

Seroprevalence of Rubella Antibodies in Women of Rawalpindi and Islamabad

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ABSTRACT

Objective: This study aimed to determine the seroprevalence of Rubella immunoglobulin G (IgG) antibodies and associated risk factors among women in the twin cities of Pakistan.

Methodology: This retrospective study involved the collection of 838 blood samples from women at Medicare Hospital Rawalpindi, Holy Family Hospital (HFH) Rawalpindi, and Pakistan Institute of Medical Sciences (PIMS) Islamabad and was conducted from May 2020 to April 2021. Rubella IgG antibodies were detected in the samples using the Chemiluminescent Microparticle Immunoassay (CMIA) method, and statistical analysis was performed using SPSS version 24.

Results: Of the 838 blood samples tested for Rubella IgG antibodies, seroreactivity and seropositivity was highest in the age group of 21-31 years. The prevalence of positive cases reached 96.82%, peaking in November, while negative cases were at 15.38%, peaking in May. These results indicate that many women possess antibodies against the Rubella virus.

Conclusion: The frequency of seropositivity and seroreactivity was elevated during winter. Socioeconomic factors may have contributed to the high prevalence of Rubella cases. Serosurveys should be conducted nationwide. Pakistan should include the MMR vaccine in its national Expanded Program of Immunization (EPI) as it is both effective and affordable.

Keywords: Chemiluminescent Microparticle Immunoassay (CMIA), Extended Program of Immunization (EPI), Measles Mumps Rubella (MMR), Prevalence, Rubella.

Authors' Contribution:

^{1,2}Conception; Literature research; manuscript design and drafting; ^{2,3}Critical analysis and manuscript review; ^{3,4}Data analysis; Manuscript Editing.

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Introduction

In 1962, the Rubella virus was first isolated from cell culture.¹ The Rubella virus is an enveloped, positive-stranded RNA virus with virions ranging from 500 to 900Å in diameter.² The Riboviria family Matonaviridae (genus Rubivirus) has only one family member that causes Rubella infection.¹ Rubella virus causes Rubella disease, also known as German measles. The symptoms of Rubella infection are mild in most cases, but become threatening when the

virus targets the fetus during the first trimester, which can cause spontaneous abortion or congenital Rubella syndrome (CRS). Children with Congenital Rubella Syndrome can experience hearing loss, microcephaly, cataract, glaucoma, heart problems, and other lifelong abnormalities, including autism and diabetes.³ It has been reported by the World Health Organization (WHO) that annually >100 000 infants are born with CRS worldwide.⁴ There is no available treatment for pregnant females or infants

with Rubella infections. Vaccination against Rubella is the only available treatment option.

Several efforts were made to develop effective vaccines using attenuated viral strains after the epidemics broke out in Europe in 1962-1963 and the USA in 1964-1965. The first successful vaccine was prepared from the HPV-77 strain, which was propagated in African green monkey kidney (GMK) cells established at the National Institutes of Health's Division of Biologics Standards.⁵ Merck quickly developed combination products; approval for the mumps-Rubella vaccine was given in 1970, whereas Measles-Mumps-Rubella (MMR) and measles-Rubella vaccines were approved in 1971. MMR comprises three live attenuated viral vaccine strains and these combinations have shown promising results in terms of broad protection against measles, mumps, and Rubella. The most widely used live attenuated Rubella vaccine is the RA 27/3 virus strain. Children should be administered the first dose of MMR at 12–15 months during the second dose between 4 and 6 years of age. Infants between 6 and 11 months of age were immunized with a single dose of MMR vaccine. Females of reproductive age who are non-pregnant and have no antibodies against the Rubella virus should be administered a single dose of MMR vaccine.⁶ Vast Rubella vaccination coverage has successfully eliminated Rubella in most European and North American countries.⁷ The incidence of Rubella is still a matter of concern in developing countries, such as Nigeria, with low immunization coverage.⁵ Pakistan has not introduced the MMR vaccine into its extended national immunization (EPI).

Specimens that are widely used for the diagnosis of Rubella by virus detection are throat swabs, nasopharyngeal secretions, or oral fluids, whereas serum samples are usually collected for the detection of IgG and IgM antibodies.⁸ Serological tests are conducted to screen for Rubella antibodies. Doctors also recommend screening for herpes virus, Cytomegalovirus, Rubella and Toxoplasma, which are collectively called (TORCH) profile, to screen for

the presence of any viral infection that can disturb the fetus or its development in pregnant women with any previous record of miscarriage, or any other abnormality in newborn babies.⁹

Commercially available equipment, such as counting immunoassays (CIAs), are used for its detection. These assays are based on enzymatic or chemiluminescent detection. The ARCHITECT chemiluminescent microparticle immunoassay (CMIA) assays for Rubella IgG and IgM utilize an indirect format (anti-human immunoglobulins conjugate), and the magnetic microparticles are coated with semi-purified Rubella virus (strain HPV 77) for IgG and viral lysate for IgM. For Rubella IgM, in predilution step, anti-human IgG was added to the reagent to lower the interference of rheumatoid factor (RF), and the results are measured qualitatively as an index value. These CIAs were adjusted according to international standards, and the results are represented in international units/ml. The cutoff value for a person being immune to the virus is mostly set at 10 IU/ml.¹⁰

The seroprevalence of Rubella varies from one region to another. According to the data available on the WHO website, the total number of Rubella cases reported in Pakistan from 2011 to 2019 was 5170, while the number of cases of Congenital Rubella Syndrome was 29 in 2018 and 12 in 2019.¹¹ In a study conducted in Rawalpindi, 696 women were screened for Rubella IgG antibodies, of which 576 (82.76%) were seropositive, 73 (10.49%) were seronegative, and 47(6.75%) showed equivocal results.¹² In a meta-analysis conducted in Iran, 7601 pregnant women were screened for Rubella antibodies, and the prevalence of immunity was 90.1%. Before the introduction of the national vaccination program, Rubella immunity was 88.6%, which increased to 91.5% after the vaccination program was introduced.¹³ In Ethiopia, 9.5% of pregnant women were positive for IgM, 79.5% were reported to be immune, and 11% were susceptible to Rubella infection.¹⁴ A high population level of immunity can be a factor in the control of Rubella

and congenital Rubella syndrome (CRS).¹⁵ A high socioeconomic status, which implies good living conditions, is associated with a minimum risk of contracting Rubella infections.¹⁶ Pregnant women with a high economic status tend to visit health practitioners regularly and are better aware of the consequences of Rubella infection or any other infection that may cause miscarriage. The chances of Rubella infection among children with a family size greater than five were 2.4 times greater than those with a household size of less than five.¹⁷

As there is no vaccination program for Rubella in Pakistan, it is of utmost importance that serosurveys be conducted all over the country regularly to determine the seroprevalence of Rubella. To highlight the importance of immunization against the Rubella virus. This study was designed to determine the seroprevalence of Rubella IgG antibodies among women of reproductive age and the associated risk factors.

Methodology

This retrospective study analyzed 838 blood samples collected from May 2020 to April 2021 from women aged 17 to 48 years who presented at the outpatient departments of Holy Family Hospital (HFH), and Medicare Hospital in Rawalpindi, and Pakistan Institute of Medical Sciences (PIMS) Islamabad. Rubella IgG antibody tests were conducted at CITI LAB on Murree Road, Rawalpindi.

Data collection and laboratory procedures: Blood samples were drawn from the patients in serum separating tubes (SST) with gel and labelled properly. Blood samples were allowed to clot and were centrifuged to separate the serum before testing. A commercially available kit (Abbott USA) was used for quantitative detection of anti-Rubella virus IgG antibodies using the Chemiluminescent Microparticle Immunoassay (CMIA) method. The tests were performed using Architect i2000SR (Abbott). These kits can detect IgG antibodies, both quantitatively and qualitatively.¹⁸ Positive values

were interpreted as IgG titers ≥ 10 IU/mL, and titers ≤ 4.9 were considered seronegative. Results ranging from 5.00 to 9.99 were considered as grey zone or borderline.

Data analysis: Following the detection of Rubella IgG antibodies, the results were entered into Microsoft Office Excel 2019. Data were statistically analyzed using SPSS version 24.

Ethical approval for the study was obtained from the Ethical Review Board (ERB) of COMSATS University Islamabad department of Biosciences. (ERB# CUI/Bio/ERB/06-21/23) on June 05, 2021.

Results

A total of 838 blood samples were collected from May 2020 to April 2021 from women attending the outpatient departments of Holy Family Hospital Rawalpindi (HFH), Pakistan Institute of Medical Sciences Islamabad (PIMS), and Medicare Hospital Rawalpindi. The age of the participants ranged from 18 to 48 years. Among the 838 selected females, 4.7% were under 20 years, 66.7% were between 21 and 31 years, 26.7% were between 32 and 42 years, and 1.8% were older than 43 years. The samples were processed for the detection of IgG antibodies. The demographic details are provided in Table I.

Table I: Frequency of socio demographic respondents along with characteristics (n=838)

Age (Yrs)	Number (Percentage)
< 20	39 (4.65)
21-31	559 (66.71)
32-42	230 (27.45)
≥ 43	10 (1.19)

Age-wise frequency of IgG antibodies: A total of 838 patients aged 18 to 48 years were tested for Rubella IgG antibodies. Among these, 39 patients were under 20 years old; 38 tested positive for IgG antibodies, while one had an equivocal result. There were 559 patients aged 21 to 31 years, with 39 showing negative IgG antibody results, 501 tested

positive, and 19 classified as borderline. Additionally, 230 patients were aged between 32 and 42 years; 207 had IgG antibodies, 12 tested negative, and 11 had equivocal results. In the group of patients aged 43 years and older, which consisted of 10 individuals, seven had positive results, and three had equivocal results. Statistical analysis revealed a highly significant difference ($p < .001$). The detailed results are presented in Table II.

Age	No	Border line	Positive	Negative	P-value
< 20	39	1	38	0	0.001
21-31	559	19	501	39	
32-42	230	11	207	12	
≥ 43	10	3	7	0	
Total	838	34	753	51	

Age	No of cases	Borderline (%)	Positive (%)	Negative (%)
< 20	39	2.56	97.4	0
21-31	559	3.39	89	6.97
32-42	230	4.78	90	5.21
≥ 43	10	30	70	0
Total	838	4.05	9.58	6.08

Age-wise prevalence of IgG antibodies: Patients aged less than 20 years had a prevalence of 97.43%, while those with borderline results had a prevalence of 2.57%. In patients aged between 21 and 31 years, a positive prevalence of 89.62% was noted, with a borderline prevalence of 3.39%. For patients aged between 32 and 42 years, the positive prevalence was 90%, and 4.78% had an equivocal prevalence. Among patients aged 43 years and older, the borderline prevalence was 30%, and the positive prevalence was 70%. The highest seroprevalence

was observed in patients aged 21 to 31 years, while the lowest was in patients younger than 20 years. Notably, the highest seropositivity of 97.43% was found in patients aged less than 20 years, whereas the lowest was observed in those aged 43 years and older. Further details are provided in Table III.

Month	No. of cases	Border line	Positive	Negative
May	13	0	11	2
June	12	1	11	0
July	79	4	72	3
August	84	3	77	4
September	87	1	76	10
October	80	1	73	6
November	63	1	61	1
December	109	6	99	4
January	82	1	76	5
February	74	5	63	6
March	68	4	62	2
April	87	7	72	8
Total	838	34	754	50

Month wise frequency of IgG antibodies: In December, there were 99 positive cases, the highest number recorded, while September had 10 negative cases, the most among all months. The lowest number of positive cases occurred in May and June, both with 11 cases. Notably, there were no negative cases in June. The highest number of borderline cases, 7, was reported in April, with no borderline cases in June. Overall, the number of positive and negative patients was highest during the winter months and lowest in the summer months. Statistical analysis revealed an insignificant value of 0.153. The additional details are presented in Table IV.

Month wise prevalence of IgG antibodies: The prevalence of positive cases was 96.82%, the highest in November, whereas May had 15.38%, the highest seronegative prevalence. The lowest positive prevalence (82.75 %) in April and November was

1.58%, which was the lowest seronegative prevalence. Detailed results are presented in table v.

Table V: Month wise prevalence of IgG antibodies

Month	No of cases	Border line (%)	Positive (%)	Negative (%)
May	1.55	0.0	84.61	15.38
June	1.43	8.33	91.76	0.0
July	9.42	3.79	91.23	5.06
August	10.02	3.57	91.67	4.76
September	10.38	1.14	87.45	11.49
October	9.54	1.25	91.25	7.50
November	7.52	1.58	96.82	1.58
December	13.00	5.50	90.82	3.66
January	9.80	1.21	92.68	6.09
February	8.84	6.75	85.13	8.10
March	8.20	5.88	91.17	2.94
April	10.38	8.04	82.75	9.19
Total	100	3.93	89.98	5.96

Discussion

Rubella, also known as German measles, is a viral infection caused by the Rubella virus. Symptoms include fever, sore throat, flu-like symptoms, bleeding, and swollen lymph nodes. One of the most serious consequences of Rubella is its ability to cross the placental barrier, potentially damaging fetal tissues and resulting in congenital Rubella syndrome (CRS). There is a lack of awareness regarding the existence and consequences of Rubella.

Current research suggests that the seroprevalence of Rubella antibodies among women of childbearing age depends on several factors, including vaccination campaigns, geographical region, and economic status. For example, a study carried out in Cameroon showed a seroprevalence of 94.4% among pregnant women, with this high level attributed to past exposure to the wild-type Rubella virus and limited immunization programs.¹⁹ Similarly, a meta-analysis of Rubella seroprevalence in sub-Saharan Africa indicated that among pregnant women, seroprevalence ranged between 68% and 98%. Despite being conducted in the sub-Saharan region, this variability highlights the inconsistency of studies.²⁰ Thus, research suggesting

that while certain groups may be immune to the pathogen, other populations remain at risk and require selective vaccination.

Another study conducted in Swabi, Khyber Pakhtunkhwa, Pakistan, found a seroprevalence of 84% for Rubella IgG among women of childbearing age, indicating that although there is a level of immunity, parts of the community are still susceptible to Rubella.²¹ This finding is consistent with the rates observed in other parts of Pakistan, where seroprevalence results range between 86% and 99%.²² These data underscores the need to maintain and improve levels of immunity, which should be pursued through constant monitoring and implementation of vaccination campaigns for women of childbearing age. Thus, the findings of these seroprevalence studies are highly significant for public health policy discussion.

Data are available on the Rubella immunity carrier rate; however, according to the WHO, 95% of women of childbearing age should have immunity against Rubella to effectively prevent Congenital Rubella Syndrome (CRS).²³

The estimated prevalence rates obtained from various surveys indicate that several areas, including Pakistan, have not achieved this goal. For instance, a study conducted in Turkey reported a seroprevalence of 94.3% among pregnant women but also noted the need for improved vaccination control to address immunity gaps.²⁴

Strategies to prevent CRS should include vaccination of Rubella in children by including MMR vaccines in the extended program of immunization (EPI) in Pakistan, susceptible women in the postpartum period, and selective vaccination of childbearing women. Two different approaches were used in Sweden to prevent CRS, with 90% of the population covered. Their first approach targeted girls attending school and women without IgG antibodies after pregnancy. The second method involved administering two doses of measles, mumps, and Rubella vaccines to girls and boys at 18 months of age. No cases of CRS have been reported since

1985.²⁵ This study was conducted to determine the seroprevalence of IgG antibodies among women in Rawalpindi and Islamabad between May 2020 and April 2021. In this study, seronegativity was high (15.38%) in May and seropositivity was high (96.28%) in November. The age group of less than 20 years had the highest prevalence of positive cases, whereas the age group of 21-31 had the highest prevalence of negative instances. The results showed that many women had antibodies against the Rubella virus. Socioeconomic factors and a lack of awareness may contribute to the prevalence of Rubella.

In this study, the age group of 21-31 had the highest seronegativity ratio of 6.97%, which means that this age group is at a high risk of Rubella infection. A similar study was conducted in Uganda for IgM prevalence in the age group 5–15 years, and age group > 15 years as compared to age < 5 years was at greater risk for Rubella virus.²⁶

In a similar study conducted in India, 1800 samples were tested for IgG antibodies against Rubella, of which 1502 (83.4%) were positive, 24 (1.3%) were indeterminate, and 274 (15.2%) were negative for Rubella IgG antibodies.²⁷ In Jeddah, Saudi Arabia, a serosurvey of Rubella showed that 8.4% of women were at risk of Rubella infection.²⁸

In another study from Rawalpindi, a total of 730 samples were tested for Rubella antibodies, of which 92.32% were seropositive and 7.70% were found to be at risk of developing infection.²⁹

Another study was conducted in Saudi Arabia on the seroprevalence of Rubella antibodies, in which 4328 pregnant women were included. The seroprevalence of Rubella immunity was 76.41%, while positive Rubella IgM antibodies were identified in 1.21% of 17 patients who underwent the test.⁵

Seropositivity was (84.7%) in 380 pregnant women, while (10%) were seronegative in a prospective multicenter study conducted in Southern Morocco. Tests were performed using an Architect i1000 chemiluminescent microparticle immunoassay (30).

The rate of seronegativity among developed countries is lower because of the wider availability and affordable healthcare systems. In American countries, owing to the universal vaccine approach, the occurrence of Rubella was reduced by more than 98%, which eventually resulted in the elimination of CRS since 2008.³¹

This study had a few limitations. As this survey did not distinguish between vaccination and vaccine-preventable infections, the vaccination status of individuals was not documented. Furthermore, because it is a predominantly random sample and was confined to only two districts of Punjab, the study sample did not reflect the entire Pakistani population. To avoid this, the rates determined from our findings may not be applicable to all pregnant women in both the provinces.

Conclusion

- The Rubella vaccine should be included in the national extended program of immunization (EPI), as it is safe and inexpensive.
- Serosurveys should be performed in diverse situations to distinguish women who are vulnerable to Rubella. Therefore, efforts should be made to increase awareness regarding the effects of Rubella. If any woman is seronegative during pregnancy, preconception Rubella screening and immunization are indicated. Owing to a lack of knowledge, there was a low level of recognition and coverage of vaccination among healthcare personnel.
- As Rubella infections are vaccine-preventable, policymakers should promote vaccination compliance among the public to eradicate them.
- Primary care doctors are responsible for counselling and educating teenage girls and women of childbearing age about Rubella infection, neonatal Rubella Syndrome, and the need for immunization to prevent these diseases.

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