SEROPREVALENCE AND RISK FACTORS OF RUBELLA VIRUS INFECTION IN HIV POSITIVE WOMEN OF REPRODUCTIVE AGE ATTENDING FEDERAL MEDICAL CENTRE, MAKURDI, BENUE STATE

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ABSTRACT

Rubella infection, also known as German measles, is a contagious, generally mild viral infection that occurs mostly in children and young adults. It usually occurs as an acute infection which is mild in children but has serious consequences in pregnant women as it causes Congenital Rubella Syndrome. It is a vaccine-preventable disease, but developing countries like Nigeria are yet to commence routine vaccination against Rubella. This study evaluates the seroprevalence of rubella antibodies in a HIV population of women of reproductive age of different socio-demographics, along with certain predisposing factors to rubella infection. The cross-sectional surveillance was carried out among 180 consenting HIV positive women between ages 15 – 49 years attending clinic at Federal Medical Centre, Makurdi, in North Central Nigeria. Questionnaires were administered to obtain information from the patients, and the serum obtained from their blood samples was analysed for rubella IgG and IgM using ELISA technique. Seroprevalence of the antibodies was analysed in association with factors such as age, marital status, parity, family size, residence, educational and occupational status, knowledge, vaccination and disease history. Rubella antibody seroprevalence of 85.6% and 22.8% was observed for IgG and IgM respectively, with a co-occurrence of 16.1%. Of the total population, 7.8% was observed to be without any of the antibodies and as such were without immunity to rubella. Educational status, history of miscarriage, and symptoms like lymphadenopathy were found to be statistically significant in association with rubella antibody seroprevalence. The tendency of Rubella to be asymptomatic was also established in this study. The high seroprevalence observed in this study suggests that Rubella is endemic in the study area, with a sustained viral circulation at the time of study. This emphasizes the need for nationwide vaccination, routine rubella screening, especially in the immunocompromised, and creation of better awareness on the disease and its risks.

Keywords: Antibodies, HIV, Immunocompromise, Rubella, Seroprevalence.
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INTRODUCTION

Rubella virus is the only member of genus Rubivirus of the family Togaviridae. They are single stranded, positive-sense RNA enveloped viruses. *In-vitro* studies with cell lines have shown rubella to have an apoptotic effect on certain cell lines [1]. The spherical viral particles have a diameter of 50 to 70nm, covered by a lipid membrane (envelop) with prominent spikes [2].

Humans are the only known host for rubella virus *in-vivo*, but the virus is capable of *in vitro* replication in a wide variety of mammalian cell types, including primary African green monkey, kidney cells, BHK21, RK13 and Vero cells [3]. The virus is transmitted by airborne droplets when infected persons sneeze or cough. Rubella infection is endemic throughout the world although the infection is vaccine-preventable, and it is included as comprehensive vaccination programmes in certain industrialized and developed parts of the world like the Americas, Western Europe, Japan and Australia. These countries have achieved reduction in incidence of Rubella and Congenital Rubella Syndrome to very low levels [3]. The disease is however, still common in other developing parts of the world in countries such as Nigeria [4].

Rubella infection is usually mild in children and immuno-competent adults, though it poses a threat to children born to women infected during pregnancy [5]. The infection and disease is known as German measles or three day measles. Rashes appear and disappear usually within three days. It differs from the commonly known measles caused by the measles virus (Rubeola) [6]. There is no specific treatment for rubella infection. However, medications can be administered to manage the symptoms associated with the disease such as the rashes, fever and joint pains and schizophrenia [7]. A hallmark of Rubella infection is progression to congenital abnormalities in the unborn child especially when infection is acquired in the first trimester of pregnancy.

As part of the TORCH (*Toxoplasma gondii*, Rubella, Cytomegalovirus, and Herpes Simplex Virus) infections, rubella infection was found as an

opportunistic infection of the immuno-deficient HIV patients. These immune-compromised patients and pregnant women are considered the two major high-risk groups affected by TORCH infections [8]. In Nigeria, vaccine for the infection is not widely and readily available and the risk remains. The work employs a prevalence studies to determine the level of infection with the virus and possible risk factors of Rubella virus infection in HIV positive women of reproductive age attending Federal Medical Centre, Makurdi, Benue State

MATERIALS AND METHODS

Study area and period

The study was carried out in Makurdi, Benue State in Nigeria. Benue State is located in the North-Central part of Nigeria, referred to as the middle belt of the country from December to February, 2017 at Federal Medical Centre, Makurdi. This healthcare facility attracts patients from all over Makurdi and its environs. It comprises 2 major centres in different parts of the town, which are: Federal Medical Centre, Wadata, Makurdi. And Federal Medical Centre, Apir, Makurdi. The healthcare facility also has an Institute of Human Virology, Nigeria clinic which caters to patients from many parts of Makurdi and nearby villages for their anti-retroviral therapy. The facility and its clinics serve as a referral centre for many cases. Hence, a study population of HIV positive women in this healthcare facility is a true representation of HIV positive women in Makurdi, and by extension, Benue State.

Study population and design

The study population included HIV positive women of reproductive age, 15 – 49 years [9] attending the healthcare facility. This included both those who were pregnant and those who were not. This was a cross-sectional study to evaluate the seroprevalence and risk factors of rubella virus in HIV positive women of reproductive ages 15 – 49 years attending FMC, Makurdi whose consent was obtained. Sample size was determined by probability sampling, using a past prevalence. Questionnaires were administered to obtain information on risk factors and socio-demographics of the participants.

Ethical consideration

Ethical approval (attached in the appendix) was obtained from the institution these women attended for their antiretroviral therapy. Informed consent was sought before commencement of sample collection.

Sample collection

The aid of a laboratory scientist was employed in the collection of all samples from patients consenting to the study. About 5 mL of blood samples was collected from patients, aseptically into plain tubes for ELISA [3]. Samples were centrifuged and separated to obtain serum. These were stored in a freezer prior to transportation to the virology lab for analysis.

Laboratory investigations

Serum samples were transported to the lab and analysed using ELISA test procedure to assay for rubella IgG and IgM antibodies, following the manufacturers' instructions for both test kits.

RESULTS

The prevalence of IgG and IgM was 85.6% and 22.8% respectively indicating that whereas 85.6% has encountered the virus, only 22.8% had current infection with the virus which may be symptomatic or asymptomatic (Figures 1 and 2). Women aged between 25 – 34 years recorded higher IgM prevalence. However, the finding was not statistically significant (Table 1). Similarly, the difference recorded in respect of the marital status, parity and family size of the women were not statistically significant (Table 2, 3 and 4). Level of education was significantly associated with IgM prevalence indication that the infection occurred more in women with secondary education followed by women without formal education while there was no association between their occupation (Table 5 and 6). Symptoms such as rash (80% - IgG), fever (81.2% - IgG; 21.9% - IgM), Lymphadenopathy (66.7% - IgG) and Arthralgia (75% - IgG) were associated with the disease with an odd of over 1 (Table 7).

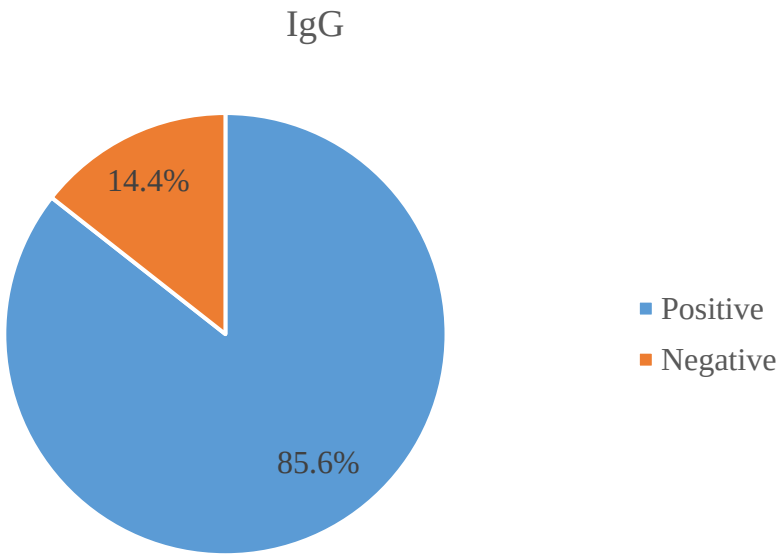

Figure 1: Seroprevalence of Rubella IgG among HIV positive women of reproductive age, attending Federal Medical Centre, Makurdi, Benue State.

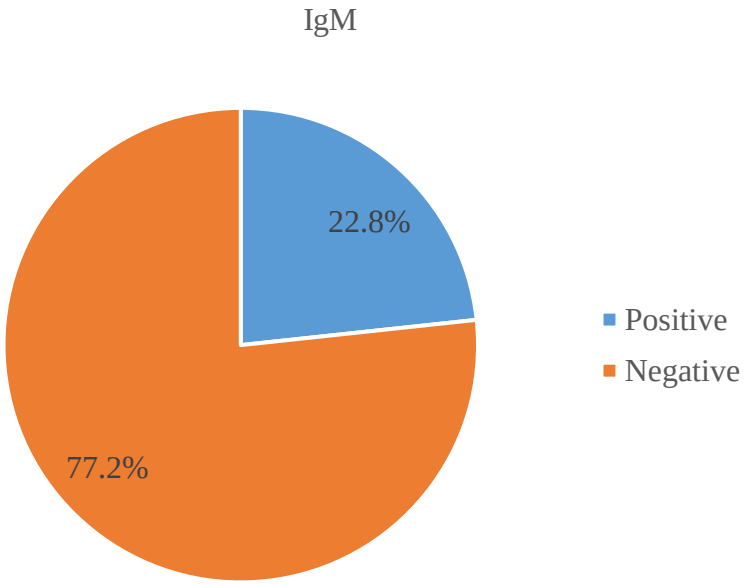

Figure 2: Seroprevalence of Rubella IgM among HIV positive women of reproductive age, attending Federal Medical Centre, Makurdi, Benue State

Table 1: Seroprevalence of rubella IgG and IgM with respect to age.

		Rubella IgG				**Rubella IgM**			
Age (years)	**No. Examined**	**No. (%) positive**	**df**	**Chi-square**	**p-value**	**No. (%) positive**	**Df**	**Chi-square**	**p-value**
15-19	1	0 (0.0)	6	9.267	0.159	1(100.0)	6	7.829	0.251
20-24	7	7 (100.0)				1 (14.3)			
25-29	35	29 (82.9)				10(28.6)			
30-34	47	38 (80.9)				13(27.7)			
35-39	35	32 (91.4)				8 (22.9)			
40-45	40	35 (87.5)				7 (17.5)			
45-49	15	13 (86.7)				1 (6.7)			
Total	**180**	**154(85.6)**				**41(22.8)**			

Key: df = degree of freedom

Table 2: Seroprevalence of rubella IgG and IgM with respect to marital status

		Rubella IgG				**Rubella IgM**			
Marital status	**No. Examined**	**No. (%) positive**	**df**	**Chi-square**	**p-value**	**No. (%) positive**	**df**	**Chi-square**	**p-value**
Married	144	121(84.0)	2	1.686	0.430	32(22.2)	2	0.209	0.901
Single	31	28 (90.3)				8 (25.8)			
Divorced	5	5 (100.0)				1(20.0)			
Total	**180**	**154(85.6)**				**41(22.8)**			

Key: df = degree of freedom

Table 3: Seroprevalence of rubella IgG and IgM with respect to parity

		Rubella IgG				**Rubella IgM**			
Parity	**No. Examined**	**No. (%) positive**	**df**	**Chi-square**	**p-value**	**No. (%) positive**	**df**	**Chi-square**	**p-value**
None	35	29 (82.9)	5	9.081	0.106	11(31.4)	5	10.109	0.072
1-2	63	50 (79.4)				19(30.2)			
3-4	54	48 (88.9)				5 (9.3)			
5-6	20	20(100.0)				4 (20.0)			
7-9	6	6 (100.0)				2 (33.3)			
>=10	2	1 (50.0)				0 (0.0)			
Total	**180**	**154(85.6)**				**41(22.8)**			

Key: df = degree of freedom

Table 4: Seroprevalence of rubella IgG and IgM with respect to family size

		Rubella IgG				**Rubella IgM**			
Family size	**No. Examined**	**No. (%) positive**	**df**	**Chi-square**	**p-value**	**No. (%) positive**	**Df**	**Chi-square**	**p-value**
<=4	64	54 (84.4)	4	0.329	0.988	17(26.6)	4	4.274	0.369
5-8	76	65 (85.5)				16(21.1)			
9-12	24	21 (87.5)				7 (29.2)			
13-16	10	10(100.0)				0 (0.0)			
>=16	6	5 (83.3)				1 (16.7)			
Total	**180**	**154(85.6)**				**41(22.8)**			

Key: df = degree of freedom

Table 5: Seroprevalence of rubella IgG and IgM with respect to educational status

		Rubella IgG				**Rubella IgM**			
Education	**No. Examined**	**No. (%) positive**	**df**	**Chi-square**	**p-value**	**No. (%) positive**	**df**	**Chi-square**	**p-value**
Primary	33	30 (90.9)	3	3.100	0.376	2(6.1)	3	10.829	0.013
Secondary	60	50 (83.3)				21(35.0)			
Tertiary	67	55 (82.1)				13(19.4)			
Informal	20	19 (95.0)				5 (25.0)			
Total	**180**	**154(85.6)**				**41(22.8)**			

Key: df = degree of freedom

Table 6: Seroprevalence of rubella IgG and IgM with respect to occupation

		Rubella IgG				**Rubella IgM**			
Occupation	**No. Examined**	**No. (%) positive**	**df**	**Chi-square**	**p-value**	**No. (%) positive**	**df**	**Chi-square**	**p-value**
Civil servant	39	31 (79.5)	4	5.000	0.287	6(15.4)	4	3.178	0.529
Business	111	97 (87.4)				30(27.0)			
Farming	15	14 (93.3)				2 (13.3)			
Students	10	9 (90.0)				2 (20.0)			
No defined regular job	5	3 (60.0)				1 (20.0)			
Total	**180**	**154(85.6)**				**41(22.8)**			

Key: df = degree of freedom

Table 7: Seroprevalence of rubella antibodies among HIV positive women of reproductive age in relation to major disease symptoms

Symptoms		Total	No. (%) positive	p-value/OR	Chi-square	df	Cl
Rash							
IgG	Yes	15	12 (80.0)	0.409/1.543	0.409	1	0.404-5.893
	No	165	142 (86.1)				
IgM	Yes	15	6 (40.0)	0.097/0.404	2.759	1	0.135-1.211
	No	165	35 (21.2)				
Fever							
IgG	Yes	32	26 (81.2)	0.445/1.477	0.574	1	0.541-4.035
	No	148	128 (86.5)				
IgM	Yes	32	7 (21.9)	0.893/1.065	0.018	1	0.424-2.677
	No	148	34 (23.0)				
Lymphadenopathy (swollen lymph nodes)							
IgG	Yes	12	8 (66.7)	0.054/3.318	3.712	1	0.921-11.949
	No	168	146 (86.9)				
IgM	Yes	12	5 (41.7)	0.106/0.382	2.608	1	0.114-1.274
	No	168	36 (21.4)				
Arthralgia (joint pains)							
IgG	Yes	20	15 (75.0)	0.154/2.206	2.029	1	0.726-6.703
	No	160	139 (86.9)				
IgM	Yes	20	7 (35.0)	0.167/0.501	1.911	1	0.185-1.354
	No	160	34 (21.2)				

Key: OR = Odds ratio, df = degree of freedom and CI = Confidence Interval

Table 8: Seroprevalence of rubella antibodies in relation to some risk factors

Risk factors		Total	No. (%) positive	p-value/OR	Chi-square	df	Cl
Residential Area							
IgG	Urban	132	112 (84.8)	0.655/1.250	0.200	1	0.470-3.327
	Rural	48	42 (87.5)				
IgM	Urban	132	34 25.8)	0.114/0.492	2.499	1	0.202-1.200
	Rural	48	7 (14. 6)				
	No	149	129 (86.6)				
Disease History							
IgG	Yes	52	45 (86.5)	0.5009	1.352	2	
	No	46	37 (80.4)				
	Unknown	82	72 (87.8)				
IgM	Yes	52	17 (32.7)	0.100	4.596	2	
	No	46	7 (15.2)				
	Unknown	82	17 (22.8)				
Vaccination status							
IgG	Yes	4	4 (100.0)	0.687	0.751	2	
	No	139	118 (84.9)				
	Unknown	37	32 (86.5)				
IgM	Yes	4	1 (25.0)	0.777	0.504	2	
	No	139	30 (21.6)				
	Unknown	37	10 (27.0)				

Key: OR = Odds ratio, df = degree of freedom and CI = Confidence Interval

Table 9: Seroprevalence of rubella antibodies with respect to pregnancy and pregnancy outcomes

Factor		Total	No. (%) positives	p-value/OR	Chi-square	df	Cl
Pregnancy							
IgG	Yes	31	25 (80.6)	0.393/1.548	0.731	1	0.565-4.241
	No	149	129 (86.6)				
IgM	Yes	31	9 (29.0)	0.361/0.669	0.833	1	0.281-1.593
	No	149	32 (21.5)				
Gestational age							
IgG	N/A	149	129 (86.6)	0.791	1.044	3	-
	First	5	4 (80.0)				
	Second	13	10 (76.9)				
	Third	13	11 (84.6)				
IgM	N/A	149	32 (21.5)	0.130	5.658		
	First	5	0 (0.0)				
	Second	13	6 (42.6)				
	Third	13	3 (23.1)				
Miscarriage(s)							
IgG	Yes	24	18 (75.0)	0.114/2.267	2.497	1	0.804-6.389
	No	156	136 (87.2)				
IgM	Yes	24	13 (54.2)	0.000/0.185	15.512	1	0.075-0.456
	No	156	28 (17.9)				
Still birth(s)							
IgG	Yes	21	18 (85.7)	0.982/0.986	0.000	1	0.269-3.615
	No	159	136 (85.5)				
IgM	Yes	21	6 (28.6)	0.501/0.706	0.454	1	0.255-1.954
	No	159	35 (22.0)				

Key: N/A – Not Applicable; OR = Odds ratio, df = degree of freedom and CI = Confidence Interval

DISCUSSION

The seroprevalence of IgG determined in the HIV positive women of reproductive age attending Federal Medical Centre, Makurdi was 85.6%. This implies that 85.6% of the study populations have been exposed to rubella infection. This high seroprevalence of rubella IgG is similar to the 88.6% reported in Yaounde, 89.4% in Ibadan and 93.1% reported in Zaria [10, 11, 12]. It is slightly lower than the 92.9%, 96.1% and 97.5% reported in Eldoret, Kenya, Turkey and in Zaria, Nigeria [13, 14, 15]. The seroprevalence in this study was however, higher than the 53%, 54.1%, 68.5% and 76.0% reported in Benin, in Maiduguri, in Ibadan and in Lagos respectively [16 - 19]. This high seroprevalence of IgG is most likely due to an increasing amount of cases of exposure to, and infection with rubella, as no routine immunization programs exists for there to be immunity due to vaccination [20, 21].

The IgM seroprevalence which indicates the percentage of the population tested had an acute, primary or current infection was determined to be 22.8%, which is lower than the 38.8% reported in Zaria [12] but higher than the 9.5% reported in Ethiopia [22],

6.8% in Jos [23] and 3.9% in Makurdi [24]. The higher seroprevalence of IgM obtained in this study may be attributed to the fact that the study group was of patients who already had HIV-compromised immune systems of varying degrees and as such were more prone to rubella infection as an opportunistic TORCH infection [25, 26]. The study population also included some pregnant women who are also considered a high-risk group for TORCH infections and its consequences [27]. The increased seroprevalence may also be a result of a possible outbreak of the disease at the time the study was carried out which is possible to have been unnoticed as a result of the tendency of the disease to be asymptomatic [5].

The co-occurrence of both IgG and IGM antibodies was observed in 16.1% of the study population. This implies that these patients either had cases of reinfection with rubella, meaning they had been infected before but failed to develop protective levels of IgG antibodies to rubella, or they had a resolving primary infection [28, 29]. The co-occurrence in the study was higher than the 10% reported in Benin City, Nigeria [16] and 5.5% reported in Cameroon [30]. The high co-occurrence of IgG and IgM observed may be as a result of the high level of immunocompromise in the study population resulting in diminishing immunity to rubella, or waning immunity to rubella as one ages [31]. It may also be as a result of late or resolving primary infection. When reinfection occurs in pregnancy however, chances of congenital infection in infant are very minimal as IgG antibody prevents placental crossing over of the virus [32].

It was observed that 7.8% of the population of women in this study had neither antibodies IgG nor IgM. This percentage implies that 7.8% of the women were without immunity to rubella and as such are susceptible to rubella infection and if pregnant already or conceive with that some immunity status, their fetuses would be at risk for Congenital Rubella Syndrome [30].

With respect to age, marital status, parity, family size and occupation and general knowledge about Rubella, no significant statistical association was found in rubella IgG and IgM seroprevalence. However, higher rates of seroprevalence was observed in patients who were likely to have more contact with children due to large family size, and those who lived or worked in areas with more likelihood of congestion and person to person contact [33].

A significant association was observed statistically ($p = 0.013$) in rubella IgM seroprevalence with respect to educational status. The highest percentage was observed in persons educated up to secondary level. This also agreed with the findings of Mangga and Yahaya [21, 34]. It could still be attributed to insufficient level of education and awareness on preventive measures or even the existence of the disease in general.

The relationship between rubella IgM seroprevalence and major rubella symptoms revealed no statistically significant association. However, with the exception of fever, the antibody was found in higher percentages in patients with all the other symptoms observed. This indicates a relationship between these symptoms and rubella infection [35]. It is however, important to note that some of the major rubella symptoms are also common symptoms of HIV/AIDS [35]. As such, it is possible that some of the patients with fever, lymphadenopathy and arthralgia may not be showing rubella but HIV/AIDS symptoms. This then implies that these patients, together with those totally without the symptoms, but with rubella IgM antibodies present, were testimonies of the asymptomatic nature of the disease in some cases [5].

Although no significant statistical association was established in the relationship between vaccination status and the seroprevalence of rubella IgG and IgM in this study ($p = 0.687$, $p = .777$ respectively), patients who claimed to have been vaccinated had the highest IgG seroprevalence (100%). This implies that despite immunocompromise due to HIV, immunity due to vaccination was still observed in these patients with protective antibody levels, which is a likely occurrence when their CD4 level is high or if they commenced antiretroviral therapy before receiving MMR [25].

For IgM seroprevalence, the highest percentage was among patients who did not know whether or not they had been vaccinated against rubella. As these patients were from a location where rubella vaccination was not available and most had never been outside the country, it is most likely that these patients were unvaccinated against rubella.

The relationship between seropositivity to rubella IgM and miscarriage was found to be statistically significant ($p = 0.000$), the relationship with stillbirths was still insignificant, although a high seropositivity indicates that the infection with virus was probably responsible for the cases recorded.

CONCLUSION

The seroprevalence of rubella antibodies observed in this study was high, which suggests both endemicity and a sustained viral infection in the population. Possible reinfections are also on-going in this immunocompromised population, and the outbreaks are going unnoticed probably due to the many asymptomatic cases observed. Awareness of rubella and its risks and effects was also observed to be almost non-existent.

RECOMMENDATIONS

There is great need for establishment of a routine national immunization plan against Rubella virus by the Nigerian Government to curb the infection. There is also need for massive campaigns to create awareness of the risks, mode of transmission and consequences of rubella infection, especially in pregnancy. Routine screening for rubella, especially in women of reproductive age should be carried out to determine immune status and act accordingly. The scope of opportunistic infections routinely screened for in HIV/AIDS patients should be broadened, especially in women, to prevent opportunistic infections with congenital consequences. Early commencement of ante-natal care for pregnant women should be advised for accurate monitoring. This would aid timely diagnosis and early arresting of any possible congenital infections for better outcomes. Finally, avidity testing should be carried out for patients with both rubella IgG and IgM antibodies to better differentiate between acute primary, resolving infection and re-infection with rubella.

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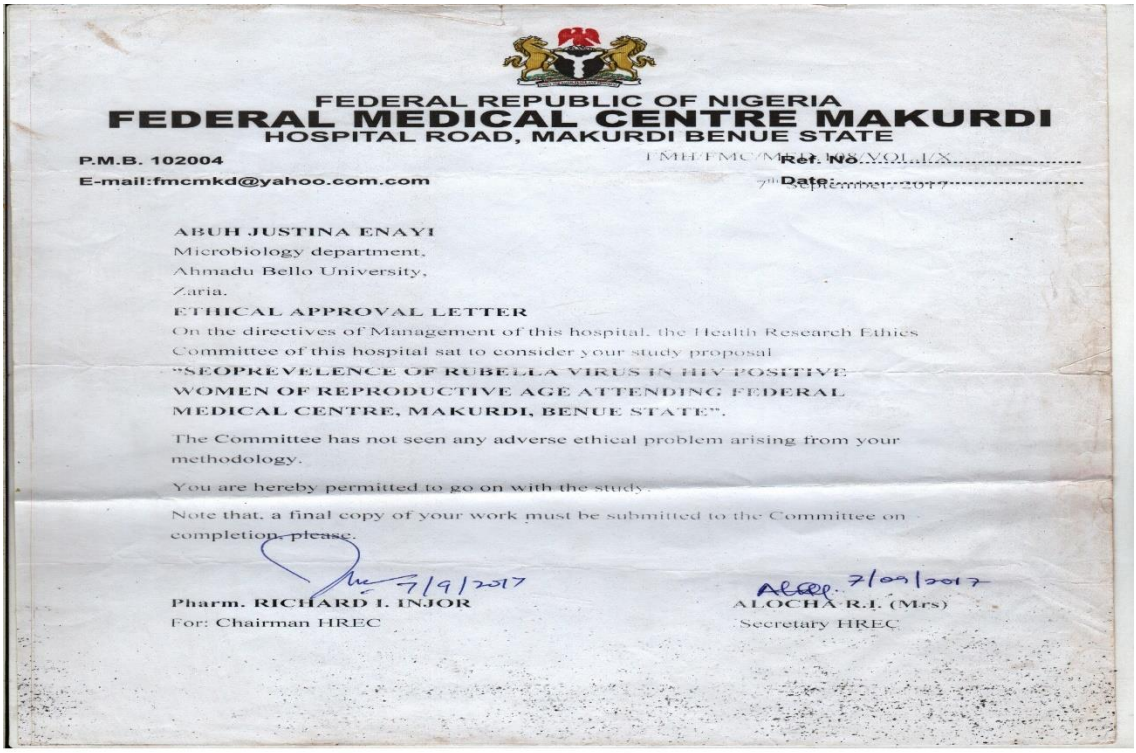

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Date: 7th September, 2017

ABUH JUSTINA ENAYI
Microbiology department,
Ahmadu Bello University,
Zaria.

ETHICAL APPROVAL LETTER

On the directives of Management of this hospital, the Health Research Ethics Committee of this hospital sat to consider your study proposal "**SEOPREVELENCE OF RUBELLA VIRUS IN HIV POSITIVE WOMEN OF REPRODUCTIVE AGE ATTENDING FEDERAL MEDICAL CENTRE, MAKURDI, BENUE STATE**".

The Committee has not seen any adverse ethical problem arising from your methodology.

You are hereby permitted to go on with the study.

Note that, a final copy of your work must be submitted to the Committee on completion, please.

7/9/2017
Pharm. RICHARD I. INJOR
For: Chairman HREC

7/09/2017
ALOCHA R.I. (Mrs)
Secretary HREC

APPENDIX I: Ethical Approval