SEROPREVALENCE OF IgG ANTIBODIES AGAINST RUBELLA VIRUS INFECTION AMONG PREGNANT WOMEN VISITING THREE HOSPITALS IN NIGER STATE FOR ANTE-NATAL CARE

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Abstract
Aim: This study was conducted to determine the seroprevalence of IgG antibodies against rubella virus infection among pregnant women visiting three hospitals in Niger State for ante- natal care.
Methods: A cross sectional type of study was conducted on 217 pregnant women. Blood samples were collected from the 217 subjects and tested for IgG anti-rubella virus, using Enzyme Linked Immunosorbent Assay (ELISA) (produced by DRG International Inc., USA).
Results: Out of the total 217, 128 (59%) were found positive for IgG anti-rubella. Pregnant women within the age 25-29 years had more prevalence 37 (17.10%) and the least prevalence 2 (0.92%) was recorded among women that are 40 years and above. It was observed that married women had the highest rate 122 (56.22%) and the lowest prevalence (0.5%) was recorded among divorced women. Similarly, pregnant women who are into farming were found to be more infected 62 (28.60%) when compare to others. Pregnant women without formal education had high 72 (56.2%) prevalence and the lowest prevalence 10 (7.8%) was recorded among women with primary education. Pregnant women in the second trimester recorded more infection rate 63(29.03%) compared to those in their first and third trimesters. Furthermore, pregnant women screened at Minna general hospital recorded high prevalence compared to those screened at Paiko and Kuta general hospitals. Both vaccinated and non-vaccinated pregnant women within the age 25-29 years were more infected compared to other age groups.
Conclusion: More awareness on the possible route of transmission of rubella should be encouraged.
Keywords: immunoglobulins G, non-vaccinated, pregnant women, seroprevalence.

Introduction

Rubella virus is a member of *Togaviridae* family. It is an enveloped virus and has a single stranded ribonucleic acid (RNA) genome. The virus has been identified as a human teratogen, capable of causing a spectrum of birth defects often collectively referred to as congenital rubella syndrome (CRS) or death of a developing foetus, especially if the viral infection is acquired at the early stages of the pregnancy (Lezan, 2015).

The virus is transmitted through the respiratory route and it replicates in the naso-pharynx and cervical lymph nodes (Lezan, 2015). During the incubation period of the virus, the patient is contagious before he/she develop rash, which may last for a week or two (Lezan, 2015). Rubella rash is a significant sign which makes it distinct from all other viruses with related signs (Elliman *et al.,* 2007; Lezan, 2015).

Measles caused by Rubella is self-limiting, mild illness that occurs during the second week after exposure to the virus (Forrest *et al*., 2002; Lezan, 2015). A prodromal illness consisting of fever, malaise and mild conjunctivitis, usually occur mostly in adults (El-Mekki, 1998). The rash is preceded by post auricular, occipital and posterior cervical lymphadenopathy by 5-10 days (Lezan, 2015).

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Rubella infected persons have a maculopapular, erythematous and often pruritic rash which occurs in 50-80% affected individuals (Lezan, 2015). The rash lasting 1-3 days starts on the face and neck regions, before progressing down to other parts of the body (Kolawole *et al*., 2014).

A pregnant infected woman within the first 20 weeks of her pregnancy, might have miscarriage, stillbirth, or baby born with Congenital Rubella Syndrome (CRS). Rubella infections can be prevented by active immunization program using live, attenuated virus vaccine. The vaccines are usually in the combination of measles mumps and *Rubella virus* (MMR) (Kolawole *et al*., 2014). Rubella virus can be detected in nasopharyngeal samples from 1 week before the onset of the rash to 2 weeks after (White *et al*., 1994; Reef *et al.*, 2002; Kolawole *et al*., 2014).

Detection of Rubella ribonucleic acid (RNA) directly from clinical samples (throat swabs) is a critical factor in early laboratory diagnosis of recent infection, in addition to the detection of Rubella specific immunoglobulin M (IgM) in blood samples (El-Mekki *et al*., 1998;Kolawole *et al*., 2014). The immunocolorimetric assay (ICA) is a technique whereby Vero/HSLAM (human signalling lymphocyte activation marker) cells are stained to identify the presence of the Rubella virus using an ordinary light microscope, since the cytopathic effect (CPE) of the virus can be difficult to detect in routine virus isolation techniques (Frey *et al*., 1998; Jin *et al.* 2007; Mwambe *et al*., 2014).

The actual burden of Rubella virus is unknown for developing countries, but it is estimated that 100 000, congenital rubella syndrome (CRS) cases occur each year world-wide (Robertson *et al*., 1997; WHO, 2000). The prevalence of rubella virus infection has been estimated in several African countries, ranging between 70-90% (Pennap *et al*., 2009; Mwambe *et al.*, 2014). The World Health Organization (WHO, 2012) reported that Rubella cases have increased in Nigeria from 450 in 2010 to 3,691 in 2011. Seroprevalence of rubella IgG in pregnant women has been reported to be 54.1% in Maiduguri (Bukbuk *et al*., 2002), 68.5% in Ibadan (Bamgboye *et al*., 2004) 83% in Zaria (Mohammed *et al*., 2010) 53% in Benin (Onakewhor *et al*., 2011) and 93.1% in Zaria (Olajide *et al*., 2012).

Rubella Virus infection has become a global challenge in most developing countries such as Nigeria (Adeboye *et al*., 2011). In Niger state, it is one of the major cause of infant mortality and deformities in children. The un-noticeable nature of rubella virus infection in many cases particularly among pregnant women in Niger State has contributed to the persistent of the disease (Adeboye *et al*., 2011). This ugly situation has contributed to the steady increase in congenital rubella syndrome and infant mortality (Adeboye *et al*., 2011). Therefore, this study was an attempt to determine the prevalence of rubella virus infection among pregnant women in some selected areas in Niger state.

Materials and Methods

Description of the study area

Niger State was created from the defunct North Western state in 1976. The State is located on latitude 3.20° east and longitude 8° and 11.3° north, with Minna as its capital. It is bordered to the North by Sokoto State, West by Kebbi State, and South by Kogi and South-West by Kwara State. Kaduna state is bordered to the North East. Niger State has a common boundary with the Republic of Benin along New Bussa, Agwara and Wushishi Local Government Area. It has a land area of 76,363 square kilometres, with human population of 4,082,558. About 85% of the State's population is farmers, while the remaining 15% are engaged in other business. Paiko and Kuta are local government headquarters in Niger state.

Study Population

The population was composed of 217 consenting pregnant women 15-45 years who are attendees of General hospitals located in Minna, Paiko and Kuta.

Ethical Approval and Consent

Ethical approval was obtained (Reference Number HMB/GHM/STA/136/Vol III/455) from the research and ethics committee of the Niger State hospital Management Board for the study. Informed consent of the participating women was also sought and obtained.

Study Design

The study was a cross sectional type, where the choice of the selected hospitals was purposeful and used as a cluster for convenient sampling method.

Questionnaire

A designed and pretested structured Questionnaire having information on socio-demographic variables such as age, marital status, occupation, level of education, gestational period of the pregnancy and risk factors that may be associated with rubella virus infection.

Inclusion criteria

Pregnant women within the age 15-45 years, who are attendees of the selected hospitals and those above 45 years, were excluded

Exclusion Criteria

Pregnant woman below the age 15years, who are not attendees of the selected hospitals and those above 45years and did not give their consent, were excluded.

Sample Size Determination

The sample size was determined, using the equation by Kuta *et al.* (2012).

$$n = \frac{Z^2(1-p)\,q}{d^2}$$

Where

n= sample size

z = statistic for level of confidence (1.96)

p= expected prevalence or proportion (83% is 0.83)

d= precision (0.05)

$$n=\frac{(1.96)^2(0.83)\ (1-0.83)}{0.05^2} = 217 \text{ samples}$$

Specimen collection

Five millilitre (5ml) of blood sample was collected from each of the 217 pregnant woman into EDTA bottles and labeled accordingly for easy identification. Before the collection of the blood samples, pretested structured questionnaire was administered to each of the pregnant woman that participated. This was done to obtain demographic information about the participants (i.e. pregnant women). The blood samples spun at 1000rpm per minute for 15 minutes. The sera were collected and transported to Centre for Genetic Engineering Laboratory, Federal University of Technology, Minna and were stored in the refrigerator at -20 $^{\circ}$ C until needed (Hermann, 1980).

Detection of Rubella virus IgG antibodies

Sera were removed from the refrigerator (-20°C) onto a work bench and thawed at room temperature (25°C). One hundred microliter 1:40 dilution of test serum, negative control, positive control and calibrator were made up by adding 5µl of sample to 200µl sample diluent and it was carefully and thoroughly mixed. One hundred microliter (100µl) of diluent sera, calibrator and controls were dispensed into the appropriate wells. One hundred microliter (100µl) absorbent solution was dispensed into 1a well and the holder taped to remove air bubbles from the liquid and was mixed thoroughly (Gravell *et al.*, 1977). The plates were incubated at 37^{o}C for 30 minutes, at the end of the incubation period, the plates were rinsed five times with diluted wash buffer (1X). Then one hundred microliter (100µl) of the enzyme conjugate was dispensed in each well and mixed gently for 10 seconds. This was further incubated at 37^{o}C for 30minutes. Then the microtiter plates were rinsed again with diluted wash buffer (IX).

One hundred microliter (100µl) of the Tetramethylbenzidine (TMB) reagent was then dispensed into each well and mixed gently for 10 seconds. This was further incubated at 37^{o}C for 15 minutes. Finally, 100µl of stop Solution was added. This was mixed gently for 30 seconds. Then using a microtiter reader, the rate of reaction was read at 450 nm within 15 minutes with a micro-well reader (Gravell *et al.*, 1977).

Data analysis

Data obtained from the study was entered into an Excel spread sheet and imported to be analysed using student's t-test and C tests hi-square statistical tests (SPSS Version 20). Descriptive summaries were calculated using proportions for categorical variables (age, educational level, employment status, vaccination history and gestational age).

Results

The two hundred and seventeen (217) pregnant women were screened for IgG antibodies specific for rubella virus, 128 women were found positive, representing 59% of the population studied, age group 25-29 years had more prevalence 37(17.10%), followed by the women within the age group 30 – 34 years 29 (13.36%) and the lowest prevalence 2 (0.92%) was recorded among those that are ≥ 40 years (Table 1).

Table 1: Prevalence of Rubella infection according to Age group

Age Group (years)	Total	IgG positive	IgG negative
15 – 19	15 (6.9)	6(2.76)	9(4.15)
20 – 24	50 (23.04)	20(9.22)	30(13.82)
25 – 29	65(30.00)	28(12.90)	37(17.10)
30 – 34	47(21.66)	18(8.30)	29(13.36)
35 – 39	34(15.67)	13(5.99)	21(9.68)
≥ 40	06(2.76)	4(1.84)	2(0.92)
Total	217(100)	89(41)	128(59)

χ^2=12.87 P = 0.025 df =5 CI=95% T=3.18

Key: IgG (+ve) = Positive, IgG (-ve) = Negative, NSS = Number of sample screened

It was observed that married pregnant women recorded higher prevalence of 56.22%, while the lowest 1 (0.5%) was observed among divorced pregnant women (Table 2).

Table 2: Sero-prevalence of Rubella IgG Antibodies according to Marital Status

Marital Status	Total	IgG positive	IgG negative
Married	205(94.47)	122(56.22)	83(38.25)
Single	5(2.30)	3(1.4)	2(0.9)
Divorced	4(1.84)	1(0.5)	3(1.4)
Widow	3(1.38)	2(0.92)	1(0.5)
Total	217 (100)	128(59)	89(41)

df=4CI=95% t=0.9984 P=0.3746χ^2=4.2392

Key: IgG (+ve) = Positive, IgG (-ve) = Negative, NSS = Number of sample screened

The Table 3 below shows the prevalence of IgG antibodies in relation to occupational status of the pregnant women. It was observed that pregnant women engaged in farming, recorded higher prevalence of 62(28.60%) and the lowest prevalence (4.15) was observed among the pregnant women who were students.

Table 3: Sero-prevalence of Rubella IgG Antibodies according to Occupation

Occupational status	Total	IgG positive	IgG negative	Total
Trading	47(21.66)	27 (12.44)	20 (9.22)	47(21.66)
Civil Servant	35(16.13)	10 (4.61)	25 (11.52)	35(16.13)
Student	21(9.68)	9 (4.15)	12 (5.53)	21(9.68)
Housewife	31 (14.29)	20 (9.22)	11 (5.10)	31 (14.29)
Farming	83 (38.25)	62 (28.60)	21 (9.70)	83 (38.25)
Total	217(100)	128 (59)	89(41)	217(100)

df = 4 cl = 95% t =0.84 p = 0.45 χ^2=24.55

Key: IgG (+ve) = Positive, IgG (-ve) = Negative, NSS = Number of sample screened

It was observed that women who had no formal education recorded higher prevalence of 72 (33.18%) and the lowest prevalence was observed among those with Primary education 10(4.61%) (Table 4).

Table 4: Sero-prevalence of Rubella IgG according to Educational Status

Educational Status	Total	IgG positive	IgG negative
Primary Education	16(7.37)	10(4.61)	6(2.76)
Post primary	67(30.88)	33(15.21)	34(15.67)
Tertiary education	43(19.82)	13(6.00)	30(13.82)
No formal Education	91(42.00)	72(33.18)	19(8.76)
Total	217(100)	128(59)	89(41)

CI=95% P=0.517 DF=6 T=0.69 χ^2=5.44

Key: IgG (+ve) = Positive, IgG (-ve) = Negative , NSS = Number of sample screened

It was observed that women in their second trimester recorded high prevalence 63(29.03%) and low prevalence was observed among pregnant women in their first trimester 28(12.90%) (Table 5).

Table 5: Sero-prevalence of IgG antibody among pregnant women according to stages of pregnancy

Marital status	Total	IgG positive	IgG negative
1st trimester	47(22.00)	28(12.90)	19(8.76)
2nd trimester	112(52.00)	63(29.03)	49(22.60)
3rd trimester	58(27.00)	37(17.10)	21(9.70)
Total	217(100)	128(59)	89(41)

χ^2=12.87df=5 P=0.0246 CI=95%

Key: IgG (+ve) = Positive, IgG (-ve) = Negative, NSS = Number of sample screened

The prevalence of IgG antibodies among pregnant women according to study areas It was observed that pregnant women from Minna recorded more prevalence 67(30.90%) and the least prevalence of 12.44% was recorded for pregnant women from Kuta (Table 6).

Table 6: Distribution of Rubella virus infection according to study areas

Location of Respondents	Total	IgG positive	IgG negative
Minna	104(48.00)	67(30.90)	37(17.10)
Paiko	60(28.00)	34(15.70)	26(12.00)
Kuta	53(24.0)	27(12.44)	26(12.00)
Total	217(100)	128 (59)	89(41)

χ^2=12.87 t=3.18 p=0.0246 df=5 CI=95%

Key: IgG (+ve) = Positive, IgG (-ve) = Negative, NSS = Number of sample screened

It was observed that 35.48% of women that had history of MMR vaccines were positive to Rubella and 32.26% were negative. On the other hand 23.50% of those without history of vaccination were positive while 8.76% of non-vaccinated pregnant women were negative (Table 7).

Table 7: Age distribution of Rubella Virus in relation to Measles Mumps and Rubella (MMR) vaccination history

Age Group (years)	MMR+	IgG(+ve)	IgG (-ve)	MMR-	IgG(+ve)	IgG(-ve)
15 – 19	10(4.61)	6(2.76)	4(1.84)	5(2.30)	2(0.92)	3(1.38)
20 – 24	36 (16.59)	14(6.45)	22(10.14)	14(6.45)	11(5.07)	3(1.38)
25 – 29	43(19.82)	24(11.06)	19(8.76)	22(10.14)	19(8.76)	3(1.38)
30 – 34	28(12.90)	19(8.76)	9(4.15)	19(8.76)	13(5.99)	6(2.76)
35 – 39	26(11.98)	12(5.53)	14(6.45)	8(3.69)	6(2.76)	2(0.92)
≥ 40	4(1.84)	2(0.92)	2(0.92)	2(0.92)	0(0.00)	2(0.92)
Total	147(67.74)	77(35.48)	70(32.26)	70(32.26)	51(23.50)	19(8.76)

χ^2=2.905 p=0.7146 t=6.50 CF=95% df=5

Key: MMR =Measles Mumps and Rubella**,** MMR+ =Number vaccinated against MMR, MMR- =Number not vaccinated against MMR, IgG (+ve) = Positive, IgG (-ve) = Negative

Discussion

In this study, the prevalence of IgG against rubella virus infection among women visiting Minna, Kuta and Paiko General Hospitals in Niger State was investigated. Our findings revealed that a high proportion 128 (59%) of the 217 women screened were positive for anti- Rubella virus IgG. However, similar prevalence (93.1%) has been reported by Onaekewhor *et al.* (2011) in Benin, Edo State, Adewumi *et al.* (2013) reported 89.4% in Ibadan, Oyo State and Olajide *et al.* (2015) reported 93.1% in Zaria, Kaduna State. The variation in the results of this study and the previous studies may be attributed to poor hygienic practices, lack of basic knowledge on the possible route of transmission of the virus, poor immunization coverage and negligence on the part of the pregnant women.

In this study, a prevalence of 17.10% was recorded among pregnant women within the age group 25-29 years which emerged as the highest prevalence among the age groups studied. Similar result has been reported by Kolawole *et al.* (2014) in Osogbo and it was attributed to depressed immunity, peculiar with malnourished pregnant women. The observation made by Kolawole *et al.* (2014) could be used as possible explanation for the result obtained in this study. However, chi square analysis revealed that there is significant association between IgG antibody titre and the age of the pregnant women.

Similarly, this study also revealed that marital status was not a significant factor in rubella seropositivity ($p>0.05$) even though high prevalence was observed among married women as compared with unmarried individual (Table 2). Similar result has been reported by Seker *et al.* (2004), Bamgboye *et al.* (2004) and Oyinloye *et al.* (2014) in Turkey, Ibadan and Maiduguri respectively.

Furthermore, this study revealed that occupation of pregnant mothers in the study areas had association with incidence of Rubella IgG. High prevalence (28.6%) was recorded among mothers who are farmers. This is similar to the report by Mohammed *et al.* (2010), who opined that occupation of women predisposed them to factors that enhance the spread of rubella virus infection, but contrary to the report of Lezan (2015) who observed that occupation is a factor in the transmission of rubella virus. The observation made by Muhammed *et al.* (2010) could be used to justify the result obtained in this study as most seropositive women were found to be farmers (Table 3).

The results of this study showed that educational status of the pregnant women was an insignificant risk factor. A finding consistent with the result of other study conducted by Kolawole *et al.* (2014) in Osogbo Nigeria (Table 4).

It is worthy to note that in all trimesters of pregnancy, Rubella IgG antibody was found to be present but was more with second trimester (Table 5). However, there was no relationship statistically between the gestation period of the pregnancy and the IgG titre levels. This result agrees with the work of Agbede *et al.* (2014), but differs from the work done by Ogbonnaya *et al.* (2012). The reason for the variation could be attributed to the level of exposure to rubella virus, coupled with weak immune system.

Pregnant women screened at Minna general hospital had more infection rate 67 (30.90%) compared to the prevalence recorded in Paiko and Kuta hospitals (Table 6). This could be attributed to cosmopolitan nature, being the hospital in the state capital coupled with referral cases from neighboring towns and villages.

It was equally observed that 35.48% of women that had history of Measles Mumps and Rubella (MMR) vaccination were positive to Rubella IgG and 32.26% were negative. This could be associated with lingering (remain for a longtime in the circulatory system) nature of the IgG in the circulatory system. Thus, MMR booster dose may require to mount long lasting immunity in vaccinated individual. On the other hand 23.50% of those without the history of vaccination were positive while 8.76% of none vaccinated were negative (Table 7). This may be associated with exposure to previous rubella infection. Chi square analysis shows that there was no relationship between IgG antibody titre and immunization history of the pregnant women.

Conclusion

The prevalence of rubella IgG antibody among pregnant women was high (59%), suggesting a sustained infection in the population and indicating endemic nature of the infection in Niger State. This may pose a challenge on pregnant women and their infants. The congenital rubella syndrome (CRS) due to rubella virus infection may likely increase in the study areas. Pregnant women within the age group 25-29 years were mostly affected. Socio-economic variables such as age, level of education, marital status and gestation period of the pregnancy were inconsequential to the level of IgG titre level.

Recommendations

It is essential for a National Rubella vaccination program to be initiated in Nigeria, particularly in Niger State. Pregnant women and women attending preconception programs should be screened for rubella, and postpartum vaccination should be done for sero-negative women to reduce morbidity, mortality and further spread.

The low level of awareness emphasizes the need for women to be enlightened about rubella infection, its dangers, and how it can be prevented.

As such, rubella should be included in the health talks given to pregnant women in antenatal education programs and women in preconception programs. Measures should be taken to ensure that outbreaks do not go unnoticed and are eventually stopped to prevent the free reign of rubella virus infection.

Finally, pregnant women should be advised to seek antenatal care earlier. This way, pregnancy can be more accurately monitored. Also congenital anomalies and their risk burdens can be better assessed and arrested in good time.

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