ORIGINAL RESEARCH PAPER

Volume-7 | Issue-1 | January-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

CLINICO-MICROBIOLOGICAL STUDY OF RUBELLA IN PREGNANT FEMALES WITH BAD OBSTETRIC HISTORY (BOH)

Microbiology

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ABSTRACT

Rubella or German Measles is a RNA virus transmitted by respiratory droplets. In itself the disease is trivial but Rubella in pregnant woman can lead to congenital malformation in the baby in 10-54% cases. Research finding indicated that Rubella IgM prevalence was 2.27% and that of IgG was 67.5%. The distribution showed increase of IgG and IgM antibodies with increase in the number of abortions. In Normal group patients (N) IgG was mostly positive for patients with Gestational age 3rd Trimester (29-40 weeks). The distribution also showed that in this group IgG was most positive for those in the age group 26-30 years. In group A1 patients again IgG was found to be most positive for those with gestational age 3rd Trimester (29-40 weeks). In this group IgG was mostly positive for those in the age group of 20-25 years. The distribution for group A2 patients showed an increase of IgG antibodies for those with Gestational age 3rd Trimester (29-40 weeks). The patients of this group also showed increased IgG antibodies in the age group 20-25 years.

High prevalence of Rubella IgG antibodies indicates prior exposure, infection or vaccination. There was only two cases of Rubella IgM antibody, one of which belonged to group A1 and the other toA2 group. The presence of rubella IgM antibodies in these two patients with history of one and two abortions indicates a close association with rubella infection.Early detection in pregnant women can reduce severity of the disease in the newborn.

KEYWORDS

Rubella, German Measles, congenital malformations

INTRODUCTION

(Rubella or German Measles is a RNA virus) is transmitted by respiratory droplets. Maternal rubella infection is manifested by rash, malaise, fever, lymphadenopathy & polyarthritis. Congenital Rubella syndrome predominantly includes cochlear, cardiac, Haematologic, liver, spleen, Ophthalmic, bone and chromosomal abnormalities. The virus predominantly affects the foetus and is extremely teratogenic if contracted within the first trimester. There is increased chance of abortion, still birth and congenitally malformed baby. [1]

Rubella has world-wide prevalence, its epidemic and pandemic outbreak occur every 6-10 years and 20-25 years respectively. It causes hypoplasia in neonates and abortion in pregnant woman. [2]

Following the rubella vaccination practises the incidence of rubella has been reduced drastically but the WHO (World Health Organization) still estimates over 100,000 children worldwide are born with congenital rubella syndrome and more so in developing countries [3]

Seroepidemiological studies have shown that 10-20% of women in child bearing age in India are susceptible to rubella infection. Infection with rubella during pregnancy may lead to congenital malformation in 10-54% of cases. [4]

AIM

The aim of the study was to assess the seroprevalence of IgG and IgM antibodies for Rubella women with normal pregnancy, pregnant women with one abortion and pregnant women with two or more abortions Bad Obstetric History (BOH).

The sero-positivity was analysed according to number of abortions, age, parity and trimester of pregnancy.

MATERIAL AND METHODS

The study was an experimental and analytical study carried out in the Department of Microbiology, MGM Medical College, Kamothe, Navi Mumbai over a period of two years from November 2012 to October 2014.Ethical clearance was obtained from institutional ethics committee prior to study. Blood samples were collected from pregnant women attending antenatal clinic and patients admitted in antenatal ward of the hospital after taking written informed consent to perform ELISA test for Rubella IgM and IgG on the serum samples.

The IgM and IgG Elisa kit was manufactured by Delta Biologicals a subsidiary of Erba Diagnostics and supplied by Transasia Biomedicals Ltd.The Rubella IgM Elisa Kit bearing lot no-224-DD with expiry date 2015-06 had a sensitivity of 90.0% and specificity of 94.9% .The Rubella IgG Elisa Kit with lot no-233 and expiry date 2014-10 had sensitivity of 100 % and specificity of 97.0%. The test was performed as per instructions of the manufacturer.

IgG Elisa -The controls consisted of one negative control and 5calibrators as positive control.Validity and test results were calculated as per literature of the manufacturer.

Validity Criteria-

a) OD of negative control was <0.6 times the OD of calibrator 2
b) OD of C2 was more than 0.2 at 450 nm.
c) OD of C5 was more than 1.5

Cut off corresponds to calibrator 2.Ratio between OD value of sample and that of cut off was > 1.3 and was considered as Positive result.

IgM Elisa-The controls consisted of two negative controls (NC),two positive controls (PC) and two cut off calibrators. The mean absorbance of NC, cut off calibrator and PC was calculated.

Validity Criteria-

a) Blankwell-Blank absorbance was < 0.050 at 450/630.
b) NC-Mean absorbance after subtracting blank absorbance was <0.100.
c) Cut off calibrator-Mean absorbance after subtracting blank absorbance was >0.150 and < 0.450.
d) PC- Mean absorbance after subtracting blank absorbance was >0.500.

The index value was calculated to obtain the qualitative specimen results

The cut-off value was obtained by subtracting the blank absorbance from the mean absorbance of the cut off calibrator.

The index value was calculated by dividing the specimen absorbance by the cut off value.

Index value was >1.1 and was considered Reactive.

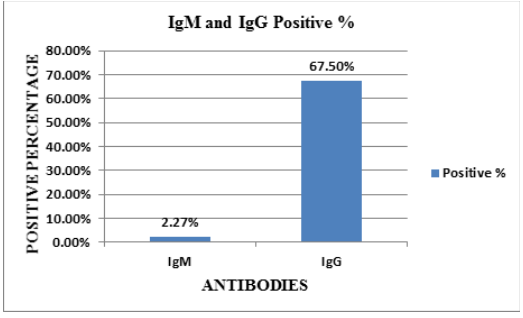
A predefined proforma was used to collect the data regarding age of the patient, gestational age (trimester) and number of abortions. The result of ELISA test was then subjected to statistical analysis.

RESULT

A total of 88 samples were taken for detection of IgM antibodies against Rubella out which 23 samples belonged to group N which consisted Normal ANC patients, 38 belonged to group A1 which consisted ANC patients with one abortion and 27 belonged to group A2 consisting of ANC patients with two or more abortions (BOH). Whereas a total of 83 samples were taken for detection of IgG antibodies against Rubella out of which 22 samples belonged to group N, 37 samples belonged to group A1 and 24 samples belonged to group A2. Prevalence of IgM positivity was 2.27% and that of IgG was 67.5% (Table 1). The distribution of abortion has shown that the prevalence of IgM increase from 0 to 3.7% and IgG from 54.54% to 83.3% with increase in number of abortions (Table 2). Only two patients out of the total of 88 subjected for determination of IgM antibodies against rubella was found to be positive. First patient belonged A1 group, age group 20-25 years (20 years),gestational age third trimester (32 weeks) and parity 1.The second patient belonged to A2 group, age group 36-40 years (40 years), gestational age third trimester (35 weeks) and parity 2. The age group distribution has shown high rate of IgG positive for group N patients belonging to age group of 26-30 years (50%), for group A1 patients high IgG positive is seen in age group of 20-25 years (41.66%) and for group A2 patients IgG is most positive for those in age group of 20-25 (45%) (Table 3). The gestational age distribution show that IgG was positive for group N patients with Gestational age 3nd Trimester (29-40 weeks) (58.33%), in group A1 patients IgG was again positive most positive with gestational age 3nd Trimester(29-40 weeks) (41.66%). The distribution for group A2 patients also showed a high positive rate of IgG antibodies for those with Gestational age 3rd Trimester (29-40 weeks) (50%).(Table 4)

Table 1: **IgM and IgG positivity in total number of subjects**

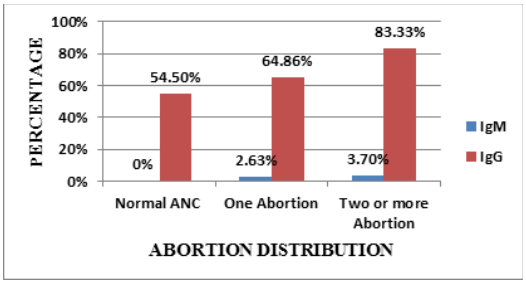
Antibody	No. of Samples	No. of Positive Samples	Positive Percentage (%)	P value
IgM	88	2	2.27%	>0.05 Not Significant
IgG	83	56	67.5%	0.0001 Significant



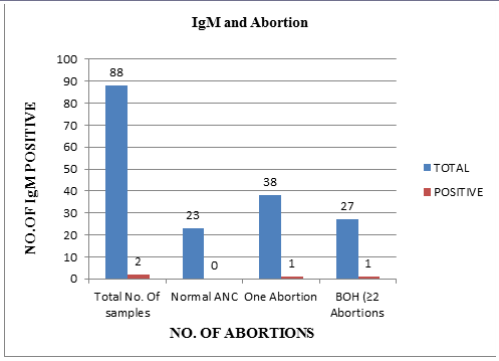
Graph 1*:* IgM and IgG positivity

Table 2: IgM and IgG positivity as per three groups of patients

Abortion Distribution	IgM	IgG
Normal ANC	0/23= 0%	12/22= 54.5%
One Abortion	1/38= 2.63%	24/37= 64.86%
Two or more Abortion	1/27 = 3.7%	20/24= 83.33%



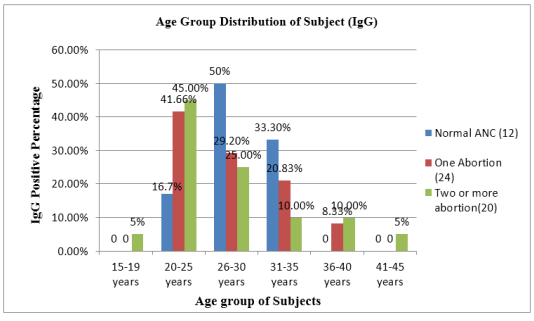
Graph 2*:* *IgM and IgG Positivity increase with increase in number of abortions*



Graph 3: IgM and abortion

Table 3: Age distribution of subjects (IgG)

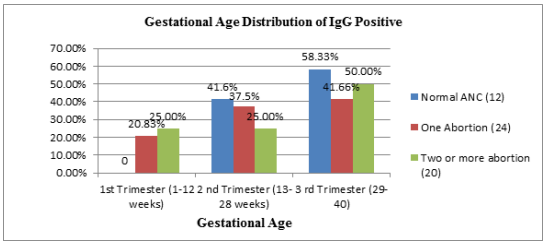
Age Distribution	Normal ANC (12)	One Abortion (24)	Two or more abortion (20)
15-19 years	0 (0%)	0 (0%)	1 (5%)
20-25 years	2 (16.7%)	10 (41.66%)	9 (45%)
26-30 years	6 (50%)	7 (29.2%)	5 (25%)
31-35 years	4 (33.3%)	5 (20.83%)	2 (10%)
36-40 years	0 (0%)	2 (8.33%)	2 (10%)
41-45 years	0 (0%)	0 (0%)	1 (5%)



Graph4: Age Group Distribution of subjects (IgG)

Table 4: Gestational Age distribution of IgG positivity

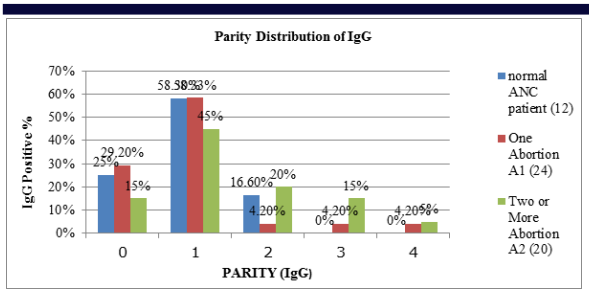
Gestational Age	Normal ANC (12)	One Abortion (24)	Two or more abortion (20)
1st Trimester (1-12 weeks)	0 (0%)	5 (20.83%)	5 (25%)
2 nd Trimester (13-28 weeks)	5 (41.6%)	9 (37.5%)	5 (25%)
3 rd Trimester (29-40)	7 (58.33%)	10 (41.66%)	10 (50%)



Graph 5 : Gestational age distribution showing IgG Positivity

Table 5: Parity Distribution of IgG

Parity (IgG)	Normal ANC Patient (12)	One Abortion A1 (24)	Two or More Abortion A2 (20)
0	3 (25%)	7 (29.2%)	3 (15%)
1	7 (58.3%)	14 (58.33%)	9 (45%)
2	2 (16.6%)	1 (4.2%)	4 (20%)
3	0 (0%)	1 (4.2%)	3 (15%)
4	0 (0%)	1 (4.2%)	1 (5%)



Graph 6: Parity Distribution of IgG

DISCUSSION

The rubella IgG and IgM antibodies were tested by ELISA method. In this study, out of 83 cases subjected to Rubella IgG ELISA, 56 cases (67.5%%) are positive for rubella specific IgG antibodies.(p value:0.0001 Significant) Out of 83 cases , 22 cases are of normal pregnancy (NP) without abortion. The seroprevalence of IgG in this group is 54.55 (12/22). Similarly, out of 83 cases 37 cases are patients with history of one abortion. The seroprevalence of IgG in the group 64.86% (24/37). Out of 83 cases, 24 patient are with a history of 2 or more abortions (A2) (BOH) with an IgG seroprevalence rate of 83.33% (20/24). The present study shows that seroprevalence for IgG increases with number of abortions(Table 2). Presence of IgG antibodies indicates prior exposure,infection or vaccination IgG seropositivity also increases with gestational age (Table 4).

A total of 88 cases were subjected to Rubella IgM ELISA and only two cases 2.27%% (2/88) was found to be positive for rubella specific IgM antibodies. The first patient belonged to A1 group with history of one abortion.The second patient belonged to A2 group with history of two abortions. The presence of rubella IgM antibodies in these patients indicates recent or current infection.

The geographical distribution of prevalence of rubella in pregnant women has been evaluated by different authors in India and abroad. The rubella IgG prevalence in normal pregnancy in our study is 54.5% which is closer to IgG prevalence of 67.8% as reported by N. Singhla et al [5] from Amritser. Higher value are reported by Deka Deepika et al [6] from New Delhi (79%) and Denoj Sebastian et al [7] from Kerala (90.4%). No Rubella IgM antibody was detected in NP group in our study. However, Mini P singh et al [3] from Chandigarh reported a somewhat similar IgM prevalence of 0.7% and a prevalence of 1% was reported by Deka Deepika et al [6].

The rubella IgG prevalence in BOH group (A2) in our study is found to be 83.33% which is closer to a prevalence of 84.1% as M.J. Golaipur et al [8] from Iran and 90.8% as reported by Padmavathy M. et al [9] from Bangalore. Other worker have reported a lower IgG prevalence in BOH group such as 67.4% and 61.3% by Kh.Sulochana Devi et al [10] and D.Turbadkar et al [4].

The rubella IgM prevalence in BOH group in our study is 3.7% which is closer to the findings reported by Kh Sulochana Devi et al [10] (3.46%), Padmavathy et al [9] (4.6%) and M.J. Golaipur et al [8] and also by Mini P. Singh et al [3]. Higher values were reported by Shashi Chopra et al [11] (9.5%), Denoj Sebastian et al [7] (11.3%) and D.Turbadkar et al [4] (26.8%).

Our study on rubella seroprevalence indicates that the prevalence of rubella IgG antibodies increases with the age, gestational age & the number of abortions. (NP=20-35 years, A1=20-40 years, A2=15-45 years). The rubella IgG antibodies were found in the 2nd and 3rd trimester for NP group but in A1 and A2 group, the IgG prevalence was found in all the three trimester of pregnancy (1st, 2nd and 3rd) . Prevalence IgG antibodies also with the parity of the patient (NP= 0-2, A1=0-4, A2=0-4).

Only two out of a total of 88 patients were found to be positive for rubella IgM antibodies. The 1st patient belonged to A1 group, age group 20-25 years, gestational age 3rd trimester and parity 1 and had a history of one abortion. The 2nd patient belonged to A2 group, age group 36-40 years, gestational age 3rd trimester and parity 2 and had history of 2 abortions.

CONCLUSION

Our study on rubella seroprevalence indicates that the prevalence of rubella IgG antibodies increases with the age, gestational age & the number of abortions. (NP=20-35 years, A1=20-40 years, A2=15-45 years). The rubella IgG antibodies were found in the 2nd and 3rd trimester for NP group but in A1 and A2 group, the IgG prevalence was found in all the three trimester of pregnancy (1st, 2nd and 3rd) . Prevalence IgG antibodies also with the parity of the patient (NP= 0-2, A1=0-4, A2=0-4). High prevalence of rubella IgG antibody indicates prior exposure / infection or vaccination.

There was only two cases of IgM antibody, one was with a history of one abortion (A1 group) and the other with 2 abortions (A2 group). These patient belonged to age group 20-25 years and 36-40 years respectively gestational age was 3rd trimester for both and A1 was with parity 1 and A2 with parity 2.The presence of rubella IgM antibody in these two patient with one and two abortions, indicates a close association with rubella infection. Early detection in pregnant women can reduce severity of the disease in the newborn. Early detection in pregnant women can reduce the severity of the disease in the newborn.

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