



Original Research Article

Serodiagnosis of Rubella in Pregnant Women

Israa Hashim Saadon
College of Medicine, University of Tikrit, Salah-El-Deen, IRAQ

*E-mail: israahs14@yahoo.com

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Abstract

This study was conducted from 15th December 2012 to 15th May 2013 and included 220 pregnant woman whose age (18-40) years , who attended Azadi General Teaching Hospital, Kirkuk General Hospital and some primary health care centers in Kirkuk Governorate. Blood sample was drawn from each woman to determine specific anti-rubella antibodies (IgM and IgG) by using Enzyme Linked Immuno Sorbent Assay (ELISA). The results revealed that anti-rubella IgG , IgM and both IgG & IgM antibodies at the same time for the 220 pregnant women were 85.90 % , 4.55 % and 1.82% respectively. The study revealed that the highest rate of seropositivity was in the age group 24-29 years, while the lowest rate was in older age group ≥ 36 years. Also the study revealed that the rate of seropositivity of rubella-IgG was higher (65.6%) among women who lives in urban areas while the highest seropositivity for rubella-IgM and both IgM and IgG at the same time were among women who lives in rural areas with significant relation $P < 0.05$. Regarding the relation of rubella antibodies among pregnant women with the number of children, highest rates for rubella-IgM and both IgM and IgG at the same time were recorded among pregnant women with no children than who had one child or more while for rubella IgG the highest rate was among pregnant women with two children with significant relation $P < 0.05$. The study revealed that the pregnant women in the second trimester had highest rate of seropositivity for anti-rubella IgG, while the highest rates for anti- rubella IgM an both IgM and IgG at the same time was recorded among pregnant women in the first trimester of pregnancy. In relation to the history of abortion and the seropositivity of rubella antibodies were the (61.58 %) of pregnant women had history of abortion with significant relation $P < 0.05$, however the abortional frequency were had no more deferent recorded among pregnant women with history of one abortion or more with non-significant relation $P > 0.05$.

Key Words: *Rubella*, pregnant women, ELISA.

التشخيص المصلي لفيروس الحصبة الألمانية في النساء الحوامل

الخلاصة

أجريت الدراسة في محافظة كركوك للفترة من 15 كانون الأول 2012 لغاية 15 ايار 2013 وشملت الدراسة 220 امرأة حامل و120 امرأة متزوجة وغير حامل كمجموعة سيطرة تراوحت أعمارهن بين 18-40 سنة واللاتي راجعن مستشفى آزادي التعليمي ومستشفى كركوك العام وعدد من مراكز الرعاية الصحية الأولية في محافظة كركوك. تم سحب عينة دم من كل امرأة للتحري عن وجود الأجسام المضادة نوع (جي) و(ام) ضد فيروس الحصبة الألمانية باستخدام فحص المترابط الأنزيمي المناعي.

وأظهرت النتائج أن الأجسام المضادة نوع (جي) و(ام) والاثنتين (جي و ام) معا في النساء الحوامل كانت موجودة بنسبة 85.90%، 4.55% و1.82% على التوالي. فقد أظهرت الدراسة أن أعلى نسبة للأجسام المضادة لدى النساء الحوامل كانت في المراحل العمرية (24-29) سنة بينما أوطأ نسبة كانت في النساء اللواتي أعمارهن أكبر أو يساوي 36 سنة. كما كشفت الدراسة أن أعلى نسبة (65.6 %) للأجسام المضادة نوع (جي) كانت بين مجاميع النساء اللواتي يسكن المناطق الحضرية , بينما كانت اعلى نسبة للأجسام المضادة نوع (ام) والاثنتين (جي و ام) معا في الوقت نفسه بين المجاميع اللاتي يسكن المناطق الريفية ويفرق معنوي $P < 0.05$. اما بالنسبة لعلاقة عدد اطفال النساء الحوامل بالانتشار المصلي لفيروس الحصبة الألمانية فقد كانت اعلى نسبة للأجسام المضادة نوع (ام) والاثنتين (جي و ام) معا في الوقت نفسه لدى النساء اللاتي ليست لديهن اطفال, بينما الاجسام المضادة نوع (جي) كانت اكثر لدى النساء اللاتي لديهن طفلين م ويفرق معنوي $P < 0.05$. وقد أشارت الدراسة ان اعلى نسبة للأجسام المضادة نوع

(جي) وجدت لدى النساء الحوامل في الثلاثة اشهر الثانية من الحمل ، بينما اظهرن أعلى نسبة ايجابية للأجسام المضادة نوع (ام) والاثنتين (جي و ام) معا في الوقت نفسه من النساء الحوامل في الثلاثة اشهر الاولى من الحمل. وأظهرت النتائج أن 61.58% من النساء الحوامل الموجبات مصليا لديهن حالات إجهاض سابقا ، وعلى الرغم من قدرة فيروس الحصبة الالمانية لحدوث الإجهاض في النساء الحوامل إلا انه لا توجد علاقة كبيرة للإصابة وتكرار الإجهاض أي انه قد يسبب إجهاض لمرة واحدة أو أكثر ويعدم وجود فرق معنوي $P > 0.05$.

الكلمات المفتاحية: الحصبة الالمانية، النساء الحوامل، تقنية الاتزيم المرتبط مناعيا".

Introduction

Rubella "Latin rubellus, reddish" is caused by the rubella virus, an enveloped, single, positive-stranded RNA virus that is a member of the family Togaviridae. *Rubella* is worldwide in distribution and occurs more frequently during the winter and spring months. Transmission of infection occurs through droplets that are shed from the respiratory secretions of infected individuals. The incubation period ranges from 12 to 23 days[1].

Rubella virus is the only member of the genus *Rubivirus* within the family Togaviridae. The outer envelope protein E1 is the viral haemagglutinin protein responsible for binding to the cell receptors to initiate infection [2].

Rubella was known initially as 'German measles' because it was first described by two German physicians in the mid eighteenth century. It is generally a mild disease and therefore received comparatively little attention until 1941, when its association with congenital defects was recognized by N. McAlister Gregg, an Australian ophthalmologist. Other studies in Australia and elsewhere confirmed these findings and suggested that a very high proportion of mothers who had had rubella during pregnancy delivered infants with congenital malformations[3].

Rubella (German measles; 3-day measles) is an acute febrile illness. It is the mildest of common viral exanthems. Most people who get rubella usually have a mild illness, with symptoms that can include a low-grade fever, sore throat, and a rash that starts on the face and spreads to the rest of the body. The infection can cause a miscarriage or serious birth defects in an developing baby

if a woman is infected while she is pregnant. The consequences of rubella in utero are referred to as the congenital rubella syndrome[4].

The significance of rubella virus is not as a cause of mild childhood disease but as a teratogen. When a non-immune pregnant woman is infected during the first trimester, especially the first month, significant congenital malformations can occur as a result of maternal viremia and fetal infection. The increased rate of abnormalities during the early weeks of pregnancy is attributed to the very sensitive organ development that occurs at that time. The malformations are widespread and involve primarily the heart "e.g., patent ductus arteriosus", the eyes "e.g., cataracts", and the brain "e.g., deafness and mental retardation"[5].

Patients with primary acquired rubella infections are contagious from 7 days before to 7 days after the onset of rash. The rash appears as immunity develops and the virus disappears from the blood, suggesting that the rash is immunologically mediated and not caused by the virus infecting skin cells. In 20–50% of cases, the primary infection is subclinical [4]. Congenitally infected infants may spread the virus to others for 6 months or longer after birth[6].

Specific IgM antibody appears within a few days of the rash, and is followed soon after by IgG. The titer of IgM increases rapidly, reaching a peak about 10 days after onset and thereafter declining to undetectable amounts over several weeks or months. The rapid appearance of specific IgM anti-body is invaluable for diagnostic purposes. IgG antibody peaks at about the same time as IgM, and persists for many years, as does IgA antibody, which appears

in the serum and nasopharyngeal secretions. The cell-mediated response precedes the appearance of anti-body by a few days, reaches a peak at about the same time, and is also detectable for many years[3, 7].

This study was conducted to detect anti-rubella antibodies (IgG and IgM) among pregnant women and to study the relation of anti-rubella antibodies with socio-demographic variables: age, residency, number of children for pregnant women, occupation, pregnancy trimester, and abortion.

Materials and Methods

A cross sectional study was carried out in Kirkuk city from 15th of December 2012 to 15th of May 2013. The number of pregnant women under study was 220 whose age between 18-40 years old. These women presented in Azadi General Teaching Hospital, Kirkuk General Hospital and some primary health care centers. Pregnant women with HBV and/or HCV infection, "as indicated by HBsAg and/or anti-HCV respectively" and those who were non-vaccinated against rubella were excluded from this study.

An interview was carried out with these pregnant women using questionnaire form consisted of the following variable; age, residency, number of children, pregnancy trimester and abortion.

Five ml of blood was collected by vein puncture using 5 ml disposable syringe from each women enrolled in this study. The blood was placed in plane tubes, left for 30 minutes at 37 °C then were centrifuged at 3000 rpm for 15 minutes then the clot removed and the remain re-centrifuged at 3000 rpm for 10 minutes twice time and the obtained sera were then aspirated using automatic micropipette and transferred into clean test tube. Label was fixed on each test tube which then stored in deep freeze at -20 °C for late serological testing for detecting specific Rubella-IgM and Rubella-IgG by using Enzyme Linked Immuno Sorbent Assay (ELISA) technique produced by Biotek Barcelona-Spain

Computerized statistically analysis was performed using SPSS (Statistical

Package for Science Services). Comparison carried out using; Chi-square (X^2) and Probability (P value). The P value ≤ 0.05 was considered statistically significant (S), and less than 0.01 considered highly significant (HS) while greater than 0.05 considered non-significant (NS).

Results

A total 220 pregnant women were examined, (their age ranged between 18-40 years old), for detection of rubella antibodies (IgG and IgM) by using ELISA technique.

Anti-rubella IgG, IgM and both IgG & IgM at the same time for the 220 pregnant women were found in 189 (85.90 %), 10 (4.55 %) and 4 (1.82%) respectively (as shown in Table 1).

Table 2 shows the seropositive of anti-rubella antibodies in relation to the age. The highest rate (31.23 %) of rubella - IgG was found in pregnant women from the age group 30 – 35 years, while the highest rate of rubella - IgM was 30 % in the age groups 18 – 29 years. The highest rate (50 %) of seropositive for both IgG and IgM at the same time was found in the age group 24 –29 years.

Table 3 shows the relation of anti-rubella antibodies seropositive with residency. The highest rate of anti- rubella IgG (65.60 %) was found in pregnant women from urban area, while 34.40 % was from rural area. The highest rate (60.00 %) of rubella- IgM was found in rural area. Also the highest rate (75.00 %) of both IgG and IgM at the same time was found in women from the rural area.

Table 4 shows the relation of rubella antibodies in pregnant women with the number of their children. The highest rate of seropositive in all rubella antibodies type was seen in pregnant women with no children, while the lowest rate of seropositive was seen in pregnant women who had three children or more.

Table 5 shows seropositive of anti-rubella antibodies in relation to gestational time of pregnancy. The highest rate (37.03 %) of rubella - IgG was found in women in the 2nd trimester of pregnancy , while the highest

rate (70.00% and 75.00%) of seropositive for both IgG and IgM at the same time and seropositive for rubella – Ig M respectively were in the 1st trimester of pregnancy.

Table 6 shows the relation of anti-rubella antibodies with abortion and number of

abortions. The total rate of abortion was 61.58%. The rate of abortion number were 21.67 %, 20.68% and 19.23 % for one, two, and three abortions or more respectively.

Table 1: Anti- Rubella Antibodies in Pregnant Women

Results	Pregnant women	
	No.	%
IgG/ +ve	189	85.90
IgM/ +ve	10	4.55
IgM and IgG/ +ve	4	1.82
IgM and IgG/ -ve	17	7.73
Total	220	100

Table 2: Relation of Seropositive Rubella Antibodies with Age

Age groups (Years)	Results					
	IgG/ +ve		IgM/ +ve		IgM and IgG/ +ve	
	No.	%	No.	%	No.	%
18-23	50	26.45	3	30	1	25
24-29	51	26.98	3	30	2	50
30-35	59	31.23	2	20	1	25
36-40	29	15.34	2	20	0	0
Total	189	100	10	100	4	100

$X^2 = 8.967$

$P = 0.092$

$P > 0.05$

Non Significant

Table 3: Distribution of Rubella Antibodies according to Residency

Residency	Results					
	IgG/ +ve		IgM/ +ve		IgM and IgG/ +ve	
	No.	%	No.	%	No.	%
Urban	124	65.6	4	40	1	25
Rural	65	34.40	6	60	3	27
Total	189	100	10	100	4	100

$X^2 = 17.01$

$P = 0.031$

$P < 0.05$

Significant(S)

Table 4: Relation of Seropositive Rubella Antibodies with Number of Children

No. of children	Results							
	IgG/ +ve		IgM/ +ve		IgM and IgG/ +ve		IgM and IgG/ -ve	
	No.	%	No.	%	No.	%	No.	%
No children	49	25.94	5	50	2	50	1	5.88
One child	48	25.39	2	20	1	25	2	11.76
Two Children	50	26.45	2	20	1	25	3	17.64
Three children or more	42	22.22	1	10	0	0	11	64.72
Total	189	100	10	100	4	100	17	100

$X^2 = 17.43$ $P = 0.028$ $P < 0.05$ Significant

Table 5: Relation of Seropositive Rubella Antibodies with Gestational Time of Pregnancy.

Gestational time of pregnancy	Results					
	IgG/ +ve		IgM/ +ve		IgM and IgG/ -ve	
	No.	%	No.	%	No.	%
1 st trimester	66	34.93	7	70	3	75
2 nd trimester	70	37.03	3	30	1	25
3 rd trimester	53	28.04	0	0	0	0
Total	189	100	10	100	4	100

$X^2 = 20.08$ $P = 0.0019$ $P < 0.01$ Highly Significant(HS)

Table 6: Relation of Seropositive Rubella Antibodies in Pregnancy with History of Abortion and Abortion Frequency

No. of pregnant women with anti-rubella antibodies	History of abortion							
	No abortion		1 abortion		2 abortions		3 abortions or more	
	No.	%	No.	%	No.	%	No.	%
203	78	38.42	44	21.67	42	20.68	39	19.23
	125 (61.58%)							
Abortion	$X^2 = 4.134$		$P = 0.029$		$P < 0.05$		Significant	
Abortion freq.	$X^2 = 7.369$		$P = 0.19$		$P > 0.05$		Non Significant	

Discussion

In the presented study the rate of rubella-IgG seropositive that indicate the past infection was 85.9% and rubella-IgM seropositive that indicate acute infection was 4.55%, while both IgG and IgM at the same time was 1.82%, that means overall seroprevalence was 92.30 % (as shown in Table 1). This high rate is due to vaccination against rubella, although some women remained susceptible to infection which may

be due to various factors including inefficient the vaccine, the differences in the immune status of the recipients or genetic factors.

Similar or closed results were reported in Baghdad (94.8%) [8], in Kuwait (92.3%) [9], in Saudi Arabia (93.3%)[10], and in Malaysia (92.3%) [11].

Higher results were reported in other countries; in Iran (98.1%) [12], in Spain

(98.1%) [13], and Mozambique (95.3%) [14].

Lower rates of infection were reported; in Taiwan (85.1%) [15], in Thailand (75.0%) [16], and in Greece (67.0%) [17].

These different results may be due to differences in efficiency of vaccine which applied in each country, or differences in hygienic, socioeconomic, and cultural factors in different countries.

Regarding the age, the present study revealed that the highest rate of women with antibodies for rubella was seen in those within the age group 24-29 years, while the lowest rate was in age group 36-40 years (as shown in Table 2). Similar results were reported by others [16, 18]. This may be due to that the antibody response declines, overtime, to below the protective level. Generally the different rates of anti-rubella IgG and IgM were detected among pregnant women within different age groups. This may be due to difference in the immunological status of women under study at the period of samples collection. However, the present study revealed non-significant relation between age and presence of antibodies against rubella ($P > 0.05$).

Regarding the residency, the present study revealed that the rate of women with IgG was higher among those live in urban areas than those who live in rural areas, while the rate of those with IgM and both IgG with IgM at the same time was higher in rural area (as shown in Table 3). This could be explained by that the rate vaccination for rubella is higher in urban area, also knowledge about the rubella as a preventable disease in urban areas is more likely, and more contact with information concerning it than in rural areas.

The current study revealed significant ($P < 0.05$) relation of rubella infection in the pregnant women with number of children (as shown in Table 4). The highest rate of seropositivity was recorded among pregnant women with low parity. This might be related to effect of rubella and other infections which lead to decrease the number of children.

In the present study rubella-antibodies were found in pregnant women within all states of pregnancy trimester, but with different rates and high significant ($P < 0.01$) relation of the distribution rubella antibodies type and pregnancy trimester. The highest rate of seropositive for rubella-IgG (37.03%) was within the second trimester of pregnancy, while the highest rate of IgM (70%) and IgG with IgM at the same time (75%) were found in women within the first trimester of pregnancy. The lower rates of seropositive (for all types of antibodies) were recorded within third trimester of pregnancy (as shown in Table 5). These findings may be due to the ability of the virus to cause infection any time during pregnancy. In regard to high rate of primary and re-infection in first trimester, this may be due to that these groups are more contact with infected individuals than pregnant women at second or third trimester. Similar result was reported by Boonruang and Buppasiri [16]. Other study reported that rubella antibodies were found in a higher rate among women in the second trimester than those in the first and third trimester [8].

The current study showed a significant relation between history of abortion and presence of rubella-antibodies (as shown in Table 6), 61.58% from the total seropositive pregnant women had a history of abortion. The abortion may be due to the effect of rubella on systemic and local (placenta) immunological response. There was non-significant relation ($P > 0.05$) between the presence of rubella-antibodies and number of abortions, this finding may reveal the ability of the rubella virus to cause abortion one time or more.

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