

# Seroprevalence of Rubella Virus Infection in Susceptible Women of Childbearing Age Group Seeking Preconceptional Counselling and Infertility Treatment- A Cross-sectional Study from Eastern India

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## ABSTRACT

**Introduction:** Rubella is an acute viral infection predominantly affecting children and adults. Although natural infection with rubella provide lifelong immunity, but, primary infection in pregnant women during the initial days of pregnancy may result in spontaneous abortion, stillbirth, or a baby born with Congenital Rubella Syndrome (CRS), which can be prevented by vaccinating the susceptible non immune females one month prior to planning conception.

**Aim:** To determine rubella virus immune status and demographic profile of women in childbearing age group, and counsel all rubella virus infection susceptible women for vaccination prior to planning pregnancy.

**Materials and Methods:** A cross-sectional observational study was done at Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, India, on 112 apparently healthy, asymptomatic women in the 18-45 years age group coming for preconceptional counselling and primary and secondary infertility treatment during April 2017 to March 2019, whose serum rubella Immunoglobulin G (IgG) antibody testing was done by Enzyme-linked Immunosorbent Assay (ELISA). The data regarding their rubella virus immune status, age, parity and occupations were coded and recorded in MS Excel spreadsheet program. The

Statistical Package for the Social Sciences (SPSS) version 23.0 (IBM Corp.) was used for data analysis.

**Results:** Out of the total 112 women {92 (82.1%) housewife and 20 (17.9%) professionals} included in this study, a total of 67 (59.8%) women tested positive for rubella IgG antibody and 45 (40.2%) tested negative for rubella IgG antibody. The mean age of the rubella virus seropositive immune patients and seronegative susceptible women were 27.72±4.83 years and 26.09±4.68 years, respectively. Among the 67 seropositive patients, maximum antirubella virus IgG seropositivity (71.4%) was seen in more than equal to 36 years age group and maximum seronegativity 23 (46.9%) was seen in 18-26 years age group. There was no direct association of occupation and parity with rubella immune status.

**Conclusion:** Higher rate of antirubella IgG seronegativity amongst younger age groups and nulliparous women renders them susceptible to primary rubella virus infection. Hence, rubella IgG antibody screening, proper counselling and prompt rubella vaccination for all primary rubella virus infection susceptible women is necessary one month prior to planning conception, to avoid foetal congenital malformations during subsequent pregnancies.

**Keywords:** Antirubella immunoglobulin G, Childbearing age, Immune status, Measles mumps rubella vaccine, Rubella containing vaccine

## INTRODUCTION

Rubella is an acute exanthematous viral infection mainly affecting children and adults, can be dangerous for a pregnant woman and her developing foetus. Although natural infection with rubella confers lifelong immunity and protection against further infection, but primary infection of the pregnant women during initial days of pregnancy, can result in miscarriage, stillbirth and have deleterious effects on the developing foetus as well. This is so, because, during the first trimester development of organs occurs and the death of any precursor cells can result in congenital defects [1]. The developmental defects related to primary rubella infection of pregnant women include CRS which results in hearing loss, heart defect, cataracts, and a variety of other permanent manifestation [2-4]. In CRS surveillance done by Murhekar M et al., from 2016 to 2018 in India, it was found that 78.8% had structural heart defects, 59.9% had one or more eye signs (cataract, glaucoma, pigmentary retinopathy), 38.6% had hearing impairment and 24.1% CRS patients died within

a period of two years [3]. The chances of developing CRS is very high if the foetus gets rubella infection during the first 12 weeks of pregnancy, and the chances decrease subsequently with mild to no deleterious effect on the foetus, if rubella infection occurs between 12 to 20 weeks and after 20 weeks of pregnancy respectively.

There is no treatment for rubella infection, and the damage done to the foetus lasts for the whole life [5]. Currently, we have Measles, Mumps and Rubella (MMR) vaccine and Rubella-containing Vaccine (RCV) including Measles Rubella (MR) vaccine for immunisation against Rubella infection [6]. In the United Kingdom (UK), rubella vaccination has been part of the routine immunisation schedule for more than 30 years and achieved rubella elimination status in 2016 by World Health Organisation (WHO), so acute rubella infections are rare there [1]. As per the recommendation of India's National Technical Advisory Group on Immunisation, RCV was introduced in 2017 with two strategies: (i) catch up campaign for single-dose MR vaccines for ages 9 months to 14 years and (ii) switch to monovalent

Measles-Containing Vaccine (MCV) versus bivalent MR vaccine in the routine paediatric immunisation program (ie, given to all children aged 9-12 months and 16-24 months) [7]. Rubella-containing vaccine has been found to be safe and effective and high RCV vaccination targets may break the chain of rubella transmission and thereby prevent CRS cases in endemic regions [2,4]. There is inadequate data available regarding immunisation coverage for RCV or MMR vaccine on national level. Only few regional studies are available from India, which shows immunisation coverage of around 45-60% in Indian pregnant women and infants against rubella infection. The coverage of MMR vaccination in Delhi, Chandigarh and Goa was found to be 42%, 30% and 5%, respectively [8]. Although rubella vaccine is available worldwide, the number of reported cases is high in countries where routine rubella immunisation is either not available or was recently introduced [9-11].

Sharma H et al., conducted a study to ascertain rubella serostatus in adolescent schoolgirls aged 11-18 years, in which a total of 90 (32.7%) girls were found seronegative and vaccinated [12]. In a developing country like India, many women of childbearing age have not been vaccinated due to illiteracy and unawareness [13]. In a study done on 200 female participants between 18 to 40 years, it was found that almost all women of reproductive age were not aware about rubella viral infection, its outcome and vaccination [14]. In different studies done in India, the rubella virus IgG seronegativity and women susceptible to primary rubella virus infection was found to range from 28.5% to 79.13% [12, 15, 16]. Since CRS and other complications can be prevented by vaccinating non immune women prior to conception, it is necessary to determine the susceptibility of women of childbearing potential to primary rubella virus infection before pregnancy is planned. All rubella IgG seronegative women planning pregnancy must be cautioned that they are susceptible to rubella virus infection and any primary rubella infection during the first trimester of pregnancy can have deleterious effects on the foetus with lifelong consequences. It can be prevented by vaccination prior to planning conception [7]. The aim of this study was to determine seroprevalence of rubella virus susceptible women of childbearing age group and counsel all rubella virus infection susceptible women for MR vaccination prior to planning pregnancy.

## MATERIALS AND METHODS

This cross-sectional, observational study was done in the Department of Reproductive Biology in association with Department of Virology of Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, India, within duration of April 2017 to March 2019 after Ethical approval (Memo no 439/IEC/IGIMS/dated 20.4.2017) from the Institutional Ethical Committee. This tertiary care centre provides services to the local community as well as for other population coming from different regions of Bihar. This study included 112 apparently healthy women and 53 women were excluded based on exclusion criteria.

**Inclusion criteria:** Women of age group 18-45 years, coming for preconceptional counselling for primary or secondary infertility at Reproductive Biology Department were included.

**Exclusion criteria:** Women not in childbearing age groups (<18 years or >45 years), not planning any pregnancy in near future and having signs and symptoms suggestive of any current/recent infection were excluded.

## Study Procedure

Data including her age, marital status, occupation, parity were collected by face to face interaction. In order to determine the rubella serology, 2 mL venous blood sample was collected and serum IgG antibody against rubella infection was determined by Enzyme-Linked Immunosorbent Assay (ELISA) using commercially available ELISA kits from Calbiotech. Serological test was conducted in the Virology laboratory according to manufacturer instructions. Good laboratory practices were followed while conducting the tests. Cut-off

value was calculated as product of caliberator Optical Density (OD) and Caliberator Factor (CF). The OD values above and below the cut-off value were taken as positive and negative for rubella IgG antibodies, respectively. Interpretations of results were based on presence and absence of rubella IgG antibody. Absence of rubella IgG antibody implied susceptibility to rubella infection and presence of rubella IgG antibody implied that such cases were not susceptible to future rubella infection.

## STATISTICAL ANALYSIS

Data were coded and recorded in MS Excel spreadsheet program. The SPSS v23 (IBM Corp.) was used for data analysis. Descriptive statistics were elaborated in the form of means±Standard Deviations (SD) and medians/Interquartile Ranges (IQR) for continuous variables, and frequencies and percentages for categorical variables. Group comparisons for continuously distributed data were made using independent sample t-test when comparing two groups. If data was found to be non normally distributed, appropriate non parametric tests in the form of Wilcoxon test was used. Chi-square test was used for group comparisons for categorical data. In case the expected frequency in the contingency tables were found to be <5 for >25% of the cells, Fisher's-exact test was used instead. Statistical significance was kept at p-value <0.05.

## RESULTS

Total 112 women tested and all were married. A total of 67 (59.8%) samples were found to be above the cut-off value and thus positive and 45 (40.2%) samples were below the cut-off value and thus negative for rubella IgG antibody. Among the 67 seropositive patients, 13 (68.4%) had parity one or more than one and 54 (58.1%) were nulliparous. Amongst the 45 seronegative patients, 39 (41.9%) were nulliparous and 6 (31.6%) were having parity ≥P1.

The maximum rubella IgG seropositivity 36 (64.3%) women were within age group of 27-35 years and maximum rubella IgG seronegativity 23 (46.9%) were found in age groups of 18-26 Years. The mean (SD) of age (years) in the Rubella IgG positive group was 27.72 (4.85) and mean (SD) of age (years) in the Rubella IgG negative group was 26.09 (4.68). There were no association of parity with the presence or absence of rubella IgG antibodies [Table/Fig-1,2].

Parameters	Rubella IgG		$\chi^2$	p-value
	Positive (n=67)	Negative (n=45)		
Mean age (years)	27.72±4.85	26.09±4.68		0.048 <sup>1</sup>
<b>Age group (years)</b>				
18-26, (n=49)	26 (53.1%)	23 (46.9%)	1.788	0.455 <sup>2</sup>
27-35, (n=56)	36 (64.3%)	20 (35.7%)		
≥36, (n=7)	5 (71.4%)	2 (28.6%)		
<b>Occupation</b>				
Housewife (n=92)	51 (55.4%)	41 (44.6%)	4.125	0.042 <sup>3</sup>
Professional (n=20)	16 (80.0%)	4 (20.0%)		
<b>Parity category</b>				
P0, (n=93)	54 (58.1%)	39 (41.9%)	0.704	0.401 <sup>3</sup>
≥P1, (n=19)	13 (68.4%)	6 (31.6%)		

**[Table/Fig-1]:** Association between Rubella IgG and different studied parameters.  
<sup>1</sup>p-value <0.05 considered significant; 1: Wilcoxon-mann-whitney u test; 2: Fisher's-exact test; 3: Chi-square test

Age (years)	Rubella IgG		Wilcoxon-Mann-Whitney U Test	
	Positive	Negative	W	p-value
Mean (SD)	27.72 (4.85)	26.09 (4.68)	1840.500	0.048
Median (IQR)	28 (25-30)	25 (23-28)		
Range	18-42	20-40		

**[Table/Fig-2]:** Comparison of the two subgroups of the variable rubella IgG in terms of age (years) (N=112).  
 Significant at p<0.05, Wilcoxon-Mann-Whitney U Test

The variable age (years) was not normally distributed in the two subgroups of the variable Rubella IgG. Thus, non parametric tests (Wilcoxon-Mann-Whitney U test) were used to make group comparisons. There was a significant difference between the two groups in terms of Age (years) ( $W=1840.500$ ,  $p\text{-value}=0.048$ ), with the median age (years) being higher in the rubella IgG: Positive group.

Among 49 participants of 18-26 years age group 26 (53.1%) were found positive for rubella IgG antibody and out of 56 participants of 27-35 years age group 36 (64.3%) were positive for rubella IgG antibody.

In [Table/Fig-1] chi-square test was used to explore the association between 'Rubella IgG' and 'occupation'. There was a significant difference between the various groups in terms of distribution of Rubella IgG ( $\chi^2=4.125$ ,  $p\text{-value}=0.042$ ). Professionals had the larger proportion of Rubella IgG positive females in comparison to housewives who had the larger proportion of Rubella IgG Negative [Table/Fig-1]. Chi-square test was also used to explore the association between 'Rubella IgG' and 'Parity (P) category'. There was no significant difference between the various groups in terms of distribution of Rubella IgG ( $\chi^2=0.704$ ,  $p\text{-value}=0.401$ ), where, 58.1% of the participants in the group P0 were Rubella IgG positive, 68.4% of the participants in the group  $\geq P1$  were Rubella IgG positive, 41.9% of the participants in the group P0 were Rubella IgG negative and 31.6% of the participants in the group  $\geq P1$  were Rubella IgG negative [Table/Fig-1].

## DISCUSSION

Rubella is a mild exanthematous illness, but primary infection during the first trimester of pregnancy can result in miscarriage or stillbirth, and the developing babies are at risk for CRS and severe birth defects with devastating, lifelong consequence [17,18]. In different studies done in India and other countries from 2010 to 2020, it has been found that the proportion of women susceptible to rubella ranged between 4% and 79% in different parts of the world [Table/Fig-3] [9,12,14-16,19-21].

S. No.	Author	Year of study	Place	Rubella IgG seropositivity	Rubella IgG seronegativity
1.	Shanmugasundaram D et al., [9]	2019-20	Six sentinel sites across India	1481/1800 (82.3%) women	308/1800 (17.1%)
2.	Sharma H et al., [12]	2010	Jammu	185/275 (67.27%)	90/275 (32.7%)
3.	Prasad D et al., [14]	2020	Patna	98/150 (65.33%)	52/150 (35%)
4.	Shahapur PR and Kandi V, [15]	2020	Karnataka	24/115 (20.86%)	91/115 (79.13%)
5	Thayyil J et al., [16]	2016	Kerala	153/224 (68.3%)	66/224 (28.5%)
6	Honarvar B et al., [19]	2013	Iran	168/175 (96%)	7/175 (4%)
7	Mazaba ML et al., [20]	2015	Zambia	114/124 (91.93%)	10/124 (8.1%)
8	Friedrich N et al., [21]	2021	Germany	6402/ 6,811 (94.0%)	299/6,811 (4.4%)
9	Present study	2022	Bihar, India	67 (59.8%)	45 (40.2%)

**[Table/Fig-3]:** Status of Rubella IgG seropositivity/seronegativity in different studies [9,12,14-16,19-21].

In this study among the 67 seropositive patients, majority of the patients were within age group of 27-35 years and amongst the 45 seronegative patients, majority 23 (46.9%) were in age groups of 18-26 years. The higher Rubella IgG seropositivity within 27-35 years age group may be due to more chances of exposure to rubella virus infection with advancing age and subsequent development of immunity against rubella virus infection. There were no association of parity with the presence or absence of Rubella IgG antibodies.

A serological study was performed by Shanmugasundaram D et al., in year 2019-2020 at six Sentinel sites in India in which it was found that approximately 80% of the pregnant women were seropositive for rubella and about 17% were seronegative and thus prone to infection with the rubella virus [9]. In another study performed by Thayyil J et al., in year 2016, Rubella IgG seropositive, seronegative and equivocal in girls found to be 68.3%, 28.5% and 3.2% respectively [16]. In our study nearly 40.02% of the females were susceptible to rubella virus infection which was significantly more in comparison to the above two studies [9,16].

The results of the study were in accordance to the study done by Prasad D et al., in 10-19 years age group of adolescent girls who had not received MMR vaccine, 34.67% adolescent girls were found to be Rubella IgG antibody negative and hence susceptible to primary rubella virus infection [14].

In New Delhi, there was 17.83% Rubella IgG seronegativity in a survey conducted by National Institute of Communicable Diseases (NICD) on adolescent girls of age group of 15 to 18 years [22]. Out of the 45 serum Rubella IgG antibody negative cases, majority (46.9%) of the patients belonged to 18-26 years age group. This was in concordance with the studies done by Singla N et al., in Amritsar [23]. Our results were in accordance with earlier studies and highlight the importance of serosurveillance and vaccination of subjects in the susceptible cohort. Rubella Vaccine (R-Vac) was tolerated well and the adverse events observed were mild and self-limiting.

In another study by Shahapur PR and Kandi V, it was found that 60.8% and 79.13% of pregnant and non pregnant women were seronegative for rubella IgG, respectively [15]. The proportion of females susceptible to Rubella virus infection was much higher in comparison to the study. Our results were in accordance with earlier studies and higher anti rubella IgG seronegativity in this part of Bihar, in younger age group, and in women with lesser exposure to the outside world, highlight the importance of serosurveillance and vaccination of the susceptible subjects [24,25]. If the data on serosurveys and incidence is considered rubella vaccine should be widely introduced in India in the second year of life, as in many other developing countries [26]. All rubella virus seronegative women, who are not pregnant, should be advised prompt rubella vaccination and should also be advised to plan pregnancy after one month of vaccination to avoid foetal congenital infection during subsequent pregnancies [7]. This can greatly reduce and help in preventing the occurrence of CRS. The finding of studies done in other nations by Honarvar B et al., [19], Mazaba ML et al., [20] and Friedrich N et al., [21], Rubella IgG seronegativity was found to be 7/175 (4%), 10/124 (8.1%) and 299/6811 (4.4%), respectively. The proportion of females susceptible to rubella virus infection was much lower in comparison to the present study. The reason for the large variation in the seroprevalence of rubella-specific IgG antibodies may be due to differential exposure to rubella virus infection, which depends on different geographic regions, age, socio-economic status, and standards of living. It may also vary depending on the vaccination status of the individual. The higher Rubella IgG seropositivity within 27-35 years age group may be due to more chances of exposure to rubella virus infection with advancing age and subsequent development of immunity against rubella virus infection.

There were no association of parity with the presence or absence of Rubella IgG antibodies. To the best of the author's knowledge, this was the first study done in Eastern Bihar to determine the seroprevalence of Rubella virus infection susceptible women of childbearing age group. Given the absence of a national immune status registry, this study therefore provides for the first time, robust and representative data on Rubella virus immune status of females planning conception and their susceptibility to primary Rubella virus infection. This is an ongoing study and in the long term, this study will generate data to monitor the progress made by the rubella vaccination program. All Rubella IgG seronegative



women should be counselled to get vaccinated with MR vaccine before planning pregnancies to reduce birth of infants with CRS and other complications.

### Limitation(s)

This study was done in tertiary care hospital of Bihar, India, on a small number of patients, prior to introduction of free rubella vaccine. None of the patient had any idea about their Rubella virus vaccination status. The authors also could not ascertain their postvaccination Rubella virus IgG serostatus, neither ascertain the incidence of CRS postvaccination in seropositive patients.

### CONCLUSION(S)

In the present study, the high prevalence of women in childbearing age group, susceptible to primary Rubella virus infection indicates that high efforts should be made to introduce the first dose of Rubella containing vaccine on a large scale in the second year of life. All women prior to planning conception should undergo rubella virus immune status determination. The rubella virus seronegative women, who are not pregnant, should be advised prompt rubella vaccination prior to planning conception and should also be advised to plan pregnancy after one month of vaccination to avoid foetal congenital infection during subsequent pregnancies which can greatly reduce the chance of development of CRS. This is an ongoing study and in long term it will generate data to monitor the progress made by rubella vaccination program.

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