Evaluation of Rubella Antibodies among Women Imminant Marriage in AL- Khalis Province in Diyala Governorate in Iraq

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Abstract

Rubella is an acute viral disease that its pathogenesis is usually mild and self-limiting. The importance of this disease its complications for fetus during pregnancy represented by congenital rubella syndrome (CRS). The present study was conducted for the period from first of November of 2014 to 30th August of 2015 in AL- Khalis province in Diyala governorate in Iraq. The study aimed to determining the rate of Rubella-specific IgM and IgG antibodies in women who get marriage. The study included 150 women who get marriage. The mean age was 22± 4.6 years (13-49). Demographic information on women regarding age, residence and level of education were collected from their women. Serum specimens were collected for each woman. Detection of Rubella-specific IgM and IgG was done by Enzyme Linked Immune Sorbent assay (ELISA) test. The results of the present study showed that the 42 women (28%) were anti-RV IgG positive and 108 (72%) were anti-RV IgG negative. While, only 13 women (8.7%) were anti-RV IgM positive and 137(91.3%) were anti-RV IgM negative. There was significant correlation in seropositivity of RV between age group (20-29) years (27%) for anti-RV IgG and (10%) for anti-RV IgM with other age groups. The results revealed insignificantly correlation between urban and rural residence, (33.3%) for anti-RV IgG and (9.5%) for anti-RV IgM. Significant correlation positivity was reported (95.2%) of women for anti-RV IgG and (23.8%) for anti-RV IgM in women with primary school of level of education. In conclusion, am recommended that working similar studies need to be done in

other parts of the Diayla governorate. Additionally, the present study recommended that rubella virus vaccination should be considered carefully in Iraq and other developing countries, because of the high seropositivity to RV in our region.

Keywords: Rubella, IgG, IgM, ELISA, Seroprevelance.

تقدير الاجسام المضادة للحصبة الالمانية بين النساء المقبلات على الزواج في قضاء الخالص في محافظة ديالى في العراق

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الخلاصة

الحصبة الالمانية هو مرض فيروسي حاد وان امراضيته عادة ما تكون خفيفة الى متوسطة. اهمية هذا المرض تكون في خطورته على الجنين اثناء الحمل و يخلق متلازمة الحصبة الالمانية الشائعة. اجريت هذه الدراسة للمدة من 2014/11/1 الى 8/30/ 2015 في قضاء الخالص في محافظة ديالى في العراق. هدفت الدراسة الى تحديد انتشار الغلوبيولينات المناعية اي جي ام و اي جي جي المضادة للحصبة الالمانية في النساء المقبلات على الزواج. شملت الدراسة 150 امراءة مقبلة على الزواج و كان متوسط اعمارهم 22 ± 4,6 سنة (13-49). جمعت معلومات ديموغرافية عن النساء فيما يتعلق بـ العمر، السكن و مستوى التعليم من كل امراءة. جمعت عينات مصل الدم لكل امراءة. اجري الكشف عن الغلوبيولينات المناعية اي جي ام و اي جي جي المضادة للحصبة الالمانية بوساطة اختبار الاليزا. اظهرت نتائج الدراسة الحالية ان 42 امراءة (28%) كانت ايجابية لمضاد الحصبة الالمانية اي جي جي و 108 (72%) كانت سلبية. في حين كانت 13 امراءة فقط (8,7%) ايجابية لمضاد الحصبة الالمانية اي جي ام و 137 (91,3%) كانت سلبية. كان هناك ارتباط معنوي في الايجابية لمضاد الحصبة الالمانية بين الفئة العمرية (20-29) سنة 27% لمضاد الحصبة الالمانية اي جي جي و 10% لمضاد الحصبة الالمانية اي جي ام مقارنة بالفئات العمرية الاخرى. النتائج كشفت عدم وجود ارتباط معنوي بين الاقامة في الريف و الحضر، (33,3%) لمضاد الحصبة الالمانية اي جي جي و(9,5%) لمضاد الحصبة الالمانية اي جي ام. سجلت ايجابية عالية 95,2% لمضاد الحصبة الالمانية اي جي جي و 23,8% لمضاد الحصبة الالمانية اي جي ام لدى النساء مع مستوى التعليم الابتدائي مقارنة مع مستويات التعليم الاخرى، مع وجود ارتباط معنوي. في الختام، انا اوصي بأن تعمل

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دراسات مماثلة نحتاج الى القيام بها في اجزاء اخرى من محافظة ديالى. بالاضافة الى ذلك توصي الدراسة الحالية بان التطعيم ضد فيروس الحصبة الالمانية ينبغي النظر اليه بعناية في العراق و غبرها من بلدان الدول النامية بسبب الانتشار المصلي المرتفع في المناطق و الدول الاخرى.

الكلمات المفتاحية: الحصبة الالمانية، اي جي جي، اي جي ام، الاليزا، الانتشار المصلي.

Introduction

Rubella virus, a member of the Tagoviridae family, is the sole member of genus *Rubivirus*. Rubella has a single stranded RNA genome; a virus envelope ranges between (60-70) nanometers [(1)]. The only natural host for the rubella virus is humans. Transmission of rubella is through direct or droplet contact with infected nasopharyngeal secretions and the virus can also be transmitted from infected mother to fetus through the placenta [(2)]. Rubella disease or sense as German measles or three-day measles is an acute viral illness caused by the *Rubivirus*. The disease is accompanied with some symptoms as fever, discrete erythematous maculopapular skin rash in all part of body and auricular, cervical and occipital lymphadenopathy in which usually is mild in children and adults, even though in most cases are asymptomatic and resolve spontaneously. While developing fetus infection occurs followed by transmission from mother to fetus, especially during the first trimester of pregnancy, creates severe side effects that cause congenital rubella syndromes known as congenital rubella syndrome (CRS) [(3,4).] The frequency of CRS varies in different parts of the world, depending on the levels of naturally acquired immunity, overcrowding and immunization policies and practices [(5,6,7)].The incidence of congenital anomalies follow the infection in the first trimester of pregnancy is 80 to 85% of cases [(8)]. Side effects may include abortion, fetal death, premature birth or live birth defect including congenital cataracts, hearing impairment, cardiac abnormalities, central nervous system involvement, and low weight at birth, the temporary effects of the adenopathy, bone lesions, enlarged liver and spleen, hepatitis, meningoencephalitis and late effects such as diarrhea [(9)].

There is no specific treatment for rubella virus infection and in some cases, 0.55 ml/kg intramuscular administration immunoglobulin within 72 hours of exposure to rubella, may reduce the risk of infection, but is not guaranteed to prevent fetal infection [10]. In countries where rubella vaccination is carried out over 95% of cases of CRS has decreased [11, 12].

Rubella immunization programmes are designed to prevent intrauterine rubella infection and lower the risk of future rubella outbreaks and CRS cases [13]. The World Health Organization suggests two methods to prevent CRS: The first approach aims to prevent CRS, suggests immunization of adolescent girls and women of childbearing age, and in the second approach aims at eradicating rubella, makes the agenda vaccination campaigns of children under supervision and assuring immunity for women at childbearing age in your agenda makes [14]. Given the importance of acquiring the disease and the complications of CRS, it is necessary to conduct routine tests to diagnosis for women at risk of pregnancy than attempting to control the CRS in children. In the study conducted in Kermanshah city on 140 premarriage girls in 2006, a seroprevelance against rubella was seen in 99.3% (139 out of 140 vaccinated girls), compared to 79.6% (191 out of 240 unvaccinated girls) [15], while in another study was carried out between 2011 and 2012 in Hanoi for testing of IgG and IgM against rubella virus in school girls and pregnant women was reported Rubella-specific IgG positive in the vast majority of school girls (91.9%) and in 85% of pregnant women. Rubella-specific IgM was positive in 27.9% of pregnant women [16]. Clinical diagnosis of acquired rubella due to the mimic many diseases associated with rash, diverse clinical pathogenesis and high levels of subclinical disease are difficult. IgG-specific antibody titer determination is evidence of immunity to rubella. Acute rubella-specific IgM antibody may indicate either acute-phase serum samples by a fourfold or greater rise IgM antibodies in acute phase samples (10.7 days after the start of disease) and convalescent phase (approximately 21-14 days after the first sample) is detected. After infection with rubella virus IgM antibodies may be detected for up to 6 weeks. Recently RNA rubella virus detection is carried out by PCR [2; 8,9,17]

Materials and Methods

Study groups:

The present study was conducted in AL- Khalis province in Diyala governorate in Iraq for the period from first of November of 2014 to 30th August of 2015. It included; the group comprises from 150 women who get marrige. The women were attended General AL- Khalis hospital for working test married. The age rang was 13 years to 49 years and the mean aged 22 ± 4.6 years.

Collection of serum specimens:

From each individual in this study, 5 ml of blood was drawn by vein puncture using disposable syringes. The blood was placed in plastic disposable tubes; it was left to stand at room temperature (20-25C°) to allow it to clot, then the sera was separated by centrifugation 10000 r.p.m for 5 minutes and stored at -20 °C till examination. The specimens were transferred to the Virology Unit / Public Health Laboratory in Baquba for detection of IgM and IgG against Rubella in serum specimens by ELISA test. All sera and reagents were allowed to stand at room temperature before use in the test.

Detection of Rubella virus:

Rubella IgM (serum) ELISA test:

This test was performed using commercially available kit (Rubella IgM ELISA Test Kit). Reactive results were indicated by the absorbance reading of 1.1 and above, while the non-reactive results were indicated by the absorbance reading less than o.9.

Rubella IgG (serum) ELISA test:

This test was performed using commercially available kit (Rubella IgG ELISA Test Kit).

Statistical analysis:

Data analyses were computer aided. Statistical analysis was done using SPSS (Statistical Package of Social Science) version 18 computer software. Frequency distribution and percentage for selected variables were done first.

Spearman correlation between screening tests and different parameters was done by evaluate r value (correlation coefficient), and an estimate was considered statistically significant if r value at the 0.01 level (2-tailed) (Niazi, 2004)[18].

Results

Using the ELISA test for detection of Rubella- specific IgM and IgG antibodies in sera women omit marrige specimens, the mean age was 22 ± 4.6 years. Additionally, none of the subjects remembered to have a history of vaccination, the results showed that 13(8.7%), 42 (28 %) of specimens were positive for IgM, IgG antibodies respectively and only 137(91.3%), 108(72 %) were negative for IgM, IgG antibodies respectively. table (1).

Table (1): Results of ELISA test for detection of IgM and IgG antibodies to Rubella Virus in women omit marrige

Results		Frequency (%) of IgM for RV	Frequency (%) of IgG for RV
Women who get mattige n= 150	Positive	13(8.7%)	42 (28 %)
	Negative	137(91.3%)	108(72 %)

Results in table (2) revealed that the detection rate of RV by ELISA IgM and IgG tests was highest in the age group (20-29) years for IgM and IgG as compared to other age groups. However, the differences between the age groups with IgG were statistically significant (r = o.23), while, the difference was failed to reach the statistical significance (r= -0.034) with IgM.

Table (2): Rubella- specific IgM and IgG antibodies among the different age groups of women who get marrige specimens

Age groups (years)	Result of IgM (RV)		Result of IgG (RV)		Total	r value	
	No. positive (%)	No. negative (%)	No. positive (%)	No. negative (%)		IgM	IgG
13-19	2 (5.5)	34 (94.4)	15 (41.6)	21 (58.3)	36	- 0.034	0.23
20-29	10 (10)	90 (90)	27 (27)	73 (73)	100	**NS	S*
30-39	1 (7.6)	12 (92.3)	0 (0.0)	13 (100.0)	13		
40-49	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)	1		
Total	13	137	42	108	150		

*** S:** significant *** * NS**: no significant

Table (3) shows the detection rate of RV by ELISA IgM and IgG tests was highest among women reside in rural areas 8 (9.5%), 28 (33.3%) respectively compared to those reside in urban areas 5 (7.6%), 14 (21.2%) respectively. The statistical difference between two groups was insignificant (r = -0.034) with IgM and (r= - o.134) with IgG.

Table (3): Distribution of Rubella- specific (IgM and IgG) antibodies results according to residence

Residence	Result of IgM (RV)		Result of IgG (RV)		Total	r value	
	No. positive (%)	No. negative (%)	No. positive (%)	No. negative (%)		IgM	IgG
Urban	5 (7.6)	61 (92.4)	14 (21.2)	52 (78.8)	66	-0.034	- o.134
Rural	8 (9.5)	76 (90.5)	28 (33.3)	56 (66.7)	84	**NS**	**NS**
Total	13	137	42	108	150		

NS: no significant

The effect of level of education in women imminent marriage is shown in table (4). It is clearly obvious that the highest detection rate of Rubella virus was among those with primary school education, followed by those literacy educations for IgM and IgG results, and the lowest rate was among those college education for IgM result and higher school education for IgG result. The difference among these groups statistically significant with IgM (r =0.77) and IgG (r= 0.59).

Table (4): Distribution of Rubella- specific (IgM and IgG) antibodies results according to level of education

Level of education	Result of IgM (RV)		Result of IgG (RV)		Total	r value	
	No. positive (%)	No. negative (%)	No. positive (%)	No. negative (%)		IgM	IgG
Literacy	3 (13.1)	20 (86.9)	15 (65.3)	8 (34.7)	23	0.77	0.59
Primary school	5 (23.8)	16 (76.2)	20 (95.2)	1 (4.8)	21	S	S
Medium school	3 (10.7)	25 (89.3)	2 (7.1)	26 (92.9)	28		
Higher school	2 (5.7)	33 (94.3)	1 (2.8)	34 (97.2)	35		
College	0 (0.00)	43 (100.0)	4 (9.3)	39 (90.4)	43		
Total	13	137	42	108	150		

S: significant

Discussion

In the present study, Rubella- specific IgM and IgG was detected in 8.7% for anti-RV IgM and 28% for anti-RV IgG of women imminent marriage specimens by ELISA test. Since, worldwide studies had reported variable results, so the present results were disagree with the

results of the study reported by Nguyen *et al*. (2014)[16] who found the highest rate of rubella IgG 91.9% and rubella IgM 27.9% in school girls in Hanoi. On the other hand, Berno *et al*. (2014)[19] found the highest rate of anti-rubella IgG 92.6% while only 0.3% was for anti-rubella IgM. In Norway, Rubella IgG seropositivities have been reported to be as 91.1% in Saudia Arabia [(20)] and 95% in Turkey [(21)]. The difference in the results of the different studies may be attributed to the some of the demographic factors (history of vaccination, history of immunity for mothers, level of education and environmental conditions), low health a awareness and neglected personal hygiene and the sensitivity and specificity of ELISA test employed.

The results of the present study revealed significant anti-RV IgG rate among age group (20-29) years (27%) and insignificant anti-RV IgM rate among the same age group (10%). The results were agreement with that reported by other Iraqi researcher [(22)]. Additionally, worldwide studies reported results consistent with present results in the RV-specific Igm and IgG rate by increased among women who aged (20-29) years [(19; 23;24;10)].

The insignificant difference by ELISA test in the Rubella-specific IgM and IgG antibodies rate among urban and rural residence women in the present study was also reported by other studies[(15;19)]. The current results with respect to rate of Rubella-specific IgM and IgG antibodies and how it rates to level of education, although it shows that the IgM and IgG seroprevalence was highest in women who had primary school education in comparison with other levels of education with statistical sagnificant, these results were consistent with other studies reported (94.4%), (93.1%), (92.5%), (99.8%), (100%) respectively ([19]; [25]; [26]; [10]; [15]).Additionally, this rate was clearly higher than 67.7% from Vietnamese young women community living in Taiwan documented by Tseng *et al*. (2006)[27].

The present study recommends that Rubella virus vaccination should be considered carefully in Iraq and other developing countries, because of the high seropositivity to RV in our region. Although, working similar studies need to be done in other parts of the Diayla governorate. Additionally, adoption of ELISA test in the hospital laboratories for routine screening for woman who get marriage and pregnant women.

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