INTERNATIONAL JOURNAL OF BIOASSAYS
ISSN: 2278-778X
CODEN: IJBNHY
ORIGINAL RESEARCH ARTICLE
OPEN ACCESS

Seroprevalence of rubella in antenatal women in and around Kirumampakkam, Puducherry, India.

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Received for publication: July 5, 2012; **Accepted**: August 25, 2012

Abstract: *Rubella* is a mild self-limiting vaccine preventable viral disease. Maternal *Rubella* infection during first trimester of pregnancy leads to abortion, still birth and *Congenital Rubella Syndrome*. Seroepidemiological studies conducted in different countries have shown that *Rubella* is a worldwide infection. The present study was conducted to assess the immunity status to *Rubella* among pregnant women in and around Kirumampakkam, Puducherry for a period of one year. A detailed history with special reference for previous bad obstetric history was collected after an informed consent. They were tested for Rubella IgG and IgM antibodies using commercially available kits. Overall seropositivity was 87.9% and 12% were susceptible to *Rubella* infection. 6.5% were positive to *Rubella* specific IgM antibodies. Percentage of seropositivity was seen to be increasing with age. The percentage of IgG negatives was higher among the cases with bad obstetric history. Cases from lower socioeconomic status had good immunity against Rubella. All the cases that had previous immunization record were positive for *Rubella* specific IgG antibodies. In conclusion, an action revamping the national immunization policy should be considered along with sero-surveillance of *Rubella* among adolescent girls and women of childbearing age before conception for the assessment and analysis of the situation, and take appropriate action to eradicate *Rubella*.

Key words: Rubella; Antenatal women; Congenital Rubella Syndrome; Bad obstetric history

Introduction

Rubella is an acute, benign and mild exanthematous disease characterized by low grade fever, lymphadenopathy and a short lived morbilliform rash. Infection during the first trimester of pregnancy leads to still births and spontaneous abortions. Infection *in utero* following transplacental transmission of virus from mother has dire consequences for the developing fetus. These are reflected in a constellation of symptoms collectively called *Congenital Rubella Syndrome* (*CRS*)[1].

Rubella is worldwide in distribution, except in countries where the disease has been eliminated and vaccination has been included in national immunization schedule. However, the extent and periodicity of *Rubella* epidemics is highly variable in both developed and developing countries. Seroepidemiological surveys of *Rubella* are an important tool to find out the proportion of population susceptible to *Rubella* and the risk of acquiring *CRS* [2]. WHO estimates that worldwide more than 100,000 children are born with *CRS* each year, most of them in developing countries.[3] In India limited few such studies have probed the problem of *Rubella*. Extrapolation based on world wide data has resulted in the claim that the estimated prevalence in India is 100-200 per 100,000 populations[4]. The reason for vehement research is simple, the condition is completely preventable. Hence, the present study was done to find out the immune status of antenatal mothers in the given region.

Materials and Methods

This prospective study was conducted over a period of one year from January 2009 to January 2010 at Aarupadai Veedu Medical College and Hospital, Kirumampakkam, Puducherry. Institutional Ethical committee approval was sought prior to collection of samples. A profoma was filled containing the subjects name, age, sex, occupation, communication address, socioeconomic status, parity and immunization status.

Women visiting the Obstetrics and Gynecology Department for regular antenatal checkup were explained about the study and an informed consent was obtained. Special reference was given to any subjects with history of rash, fever, lymphadenopathy or arthralgia in the past. Cases with Bad Obstetric History (BOH) which implies previous unfavorable fetal outcome in terms of

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http://dx.doi.org/10.21746/ijbio.2012.10.002

two or more consecutive spontaneous abortion, history of intrauterine fetal death, intrauterine growth retardation, still births, early neonatal death and/or congenital anomalies were also given importance. Subject's date of last menstrual period and expected date of delivery were also noted. Any significant medical, surgical illness was also given consideration. Socioeconomic status of the subjects in the study was given scores based on the Kuppuswamy's criteria[5] for classification.

Sample collection and storage: Blood samples were collected from 182 pregnant women who were asymptomatic and women with BOH irrespective of gestational age, immunization status. 5ml of blood was collected aseptically and serum was separated and stored at -20^{0}C until it was tested.

Processing of samples: *Rubella* specific IgG and IgM ELISA kits were purchased from CALBIOTECH Inc; CA. Manufactures guidelines were strictly followed while doing the procedure. Samples were tested for *Rubella* specific IgG and IgM. The optical density of each sample was read at 450nm and the reader was of dual wave length with an automatic washer attached to it.

Reagent preparation and assay procedure: All the specimens and the kit reagents were brought to room temperature and gently mixed before being used. 1:21 dilution of the test samples was prepared. Desired number of coated strips was placed into the holder. 100µl of the diluted sera along with the calibrator, positive and negative controls were dispensed into appropriate wells. For the reagent blank 100µl of the sample diluent was dispensed in 1A well position and the plate incubated at room temperature for 20mins. All the wells were washed three times with 300µl of 1X wash buffer in an automatic washer. Wells were blot dried with absorbance paper. 100µl of enzyme conjugate was added to each well and incubated at room temperature for 20mins. Enzyme conjugate were washed three times with 300µl of 1X wash buffer. Wells were blot dried with absorbance paper. 100µl of TMB substrate was added to each well and again incubated at room temperature for 10mins. 100µl of the stop solution was added to stop all the reactions. Read at 450nm in an ELISA reader within 15mins.

Note: Reagent and assay procedures were strictly followed according to manufacturer's instructions. Assay procedures were the same for IgG and IgM ELISA.

Calculation of antibody index

Cut off value = calibrator mean OD X calibrator factor
Calibrator factor (IgG) = 0.55
Calibrator factor (0.50
Calibrator mean OD = the mean of the calibrator in the well D&E
Antibody Index = Sample OD divided by cut off value
An antibody index > 1.1 was taken as positive
= 0.9 - 1.1 was taken as equivocal
< 0.9 was taken as negative

(Instructions as per the manufacturer's catalogue was followed)

Statistical Analysis:

Properties of outcome were calculated as percentages. Variables like age, parity, antibody index, outcome of pregnancy, socioeconomic status, occupation, immunization status and status of IgG and IgM were compared. Mean, standard deviation and T test were calculated for the data, a *P* value of < 0.05 was considered significant.

Results

Table I: Seroprevalence of *Rubella* specific IgG and IgM antibodies in different age groups.

AGE	IgG positive	IgG negative	IgM positive	IgM negative
18 – 22 (n = 44)	34 (77.2%)	10 (22.7%)	4 (9%)	40 (90.9%)
23 – 27 (n = 92)	80 (86.9%)	12 (13%)	6 (6.5%)	86 (93.4%)
28 – 32 (n = 28)	28 (100%)	0	0	28 (100%)
≥ 33 (n = 18)	18 (100%)	0	2 (11.1%)	16 (88.8%)
TOTAL (n = 182)	160 (87.9%)	22 (12%)	12 (6.5%)	170 (93.4%)

In Table I, the subjects were grouped into different age groups and Rubella specific antibodies were compared. 160 (87.9%) subjects were immune to *Rubella* infection and 22 (12%) were susceptible. Maximum IgG seropositivity was seen in the age group 28 yrs and above (100%). Also, 12 (6.5%) subjects were IgM positive and 170 (93.4%) were IgM negative. Maximum positivity was observed in the age 33 yrs and above. *P* value was < 0.05.

Table II: Comparison of IgG antibody index among different age groups.

AGE	IgG antibody index ≥ 1.5	IgG antibody index ≤ 1.5
18 – 22 (n = 44)	32 (72.7%)	12 (27.2%)
23 – 27 (n = 92)	72 (78.2%)	20 (21.7%)
28 – 32 (n = 28)	26 (92.85%)	2 (7.14%)
≥33 (n = 18)	17 (94.4%)	1 (5.5%)
TOTAL (n = 182)	144 (80.7%)	35 (19.2%)

In this table II, the percentage of IgG antibody index (≥ 1.5) was seen to be higher in the age group 28 yrs and above (92.85%, 94.4%), it was also seen that antibody index increased with age. The numbers of subjects with an antibody index greater than 2 were also in this group, *P* value was 0.04.

Table III: Seroprevalence of *Rubella* specific IgG & IgM antibodies in Normal & BOH subjects.

Pregnancy outcome	IgG positive	IgG negative	IgM positive	IgM negative
Normal (n =62)	56(90.3%)	6(9.6%)	0	62(100%)
*BOH (n = 38)	30(78.9%)	8(21%)	12 (31.5%)	26(68.4%)
TOTAL (n=100)	86(86%)	14 (14%)	12(12%)	88(88%)

* BOH – Bad Obstetric History

In Table III, the percentage of IgG negativity was found to be higher in subjects with BOH (21%) when compared to subjects without BOH (9.6%).

Table IV: Comparison of IgG & IgM seroprevalence in BOH subjects.

BOH	IgG positive	IgG negative	IgM positive	IgM negative
A 1 (n = 30)	26 (86.6%)	4 (13.3%)	12 (40%)	18 (60%)
A 2 (n = 4)	2 (50%)	2 (50%)	0	4 (100%)
A 3 (n = 4)	2 (50%)	2 (50%)	0	4 (100%)
TOTAL (n=38)	30 (78.9)	8 (21%)	12 (31.5%)	26 (68.4%)

Subjects with BOH were grouped into first abortion (A1), second abortion (A2) and third abortion (A3) depending on the number of abortions. Percentage of IgG positivity was higher in A1 (86.6%). All the 12 subjects who were IgM positive also belonged to A1.

Table V: Comparison of IgG antibody index in BOH subjects.

BOH	IgG antibody index ≥ 1.5	IgG antibody index ≤ 1.5
A 1 (n = 30)	24 (80%)	6 (20%)
A 2 (n = 4)	2 (50%)	2 (50%)
A 3 (n = 4)	2 (50%)	2 (50%)
TOTAL (n=38)	28 (73.6%)	10 (26.3)

When the IgG antibody indexes were compared among first abortion (A1), second abortion (A2) and third abortion (A3) the percentage of subjects with an IgG antibody index of ≥ 1.5 was seen to be higher in the A1 group when compared to the other groups.

Table VI: Comparison between parity and IgG seroprevalence.

Parity	IgG positive	IgG negative	IgM positive	IgM negative
G1 (n = 82)	74 (90.2%)	8 (9.7%)	0	82 (100%)
G2 (n = 68)	60 (88.2%)	8 (11.7%)	6 (8.8%)	62 (91.1%)
G3 (n = 24)	22 (91.6%)	2 (8.3%)	6 (25%)	18 (75%)
G4 (n = 8)	4 (50%)	4 (50%)	0	8 (100%)
TOTAL (n=182)	160(87.9%)	22 (12%)	12 (6.5%)	170 (93.4%)

In this study, the total number of IgG positive was 87.9% and 12% were IgG negative. 9.7% of the primis and 14% of the multiparous subjects were susceptible to *Rubella* (IgG negative). Maximum percentage of IgG positivity was seen among G3 (91.6%). *P* value for IgG was 0.03 which is statistically significant. The total numbers of IgM positive subjects were 12 (6.5%). They were equally distributed among G2 and G3. There were no IgM positive subjects among the primi. *P* value for IgM was 0.03 which was statistically significant.

Table VII: Comparison between IgG & IgM seroprevalence with socioeconomic status.

Socioeconomic Status	IgG positive	IgG negative	IgM positive	IgM negative
Upper Socio-economic (n = 2)	2(100%)	0	0	2(100%)
Middle upper (n = 8)	8(100%)	0	0	8(100%)
Middle lower (n = 14)	12(85.7%)	2(14.2%)	0	14(100%)
Lower Socio-economic (n = 158)	138(87.3%)	20(12.6%)	12(7.5%)	146(92.4%)
TOTAL (n = 182)	160(87.9%)	22(12%)	12(7.5%)	170(93.4%)

In the study, 158 (86.8%) subjects were from the lower socio economic status and when the subjects in the middle upper and lower socioeconomic status were also taken into account their immune status to *Rubella* IgG was 87.3%, 100% and 85.7% respectively. There was also a sizeable population in this group who were susceptible (12.6% and 14.2%). However, all the 12 IgM positive subjects also belonged to the lower socioeconomic group.

Table VIII: Comparison of IgG & IgM seroprevalence with occupation.

Occupation	IgG positive	IgG negative	IgM positive	IgM negative
House wife (n = 166)	146 (87.9%)	20 (12%)	12 (7.2%)	154 (92.7%)
Professional (n = 8)	8 (100%)	0	0	8(100%)
Medical (n = 6)	4 (66%)	2 (33%)	0	6(100%)
Student (n = 2)	2 (100%)	0	0	2(100%)
TOTAL (n = 182)	160 (87.9%)	22 (12%)	12 (6.5%)	170 (93.4%)

The Table VIII shows the prevalence among various occupational groups. It was found that medical professionals had the highest number of IgG negative cases (33%) followed by housewives (12%). All the IgM positive cases were among the housewives (7.2%).

Discussion

Congenital abnormalities following maternal *Rubella* infection was first recognized 50 years back. Despite this, *Rubella* immunization rates are not optimal and infections during pregnancy still occur. Lack of *Rubella* IgG antibodies in the childbearing age is susceptible to primary infection (5-25%)[6]. Extrapolated data show *Rubella* has been linked to the etiology of 26% of cataracts, 12-17% of congenital malformations and upto 29% of sensorineural deafness among infants in India.[4] *Rubella* and *CRS* is not yet a notifiable disease in India. Seroconversion of pregnant women has been considered as one of the reasons for medical termination of pregnancy. The diagnosis of *Rubella*

is very often missed as the infection is mild and the rash and lymphadenopathies are transient. Serodiagnosis is the most useful and reliable method to detect the infection.[2]

In our study, 87.9% of the pregnant women were having *Rubella* specific IgG antibodies and 12% of them were virgin to *Rubella* infection. In India, the immunity status of *Rubella* was screened in antenatal cases, BOH and children with congenital deformities from 1998-2002 and later in 2003-2006[7],which revealed a seropositivity of 83.06% among antenatal cases and 86.90% among BOH during 1998-2002 which rose to 95.15% among antenatal cases during 2003-2006[7,8]. Other studies from Western Cape Town and Sri Lanka have shown a seropositivity of 89.5% and 76% respectively[9,10]. In our study the percentage of seropositivity is also within this range indicating that immunity to *Rubella* is still not optimal. When considering the IgM seropositivity in the studied population, 12 (6.5%) cases were positive for *Rubella* specific IgM antibody, none of them in the study remembered having any symptoms of *Rubella;* neither did they have any *Rubella* vaccination in the recent past and all had history of abortion.

Seropositivity was found to be maximum in the age group of 28-33 yrs, also it was seen that the percentage of seropositives increased with age. The *P* value was 0.02, which was statistically significant. Possibly this increase in seropositivity with age could be due to more frequent exposure of the older age groups to *Rubella* and thus an acquired immunity to natural infection. Similar study in Sri Lanka also indicated an increase in seropositivity in the age group of 25-29 yrs.[10]

IgG antibody index when compared among the various age groups in our study revealed antibody index more than 1.5 (mean antibody index of 1.7) among the older age group. The higher percentage of IgG seropositivity was also seen among the older age group, *P* value was 0.03 which was statistically significant. Similar findings were also observed in other studies[6,11]. A possible reason could be reinfection (frequent exposure) in these age groups by *Rubella*. Reinfection is associated with a rise in antibody concentration, sometimes to very high levels. An IgM response may also be present, but is usually lower and more transient than that following primary infection[12].

IgM seropositivity was compared with the different age groups. Maximum seropositivity was seen among older age groups, *P* value was significant ($P = 0.02$). Possibly, these subjects could have had a subclinical infection which went unnoticed. Studies conducted in various regions in India showed an IgM seropositivity of 6.5%, 4.9%, 9.69%, 11.3% and 4.3% respectively[7,13,14,15,16].

Immune status was compared with socioeconomic status using Modified Kuppuswamy's scale.[5] 86.8% subjects belonged to lower socio economic status and their immunity to *Rubella* was 87.3%. All the 12 (7.5%) IgM positive subjects also belonged to the lower socioeconomic group. The *P* value was statistically significant ($P < 0.05$). This could be explained on the basis of high chances of Rubella infection due to close contact or overcrowding and acquisition of natural immunity in the lower economic group. Findings of earlier studies were similar to the present study [5,17].

Out of total 182 subjects, 8 (4.3%) knew that they received MMR in their childhood and all the 8 were IgG positive. 174 (95.6%) were not sure of their immunization status, 12% among this group were seronegative and all the 12 (6.5%) IgM positive subjects were also in this group. Our study shows that the knowledge about *Rubella*, its consequences and preventive measures are inadequate. Hence public awareness has to be created.

It is high time to generate our own data to know the incidence and prevalence of *Rubella* and *CRS* in our country without depending on extrapolated data, which does not reflect our actual burden of disease. All the concerned specialties should come to a common platform and should come out with a new policy to control, prevent and eradicate the disease. Exact magnitude and extent of the problem have to be studied yet, because of the simple reason that the disease is completely vaccine preventable.[4]

Conclusion

India should consider serosurveillance of *Rubella* among adolescent girls and women of childbearing age before conception. We should not further delay the assessment and analysis of the situation, and take appropriate action to eradicate *Rubella*, only then we can prevent innumerable stillbirths, abortions and the most devastating *CRS*.

Acknowledgement

The Authors are thankful to The Management and The Dean, Aarupadai Veedu Medical College and Hospital, Kirumampakkam, Puducherry for providing the necessary facilities and permitting to carry out this research work. Also the authors are also very much thankful to Dr. Rajeshwar Lall, Former Head of the Department, Department of Microbiology, Aarupadai Veedu Medical College and Hospital, Kirumampakkam, Puducherry for his constructive criticism throughout the period of the study. The Authors are also very much

thankful to Dr. Sageera Banoo, Assistant Professor, Department of Microbiology, Aarupadai Veedu Medical College and Hospital, Kirumampakkam, Puducherry, for her timely guidance and moral support.

Abbreviations

CRS – Congenital Rubella syndrome
BOH – Bad obstetric history
OD value – Optical Density value
WHO – World Health Organization

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Cite this article as:
Vinod Raveendran, Dhandapany Senthil Pragash, Manju, Ivvala Anand Shaker, Vijaya Rayapu. Seroprevalence of rubella in antenatal women in and around Kirumampakkam, Puducherry, India. *International Journal of Bioassays* 1.10 (2012): 74-78. http://dx.doi.org/10.21746/ijbio.2012.10.002

Source of support: Nil
Conflict of interest: None Declared