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Original Article

Microbiology Section

Seroprevalence of Rubella Antibodies in Infertile and Pregnant Sudanese Women

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ABSTRACT

Introduction: Primary rubella leads to serious consequences in pregnant women such as abortion, stillbirth and severe birth defect. Vaccination is the best strategy to give acquired immunity and to prevent the disease. However, screening women at reproductive age for rubella is not routinely used in many resource-limited countries like Sudan and data on prevalence of the disease lacks accurate information.

Aim: We aimed to determine anti-Rubella IgM and IgG antibody profile of infertile women in Sudan to assess possible association of rubella with women infertility.

Materials and Methods: This was a cross-sectional study conducted during April-July 2016 where a total number of 184 serum specimens were collected. Specimens included 92 sera of infertile women collected in antenatal care hospital for fertility in Khartoum and 92 sera collected from healthy pregnant women, as controls. Sera were screened for anti-Rubella IgM and IgG antibodies using ELISA kits (Fortress Diagnostics, UK).

Results: Socio-demographic and clinical information were obtained from infertile women using a structured questionnaire and were analysed using Pearson's Chi-square test by SPSS program version 16. Seropositivities of anti-Rubella antibodies among infertile women were found to be 75% (69/92) for IgM and 94.5% (87/92) for IgG. These values increased with age; however, there were no statistical differences among the different age groups. Interestingly, all infertile women at the age above 46 years demonstrated presence of IgM and IgG, accounting 100% seropositivity. A statistically significant difference was observed in the seropositivity of anti-Rubella IgM antibodies among infertile women of different duration of marriage (p-value=0.025). Among pregnant women (control group), 14.1% (13/92) and 92.40% (85/92) were positive for anti-Rubella IgM and IgG antibodies, respectively.

Conclusion: The high anti-Rubella IgG antibody titres among the two cohorts in the absence of routine vaccination in the country suggest high endemicity and sustained transmission. Additionally, the higher IgM antibody titers among the infertile women suggest potential role of rubella to impair women fertility. Further studies are needed to determine high risk population and the need for an immunization policy for rubella in Sudan.

Keywords: Fertility, Immunization, Stillbirth, Vaccination

INTRODUCTION

Rubella is an important viral infection that has worldwide prevalence. It is caused by rubella virus, which is a member of the togaviridae family with a single-stranded RNA genome. The disease usually appears with mild symptoms including transitory skin rashes, respiratory involvement and swollen glands [1]. Infection during early pregnancy increases the chance of passing the virus to the foetus, increasing the risk for intrauterine death and spontaneous abortion. If contracted during the first trimester, severe birth defects, known as Congenital Rubella Syndrome (CRS), can develop with multiple organ involvement and serious complications such as encephalopathy and thrombocytopenia [2].

Rubella remains without specific treatment; however, the disease is preventable by vaccination. The vaccine has been implemented in many industrial countries and significantly reduced incidence of the disease in these countries and prevent epidemics [3,4]. In countries where the vaccine is not yet included in their national vaccination schedule, rubella becomes a frequent disease and epidemics occur due to the increased susceptibility. Rubella vaccine is available at affordable price and proven high protective rate. However, it seems that the commitment of the governments in developing countries for introduction of rubella vaccine is not satisfactory [3,5].

Following rubella infection, IgM antibodies increase rapidly during 7–10 days and decline after several weeks, thus elevated IgM antibody level indicates a recent rubella infection. IgG antibody develops more slowly and can remain positive for life, providing protection and immunity against further infection and indicating previous exposure to the virus [6,7]. The World Health Organization (WHO) recommends assessing rubella immune status and introduction of rubella vaccine to reduce the risk of the infection and CRS [8]. In Sudan, like in most other African countries, rubella vaccine is not yet included in the national immunization program and the seroprevalence of rubella is high, reaching 95.1% in women of childbearing age [9,10]. Most of studies on seroprevalence of rubella in Sudan were performed on IgG, giving little information about active rubella infection in the country and data on the prevalence of rubella among infertile women are lacking. We conducted this study to detect the presence of anti-Rubella IgG and IgM antibodies in sera collected from infertile women in an antenatal care hospital in Khartoum, Sudan to explore possible association between rubella infections and women infertility in highly endemic countries.

MATERIALS AND METHODS

Study design and population: This was a cross-sectional study in which sera was collected during April-July 2016 at antenatal care hospital in Khartoum. The study included collection of blood specimens to detect presence of anti-Rubella IgG and IgM antibodies among infertile women and healthy pregnant women, as control. One hundred eighty-four women, who expressed their interest of participation in the study and have given their written consent for obtaining their blood samples, were included. Collection of data was done through a structured interview of participants' first visit to the center. A data collection form was used for collection of socio-demographic and clinical data. The form was pre-submitted to the

health authority of the antenatal care hospital and was approved. Some women were excluded from the study on the basis of their pregnancy, having less than one year of marriage or absence of regular sexual relationship due to the absence of their husbands. Those women with hormonal imbalance or if their husband have low sperm count or other abnormalities were also excluded. All women were within the reproductive age (15-49 years) according to the WHO definition of infertility [11]. An exception was a woman of the age 51; this woman was included in the study because she was considered infertile during her first visit to the center at the age 45 years and was visiting the center since that time.

Ethical consideration: This study was approved by the Institutional Review Board at Faculty of Medical Laboratory Sciences, Sudan International University. The protocols and experimental procedures were in accordance with the ethical standard of the national ministry of health and the M.Sc. program at Faculty of Medical Laboratory Science, Sudan International University. Approval was also obtained from the Health Authority at antenatal care hospital in Khartoum for Fertility- Khartoum (No. 2015/3/2 and 2015/3/22). Participants were informed about the study and signed consent was obtained before enrolling in the study.

Sample collection and processing: A structured questionnaire was designed and distributed to the participants to obtain information on the following: age, duration of marriage, medical history of abortion, still birth, residence, history of pregnancy and socio-demographic features. Collection of the data was done before sample collection. Venous blood specimens were collected after filling informed consent. The specimens were collected using standard aseptic technique and allowed to stand at room temperature and serum specimens were separated by centrifugation at 2,500 rpm for 5 minutes. All sera were stored at -20°C until the serological testing.

Detection of Anti-Rubella IgG and IgM antibodies: Rubella IgG and IgM EIA commercial test kits (Fortress Diagnostics, UK) were used to assess antibodies to Rubella. The kits were purchased from the local market at Khartoum. These tests are based on indirect principle for the qualitative and quantitative detection of the presence of IgG and IgM antibodies to Rubella in serum specimen [12]. Performance of the test and interpretation of results were done according to instructions of the manufacturer and in duplicate within the same batch of kits. Test results were interpreted qualitatively, as positive, negative or equivocal. Anti-Rubella IgG titers greater than 10 IU/mL were considered positive, 5.0-10 IU/mL equivocal, and <5.0 negative. Anti-Rubella IgM titers of >1.1 IU/mL were considered positive, >0.9 and <1.1 IU/mL equivocal, and <0.9 negative. In case of equivocal results, tests were repeated twice on the same specimens. Optical densities were measured in microplate reader (Human, Germany) at 450 nm and results were expressed as international units per mL. To obtain valid results, the following validation requirements were met: blank absorbance <0.05, calibrator 1 mean absorbance <0.100 after subtraction of bank absorbance, calibrator 2 mean absorbance >0.200 and <0.700 after subtraction of bank absorbance, calibrator 3 mean absorbance >1.500 after subtraction of bank absorbance. The test results were considered invalid if the above validation requirements were not met and tests were repeated.

STATISTICAL ANALYSIS

All obtained data and results of the laboratory analysis were analysed using SPSS version 16. The p-values was calculated using Pearson's Chi-square test at a 95% confidence interval. The p-values of <0.05 were considered significant.

RESULTS

The study was carried out in antenatal care hospital for Fertility in Khartoum on 92 sera of infertile women and 92 sera of pregnant healthy women, as controls. The mean age was 34.7 years (median 35) for infertile women and 27.2 years (median 29) for pregnant women. The overall seropositivities of anti-Rubella IgM and IgG among the infertile women were 75% and 94.5%, respectively. The seropositivity increased with the age; however, there were no statistically significant differences among the different age group [Table/Fig-1]. Interestingly, all infertile women of the age group 46-51 (n=4) demonstrated presence of IgM and IgG, accounting for 100% seropositivity. Out of the 92 infertile women, only 3 (3.26%) showed no detectable IgM and IgG antibody responses to rubella, indicating high susceptibility and the need for vaccination. Among pregnant women, 13 (14.10%) and 85 (92.40%) were positive for anti-*Rubella* IgM and IgG antibodies, respectively [Table/Fig-1].

Age/year (number examined)	Anti-Rubella IgM >1.1 IU/mL			Anti-Rubella IgG >10 IU/mL		
	Infertile Women	p-value	Pregnant Women	Infertile Women	p-value	Pregnant Women
18-25	19/25 (76%)	0.562	5/26 (19.2%)	24/25 (96%)	0.783	24/26 (92.3%)
26-35	25/36 (69.4%)		4/36 (11.1%)	33/36 (91.6%)		33/36 (91.7%)
36-45	21/27 (77.7%)		4/30 (13.3%)	26/27 (96.3%)		28/30 (93.3%)
46-51	4/4 (100%)		0/0 (00.0%)	4/4 (100%)		0/0 (00.0%)
Total	69/92 (75%)		13/92 (14.1%)	87/92 (94.5%)		85/92 (92.4%)

[Table/Fig-1]: Detection of anti-Rubella antibodies (IgM and IgG) in infertile and pregnant Sudanese women of different age groups.
Values are number positive/total number (%) for both IgM and IgG antibodies. Anti-Rubella IgG antibody titers greater than10 IU/mL were considered positive for IgG and titers >1.1 IU/mL considered positive for IgM. Age effect on antibody levels of infertile women was assessed using Pearson's Chi-squared test at a 95% confidence interval and p-values of <0.05 were considered significant.

A summary of seropositivity of anti-Rubella IgM and IgG antibodies of infertile women in relation to clinical and socio-demographic characteristics is shown in [Table/Fig-2]. The participants had different marriage duration up to ≥ 10 years. A statistical significant difference was observed in the seropositivity of Rubella IgM antibodies among infertile women of different duration of marriage (p-value=0.025). However, the different in seropositivity of IgG among the two groups was not significant (p-value=0.181).

Characteristics	Anti-Rubella IgM			Anti-Rubella IgG	
	Number Examined	Number Positive (%) >1.1 IU/mL	p-value	Number Positive (%) >10 IU/mL	p-value
Duration of Marriage (years)					
1-2	12	10 (83.3%)	0.025*	11 (91.6%)	0.181
3-5	23	13 (56.5%)		20 (86.9%)	
6-9	36	26 (72.2%)		36 (100%)	
≥10	21	20 (95.2%)		20 (95.2%)	
Educational Status					
None educated	4	2 (50%)	0.550	4 (100%)	0.614
Primary	8	6 (75%)		8 (100%)	
Secondary	32	26 (81.2%)		31 (96.8%)	
University or higher degree	48	35 (72.9%)		44 (91.6%)	
Resident					
Khartoum state	48	38 (79.1%)	0.335	45 (93.7%)	0.719
Other states	44	31 (70.5%)		42 (95.4%)	

[Table/Fig-2]: Detection of anti-Rubella antibodies (IgM and IgG) of infertile Sudanese women in relation to clinical and socio-demographic characteristics.
p-values were calculated using Pearson's Chi-squared test at a 95% confidence interval. p-values of <0.05 were considered significant. *Significant p-values

Significant differences were also not observed in the seropositivity of anti-Rubella IgM and IgG (p-values >0.550) among infertile women of different educational levels or from different geographical

locations (p=values >0.335). The percentage of the positive anti-Rubella antibodies among women resident in Khartoum state was 79.1% (38/48) for IgM and 93.7% (45/48) for IgG [Table/Fig-2].

Anti-Rubella IgM and IgG antibodies among infertile women were also studied in relation to rubella-specific complications [Table/Fig-3]. The percentage of positive anti-Rubella antibodies among primary infertile women was 81.03% (47/58) for IgM and 98.27% (57/58) for IgG; these values were 67.64% (23/34) and 91.17% (31/34) for IgM and IgG, respectively among women with secondary infertility. However, there were no significant differences in seropositivity of anti-Rubella IgG and IgM among infertile women of different type of infertility (p-values >0.107). In addition, high percentage of infertile women with history of abortion demonstrated seropositivity to anti-Rubella IgM (71.87%) and all the women in this group showed presence of anti-Rubella IgG antibodies, 32/32 (100%). The presence of these antibodies were not statistically significant with the history or number of abortion (p-values >0.317). In particular, all infertile women with history of still birth (n=3) showed high anti-Rubella IgM and IgG antibodies, nevertheless, presence of these antibodies was not of statistical significant (p-values 0.309 and 0.673 for IgM and IgG, respectively).

Rubella specific-complication	Anti-Rubella IgM			Anti-Rubella IgG	
	Number Examined	Number Positive (%) >1.1 IU/mL	p-value	Number Positive (%) >10 IU/mL	p-value
Type of infertility					
Primary	58	47 (81.03%)	0.146	57 (98.27%)	0.107
Secondary	34	23 (67.64%)		31 (91.17%)	
Abortion					
Yes	32	23 (71.87%)	0.317	32 (100%)	0.455
No	58	47 (81.03%)		57 (98.27%)	
Number of abortion					
0	60	47 (78.34%)	0.622	58 (96.67%)	0.984
1	19	15 (78.94%)		18 (94.73%)	
2	7	4 (57.14%)		7 (100%)	
3	3	3 (100%)		3 (100%)	
4	2	2 (100%)		2 (100%)	
5	1	1 (100%)		1 (100%)	
Stillbirth					
Yes	3	3 (100%)	0.309	3 (100%)	0.673
No	89	66 (74.1%)		84 (94.3%)	

[Table/Fig-3]: Anti-Rubella antibodies (IgM and IgG) among infertile Sudanese women in relation to Rubella -specific complications.
p-values were calculated using Pearson's Chi-squared test at a 95% confidence interval. p-values of <0.05 were considered significant.

DISCUSSION

This study determined for the first time data on seroprevalence of anti-Rubella IgM and IgG antibody profile among infertile and fertile women in Sudan. This data helps ascertain endemicity and transmission of the virus and the need for rubella vaccination [13]. The factors known to be associated with infertility were considered [14,15].

Most of collected socio-demographic data and the observed clinical characteristics of the infertile women were not significantly associated to rubella. However, of interest is the higher seropositivity rate of anti-Rubella IgM antibodies among the infertile women. The high IgM antibodies are usually associated with recent infections that develop after onset of the clinical disease and can or can't be associated with IgG positivity [16-18]. Of note, none of the cases with IgM positivity in the two cohorts was identified as active infection and were not treated, accordingly. The reason might be due to the nature of the disease as most of rubella infection can be mild or even subclinical.

The presence of high level of IgG antibodies in the two cohorts indicates previous exposure to the virus and protection. This is also suggesting endemicity of the disease in the area. The local health authorities should take responsibility towards establishing a national immunization program for rubella. Indeed, large-scale rubella vaccination programs will reduce or even eliminate rubella and their clinical effects. The negativity in both IgG and IgM indicates lack of immunity and high susceptibility to the virus. In this study, highly susceptible population is not large (3.26%).

Previous works from Sudan reported comparable high prevalence of Rubella IgG antibodies (65.3-95.1%) among pregnant women [9,19]. In agreement with the present results; the authors couldn't correlate between clinical and socio-demographic characteristics of the patients and seropositivity to anti-Rubella antibodies. Worldwide, considerable variation has been shown in the seroprevalence of immunity (IgG) among pregnant and non-pregnant women in reproductive age from various countries. In most African and Arab countries, the seropositivity to rubella was greater than 90% [20,21]. Protective immunity of rubella vaccine is clearly demonstrated in the developed countries like UK and USA where the disease has become quite rare following implementation of national programs for vaccination [22,23].

Data on direct association between rubella and women infertility is lacking [24-29]. However, present data clearly shows, for the first time, high positivity rates for both IgM and IgG among infertile women. Since rubella vaccine is not used in Sudan, prevalence of these antibodies is more likely related to natural 'previous' infection-exposure. However, interpretation of these findings should be done with care. It is obviously clear that rubella serology can have poor diagnostic outcomes in areas with low disease prevalence. So, the high positivity in rubella IgM serology might be due to false results, which can occur in apparently-healthy individuals [30]. Nevertheless, this data suggests possible association between rubella and women infertility and highlights the need for introduction of a national strategy against rubella in Sudan.

LIMITATION

This study had some limitations. Authors couldn't obtain all demographic details of our study groups. Moreover, follow-up of the patients for possible change in the IgM level was not possible because the patients lived in rural areas which are far from the treatment center. Giving the high endemicity of the rubella in the two population and high IgG levels, authors couldn't make an association between rubella and infertility.

CONCLUSION

These data suggest high endemicity of rubella in Sudan and its potential role to impair women fertility. Further studies are needed to determine high risk population and the need for new immunization policy for rubella in Sudan.

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