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Rubella Virus IgG antibodies among patients attending a Teaching Hospital in Port Harcourt, Nigeria.

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Abstract: Rubella is a disease with a significant human public health problem in developing countries. This study aimed to determine the Rubella Immunoglobulin G (IgG) antibody prevalence among patients attending a teaching hospital in Port Harcourt, Nigeria. Serum samples from 80 patients were analyzed using ELISA Kit containing IgG for Rubella Virus detection. The overall prevalence of Rubella Virus IgG antibody was 72(90.0%). The gender-specific prevalence was (89.7%) for males and 46 (90.2%) for females. The seroprevalence of IgG was highest (90.9%) in the age group 2-25 years and least (88.2%) in the age group 41-70. The seroprevalence of Rubella virus IgG antibodies among patients in Port Harcourt is high. This high prevalence suggests that a sustained viral circulation exists in children and infection occurs early in infancy, underscoring the importance of continual vaccination to eliminate possible transmission of Rubella to the general populace.
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1.0 Introduction

Rubella is a mild disease with a significant human public health problem in developing countries (Olabode et al., 2021). It is caused by Rubella virus, a non-arthropod-borne member of the family *Togaviridae*. Although many infections with the agent are subclinical, this virus can potentially cause fetal infection, resulting in congenital disabilities (Gershon, 2005). Primary infections caused by Rubella can lead to severe complications in pregnancy (Uyar *et al*., 2008).

Rubella and Congenital Rubella Syndrome (CRS) remain essential health problems in many countries (Best et al., 2005). Rubella is a viral exanthema of childhood that is generally sub-clinical and inconsequential (Kaushal and Baxi 2007). Devastating congenital rubella syndrome makes Rubella virus of significant public health importance during pregnancy (Palihawadana *et al*., 2003; Bamgboye *et al*., 2004; Seker *et al*., 2004; Kaushal and Baxi, 2007).

At least half of all primary infections go undiagnosed because of the subclinical nature of the infection (Shukla and Maraqa, 2021). Nevertheless, Rubella poses a particular threat to the developing fetus if contracted during early pregnancy. *In utero* infection of the fetus may result in congenital deformity or other consequences of Congenital Rubella Syndrome (Lambert *et al*., 2015; Mawson and Croft, 2019). Therefore, girls must develop immunity to Rubella by the time they reach childbearing age to prevent such an outcome.

Rubella epidemics are or have been a worldwide phenomenon among the general population. However, concern for congenital Rubella infection has made the survey of infection among the female population critical, as about 60% of women of childbearing age worldwide remain susceptible to Rubella. The risk of congenital Rubella in seronegative pregnant women has been found to produce congenital abnormalities even in developed countries (Bamgboye *et al*., 2004). Anti-Rubella IgG seropositivity varies widely in different countries in the world. One study in Sri Lanka reported seropositivity of 76% in pregnant women (Palihawadana *et al.,* 2003). In Iran, the antibody prevalence among women under 25 years old has been reported to be 88.9%% (Ghafourian *et al*., 2015). Desinor *et al.* (2004) reported Rubella seropositivity of 95.2% in pregnant women in Haiti, a country without a vaccination program against Rubella. In Nigeria, Rubella antibody prevalence in women of childbearing age has been reported to be 77% (Onyenekwe *et al*., 2000). Another study in Nigeria reported a Rubella seroprevalence of 54.1% in pregnant women (Bukbuk *et al.,* 2002). Elsewhere

in Africa, a study in Ghana reported a 92.6% prevalence among pregnant women, with susceptibility linked with younger age (Lawn *et al*., 2000), while another in Eritrea reported a prevalence of 99% among the female population.

Although most women are immune to Rubella at childbearing age, studies continue to show early age of exposure to Rubella and congenital Rubella (Lawn *et al*., 2000; Baio *et al*., 2018). Indeed, Rubella infection is primarily common in childhood but can occur at any age worldwide (Best *et al.,* 2005; Santis *et al.,* 2006). An increase in immunization coverage has decreased the incidence of Rubella globally, but the incidence and mortality among children in low and middle-income countries are still high (Portnoy *et al*., 2019). A study of Rubella antibodies among children in Jos, Nigeria, reported a seroprevalence of 45.2%, of which 66.7% were female (Junaid *et al*., 2011). A seroprevalence of 74.8% was reported among children aged 3-14 years in Sudan (Ahmed *et al*., 2019). Because the Rubella Virus IgG antibody test does not discriminate between vaccine-induced and naturally acquired immunity, seropositivity to Rubella IgG is likely to be a surrogate for vaccine status (Abu-Madi *et al*., 2010).

Most nations in the Middle East, including Qatar, have Rubella in their national immunization schedules, whereas almost all African nations do not (Robertson *et al*., 2003). Rubella immunization rates are not optimal, and infections during pregnancy still occur since many countries incorporate no Rubella vaccine in their national immunization program (Seker *et al*., 2004).

In most countries, the evaluation of immunity to the Rubella virus relies on specific antibodies and its titers in the blood (Kaushal and Baxi, 2007). Detecting Rubella-specific IgG and IgM antibodies is crucial for the serological diagnosis of both congenital and primary postnatal Rubella infections as they can lead to severe congenital disabilities (Okonko et al., 2020). The absence of Rubella-specific IgG antibodies in sera characteristically of long-term duration after primary infections, presence of virus-specific IgM is indicative of the risk of defects in newborn infants.

There is a dearth of information on the national incidence and prevalence of Rubella infection (Adim et al., 2020). In this study, we explored the immune status, sex, and the likely age of exposure to Rubella virus among patients attending a teaching hospital in Port Harcourt, Nigeria. Precise Rubella IgG assays provide the clinician with a helpful and reliable test for monitoring these risks in pregnancy and the immunological response upon vaccination.

2.0 Materials and Methods

2.1 Study Area

This study was carried out among patients attending the University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria. Port Harcourt (Igbo: *Diobu*, *Iguocha* or *Ugwuocha*; Pidgin: "Po-ta-kot") is the capital of Rivers State, Nigeria. According to the 2006 Nigerian census, Port Harcourt has a population of 1,382,592 (NPC, 2006). The Port Harcourt Urban Area (Port Harcourt metropolis) comprises the city and parts of the Obio/Akpor Local Government Area. It lies along the Bonny River in the Niger Delta. Coordinates: 4°53'23"N 6°54'18"E and located in a city 360 km^2 (139 sq mi). (Ogbonna *et al*., 2007; Mbakwem-Aniebo *et al*., 2012a,b).

2.2 Study design

A hospital-based cross-sectional survey design was adopted for the present study, which seeks to survey the prevalence of Rubella Virus IgG antibodies in patients attending the Lulu Briggs Health Centre, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

2.3 Study Population

Eighty patients (29 males and 51 females) of different ages and socioeconomic statuses attending the Lulu Briggs Health Centre, University of Port Harcourt, were enrolled in this study. The study was conducted from June 2013 to March 2014 by recruiting consecutive consenting patients until 80 participants were attained. Other relevant information from all participants was obtained using a questionnaire designed for this purpose.

2.4 Specimen collection

The method of sample collection employed was the venipuncture technique (Okocha *et al*., 2005). The samples of blood were collected into an EDTA bottle. The specimens were transported in a commercially available collection and transport system for viral parasites to the Virus Research Unit of the Department of Microbiology, University of Port Harcourt, Nigeria, for analysis and processed using standard laboratory procedures.

2.5 Specimen Preparation

From aseptically drawn blood samples was prepared plasma or serum using standard techniques of preparation of samples for clinical laboratory analysis. Samples were identified with codes or names to avoid misinterpretation of results. Sera and plasma were stored at +2°-8^{0}C for up to five days after collection. Samples were centrifuged at 3000 rpm for 20 min.

2.6 Serological Analysis

ELISA Kit containing IgG for Rubella virus (manufactured by DIA.PRO Diagnostic Bioprobes, Milano – Italy) was used to screen for Rubella-

specific–IgG antibody according to the manufacturer's instructions. About 5ml blood samples were collected from the participants by venipuncture into sterile plain bottles. The samples were centrifuged at 1500g for 20 minutes, and the sera were aspirated into sterile labelled plain micro vials and kept frozen at minus 20^0C until tested.

2.7 Data Analysis

Data were analyzed using Microsoft Excel 2007 version to calculate the International Unit (IU) from Optical Density (OD). Values below ten were considered negative, and values above ten were considered positive. Results were expressed as numbers and percentages. Chi-square tests were performed to assess differences between groups. A p-value of < 0.05 was considered statistically significant. Identification of factors that may correlate with seroconvert- ion was carried out by Chi-square (x^2) distribution method using Microsoft Excel.

3.0 Results

3.1 Subjects characteristics

In this study, 80 (100.0%) patients were tested for Rubella Virus IgG antibody. The age range of the subjects used in this study was two years to 69 years. Majority of the subjects were females [51(63.7%)] while 36.3% (n = 29) were males (Table 1).

Table 1: Socio-demographic characteristics of the patients

Characteristic	Frequency
Age Group	
Two -25 years	22
26-40 years	41
41-70 years	17
Sex	
Males	29
Females	51
Total	**80**

3.2 Seroprevalence of Rubella Virus IgG antibody

Of the 80 subjects tested for Rubella Virus IgG antibody, 72(90.0%) were positive, and 8(10.0%) were negative (Table 2).

3.3 Seroprevalence of Rubella Virus IgG antibody with age

Table 2 shows the seroprevalence of Rubella Virus IgG antibody with the subjects' ages. It showed that seroprevalence of anti-rubella virus IgG was higher in the age group 2-25 years [20(90.9%)] and 26-40 years [37(90.2%) than their counterparts in the age group 41-70 years with 88.2% prevalence of Rubella Virus IgG antibody. It also showed that of the 8(10.0%) that had no Rubella Virus IgG antibody, 2(9.9%) were from the age group 2-25 years of age, 4 (9.8%) were from the age group 26-40 years of age, and 2(11.8%) were from age group 41-70 years of age (Table 2). The study showed no age ($p>0.05$) association in the seroprevalence of Rubella Virus IgG among the subjects studied.

Table 2: Seroprevalence of Rubella Virus IgG Antibody with Age

Age groups	No. Tested (%)	No. Positive (%)	No. Negative (%)
Two months -25 years	22(27.5)	20(90.9)	2(9.1)
26-40 years	41(51.3)	37(90.2)	4(9.8)
41-70 years	17(21.2)	15(88.2)	2(11.8)
Total	**80(100.0)**	**72(90.0)**	**8(10.0)**

3.4 Seroprevalence of Rubella IgG Virus antibody with sex

Table 3 shows the seroprevalence of Rubella Virus IgG antibody with sex. Twenty-six (89.7%) males tested positive for anti-rubella virus IgG, and 46 (90.2%) females tested positive for anti-rubella virus IgG (Table 2). It also showed that 10.3% (n = 3) of the male subjects tested negative for Rubella Virus IgG antibody, while 9.8% (n = 5) of the females also tested negative for Rubella Virus IgG antibody. However, sex ($p<0.05$) was significantly associated with seropositivity of Rubella Virus IgG antibody (Table 3).

Table 3: Seroprevalence of Rubella IgG Antibody with Sex

Sex	No. Tested (%)	No. Positive (%)	No. Negative (%)
Males	29(36.3)	26(89.7)	3(10.3)
Females	51(63.7)	46(90.2)	5(9.8)
Total	**80(100.0)**	**72(90.0)**	**8(10.0)**

4.0 DISCUSSION

This study has shown that antibody to Rubella Virus-IgG was detected in 90.0% (n=72) of patients tested, 90.2% (n=46) of females and 89.7% (n=26) of males. This is similar to the previously reported by many authors within and outside Nigeria (Onyenekwe *et al*., 2000; Sallam *et al*., 2003; Seker *et al*., 2004; Kaushal and Baxi, 2007; Uyar *et al*., 2008; Mohammed-Durosinlorun *et al*., 2010).

The 90.0% seroprevalence reported in this study is comparable to 91.6% reported for Rubella IgG by Sallam *et al*. (2003). However, our value is lower than the seroprevalence of 94.3% anti-IgG against Rubella reported by Uyar *et al*. (2008) in a similar study. It is lower than 97.9% reported by Mohammed-Durosinlorun *et al*. (2010) and 92.4% reported by Adim and Okonko (2022) in similar studies.

Noticeable variability ranging from <10 to >250IU/ml was observed in this study. Seker *et al*. (2004) showed similar variability in serum IgG levels ranging between 24 and 143 IU/ml. Higher values occurred in women more than 28 years. Seropositivity in pregnant females increases with age. Bamgboye *et al*. (2004) found a statistically significant higher prevalence of antibodies in rural populations than urban areas.

In this study, the Rubella-IgG antibody titres of between <10 IU/ml and >250 IU/ml were encountered among the female subjects. This finding is a deviation from what has been previously reported by Onyenekwe *et al*. (2000). In a similar study, 91.0% of women had Rubella IgG levels of above 50 IU/ml in a study by Seker *et al*. (2004). Among Indian women, Rubella IgG antibody titers ranged between 15-272 IU/ml in a study by Kaushal and Baxi (2007). Similarly, 90.2% of all the female subjects were positive for Rubella-IgG antibody, while 9.8% had no detectable Rubella IgG antibody in this study. Kaushal and Baxi (2007) reported Rubella IgG in pregnant women (91.73%), non-pregnant women (88.93%) and women who had a miscarriage (92.65%).

Positive Rubella-specific IgG antibodies prevalence was slightly higher in two age groups (age group 0 to 25 years and 26 to 40 years). Antibody prevalence ranged between 88.2% and 90.9% for the different age groups. The relatively high prevalence in the youngest group (≤25 years) may indicate a lack of age-dependent association. Comparable antibody prevalence occurred in all age groups in this study. Although, seropositivity increases with age, according to some researchers (Nessa et al., 2008; Gupta et al., 2015). According to Hassan et al. (2016) this tendency differed from that observed in other locations.

This study had no statistically significant correlations between immunity to Rubella and demographic characteristics. This result agrees with the findings of Barreto *et al*. (2006), who reported that Rubella IgG antibodies occurred in 95.3% of pregnant women, and age and residence did not significantly affect the prevalence of Rubella IgG antibodies. It also agrees with our recent findings (Adim and Okonko, 2022). Caidi et al. (2009) reported that the highest seropositivity was identified in the age group of >40 years in an Oyo State, Nigeria, while the lowest rate, in the age range of 20-29 years, revealed a decreasing seroprevalence of rubella IgG antibodies as age increases (Adesina et al., 2008; Adim and Okonko, 2022). Thus, agreeing favorably with our present finding.

In this study, the prevalence of Rubella Virus IgG among females was 90.2%. Similarly, high antibody prevalence has occurred in several European countries where vaccination programmes against Rubella exist. In Iran, 85.3% had high-avidity anti-Rubella IgG and were regarded as cases of reinfection (Hamkar *et al*., 2009).

Also, this study determines the immune status of the patients studied. In the studied population, 90.0% of them were immune to Rubella. This observation is comparable to other studies. Reis *et al*. (2004) reported positive results in 84.0% of the patients, most of whom had high antibody levels. Palihadwadana *et al*. (2003) and Onyenekwe *et al*. (2000) reported 76% positive results. Bamboye *et al*.

(2004) reported positive results in 68.5% of the female subjects.

In this study, 9.8% of the females were non-immune to Rubella. The incidence is similar to that of several authors (Onyenekwe *et al*., 2000; Palihadwadana *et al*., 2003; Bamgboye *et al*., 2004; Reis *et al*., 2004; Kaushal and Baxi, 2007). Although most of the studied population appeared to possess protective levels of Rubella IgG antibodies, screening for protective immunity is always necessary for future protection against reinfection (Kaushal and Baxi, 2007).

In Nigeria, the Rubella seroprevalence has been between 86.5% and 100.0% among pregnant women in different studies reported in the last five years. The value reported in this study is similar and falls within the range of 86.5 and 100.0%. In the same vein, the value reported in this study compared favourably with the high seroprevalence of Rubella Virus reported elsewhere, in Turkey, with 86.5% - 95.1% prevalence in pregnant women (Yilmazer *et al.,* 2004; Pehlivan *et al.,* 2007) in one study and up to of 100% in another (Karakoc *et al.* (2003).

5.0 Conclusion

The seroprevalence of Rubella virus IgG antibodies among patients in Port Harcourt is high. Age and sex were not significantly associated with the seropositivity of Rubella virus IgG antibodies among patients in Port Harcourt, Nigeria. This high prevalence suggests that a sustained viral circulation exists in children and infection occurs early in infancy, underscoring the importance of continual vaccination to eliminate possible transmission of Rubella to the general populace.

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