ORIGINAL ARTICLE

CLINICAL STUDIES ON SEROPREVALENCE OF RUBELLA VIRUS IN PREGNANT WOMEN OF CAMEROON REGIONS.

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ABSTRACT
A study was conducted to investigate the seroprevalence of the rubella virus amongst pregnant women and the relationship it has with the duration of pregnancy, premature delivery, and past history of abortion in pregnant women visiting the Yaoundé Gynecological, Obstetric and Pediatric Hospital (HGOPY). 211 pregnant women attending the prenatal consultation of mean age 27±5.99 years were randomly selected and screened for rubella IgG antibodies. 39.3% of them were in their third trimester of pregnancy while 25.6% and 35.1% were in their first and second trimester of pregnancy respectively. 11.73% of the women had a history of premature delivery and 40.3% had a history of at least one abortion. Spearman's correlation was calculated between antibody titre and age. 88.6% of pregnant women were seropositive while 9% (susceptible) were seronagative and 2.4% had equivocal results. The most susceptible women to rubella infection were in the age group 26-30 years while women in the age group 21-25 years band were the most seropositive. There was a strong correlation between the antibody titre and age (r=0.549 p<0.01). There was no statistical difference between the pregnancy in trimesters and antibody titres (p=0.0926) as well as between the number of previous abortions and the antibody titre (p<0.01, r=0.246). No correlations between antibody titre and pregnancy duration, or occurrence of premature births. There was a weak correlation between the antibody titre and number of previous abortions.

INTRODUCTION

Rubella virus is an infection caused by a virus of the genus Rubivirus of the Togavirus family [1]. It has a simple architectural structure of single stranded RNA genome enclosed by an icosahedral nucleocapsid, protected by a lipid bilayer membrane [2-4]. Rubella (which means "little red" and is also known as German measles) was originally thought to be a variant of measles. It is a mild disease in children and adults, but can cause devastating problems if it infects the fetus, especially when the infection occurs during the first weeks of pregnancy [1, 2]. This is known as congenital rubella syndrome (CRS). When a woman is infected

with the rubella virus early in pregnancy she has a 90% chance of passing the virus unto

the fetus [2, 3]. This can cause the death of the fetus, it may cause CRS. The complications include hearing loss, congenital heart defects, neurologic problems (psychomotor retardation), ophthalmic problems (cataract, glaucoma, and retinopathy) intrauterine growth retardation, hepatomegaly, splenomegaly [2, 3, 4]. There may also be variety of other problems including bone lesions [1, 3]. Virus from congenital infections persists after birth and persons with congenital infections has

the potential to infect others after birth for a year or more [2, 5].The virus occurs in naso-pharyngeal secretions, urine and feces. Later on, patients with congenital syndrome may develop additional complications including diabetes mellitus (up to 20%), thyroid dysfunction, growth hormone deficiency; ocular complications [2, 3, 6].When a woman is infected with the rubella virus, the body produces both immunoglobin G (IgG) and immunoglobulin M (IgM) antibodies to fight against infection [2, 4, 7]. Once IgG exists, it persists for a lifetime, but IgM antibody usually wanes over six months [3].

If rubella IgG is present it can be confirmed that a patient has immunity to rubella. Specific IgG determination is performed through enzyme Linked Immunosorbent Assay (ELISA) techniques. The results are expressed in IU/ml. [1, 4, 8]. The microbial world is complex and constantly evolving and despite scientific efforts to contain diseases with microbial etiology, the growth of international travel has increased the ease with which microbes formerly restricted to certain geographical areas are spread across continents [5] For instance the recent movement of people fleeing the war in Chad to Cameroon is a situation that could trigger the spread of the rubella virus amongst unvaccinated population. Rubella is one of the most common causes of birth defects in the world, resulting in spontaneous abortions, stillbirths, and congenital rubella syndrome (CRS) rubella rashes [2, 3, 8, 9]. The manifestations of CRS include hearing

impairment, blindness, heart defects, and mental retardation. According to the World Health Organization, in 1996, two thirds of the world's population live in countries where rubella vaccination was not practiced routinely, and the number of infants with CRS born each year worldwide was estimated to be 110,000 in 1999 [3]. About 5 to 25% of women of childbearing age lack rubella IgG antibodies and are susceptible to primary infection [2, 7]. Rubella is transmitted by the respiratory route. The incubation period is 13 to 20 days, during which a viraemia occurs and virus disseminates throughout the body [12]. In adults a prodromal phase may be present with fever and malaise for a day or two before the rash develops [13, 14]. The rash is typically a maculopapular rash, which first appears on the face and then spreads to the trunk and the limbs. The rash seldom lasts more than 3 days. The exact mechanism of how the rash is induced is uncertain but an immunopathological mechanism may be present [15]. Lymphadenopathy may precede the rash by up to a week and persists up to 2 weeks after the rash has gone [16].

Rubella has a worldwide distribution. Before the introduction of vaccination outbreaks tend to occur in spring and summer [6]. Infection is uncommon in preschool children but outbreaks involving school children and young adults are common (7, 8). In general, about 50% of 10 year olds have rubella antibodies. About 80% of women of childbearing age were found to be immune

in the pre-vaccination era [10] Children 3 to10 years are most frequently affected. Despite the vaccination program 5 to 10 % of women of child bearing age are susceptible to Rubella infection [11].So far, no vaccination programme has been put in place in Cameroon. Statistics from the World Health Organization (WHO) show that this virus is present in Cameroon with confirmed cases: 83 in 2004, 159 in 2005, 58 in 2006, and 126 in 2007[4, 7]. These cases were initially suspected cases of measles which turned out negative and rather tested positive for rubella. Considering the fact Cameroon is one of the countries not implementing a vaccination scheme, the danger of an eventual outbreak cannot be over emphasized. There is the need to know the epidemiology of rubella in pregnant women because of the congenital rubella syndrome (CRS), and the *de novo* infection in the first trimester of pregnancy. The purpose of this study was to identify the susceptibility of women to the rubella virus in Yaoundé through the assessment of the Immunoglobulin IgM protective antibody level in Pregnant women at the Yaoundé Gynecology, Obstetric and Pediatric Hospital (HGOPY) in Cameroon Samples were collected randomly at (YGOPY) Cameroon, so that the data generated from the study would be useful for introducing vaccination in Cameroon.

MATERIALS AND METHODS

Study Design

A Cross-sectional descriptive study was carried out in pregnant, outpatient's women visiting the Yaoundé Gynaeco-Obstetric and Pediatric Hospital (YGOPH). This hospital was chosen because of its high patient's attendance as well as logistic and administrative facilities.

Collected blood specimens were analyzed at the Center for the Study and control of Communicable Diseases (CSCCD), of the Faculty of Medicine and Biomedical Sciences (FMBS), University of Yaoundé. This study was for 3 months and ran from April to July 2008.

The Inclusion Criteria, was basically to be a pregnant woman, sign the consent form as a volunteer, with no cash involvement and the acceptance to participate in the study.

The Exclusion Criteria included the refusal to participate in the study

Sample Size: The minimum acceptable sample size was 207 as calculated using Lorenz formula for two-tailed dichotomous variables.

Where N = sample size, Za= the normal distribution value for which a=0.05 (the standard normal deviate=1.96) 95%, confidence interval; a=level of statistical significance (a=0.05), p=prevalence (9), Q=1-p, D=degree of precision= level of error we want to accept (D=0.05 for a 95% confidence interval)

Using Z a= 1.96, P = 84 % (9), D = 0.05,

$$N= \frac{(1.96)^2 \times (0.84) \times (0.16)}{(0.05)^2} \approx 207$$

Sampling Method

Consecutive sampling was used whereby subjects who satisfy the inclusion criteria during the study period were included in the study.

Data and blood specimen collection

Each patient was made to sit comfortably, then the arm region intended for the venupuncture was cleansed with an alcohol swab, the selected vein pricked with a sterile needle attached to a syringe (10 ml) and 4-5 ml of blood drawn. The needle was then withdrawn under a dry cotton and brief haemostasis effected by digital pressure with the swab at the puncture site. The blood sample was put in a sterile dry tube. Centrifugation was done at 2000 rotations per minute (rpm) for 5 minutes. Serum was collected in cryotubes and stored in refrigerator at -20 degree Celsius. Cryotubes were put inside a cold box and transported to the CSCCD of the FMBS. Laboratory analysis was done at the end of the month.

Laboratory analysis of specimens:

Reagents and specimens were brought at room temperature before use. Testing for the presence of rubella virus was done using Human-Rubella IgG ELISA (26) this is an enzyme immunoassay for the detection of rubella antibodies in the plasma or serum. 10µl of patient serum were diluted to 1ml of buffer and mixed properly. Well A1 was left blank while B1/C1 100 ml of negative control (NC) was put. Dl/E1 100µl of cut off control (CC) and F1/G1 100 ml of positive control (PC).100µl of each serum to be tested was added to the microtitre plate. The microtitre plate was the covered with adhesive foil and allowed to incubate for 30 minutes at 25 degree Celsius. They were then washed 4 times with 350µl washing solution using an automatic washing device. Each well was filled with 100 µl conjugate solution (Anti-human IgG rabbit, peroxidase-conjugated). Then, the plate was covered, and incubated at 25 degree Celsius for 30 minutes, then washed 5 times as above. Then, each Well was filled with 100µl of substrate reagent (3 3`, 5, 5` tetramethylbenzidin (TMB hydrogen peroxide). The plate was covered and incubated for 15 minutes at 25 degree Celsius in a dark room. 100 µl of STOP solution was added to each well. The Wells were read using a zero-balanced photometer at 450 nm within 30 minutes after termination of the reaction, using a reference wavelength of 690nm.

Calculation of control values and cut-off:

Mean absorbance values of negative control (NC) in wells B1 and C1, mean negative control (MNC) in wells D1 and E1, mean cut-off control (MCC), and Positive control (PC) in wells F1, and G1 mean positive control(MPC) were calculated according to:

$MNC=A_{450}$ (B1) $+A_{450}$(C1) /2; $MCC=A_{450}$ (D1) $+A_{450}$(E1) /2; $MPC=A_{450}$ (F1) $+A_{450}$(G1) /2

The test was considered valid as the following criteria were met: **Substrate blank in well A1 <0.150; MNC ≤MCC; MPC >0.750; MPC: MNC >2.5 . Interpretation of results: A_{450}(patient) >MCC+15% anti RV-IgG-Ab-positive A_{450} (patient) >MCC-15% antiRV-IgG-Ab- negative**

Due to physiological and analytical variations, patient's results lying at 15% above or below the calculated cut-off were considered equivocal [26].

Quantitative Estimation of rubella IgG in patient samples

Each plate test was validated when the absorbance of the mean cut off control were <10 iu/ml and the absorbance of the positive control >15 iu/ml and values in-between were considered equivocal.

Data Quality Control: To guarantee the authenticity of the information collected,

A standardized questionnaire was used to record the information obtained from every patient, to ensure uniformity.The questionnaire were pre-tested during a short pilot study on few (10) subjects before recruitment proper. The questionnaire was then revised following the results of this pilot study before the main study started.

The data was filled by the researcher personally to ensure precision of information.

Data Presentation and Analysis: The data collection forms were first of all cross-checked to make sure all the relevant information was appropriately entered. The EPI INFO version 3.3.2, February 09, 2005 (Centre for the Disease Control and Prevention, Atlanta, Georgia, USA) statistical software was used for the data entry, validation and analysis. To ensure accuracy of entry a CHECK programme was created. This programme ensured that only legal entries and data in specified ranges and codes were entered. Discrepant records were subsequently reviewed and corrected. All entries on computer were further checked against that on paper, item by item. Finally, frequency tables were generated for variables in order to examine for unusual entries. Spearman correlations were used to calculate the various variables. The prevalence of rubella virus among pregnant women was calculated as:

$P = N1/N2*100\%$: Where P is prevalence; N1 the total number of women presenting antibodies to the rubella virus; N2 the total number of women tested for antibodies.

Ethical considerations

Institutional Ethical Clearance was procured from the Faculty of Medicine and Biomedical Sciences (FMBS) ethical committee. Informed, written and signed consent was obtained from subjects by way of a consent form, after the purpose and the procedure of the study had been explained. Non-consenting individuals were excluded from the study. Records were kept strictly confidential with code numbers used at the registration of each participant and records accessible only to members of the immediate research team. The entire procedure was of minimal risk to the subjects. Each needle was used once and properly discarded after use. The informed consent of each subject was sought systematically before recruitment. The aim and the nature of the study were explained to each patient and her role in the study clarified. Confidentiality was strictly respected and all records were accessible only to members of the immediate research

team. Questionnaires were coded to ensure anonymity.

RESULTS

From April to July 2008, two hundred and eleven (211) pregnant women were recruited in our study population from the Yaoundé Gynecology, Obstetric and Pediatric Hospital.

**General Characteristics of the subjects of study**

The age of the subjects ranged from 14 to 46 years. The 21-25 years and 26-30 years were the most represented, with 29.4% and 33.6% respectively, as shown in Table 1, the mean age was 27±5.99 years. The subjects were distributed in first, second and third trimesters as shown in figure 1.

Table 1: Distribution of subjects into age groups

| Age	Frequency	Mean percentage
<15	1	0.5±0.0
16-20	15	7.1±1.3
21-25	62	29.4±6.0
26-30	71	33.6±5.9
31-35	36	17.1±3.2
36-40	18	8.5±2.2
40+	8	3.8±0.8
Total	211	100±7.6
P-value	0.3922	

Fifty-four (54) of the subjects were in the first trimester of pregnant. The partition of subjects based on history of premature delivery showed that (Figure 2) showed that a low incidence of subjects 24

(11.37%), with any history of premature delivery. There was a high subject population 187 (88.63) with no history of premature delivery in the study.

Figure 1: Distribution of subjects by pregnancy duration in trimester
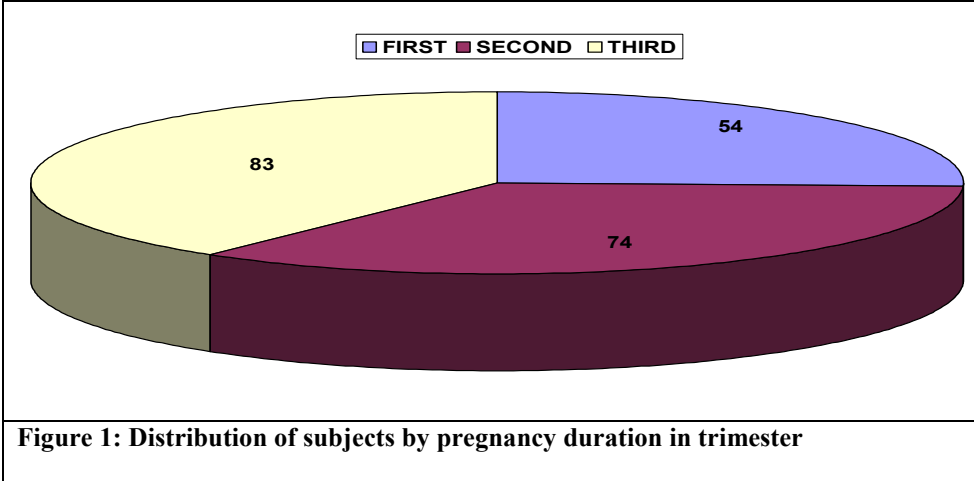
The calculated percentage shown is with respect to the Sero status. (Figure 3) The age group 21 to 25 and 26 to 30 years had the highest prevalence of the antibodies against the rubella virus, with a mean age of 27.0±5.99 years. No statistical difference was obtained between the age groups (P=0.403).
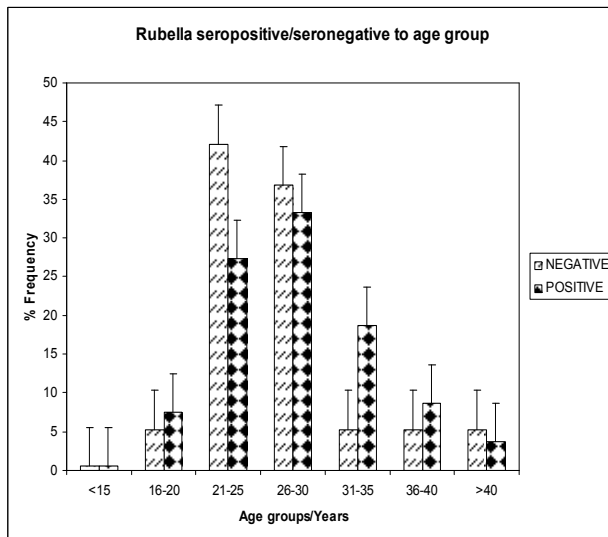

Figure 2: Distribution of subjects according to premature delivery
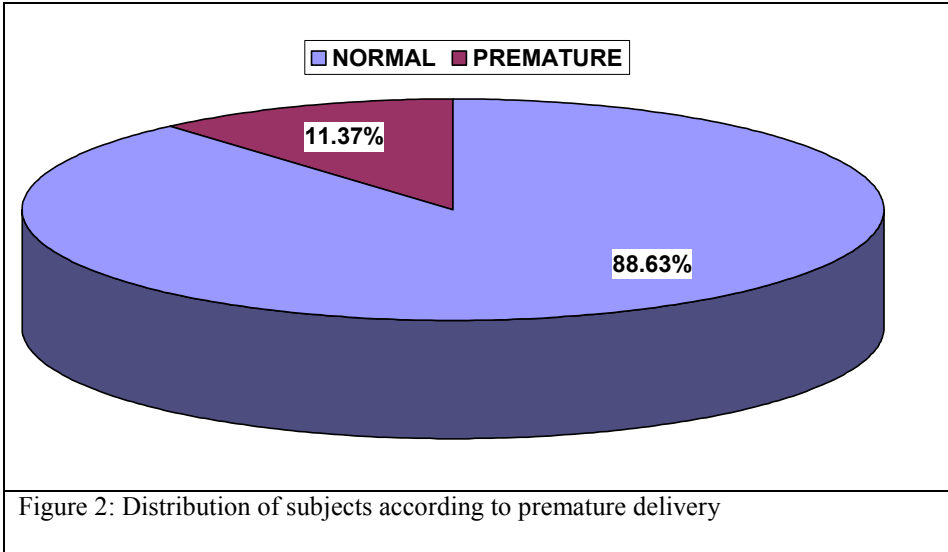
Figure 3: Classification of rubella seropositivity/negativity with respect to age group
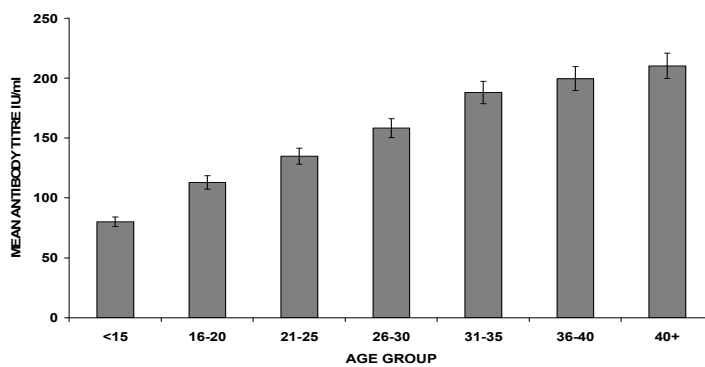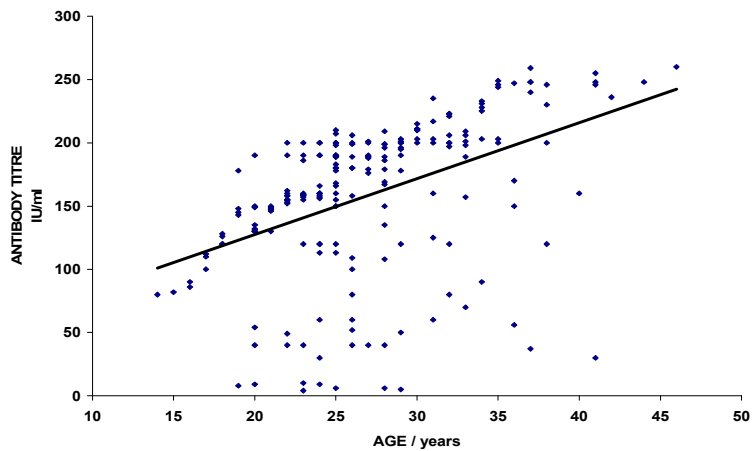

Fig 4: Mean Antibody titre and age distribution

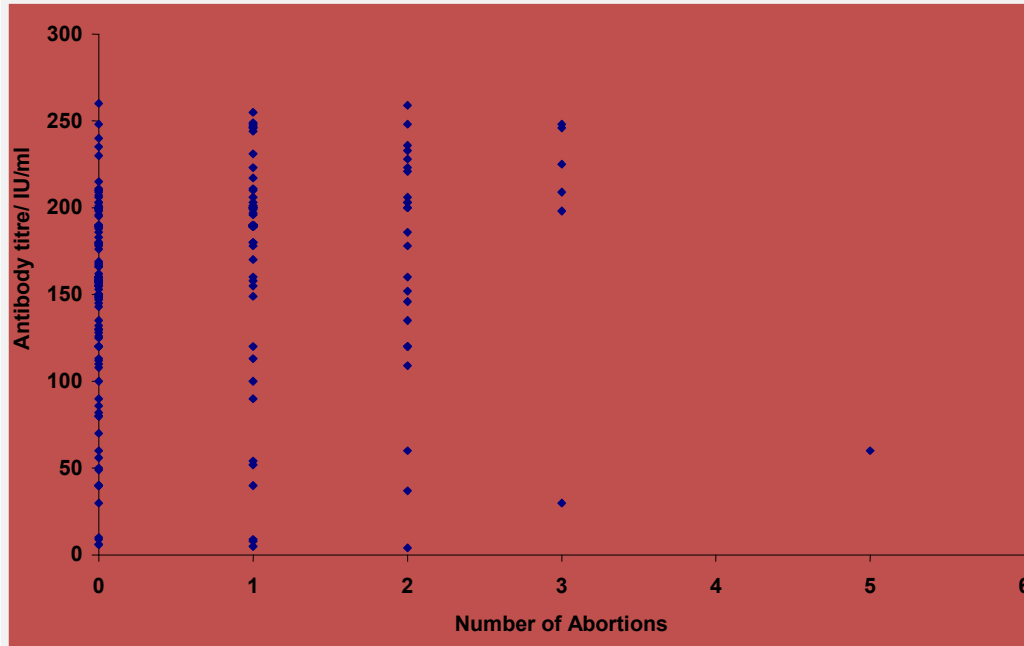**Figure 5: Correlation between age and antibody titre of subjects**

Figure 6: Correlation between antibody titre and number of abortions

There was a steady increase in the mean antibody titre levels with increase in age (Figure 4) There is a significant difference between mean antibody titre of the women age values (P<0.0001).This showed that as the age increases, the antibody titre significantly increased. Investigation of the relationship between age and antibody titres by the Spearman Correlation analysis values showed that there was a significant positive correlation (p<0.01, r=0.549, N= 211) between the subject ages and the antibody titre. This means that as the age increases, the antibody titre increases. (Figure 5). Investigation to establish any relationship between antibodies titre and the number of abortions by the Spearman correlation showed that there was a positive but weak correlation (P<0.01, r=0.246

N=211) between the number of abortions and the antibody titre. (Figure 6).

DISCUSSION

The investigation conducted on the seroprevalence of rubella virus amongst pregnant women visiting the Yaoundé Gynecological, Obstetric and Pediatric Hospital (HGOPY) showed that of the 211 pregnant women randomly selected visiting prenatal consultation. The seroprevalence of the rubella virus was found to be 88.6% while 9% were seronegative (susceptible) to the rubella virus. 5 women (2.4%) were found to be equivocal. The latter may be due to re-infection cases, the IgG is highly elevated whilst IgM may be demonstrable, giving equivocal results [8]. For such cases, it is recommended to collect fresh samples taken within 7 to 14 days and repeat the

assay in parallel [25], to confirm these equivocal cases. However, it was not possible to repeat the tests for these samples due to the time allocated for this study and also difficulties involved in scheduling another meeting with the subjects.

The seroprevalence of (88.6 %) recorded in this study is similar to those reported in other African countries in pregnant women, women of childbearing age, women and men [40, 41, 42,43, 44, 46, 47, 48, 48, 49, 38, 51, 52].The assays for rubella-specific IgG varied between studies, as did the titre that was considered positive. The Haemagglutination Inhibition (HI) test, which is considered the reference stardard was used in most of these studies, but some used Single Radial haemolysis (SRH), latex Agglutination, or Enzyme-based Immunoassay (EIA). Although there is a general agreement between these tests, the results vary between laboratories, and those of different assays or different commercial kits may not be strictly comparable [2, 10, 17].

None of these women had previous history of vaccination of rubella virus. This high prevalence might suggest the presence of the wild type virus [12, 13, 22, 25] also, since it is a hospital based study, and most of the women were living in urban areas, the seroprevalence might be higher than normal due to overcrowding and the ease with which the virus spreads amongst unvaccinated population [16-21, 36). It might also be as a result of selection bias due to exclusion of women who did not come for prenatal checks. Previous studies performed in different populations and study zone reported seroprevalences ranging from 59% to 94% [22, 25, 26, 40, 41, 42]. Seroprevalence of up to 90% in countries without any mass vaccination program, are generally a reflection of post-epidemic immunity (37). We cannot conclude that these cases were from post epidemic immunity since no data is available for epidemics in Cameroon Rubella natural infection is followed by a high level of protection from re-infection [19] However, re-infection can occur which is generally asymptomatic and in pregnancy it poses minimal risk to the fetus [18]. Studies to investigate any relationship between maternal age and the mean antibody titre (IU/ml) within the subjects ranging from 14 to 46 years showed that the 21 to 25 years and 20 to 30 years band were the most represented with mean values of 29.4% and 33.6% respectively. An observation of a steady increase in the antibody titres levels and the mean ages was recorded. This increase, was significant (p<0.0001). Also a significant spearman moment product correlation (P<0.01, r=0.549,N=211) was observed between the age and antibody titre levels (iu/ml).This suggests that as the age increases, the antibody titre significantly increases as confirmed by other publications [37, 39].

The majority of pregnant women were in their third trimester of pregnancy (39.3%)

the mean antibody titre was higher in the first trimester no statistical significant difference was observed between the first, second and third trimesters and mean antibody titre (P=0.0926) and also no Spearman correlation between antibody titre and pregnancy duration in trimesters (p=0.07). This may imply the rubella virus does not affect pregnancy duration. This correlates with previous datum that shows that there was no relationship between pregnancy duration prevalence of rubella [38]. 59.7% of the subjects did not have any history of abortion and 40.3% had previous history of abortion. The higher the antibody titre, the greater the chances of abortion occurring [16, 26]. A look into the variation of number of abortion with antibody titre was necessary. Looking at the relationship between number of abortions and the prevalence of rubella, we observed that the prevalence of rubella significantly increased with the number of abortions (P<0.05) furthermore, there was a significant but weak Spearman correlation (P<0.01, r=0.246 N=2.) between the number of abortions and the Antibody titre. This means that the higher the antibody titre, the higher the probability of abortion, implying that those with higher rate of abortions had higher antibody titre. Rubella virus enters the fetus during the maternal viraemic phase through the placenta [3, 21, 29] The damage to the fetus seems to involve all germ layers and results from rapid death of some cells and persistent viral infection in others [22, 48, 49]. However, since the study was cross

sectional, it is difficult to say whether the occurrence of high antibody titre preceded or followed the abortions recorded. Generally, the rubella virus plays a significant role in the occurrence of abortion in the study population. [2, 7, 53]. There was no correlation between the antibody titre and the occurrence of premature births (P=0.947, r=0.012, N=24). This either suggests that there were no enough rubella antigens to induce active immunity against the virus and since it was a cross sectional study, no information was present to say if the occurrence of premature birth was due to high antibody titres. Thus these premature cases might have either resulted from low titre of antibody with encountered with the virus or from other sources.

Rubella virus enters the fetus during the maternal viraemic phase through the placenta [21]. The damage to the fetus seems to involve all germ layers and results from rapid death of some cells and persistent viral infection in others [22]. Chromosomal aberrations and reduced cell division are present. The fetus is almost invariably infected if the mother is infected during the first trimester. After the first trimester, the virus is isolated infrequently from the neonates, probably because fetal immune mechanisms can be activated and infection can be terminated. [23, 45].

Rubella virus is seldom isolated from infants whose mothers acquired rubella after the first trimester. However rubella- specific IgM can be detected in a high proportion of these infants which means that they were infected.

Major abnormalities are very rare because organogenesis is complete by 12 weeks and the immune response may be more developed [29]. Deafness and retinopathy (which does not affect vision), are likely to be the only abnormalities associated with post first trimester rubella. Deafness is usually the sole clinical manifestation of fetal infection occurring between 13 and 16 weeks [30].

Rubella virus specific IgM antibodies are present in people recently infected by Rubella virus but these antibodies can persist for over a year and a positive test result needs to be interpreted with caution [6].The presence of these antibodies along with, or a short time after, the characteristic rash confirms the diagnosis [2, 11, 35]. Serology is the mainstay of diagnosis of rubella infection. A recent rubella infection can be diagnosed by [26] detection of rubella-specific IgM, [8] rising titres of antibody in HAI and ELISA tests, and seroconversion [27]. It is essential to obtain accurate information relating to the date and time of exposure, the date of onset of illness, a history of previous rubella vaccination, as well as previous results of rubella screening tests. Blood should be collected from pregnant women with features of rubella-like illness as soon as possible after onset of symptoms. [26]. A significant rise in HAI antibodies can often be demonstrated. However, rubella-specific IgM is the test of choice for demonstrating patient vaccinated postpartum [1, 5, 8]. This study certainly has certain limitations since it

current infection. It has been shown though that low and transient level of IgM can be detected in cases of reinfection [26, 31, 50]. Furthermore, low levels of rubella IgM may persist for a few months to 4 years following rubella vaccination.

Typical serological events following acute rubella infection [8], note that in reinfection, rubella-specific IgM is usually absent or present at a low level transiently ELISA is now the test of reference in many laboratories but it is considerably more expensive than the SRH. An antibody titre of equal or greater than 15 IU/ml is regarded as being immune to rubella. However, there is some controversy as to the 15 IU/ml cutoff since it was arrived at empirically in the first place. It is quite clear that lower levels of antibody, such as 10 IU/ml would probably be protective as well. HAI is not used for rubella antibody screening because it is not sensitive enough [18].

It is important that women are vaccinated prior to their first pregnancy [12]. United States recommendations are for childhood vaccination to prevent epidemics, combined with vaccination of susceptible, non-pregnant adolescent and adult females [37]. The vaccine is contraindicated for pregnant women, but when unwittingly used, no problems have been seen. If the patient is pregnant and seronegative, the pregnancy should be monitored carefully and the

is a hospital based study selection bias could have occurred due to the criteria of

selection since some women might not have visited prenatal consultation. The study is limited to females visiting the prenatal consultation and it is difficult to extrapolate the results to the general population. There are constraints on the use of data from a cross-sectional survey to estimate the transmission dynamics of rubella. The duration of study was too short to give a strong conclusive finding. However, this preliminary investigation has provided a platform form a wider and long duration project as a follow up by a team of PhD students.

CONCLUSION:

The majority of pregnant women attending the Gynecology Hospital possess a protective level of Rubella IgG antibodies. However, 9% are susceptible to rubella. Furthermore, rubella antibodies increase with increasing number of previous abortions and with maternal age. Some recommendations to be made is geared towards encouraging the ministry of Public Health in Cameroon the necessity for a mass vaccination program, Increase awareness through media. There is also the need for the clinician to systematically check rubella serology in all female desiring pregnancy and in women of child bearing age, and also prenatal screening of pregnant women and vaccination of those who are seronegative to reduce the morbidity and mortality related to rubella virus in new born babies.

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