

Research Article

Seroprevalence of Rubella IgM and IgG Antibodies and Associated Risk Factors among Pregnant Women Attending Antenatal Clinic at Bafoussam Regional Hospital, West Region of Cameroon

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Received date: August 10, 2018; Accepted date: September 10, 2018; Published date: September 18, 2018

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Abstract

Background: Rubella is a contagious vaccine-preventable viral infection. Its etiologic agent, Rubella virus has been identified as a human teratogen capable of causing a spectrum of birth defects known as Congenital Rubella Syndrome (CRS) if the infection occurs within the first trimester of pregnancy. Rubella continues to be a serious public health problem in developing countries. This study focused to find out the seroprevalence and risk factors associated with Rubella infection in pregnant women at the Bafoussam Regional Hospital.

Materials and Methods: This was a cross-sectional study carried out among pregnant women attending the antenatal clinic. Clinicians carried out screening of 91 pregnant women for Rubella IgG and IgM antibodies from July to September 2016, using sandwich ELISA kit (SinoBiological Inc, India). A questionnaire captured known risk factors for Rubella among all participants. Risk factors for Rubella were analyzed in a binary logistic model in which not being positive for Rubella was considered the reference condition. Odd ratios calculated at 95% CI, were used to measure the strength of association between the risk factors and Rubella antibodies seropositivity. A $p \le 0.05$ was considered statistically significant.

Results: Of the 91 pregnant women who accepted to participate in the study, 93.40% (85/91) were seropositive for Rubella IgM and IgG antibodies. Among the Rubella seropositive women, 87.91% (80/91), 5.49% (5/91) and 5.49% (5/91) were seropositive for IgG antibody, IgM antibody, and both IgM and IgG antibodies respectively. Among the risks factors evaluated, none was found to be an independent risk factor of Rubella infection.

Conclusions: The findings from this study show that Rubella virus is circulating in the study area and more women have been and are perhaps still being exposed to this potentially hazardous childbearing age infection and there is a need to inform them on how they could protect themselves from contracting this virus.

Keywords: Rubella; Prevalence; IgM; IgG; Antibodies; Pregnant women; Cameroon

Clinical diagnosis of Rubella infection is not easy to establish because of the transient symptoms [2].

Introduction

Rubella (which means "little red") commonly known as "German Measles" was first thought to be a variant of measles [1,2]. It is a viral infectious disease caused by the Rubella virus which is the only member of the genus *Rubivirus* in the *Togaviridae* family [3] and has humans as its sole reservoir [2]. The disease commonly occurs in childhood and is characterized by a mild, maculopapular rash. It is the mildest of common viral exanthema [4]. Other symptoms include low-grade fever, swollen glands (suboccipital and posterior cervical lymphadenopathy), joint pains, headache and conjunctivitis [5].

The Rubella virus has an incubation period of 2-3 weeks and is transmitted through the respiratory route [2,6,7]. This syndrome results in cerebral, cardiac, ophthalmic and auditory defects [4,6-8]. The risk of fatal manifestation is 85%, 52% and rarer if the maternal infection occurs before 9 weeks, between 9-12 weeks, and after 16 weeks of gestation respectively [9]. Rubella vaccines are live attenuated vaccines and a single dose of the vaccine confers long-lasting immunity in more than 95% of the vaccine recipients. In addition, immunity is naturally induced after Rubella infection. Hence, the presence of immunoglobulin G (IgG) antibody in serum serves as a seromarker of Rubella immunity. So, IgG antibodies ≥ 10 IU/ml is

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generally considered protective [6,8]. Since Rubella infection can be prevented by a vaccine, some countries have carried out active immunization with live vaccine combined with measles and mumps vaccines [2,10]. Despite the fact that Rubella infection has reduced with the implementation of Rubella vaccination over the years, it is still considered as an important public health problem around the world especially in the third world countries. In their efforts to eliminate Rubella virus infection and its attendant sequel, many countries have introduced the vaccine in a vaccination program [11]. Despite the availability of a vaccine against Rubella virus, most countries in Africa are yet to include it in their national public health vaccination programs [12]. Thus, the Rubella virus is circulating freely in most African regions. Limited data is also available on the seroprevalence of this virus in most African population although World Health Organization (WHO) had earlier advised that countries should key into the accelerated measles control and elimination programs so as to introduce Rubella-containing vaccines [6]. So far, the vaccination program was put in place in Cameroon less than five years ago. WHO statistics show that this virus is present in Cameroon with 83, 159, 58, and 126 confirmed cases in 2004, 2005, 2006, and 2007 respectively [13,14]. These cases were first suspected cases of measles which turned out negative and instead tested positive for Rubella. Taking into account the fact that Cameroon stands out as one of the countries with less than five years implementation of a vaccination scheme, the risk of a possible outbreak cannot be over-emphasized. Thus, it is necessary to know the epidemiology of Rubella in pregnant women because of the Congenital Rubella Syndrome (CRS). This study sought to identify the susceptibility of women to the Rubella virus in Bafoussam through the estimation of IgM and IgG protective antibodies levels in pregnant women at the Bafoussam Regional Hospital, in order that the data obtained from the study would be useful for enlarging vaccination in Cameroon.

Materials and Methods

Study area and period

This study was conducted from July to September 2016 at Bafoussam Regional Hospital. Bafoussam is the capital of the West Region of Cameroon. It is situated at 200 km from the Capital Yaoundé and 285 km from Douala the economic city of Cameroon. Bafoussam is located between the Longitude 10°25' East and Latitude 5°28' north and situated on an altitude of 1310-1470 m above sea level.

Study population and design

A cross-sectional study was conducted among pregnant women attending the antenatal clinic at the Bafoussam Regional Hospital. Enrolled in this study were 91 pregnant women. Oral interviews were conducted to obtain socio-demographic information of the respondents.

Sample Collection

About 5 ml of venous blood was aseptically collected and transferred into a plain tube then allowed to clot at room temperature. The samples were transported in a cold box to the Biochemistry Laboratory of the Regional Hospital Bafoussam. The blood was centrifuged at 3000 rpm for 5 minutes. Each supernatant serum was carefully collected into a labeled cryo tube using a Micro-pipette.

Approval for this study was acquired from the National Ethical Review Committee on Human Research and Health, (CNERSH), Yaoundé.

Laboratory investigations

The Rubella virus-specific IgM and IgG ELISA kits (Cortez Diagnostic Inc. USA) were used to detect the Rubella virus IgM and IgG antibodies in the sera following the manufacturer's instructions.

Test principle: On the surface of microwells is coated purified Rubella antigen. The diluted patient's serum is added to the wells. The Rubella IgM and IgG specific antibody binds to the coated antigen in the wells, and all unbound materials are washed away. After adding an enzyme conjugate, it binds to the antibody-antigen complex. A chromogenic substrate is added after excess enzyme conjugate is washed off. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the quantity of specific IgM and IgG antibody in the sample. The results are read using a microwell reader and compared in a parallel manner with calibrator and controls.

ELISA procedure

The test procedure was performed according to the manufacturer's instructions.

Negative result

Rubella IgM and IgG Index \leq 0.90 was considered serone gative for IgM and IgG antibody to Rubella.

Positive result

Rubella IgM and IgG Index of \geq 1.00 were considered positive.

Equivocal result

Rubella IgM and IgG Index of 0.91-0.99 were considered equivocal, and the sample was retested.

Statistical analysis

Data gathered from this study was analyzed using SPSS version 20 and the association of viral prevalence of infection among pregnant women with the studied risk factors was determined by Chi-Square test. Values obtained were considered statistically significant at $p \leq 0.05.$

Results

In all, 91 consented women aged 16 to 44 years were recruited for this study. The mean age of participants was 27.60 ± 5.74 years. Most of the women were aged between 22 and 27 years (32/91, 35.16%). A total of 67.03% (61/91) of the women were married, and 2.20% (02/91) never attended school. All the participants were pregnant with 19.78% (18/91), 37.36% (34/91) and 42.86% (39/91) being respectively in the first, second and third trimesters. 21.98% (20/91) were pregnant for the first time, 21.98% (20/91) have had an abortion, 2.20% (02/91) had given birth prematurely and 92.31% (84/91) had been in contact with a child not belonging to them with the status of the child unknown concerning Rubella. 5.49% (05/91) had received a blood transfusion.

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None of the participants had been vaccinated for Rubella, 49.45% (45/91) knew about Rubella and 13.2% (12/91) were living in the rural area (Table 1).

		Anti-Rubella IgG		Anti-Rubella IgM		Anti-Rubella IgG and IgM				
Characteristics	Number n(%)	lgG (+ve)	COR (95% CI)	p Value	lgM (+ve)	COR (95% CI)	P Value	lgG and lgM (+ve)	COR (95% CI)	p Value
Age category (ye	ars)									
[16 - 21]	14 (15.38%)	14 (100%)	1.0449	0.574	01 (7.14%)	0.9801 (0.8337-1.1522)	0.8077	01 (7.14%)	0.9801 (0.8337-1.1522)	0.8077
[22 - 27]	32 (35.16%)	28 (87.5%)			01 (3.125%)			01 (3.125%)		
[28 - 33]	30 (32.97%)	29 (96.66%)	-		03 (10%)			03 (10%)		
[34 - 39]	13 (14.29%)	12 (92.30%)			00 (00%)			00 (00%)		
[40 - 44]	02 (2.20%)	02 (100%)	-		00 (00%)			00 (00%)		
Knowledge										
Yes	73 (80.22%)	67 (91.78%)	0.0000	0.973	3 (4.10%)	0.3427	0.2616	3 (4.10%)	0.3427 (0.0528-2.2227)	0.2616
No	18 (19.78%)	18 (100%)	(0.0000->1.0 ¹²)		2 (11.11%)	(0.0528-2.2227)		2 (11.11%)		
Married			1		-	1	1	1	1	
Yes	61 (67.03%)	59 (96.72%)	2.2693	0.334	4 (6.55%)	1.9236 0.566	4 (6.55%)	1.9236	0.566	
No	30 (32.97%)	26 (86.66%)	(0.4291-12.0003)		1 (3.33%))		1 (3.33%)	(0.2060-17.9612)	
Working status			1		-		1	1		
Employed	45 (49.45%)	42 (91.30%)	0.9767	0.977 3 (6.66	3 (6.66%)	1.5712	0.63	3 (6.66%)	1.5712	0.63
Unemployed	46 (50.55%)	43 (95.55%)	(0.1865-5.1154)		2 (4.34%)	(0.2500-9.8763)	2 (4.34%)	(0.2500-9.8763)		
Gestation		1	1	1		1	1	1	I	
1st trimester	18(19.78%)	16(88.89%)	0.2519 (0.026-2.745)	0.269	1	0.7500 0. (0.117-4.776)	0.7607	1	0.7500 (0.117-4.776)	0.7607
2nd Trimester	34 (37.36%)	31 (91.17%)	-		2 (5.88%)			2 (5.88%)		
3rd Trimester	39 (42.86%)	38 (97.43%)	-		3 (7.69%)			3 (7.69%)		
Abortion	1	1	1					1		
One	20 (21.98%)	18 (90%)	0.0000 (0.0000-	0.966	1 (5.55%)	0.0000 (0.0000- >1.0 ¹²)	0.9721	1 (5.55%)	0.0000 (0.0000- >1.0 ¹²)	0.972
More than one	11 (12.09%)	11 (100%)	- >1.0'-)		1 (9.09%)			1 (9.09%)		
Pregnancy delive	əry	1					1	1		
Normal	89 (97.80%)	83 (93.25%)	25141.8239 (0.0000-		5 (5.61%)	0.0000(0,0000- >1.0 ¹²)	0,9708	5 (5.61%)	0.0000(0,0000-	0.971
Premature	02 (2.20%)	02 (100%)	- >1.0)		0 (00.00%)			0 (00.00%)	31.0)	
Status of the chi	d	1	1				1	1	1	
Normal	91 (100%)	85 (93.40)								
Mal formation	00 (00%)	0								
Contact with child										
Yes	84 (92.31%)	78 (92.85%)	0.0000 (0.0000-> 1.0E12)		4 (4.76%)	0.3000 (0.0289-3.1158)	0.3133	4 (4.76%)	0.3000 (0.0289-3.1158)	

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No	07 (7.69%)	7 (100%)			1 (14.28%)			1 (14.28%)		
Blood transfusio	Blood transfusion or Organ transplantation									
Yes	05 (5.49%)	5 (100%)	70905.6131(0.0000-	0.966	1 (20%)	5.1250	0.1838	1 (20%)	5.1250	0.183
No	86 (94.51%)	80 (93.02)	>1.0'-)	>1.0**)	4 (5%))		4 (5%)))	
Rubella vaccine										
Yes	00 (00%)	0								
No	91(100%)	85(93.40%)								
Number of pregn	Number of pregnancies									
First	20 (21.98%)	20 (100%)	1.5385 (0.1698-13.9392)	0.701	0 (00%)	0.0000 (0.0000- >1.0 ¹²)	0.9721	0 (00%)	0.0000 (0.0000- >1.0 ¹²)	0.972
More than one	71 (78.02%)	65 (91.54%)			5 (7.04%)			5 (7.04%)		
Knowledge of Rubella										
Yes	45 (49.45%)	41 (91.11%)	0.4659	0.391	1 (2.22%)	0.2386	0.2081	1 (2.22%)	0.2386	0.208
No	46 (50.55%)	44 (95.65%)	(0.0811-2.6766)		4 (8.69%)	(0.0230-2.2210)		4 (8.69%)	(0.0230-2.2210)	
Residential area										
Rural	12 (13.2%)	10 (83.33%)	1.5000 0.723 (0.1589-14.1624)	0.723 1 (8.	1 (8.33%)	0.5263 0.58	0.5825	1 (8.33%)	0.5263	0.582
Urban	79 (86.8%)	75 (94.93%)		4 (5.06%)	(0.0334-5.1000)		4 (5.06%)	(0.0004-0.1000)		

Table 1: General characteristics of study participants and distribution of Anti-Rubella antibodies among pregnant women.

Prevalence of anti-Rubella antibodies

The combined seroprevalence of Anti-Rubella antibodies among the 91 pregnant women in this study was 93.40% (85/91). Among the seropositive women, 87.91% (80/91) were seropositive for IgG antibodies, while 5.49% (5/91) were seropositive for IgM antibodies. The same patients that were seropositive for IgM were also seropositive for IgG antibodies, giving a prevalence of 87.91%, and 5.49% respectively (Table 2).

	Stage of preg	Total [n (%)]		
	1st Trimester	2nd Trimester	3rd Trimester	
lgG⁺	16 (20%)	29 (36.25%)	35 (43.75%)	80 (100%)
lgM⁺	00 (00%)	02 (40%)	03 (60%)	05 (100%)
IgG ⁺ and IgM ⁺	00 (00%)	02 (40%)	03 (60%)	05 (100%)

Table 2: Seroprevalence of antibodies to Rubella according to the stage of pregnancy.

The seroprevalence of antibodies to Rubella was higher in pregnant women at third trimester 45.05% (41/91) than second trimester 36.26% (33/91) and first trimester 17.58% (16/91) (Table 2). Seroprevalence of Anti-Rubella IgG antibody was 20% (16/80) in the first trimester, 36.25% (29/80) in the second trimester and 43.75% (35/80) in the third trimester. Similarly, a higher seroprevalence of IgM antibody to Rubella was observed in the pregnant women at third trimester 60% (3/5) than those in their second 40% (2/5) and first trimesters 0.0% (0/5). The same result was obtained with pregnant seropositive to both IgG and IgM.

Risk factors for Rubella seropositivity

A simple logistic regression analysis with suspected variables indicated that no suspected risk factor was significantly associated with Rubella seropositivity irrespective of the antibody type (Table 3). In addition, multiple logistic regression analysis of selected risk factors further showed that no suspected risk factor of Rubella infection was an independent predictor of Rubella infection irrespective of the antibody type. No significant association was found between Rubella seropositivity and residential area (P=0.64), marital status (P=0.31), cat ownership (P=0.91), contact with the child (P=0.31) and cutaneous eruption (P=0.64) (Table 4).

Variable	No of subjects tested	Odd ratio	p value
	(n, %), N = 91	(95 % CI)	
Age (Years)			
[16 - 21]	14 (15.38)	1.0449	0.5741
>21	77 (84.62)	(0.8964-1,2180)	
Marital status			
Married	62 (68.13)	22,693	0.3349
Single	29 (31.87)	(0.4291-1.2180)	

No

Yes No

with Rubella

Discussion

Cutaneous eruption

cataract, and deafness known as congenital Rubella syndrome. The Rubella virus plays an important role in the occurrence of abortion [14-16]. The data presented in this study has found that the seroprevalence of Rubella virus was 93.40% with the prevalence of IgG being 87.91%. This may be due to antibodies developed in response to the Rubella virus (natural Rubella infection) as none of the women was vaccinated. Similar results have been reported in other African countries in pregnant women. In Sudan, it was reported that the seroprevalence of Rubella was 95.1% [17] and in Mozambique, 95.3% was reported [18]. This overall prevalence obtained in our study was higher than the overall prevalence obtained in Federal Medical Centre, Keffi, Nigeria where this prevalence was observed to be 11.4% [19] and slightly higher than the prevalence of 88.4% obtained in Southern Ethiopia [20]. The high seropralence observed in our study as compared to others studies carried out in another part of Africa suggest the natural virus circulation within the community [21,22]. The 87.91% IgG prevalence found in this study suggests that Rubella virus is present in the study area with most pregnant women previously been exposed to the virus. This result corroborates with the study conducted in Southern Ethiopia where the prevalence of 86.3% was obtained [20]. Similar results were obtained in Nigeria where the prevalence of IgG was found to be 83.3% among women [23]. The 87.91% IgG prevalence obtained in this study is also comparatively higher than the 53%, 62.7%, 68.5% prevalences in Benin, Zaria and Ibadan [24,25]. The difference between these studies may be due to the level of circulation of the virus within the population of each study that was high in our population. 5.49% of pregnant women were positive for IgM antibody. All these pregnant women were as well positive for IgG antibody, either suggesting a reinfection or resolving the primary infection. This could be attributed to the fact that they were not actually immune as earlier concluded, but were still in the recovery stage, although this was not confirmed with an avidity test. This result corroborates with results obtained in a study conducted in pregnant Sudanese women with a history of abortion where the prevalence of IgM was found to be 5.4% [26]. The prevalence of 5.49% obtained in our study was high compared to the 2.1% obtained in a study performed in Southern Ethiopia [20] but low compared to the 10%, 14.3%, 16.3% and 37.8% prevalence obtained in Benin, Ilorin, Abuja, and Maiduguri respectively [24,25,27,28].

0.5694 (0.0549- 5.9091)

*p < 5 %: Statistical significance; OR > 1 and OR < 1: Association of a variable

Maternal infections, particularly during the first trimester, are adversely associated with stillbirth, spontaneous abortion, or antagonistic neonatal outcome that encompasses heart disease,

Table 4: Multiple logistic regression of selected variables.

The age-stratified prevalence of Anti-Rubella antibodies among the pregnant women within the 28-33 years revealed the highest prevalence of 31.87% (29/91). We observed also that a combination of the 16-21 years and 22-27 years age brackets constituted 46.15% of the pregnant women sampled in this study. This is instructive in that it

Urban	79 (86.8)	(0.1591-14.181 0)	0.7225	
Contact with child				
Yes	84 (92.31)	0		
No	07 (7.69)	(0.0000 - >1.0e12)	0.9757	
Blood transfusion or Organ transplantation				
Yes	05 (5.49)	26084.64	0.9692	
No	86 (94.1)	(0.0000- >1.0e12)		
Fever				
Yes	25 (27.47)	1.9672		
No	66 (72.53)	(0.2183-17.724 7)	0.5464	
Adenopathy				
Yes	13 (14.29)	0.8219	0.8632	
No	78 (85.71)	(0.0882- 7.6593)		
Cutaneous Eruption				
Yes	11 (12.09)	0.6658		
No	80 (87.91)	(0.0705- 6.2862)	0.7225	
Articular pain				
Yes	30	0.2203		
No	61	(0.0379-1.2789)	0.0918	
*p < 5 %: Atatistical signific with Rubella	cance; OR > 1 and (DR < 1: Associatio	on of variable	
Table 3: Simple logistic re	egression with susp	pected variables.		

12 (13.2)

15.020

0.7225

Residential area

Rural

Veriekle	Adjusted odd ratio		
Variable	(95 % CI)	p value	
Residential area			
Rural	1.7312 (0.1695- 17.6781)	0.64	
Urban	-		
Marital status			
Maried	2.3992 (0.4387- 13.1223)	0.31	
unmaried	~		
Contact with child	0.0000 (0.0000 >1.0E12)	0.97	
Yes	0.0000 (0.0000- >1.0E12)		

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0.64

shows that majority of the women in the area of study enter into childbearing early i.e. between 16-27 years of age moreover, the 87.91% IgG prevalence found in this study corroborate with the relationship between Rubella seroprevalence and age and the prevalence of 88.6% of IgG reported by Fokunang et al, in Cameroon [1].

The predominance of non-vaccinated pregnant women (100%) of first-time visitors (42.86%) to antenatal clinics during the third trimester of pregnancy was observed in this study (Table 1). This implies that majority of pregnant women either do not attend at all or present late at the antenatal clinics for routine medical attention or the low expansion of vaccine that was newly introduced to the antenatal vaccination program. This attitude would impact negatively on any possible future effort to conduct surveillance on the prevalence of and mitigate the occurrence of children born with congenital Rubella syndrome in the study area in particular and Cameroon in general. This highlights the utmost importance of educating women of childbearing age in the study area on the need to enroll immediately in antenatal clinics whenever they are pregnant and also accept to receive the vaccine during enlarged vaccination program.

Rubella virus infection often causes a mild disease in humans, whereas infection during early pregnancy usually leads to severe congenital abnormalities [29]. Therefore, the fetuses of 20% (16/80) pregnant women in the first trimester who were seropositive for IgG are at risk for severe congenital abnormalities.

It also implies that if the 36.25% (29/80) and 43.75% (35/80) pregnant women in the second and third trimesters respectively who were seropositive for IgG contracted this virus earlier, their fetuses would have also been at great risk of malformation (Table 2).

In this study, 6.59% (6/91) of the pregnant women were IgM and IgG Anti-Rubella antibodies negative. This means that they constitute a population at risk. They are susceptible to infection by the Rubella virus. Among these six, two (33.33%; 2/6) had a history of pregnancy loss. This is indicative of miscarriage of non-Rubella aetiology. The loss could only be attributed to other teratogenic organisms. However, 31.87% (29/91) of the pregnant women seropositive for Rubella IgG had a history of miscarriage. Further research revealed an association between miscarriage and IgG antibody level in this category of pregnant women. In addition to age, other risk factors associated with seropositivity of Rubella antibodies as marital status, educational level, place of residence, and vaccination history were investigated. Our findings showed that marital status was an insignificant predictor of Rubella seropositivity. This result corroborates with the study carried out among Egyptian females [30].

This study also showed that there was no significant difference between the proportion of seropositivity in urban and rural areas indicating that virus circulation is similar in urban and rural zones and that the women of both areas are at similar risk of infection to Rubella virus. Most studies were in agreement with our finding [31].

It is important to note that, the sample size constitute the most important limit of this study and thus inferences that can be drawn at the population level are limited

Conclusions

Due to the absence of serious clinical symptoms in the disease coupled with a high seroprevalence found in this study, a national program on prevention and elimination of Rubella Virus in Cameroon must first promote a free antibody testing in populations and secondly proceed to vaccination of non-protected individuals.

Contributors

MN and LFS designed the study; DGKT, SS, and HD carried out the laboratory work; BK and CBT supervised the field study while ECW, SS performed the statistical analyses. All authors took part in the write-up and approved the final version of the manuscript.

Acknowledgments

We sincerely thank the study participants for their time and blood donated for this study. In a special way, we thank the Director and Nurses of the Bafoussam Regional Hospital for their contributions, and collaboration during sample collection and analysis.

Competing Interests

None declared.

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