education level, their occupation, rural/urban background, approximate family income, State to which they belonged, relevant medical history, immunization history, history of past illness, history of any congenital anomaly etc. General examination was carried out to examine their pulse, body weight, general nutritional status, BCG scar and status of personal hygiene. With informed consent of parents, blood samples were collected for testing of Rubella antibodies. Blood sample collected were tested at national accredited lab at Pune. Rubella IgG was tested using Quant kit, manufactured by Globe Diagnostics S.r.l in Milan, Italy.

Fresh sera or plasma samples were collected and stored at 2-8^{0}C and same was tested within a week.

Principle of the Assay: Micro plates are coated with purified and inactivated Rubella antigens that in the first incubation capture specifically anti-virus antibodies if present in the sample. After washing out the other components of the sample, specific anti-rubella antibodies are detected with a goat anti-human IgG antibody, conjugated with peroxidase (HRP). The intensity of the color, generated by the enzyme on the substrate / chromogen mixture in the last incubation, is proportional to the content of anti-Rubella antibodies in the sample.

The samples are diluted 1:50 with the Sample Diluent provided with the kit. Firstly blood samples were centrifuged to separate the sera. Then 10 µl of serum was diluted with 500 µl of diluent provided with the kit and 100 µl of this solution was added to the well and incubated at 37^{o}C for one hour. Micro titer plate was provided with the kit. It is of 96 wells each of 350µl capacity and used for 90 samples, 1 blank and 5 controls. Then 100 µl of conjugate is added to solution and it is again incubated for one hour. Solution is again washed and chromogen substrate (made by mixing reagent chromogen and reagent substrate in equal volume) is added in 1:1 ratio. 100 µl of this solution is again incubated at room temperature for 20-30 minutes and then stop solution (contains a mixture of 1M HCl and phosphoric acids) is added to stop further reaction. The resultant solution is then read with an ELISA automatic reader at 450 nm filter and results are calculated as per Performa available with kit.

CALCULATION OF RESULTS

Values are expressed in international units per ml (IU/ml). From the study of a normal donor's population, the 10 IU/ml standard can be considered as the cut-off to distinguish the negative from the positive samples. In the clinical international literature, a subject having an anti Rubella IgG concentration higher than 20 IU/ml is considered to be immunologically protected. Results are calculated by means of a standard curve calibrated on the WHO standard, providing a quantitative determination of Rubella-specific IgG.

Data collected was analyzed by applying the suitable tests of significance which included paired and unpaired 't' test, Chi square test, 2x2 Table Analysis, using spss version 20. Ethical clearance was taken from institutional ethics committee. The study protocols were

RESULTS

Girls under study were divided into various age groups, maximum girls were between 13-14 years of age with mean age of the girls was 13.94 (+ 3.5) yrs and median 13 years.

Maximum number of girls i.e. 123 (45.72%) had only one more sibling, followed by 105 (39.03%) girls with two siblings, 23(8.55%) girls with three, 5(1.85%) girls with four and 4 (1.48%) girls with five siblings. 9 (3.34%) girls gave history of being only child in the family. 131(48.70%) families had up to 4 members in their family, 128(47.74%) had 5-6 members and 10(3.54%) families had more than 6 members.

Among Fathers, 5(1.85%) were studied up to 5th standard, 3(1.12%) up to 6th-8th standard, 56 (20.81%) were educated up to Higher Secondary, 73 (27.13%) up to Intermediate, 102 (37.91%) were Graduates and only 30 (11.15%) Post Graduates.

In Mothers, 54 (20.07%) were Graduates and 15 (5.57%) Post Graduates, 88 (32.71%) ladies up to Higher Secondary level and, 14(5.20%) were educated up to 5th standard, 23(8.55%) up to 6th--8th standard and 75(27.88%) up to 11th- 12th standard. Although educated, only 22(8.17%) ladies were working while remaining 247(91.83%) were housewives.

There was strong association between Mother's education and Rubella immunization ($p<0.0000$). As majority of girls whose mothers were educated upto secondary or higher secondary level, gave negative history for Rubella Immunization, as many as 26.2% & 41.2% girls gave positive history where mothers were either graduates or postgraduates.

In our study findings, majority of the girls 176(65.42%) had their father working in defense forces. Remaining 93 (34.57%)

worked in various civil establishments, private companies, businessmen etc. Income of the family and its association with immunization status is depicted in table1.

Table 1 Correlation between Family Income and Rubella Immunization

Income	Rubella IgG (%)			Chi square for trends
	Positive	Negative	TOTAL	
<Rs5000	59(73.8)	21(26.2)	80	p=0.9345
55001-10000	90(69.8)	39(30.2)	129	
Rs10001-15000	29(72.5)	11(27.5)	40	
>Rs15000	14(70)	6(30)	20	
TOTAL	192(71.37)	77(28.62)	269	

55.01% girls mentioned their family background as Urban, while remaining 44.98% as Rural. In our study, no significant difference was found in seropositivity for Rubella antibodies between girls from Urban or Rural background. (p value-0.2636)

220(81.78%) gave history of complete immunization and 49(18.21%) girls gave a negative history i.e. either none or incomplete immunization, out of them 31 (63.26%) girls were from rural background.

Only 56 (20.81%) girls gave history of having proper immunization record maintained by their parents, while remaining 213 (79.18%) had either incomplete or no record available. Fig 1 showing presence of Rubella Antibodies in study subject (Total 269).

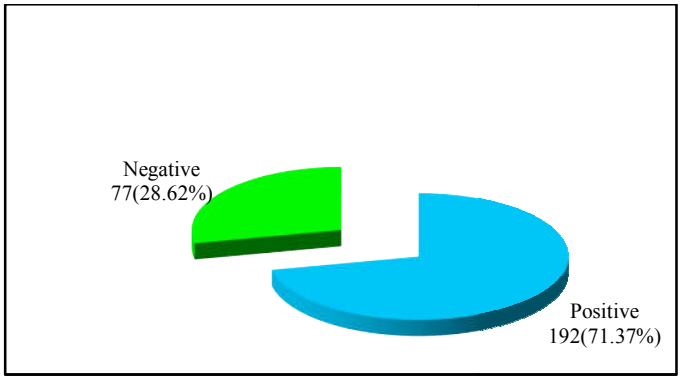

Fig 1 Distribution of Rubella antibodies in study subjects

DISCUSSION

In this study girls were between 10 to 19 years of school going girls. Which was similar to many studies like Rustgi Rachna *et al* in Delhi (55) and Seth *et al* in Delhi in 1985 [12].

About 50% of children acquire rubella antibodies by the age of 5 years and 80 to 90% become immune by the age of 15 [12]. In this study 71.3 % girls were sero positive for Rubella, which is similar to study by Seth *et al* in Delhi in 1985 [12] where it was79.5 % in 15-19 years in urban girls and 70% in rural girls was, 83.9% in Chandigarh [18], 79.3% in Lucknow [26], and 60% in Calcutta [13].

In our study seronegativity to Rubella IgG in the girls of age group of 16 yrs was 21.2% while maximum percentages of girls seronegative to Rubella antibodies were found in age group of 17 yrs (37.5%). Although, no significant difference was found in seropositivity in relation to various age groups (p< 0.9130). The overall seronegativity of adolescent girls in the age group of 10 to 18 years of age was 28.62%.

In study of Rustogi Rachna *et al*, in Delhi in 2005, the seronegativity of rubella IgG in different age groups of 15 to 18 yrs varied from 15.12% in 16-year group to 23.08% in 18-year group, the overall seronegativity being 17.83%. There was no statistical correlation [15].

In a study by Nalini Ramamurty *et al* in Tamilnadu in 2006, on age group of 1-5 yrs (both sexes) & 10-16 yrs Girls, it was found that 48.3% were negative for Rubella IgG antibodies. The seronegativity was 82.2 per cent in 1-5 yr and 13.5 per cent in the 10-16 yr age groups; the difference was statistically significant (P<0.001) [14]. This showed that children have contracted natural rubella infection between 5 and 10 yr of age, which. [14]

In our study, of the total **310** girls, **41** girls, this included 36 girls with positive history of Rubella vaccination and 1 girl with history of Rubella infection. Out of the total **269** blood sample tested, **192** (71.37%) tested positive which is very much coinciding with various studies across India.

In this study 73% girls with rural background and 69.5% girls with urban background were found seropositive. However study by Yadav *et al* showed that seropositivity rate was much higher (76.6%) in urban girls as compared to those residing in rural areas (58.1%) [10].

In our study, there was no significant difference in distribution of girls who tested negative for Rubella IgG, as per income groups of their parents it was similar to study carried out in Turkey, there was no correlation found between socioeconomic status and rubella seropositivity [16].

A study done at Amritsar the Rubella seropositivity rates were also found to be higher in women of lower socio-economic class (71.8%) than in women of upper class (55.9%) as was reported by Yadav *et al* [10]. Similarly, in a study at AIIMS by Rustgi Rachna *et al* in Delhi in 2005, the percentage of seronegativity (susceptibility) for rubella infection in the higher socioeconomic status was 26.09% while in the lower socioeconomic status it was 9.56%. The difference between the two was statistically significant (p > 0.001). Possible reason of this could be due to close contact or overcrowding and acquisition of natural immunity in the lower socioeconomic groups [15].

Study by Gutierrez *et al* in Mexican women found that 79.96% women were seropositive and the figure increased with age. They also found that it was 82.5% in the higher socioeconomic group and 77% in the lower socioeconomic group [17]. These contradictory results of rubella immune status with respect to the socio-economic status in the western countries and in our study can be explained on the basis of wider availability, affordability, acceptability and greater knowledge of rubella vaccine in the West.

Dropout rates remain troubling for multiple-dose vaccines. Even in our study, proper BCG vaccination scar was found only in 212 (78.81%) girls. As per WHO estimates India's BCG coverage is approx 74%. 57 (21.18%) girls without vaccination with BCG, is a matter of concern for health Programme implementers as 43(75.43%) of these girls, were from rural background.

As Rubella immunization is not a part of our National immunization programme and awareness about rubella infection and its consequences is also minimal, only

36(11.61%] girls of the total 310 selected for our study, gave a positive history of Rubella vaccination and remaining **274**[88.39%] were not immunised against Rubella. The income group distribution of girls not immunized against Rubella also indicates toward a higher level of awareness as well as immunization by members of upper income group. Apparently this is because of better accessibility to information and affordability by parents of this group. As a consequence of this even the burden of CRS will be borne by the weaker section of society.

WHO has stated that introduction of MMR and rubella vaccines should primarily aim to prevent CRS. Interruption of rubella transmission by universal vaccination with MMR vaccine is a good strategy if high coverage can be assured.[8] Despite a safe and effective vaccine being available for more than two decades, in India so far there has been no clear-cut policy regarding rubella immunization of children either at 15 months or young girls at 9-12 years.

CONCLUSION

In India, pregnant women belonging to low socio-economic group may be exposed to a variety of infections due to poor environment and hygiene. Maternal infections which have been considered as significant factors in adverse pregnancy outcome elsewhere but have not assumed much significance in India since their prevalence and effect on pregnancy outcome have not been studied so far. No official data is available regarding the prevalence of acquired and congenital rubella infection in India, as it is not a notifiable disease. About 50% of children acquire rubella antibodies by the age of 5 years and 80-90% becomes immune by the age of 15. Between 6-12% of babies born with congenital malformations have serological evidence of rubella. There it is mandatory to increase the coverage of universal immunization program and also to intensify the 2 dosage of MR vaccine though ongoing campaign.

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How to cite this article:

Dr Vipul Dutt, Dr Vineet Rastogi and Dr Barun Bhai Patel (2020) 'Seroprevalence of Rubella Antibodies Among School Going Adolescent Girls in the Age Group of 10 to 18yrs of Pune', *International Journal of Current Advanced Research,* 09(02), pp. 21298-21301. DOI: http://dx.doi.org/10.24327/ijcar.2020.21301.4181

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