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Screening the Prevalence of Rubella Virus Infection Using Enzyme Linked Immunosorbent Assay (ELISA) and Electrochemiluminescence (ECL) Diagnostic Techniques among Pregnant Women in Mukalla City, Hadhramout/Yemen

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Abstract: Background: Infection with Rubella virus is a public health concern because it may lead to serious consequences such as congenital rubella syndrome (CRS) during early pregnancy. The sero-prevalence rates of the Rubella virus among pregnant women vary widely throughout the world. Objective: This study was aimed to determine the prevalence of Rubella virus infection and associated risk factors among pregnant women in Mukalla city, Hadhramout/Yemen. Materials and methods: In this cross-sectional and hospital-based study, the serum samples were collected from 190 pregnant women, then screened for Rubella virus antibodies (IgG and IgM) using enzyme linked immunosorbent assay (ELISA) and electrochemiluminescence (ECL) immunoassay techniques. Qualitative data regarding risk factors for rubella infection were assessed in participants interview using a questionnaire including socio-demographic and reproductive variables. Findings: The results indicated that among the tested pregnant women, there was a high seropositive cases of anti-rubella IgG 136(71.6%), while the seropositive cases of anti-rubella IgM was 17(8.9%). The proportion of pregnant women who were rubella IgG positive was significantly dependent on the age groups 15–30 years of the pregnant women (COR=0.749, 95%CI=0.113-0.557, *P*=0.001) and the moderate level income (COR=0.761, 95%CI=0.075-0.760, *P*=0.015). Anti-IgM positive rubella infection had a significant relationship with the pregnant women miscarriage (COR=0.925, 95%CI=0.020-.283, *P*=0.00), and the risk of contracting Rubella virus infection was found to increase with history of live births with a statistical significance (COR=1.942, 95%CI=1.020-3.695, *P*=0.043). ELISA technique proved to be high sensitivity, specificity and accuracy for detecting Rubella virus infection.

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Conclusion: the prevalence rate of rubella infection was relatively high in Mukalla city, Hadhramout and are significantly associated with an increase in age and income level, and the risk of contracting rubella infection was found to increase with gestational age and associated with miscarriage. Screening of rubella and immunization of women are highly recommended in this setting.

Keywords: Prevalence, Rubella virus, Pregnant women, ELISA technique, Electrochemiluminescence immunoassay

1. Introduction

Rubella is a mild childhood viral infection that causes illness worldwide, and caused by a non-arthropod-borne member of the Togaviridae family[1]. The rubella infection may be subclinical or cause self-limiting illness with clinical features; these are as low-grade fever, lymph nodes atrophy and febrile rash illness in children and adults[2]. However, if the Rubella virus is infected during pregnancy, particularly during the first trimester can result in congenital rubella infection (CRI)[3]. CRI has outcomes including stillbirth, miscarriage, abortion, congenital rubella syndrome (CRS) as well as asymptomatic infection in the infant[2]. The manifestations of CRS included cerebral, cardiac, auditory and ophthalmic defects according to the center of diseases control and prevention (CDC)[4].

The sero-positivity of rubella infection among pregnant women are varies widely in different countries of the world. In fact, in many developing countries, the rubella sero-positivity among pregnant women has been reported to range from 54.1% to 95.2%[5-6]. Hence, serological screening of rubella based on the detection of IgG and IgM antibodies remains the mainstay for rubella diagnosis[7]. There is no specific treatment for rubella disease, maternal or CRI. The primary means of preventing CRS is rubella immunization. Many developed countries have been able to utilize the vaccine effectively to reducing the prevalence of rubella and preventing the consequences of CRS[8]. Rubella is a vaccine-preventable infectious disease, and considered to be potentially eradicable. Women vaccination before pregnancy is the only mean to prevent congenital infection. In developed countries, rubella infections are indeed protected by active immunization of measles, mumps and rubella (MMR) vaccine[5]. The data of World Health organization (WHO) revealed that more than 100,000 children are born with CRS each year in developing countries[9], and as a result of the vaccination schedule in many high-income and in some low-income and middle-income countries, the estimated number of CRS cases globally decreased from 119000 cases in 1996 to 105000 cases in 2010[10].

In Yemen, the rubella vaccination is not included in the national immunization program until the year 2010, and there is no a clear strategy for the rubella infection surveillance in pregnant women. Although rubella sero-prevalence in pregnant women has been studied elsewhere in Yemen, but in Hadhramout governorate there is no published data describing the prevalence of the rubella disease. Therefore, the present study was aimed to determine the sero-prevalence of Rubella virus infection using the enzyme linked immunosorbent assay (ELISA) and Electrochemiluminescence (ECL) immunoassay techniques and their associated risk factors among pregnant women attending antenatal care clinics in Mukalla city, Hadhramout, Yemen.

2. Materials and Methods

2.1 Study design, area and duration

A hospital-based cross-sectional study was carried out to detect Rubella virus infection among pregnant women in Mukalla city-Hadhramout, Yemen during the period from November 2019 to June 2020.

2.2 Study population and inclusion criteria

The study population were the pregnant women referred to some health care centers and gynecological clinics in the main hospitals of Mukalla city/Hadhramout. The range age of population study were 15-45 years. Pregnant women confirmed to be in their first, second or third trimester who referred to clinics were included in this study. Women excluded when were they are non-pregnant.

2.3 Sample size and data collection tool

A total of 190 pregnant women participants were included in this study. A standardized, interviewer-administered, structured questionnaire was developed to obtain data regarding Rubella virus infection risk factors. It consisted of systematic questions on socio-demographic and the reproductive characteristics. The questionnaire filled with the aid of an interviewer.

2.4 Laboratory investigation

The blood samples were collected in plain tubes, allowed to clot and centrifuged at room temperature. Then the sera were separated and stored at -20°C until transported in dry ice to the National Center of Public Health Central Laboratories in Mukalla city for analyses. ELISA immunoassay technique was used for detection of anti-rubella IgG and IgM using commercial diagnostic kits according to the instructions supplied by PerkinElmer Company, USA. Manufacturer reference values for positive results were Rubella IgG index of 1.00 or greater, or >15 IU/mL and Rubella IgG index of 0.90 or less, <13 IU/mL for negative results, while Rubella IgM index of 1.00 or greater was considered as positive and Rubella IgM index less than 0.9 as negative result. IgM and IgG were also detected by ECL immunoassay technique using the full automated closed system Cobas e411 instrument according to the instructions of the manufactures company Roche.

2.5 Evaluation of diagnostic tests for Rubella virus

For evaluation the diagnostic tests for Rubella virus infection, parameters like sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of ELISA technique were calculated and compared with ECL technique as standard method.

2.6 Statistical analysis

Statistical Package for Social Sciences (SPSS) version 20 (SPSS Inc, Chicago, IL, USA) was used for data analysis. The association between different variables and outcome of Rubella virus infection was calculated and compared using Pearson Chi-square ($\chi 2$) test. Binary and multiple regression tests of crude odds ratio (COR) / confidence interval 95% (CI) were used to detect independent predictors of Rubella virus positivity. The level of statistical significance was set at p-value < 0.05.

2.7 Ethics approval and consent to participate

Research ethical approval of this study was obtained from Hadhramout University, Faculty of Sciences. Written consent was obtained before commencing the study. Permission letter was obtained from the hospital's administrations. The information was taken from the participants after they agreed to it verbally according to the informed consent with confidentiality of each study participant's result.

3. Results and Discussion

The sero-prevalence of Rubella virus infection among pregnant women screened by ELISA immunoassay technique of rubella-specific IgG antibody was 136(71.6%) which indicates prior exposure to the Rubella virus (immune as a result of previous wild-type rubella infection), and 17(8.9%) were positive for rubella-specific IgM antibody which indicates acute (recent) Rubella virus infection. ECL technique detected 140(73.6%) cases for IgG and 17(8.9%) for IgM as presented in **Table 1**.

Table 1: Sero-prevalence of Rubella virus infection in pregnant women

Antibody assay	ELISA technique				ECL technique			
	Sero-positivity		Sero-negativity		Sero-positivity		Sero-negativity	
	No.	%	No.	%	No.	%	No.	%
Rubella IgG	136	71.6	54	28.4	140	73.6	50	26.4
Rubella IgM	17	8.9	173	91.1	17	8.9	173	91.1

In our validation for Rubella virus infection detection, the sensitivity, specificity, PPV, NPV and accuracy of ELISA IgG and IgM technique were measured and compared with ECL standard technique. The sensitivity, specificity, PPV, NPV and accuracy of ELISA IgG were 98.5%, 81.5%, 98.5%, 81.5% and 93.7% respectively, and for ELISA IgM were 100% for each, as shown in **Table 2**.

Table 2: Evaluation of ELISA technique for Rubella virus detection

Antibody assay	Sensitivity	Specificity	PPV	NPV	Accuracy
ELISA IgG	98.5	81.5	98.5	81.5	93.7
ELISA IgM	100	100	100	100	100

The anti-Rubella IgG sero-prevalence in Mukalla city is relatively high, suggesting a continuous transmission of endemic Rubella virus in the region. This prevalence was comparable to a study recorded 91.64% positive for rubella IgG among schoolgirls in Sana'a city, Yemen[11]. Other studies revealed high levels of anti-Rubella IgG exposure reported in pregnant women in Turkey 95.0%[12] and 96.1%[13], Nigeria 83.3%[14], India 86.8%[15], Ethiopia 79.5%[16], Egypt 88.2%[17], Saudi Arabia 91.6%[18], Sudan 95.1%[19], Tanzania 92.6%[20], Kenya 92.9%[21], Zimbabwe 92%[22] and Ghana 93%[23]. These findings showed that a high proportion of the population has an immunity which confirms exposure to previous natural infection of Rubella virus. The current study suggests that Rubella virus is prevalent in the study area which may be attributed to sustained transmission hence development of the antibodies. The reported sero-prevalence of Rubella IgG antibody in this study is higher than reported in Sudan 65.3%[24] and 51.6%[25], Ethiopia 46.4%[26], Nigeria 68.6%[14], India 39.20%[27] and Libya 44.2%[28]. These data suggest that there is a high transmission rate of the Rubella virus in Mukalla city, Hadhramout. However, this might not reflect the true picture as women were not screened during early pregnancy and followed-up. Also, the differences in the rate of past exposure to rubella between these countries may indicate a varying epidemiology of the infection in different localities.

The current study recorded anti-Rubella IgM sero-positivity 8.9% among pregnant women that represents recent infection, which is comparable to 9.5% were positive for rubella-specific IgM antibody among pregnant women before the introduction of rubella vaccine in Ethiopia[16]. Other studies results reported high levels of Rubella IgM positive in pregnant women in Libya 18.9%[28], Ethiopia 39.4%[29] and 46.5%[15]. The sero-positivity of anti-Rubella IgM in this study was higher than that recorded in Turkey 0.54%[13], Bangladesh 0.75%[30], India 5.26%[27], Nigeria 3.9%[14]. The variations in sero-prevalence could be as a result of epidemics which go unnoticed due to the gentle nature of the infection. This IgM sero-positivity could have been attributed to lack of introduction of rubella vaccine into routine national immunization program in some countries.

Regarding the statistical analysis of the socio-demographic variables, the age group of 21-30 years was significantly associated with rubella infection when detected by IgG test (COR=0.749, 95%CI=0.113-0.557, P=0.001), while for IgM test was significant association of age group 15-20 years (COR=8.750, 95%CI=1.016-75.374, P=0.048). Education level was not significantly associated with rubella infection for IgG and IgM antibodies tests (P>0.05). For residence, there was no significant association of rubella infection detected by IgG and IgM antibodies tests (P>0.05). There was

significantly association of rubella infection when detected by IgG antibody test with moderate income level (COR=0.761, 95%CI=0.075-0.760, *P*=0.015), while for IgM antibody test was insignificant association (*P*>0.05). Also, there was no significantly association for anti-Rubella IgG and anti-Rubella IgM tests (*P*>0.05) with an occupation. Multivariate logistic regression analysis was performed for these associated socio-demographic variables that showed significant at the crude odds ratio calculation for positive cases ELISA IgG and IgM. The association remains significant between Rubella virus infection, age groups and income level as shown in **Table 3**.

Table 3: Prevalence of Rubella virus infection in relation to the demographic characteristics of the pregnant women

Character	Categories	Sero-status IgG		COR	CI(95%)	P-value	Sero-status IgM		COR	CI(95%)	P-value
		No.	%				No.	%			
Age group	15-20 years	25	13.2	0.584	0.157-1.097	0.076	1	0.5	8.750	1.016-75.374	0.048*
	21-30 years	94	49.5	0.749	0.113-0.557	0.001*	9	4.7	2.725	0.952-7.98	0.062
	31-45 years	17	8.9	1			7	3.7	1		
Educational level	Illiterate	19	10	1			5	2.6	1		
	Primary	64	33.7	0.456	0.228-1.299	0.171	9	4.7	1.645	0.505-5.355	0.408
	High school	46	24.2	0.553	0.173-1.156	0.097	2	1.1	5.481	0.997-30.127	0.050
	Bachelor	6	3.2	1.583	0.413-6.063	0.502	1	0.5	0.038	0.160-5.786	0.966
	Postgraduate	1	0.5	1.583	0.090-27.771	0.753	0	0	310668243.1	0.000	0.999
Residence	Rural	22	11.6	1			1	0.5	1		
	Urban	114	60.0	1.110	0.461-2.672	0.816	16	8.4	0.357	0.140-2.953	0.570
Income level	High	15	7.9	0.143	0.233-3.159	0.817	7	3.7	1.950	0.369-10.309	0.432
	Moderate	115	60.5	0.761	0.075-0.760	0.015*	9	4.7	3.709	0.888-15.484	0.072
	Low	6	3.2	1			1	0.5	1		
Occupation	Student	4	2.1	80774441.0	0.000	0.99	1	0.5	1.00	0.000	0.99
	Employee	8	4.2	80774441.0	0.000	0.99	1	0.5	1.00	0.000	0.99
	Housewife	122	64.2	635602183.1	0.000	0.99	15	7.9	1.00	0.000	0.99
	Unemployed	2	1.1	1			0	0	1		

*Statistically significant at P-value < 0.05

In the current study, the most pregnant women were within the accepted child bearing age infected with anti-Rubella IgG for the age group 21-30 years. A study carried out in Nigeria revealed that most infections of rubella were acquired before the age of 35 years[31]. Also, the prevalence of rubella infection was 83.9% among women aged 20–25 years and 93.9% among those aged 25–30 years in Egypt[17]. Age groups showed no significant association with Rubella-specific IgM and IgG antibodies among pregnant women before the introduction of rubella vaccine in Ethiopia[16]. Some studies revealed the proportion of women with the highest sero-positivity with rubella infection was in the age group 20-30 years in Kenya[20], the age group 14-20 years in Tanzania[21], the age group 20-29 years in Nigeria[6], the mean age 30.9 years in Zimbabwe[22], the mean age 29 years in Colombia[32], the mean age of 25.7 years in Western Sudan[26], the age group 20-29 years in Brazil[33].

In this study, primary and high school levels were at higher risk for rubella infection with no statistically significant association between the level of education of the pregnant women and rubella infection sero-positivity, similar results of studies conducted in Iran and Zimbabwe showed a relationship between rubella infection and secondary level of pregnant women education[22,32]. There was a significant relationship between rubella infection and illiteracy in Western Sudan[24]. Other studies showed incidence of anti-rubella IgG and IgM was not associated with education of pregnant women in Ethiopia[16] and Egypt[17]. In the present study, the majority of positivity rubella infection of IgG and IgM of pregnant women were from urban area with insignificantly associated differences. Different results reported the prevalence in rural areas was 51.5% and in urban areas was 44.7%[26]. Also, residence was not significantly associated to the prevalence of rubella antibodies[17]. The residence showed no significant association with Rubella-specific antibodies among pregnant women before the introduction of rubella vaccine in Ethiopia[16]. A significant association between residence site and IgG sero-positivity was observed in Ethiopia, where urban dwellers had higher past rubella exposure compared with rural residents[34]. Likewise, moderate socioeconomic status has been found as a strong risk factor for acquisition rubella infection in the current study, and there was a significant relationship between pregnant women income level and rubella infection sero-positivity. Lower social economic status puts people at higher risk of having poor health due to poor housing conditions which are overcrowded[16].

The majority of pregnant women infected with rubella in this study were housewives with a percentage of 64.2% and 7.9% of anti-Rubella IgG and IgM respectively, and there was insignificant statistical association. Mothers who were in small business enterprises had highest IgG positivity 31.5% followed by farmers 29.5% found in Tanzania[21] and South Africa[35]. In Western Sudan, most of the participants were farmers 49.4% followed by housewives 45.2%[24], while in Southern Sudan, 94.2% of women were unemployed[36]. Rubella-specific IgM and IgG sero-prevalence was not associated with occupation among pregnant women in Ethiopia[16]. These findings could have been attributed by the interactions or contact with infected persons in populated areas.

For reproductive characteristics, the trimester at the time data collection was insignificantly associated with Rubella virus infection of both anti-Rubella IgG and IgM (P>0.05). The parity of 1-3 times was significantly associated with Rubella virus infection detected by IgG antibody test (COR=1.942, 95%CI=1.020-3.695, P=0.043), and IgM antibody test (COR=0.668, 95%CI=0.122-0.902, P=0.031). Moreover, history of miscarriage of 1-2 times was significantly associated with Rubella virus infection detected by IgM antibody test (COR=0.925, 95%CI=0.020-.283, P=0.00), and for > 2 times (COR=0.971, 95%CI=0.005-.164, P=0.00), while insignificantly associated for IgG antibody test (P>0.05). The past history of rubella infection was insignificantly associated with Rubella virus infection of both anti-Rubella IgG and IgM tests (P>0.05). All pregnant women enrolled to the study were not protected against Rubella virus. Multivariate logistic regression analysis was performed for these associated reproductive variables that showed significant at the crude odds ratio calculation for positive cases ELISA IgG and IgM. The association remains significant between Rubella virus infection, parity and history of miscarriage as shown in **Table 4**.

Table 4: Prevalence of Rubella virus infection in relation to the reproductive characteristics of the pregnant women

Character	Categories	Sero-status IgG No.	%	COR	CI(95%)	P-value	Sero-status IgM No.	%	COR	CI(95%)	P-value
Gestation age	1st Trimester	42	22.2	1			5	2.6	1		
	2nd Trimester	43	22.6	0.24	0.335-1.721	0.510	3	1.6	1.205	0.307-4.730	0.790
	3rd Trimester	51	26.8	1.007	0.478-2.120	0.986	9	4.7	0.354	0.204-2.044	0.458

Parity	1-3	92	48.4	1.942	1.020-3.695	0.043*	6	3.1	0.668	0.122-0.902	0.031*
	>3	44	23.2	1			11	5.8	1		
History of miscarriage	1-2	32	16.8	1.155	0.555-2.403	0.700	15	7.9	0.925	0.020-0.283	0.00*
	> 2	5	2.6	2.178	0.554-8.563	0.265	1	0.5	0.971	0.005-0.164	0.00*
	None	99	52.2	1			1	0.5	1		
History of rubella infection	Yes	4	2.1	1.941	0.420-8.978	0.396	0	0	176233621.6	0.000	0.999
	No	132	69.5	1			17	8.9	1		
Immunization against Rubella	Yes	0	0	-	-	-	0	0	-	-	-
	No	136	71.6	-	-	-	17	8.9	-	-	-

*Statistically significant at P-value < 0.05

In the current study, there was no significant association of the incidence anti-Rubella IgG and IgM with gestation age of the pregnant women, and the sero-prevalence in various trimesters is still higher than that from other countries with no immunization program in Kenya[20] and Sudan[24]. Other study revealed that the risk of contracting Rubella virus infection was found to increase with gestational age[21]. Rubella IgM and IgG antibodies prevalence in relation to gestation age showed no significant association in a study carried out in Ethiopia[68], while pregnant women at first trimester was risk factor found to be significantly associated with rubella anti-IgG sero-prevalence in Northwest Ethiopia[26]. This study showed that the risk of contracting Rubella virus infection was found to increase with history of live births (parity) of 1-3 times 48.4% with a statistical significance relationship of anti-Rubella IgM, whereas rubella IgM and IgG antibodies prevalence in relation history of live births showed no significant association in a study carried out in Ethiopia[16]. In this study, IgM positive rubella infection had a significant relationship with the pregnant women miscarriage of 1-2 times 16.8%, whereas other result study of rubella IgM and IgG antibodies prevalence in relation to history of abortion and stillbirth showed no significant association[22]. Very small proportion of pregnant women had a history of rubella infection were positive for rubella IgG antibody in this study compared with those has no history 69.5%. Other similar study results showed 86.6% of women reporting no history of rubella were positive for rubella antibody[17]. In the current study, all pregnant women participants were not protected against Rubella virus. Other study showed none of the women ever had previous rubella vaccination[31].

4. Conclusion

Sero-prevalence of anti-IgG rubella is relatively high in Mukalla city, Hadhramout with a significant proportion of women at risk of contracting primary rubella infection. The rubella infection was significantly associated with an increase in age and income level, and the risk of contracting rubella infection was found to increase with gestational age and associated with miscarriage. ELISA technique proved to be high sensitivity, specificity and accuracy for detecting Rubella virus infection when compared to standard ECL technique. Screening of rubella and immunization of women are highly recommended in this setting.

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