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Prevalence of rubella virus in pregnant women in Kirkuk city-Iraq

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ABSTRACT

0bjective:Determination of anti-rubella IgM and IgG seropositivity and susceptibility rates among pregnant women with the history of abortion & women without the history of abortion. Subjects and method: A total of ninety pregnant women (Sixty-four pregnant women who had previous abortion; and twenty-six pregnant women without the history of abortion), there age was ranged between (18-40) year; were enrolled in the present study which was conducted during the period of 15 March to 23September /2013. All obtained sera from all participants were tested using ELISA method for determine of rubella virus antibodies. Data was analyzed, using SPSS software (chi square) and Anova test.

Results: The results revealed that the rubella virus-IgG seropositivity among pregnant women with previous abortion was 37(57.8%).In addition, the results showed that the percentage rate of the rubella virus-IgM seropositivity among pregnant women with previous abortion and without the history of abortion were 17(26.6%) & 1(3.8%) respectively. The study showed that there was no significant correlation between anti-rubella virus antibodies (IgM,IgG) and the age, also in the current study , among the studied groups there was a significant positive correlation between anti-rubella virus antibodies(IgM,IgG)and the number of abortion.

Conclusion: There was no effect of age on the seroprevalence of anti-rubella IgM and IgG in the studied groups.

Keyword: *Rubella virus, Pregnancy, Enzyme Linked Immune-Sorbent Assay (ELISA).*

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انتشار فايروس الحصبة الالمانيـة في النسـاء الحـوامـل في محـافـظـة كـركـوك - العراق

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الملخص

تقدير اضداد فايروس الحصبة الالمانية(IgG و IgM) ايجابية المصل بين النساء الحوامل مع تاريخ الاجهاض وحوامل دون تاريخ الاجهاض . اشتملت عينة الدراسة على تسعين من النساء الحوامل (64 من النساء الحوامل اللاتي تعرضن للإجهاض سابقة و 26 امرأة حامل دون تاريخ اجهاض)وقد تراوحت اعمارهم بين (18–40)سنة . اجريت هذه الدراسة في محافظة كركوك للفترة من 15 اذار ولغاية 23 ايلول–2013 . جميع الامصال التي تم الحصول عليها من جميع المشاركات تم اختبارها باستخدام طريقة الELISA لتحديد الاجسام المضادة لفايروس الحصبة الالمانية ،اظهرت الدراسة ان معدل اضداد فايروس الحصبة الالمانية ايجابية المصل (IgG , IgM) في النساء الحوامل اللاتي لهن اجهاض مسبق (,37(57.8% 17(26.6%)على التوالي . حيث بينت الدراسة انه لا توجد دلالة معنوية ضمن الفئة العمرية في نسبة اضداد فايروس الحصبة الالمانيةIgM,IgG)(في النساء الحوامل اللاتي لهن تاريخ اجهاض سابقة والنساء اللاتي يبدون اصحاء أي ليس لديهن اجهاض سابق بالنسبة الى العمر ووجود علاقة معنوية بين اضداد فايروس الحصبة الالمانية(IgM,IgG) في النساء الحوامل اللاتي لهن تاريخ اجهاض سابقة والنساء اللاتي يبدون اصحاء أي ليس لديهن اجهاض سابق وتعدد الاجهاضات.

الكلمات الدالـة : فايروس الحصبة الالمانية، الحمل , تقنية الامتزاز المناعي المرتبط بالأنزيم.

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1.INTRODUCTION

Rubella is an infection caused by a rubella virus [1].Rubella virus is the sole member of the genus Rubivirus in the Togaviridae family, only one serotype has been identified [2].Rubella virus is a spherical (40-80) nm, positive-sense, single-stranded RNA virus with spike-like haemagglutinin-containing surface projections. An electron-dense (30-35) nm core is surrounded by a lipoprotein envelope [2, 3].The name Rubella comes from the Latin word meaning “little red”. In 1814, it was first discovered to be a separate disease from measles in German medical literature thus receiving its nickname the “German measles”, In 1914,Hess postulated a viral etiology and in 1914 Norman Gregg reported congenital cataracts in 78 infants whose mothers had maternal rubella in early pregnancy[4].Rubella virus is transmitted (congenital) to a fetus by an infected mother [5] Acquired Rubella(non congenital) is transmitted via airborne droplet emission from the upper respiratory tract of active cases; can be passed along by the breath of people sick from rubella; also can be spread by coughing or sneezing .The virus may also be present on the skin , there is no carrier state , the reservoir exists entirely in active human cases. The disease has an incubation period of two to three weeks [6, 7]. Rubella generally is a mild rush fever disease when acquired in childhood , but when infection occurs during the first months of gestation leading to fetal death, miscarriage ,stillbirth or infants with a pattern of birth defects, known as congenital rubella syndrome CRS [8].Rubella has symptoms that are similar to those flu. , however, the primary symptom of rubella virus infection is the appearance of rash (exanthema) on the face which spreads to the trunk and usually fades after three days. Other symptoms include low-grade fever, swollen glands(sub occipital & posterior cervical lymphadenopathy),joint pains , headache and conjunctivitis .The virus has a tetatogenic properties and is capable of crossing placenta and infecting the fetus [9].20-50% of infected persons are asymptomatic, the laboratory diagnosis of rubella is required, since clinical diagnosis is often inaccurate [10].Viral isolation may occasionally be warranted particularly during infections in pregnancy[11], and as a result of the non-cytopathic effect of rubella virus in cell cultures, it is not Usually recommended[12].Nucleic acid amplification techniques have been developed since the 1990 for the detection of rubella virus RNA in clinical samples of orophangeal sources in serum or saliva[12,13].The definitive diagnosis of rubella virus is suspected case with a positive test for rubella-specific IgM in all ages by Enzyme Linked Immuno-sorbent Assay ELISA .Test results for the detection of rubella virus-specific IgG&IgM antibodies by ELISA are presented

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as positive, borderline and negative , which is a reflection of the spectra of the photocell detected light of the spectrophotometry , which determines both qualitative and quantitative results in a given run[11,12,14].Viral specific IgM antibodies first detected ten days post infection, and peaks at about 4 weeks post infection. This may persist for over 7months after an acute infection. By 3weeks post infection, anti-rubella virus antibodies are present in all

Aims of the study: - This immunoglobulin classes, including IgG, IgA, IgD, and, IgE [11). study was conducted to shed a light on the detect the present of rubella virus at a positive agent for recurrent abortion, and how many normal pregnancy areat risk of giving birth to congenitally malformed child in our population.

2.PATIENTS AND METHODS

A total of 90 women comprising of 64 pregnant (with the history of abortion) and 26 pregnant (without the history of abortion) as a controls group, were screened for anti-rubella antibodies of IgG and IgM type by ELISA(Biocheck, Foster City). Criteria for selection were women with the history of abortion, without history of abortion and normal age .Blood samples were withdrawn from the antecubital vein in all women. All blood samples were centrifuged at 2000g for 15 minutes. Serum was separated and stored at -20ºC until assayed

***ELISA kit for the detection of IgM antibodies to Rubella virus** Purified rubella antigen is coated on the surface of micro wells. All sera were diluted (1:40),(100µl) of diluted sera , calibrators , and controls were added into the appropriate wells , and the rubella IgM specific antibody, if present , it binds to the antigen during incubation . After washing the wells with diluted wash buffer 5 times, to remove unbound samples. Antibody to human IgM conjugated with horseradish peroxidase (HRP)(100µl) is added and incubated at 37ºC for 30minutes; unbound conjugate is removed by a subsequent washing step. A solution of TMB (100µl) is then added to all micro wells. The enzyme conjugate catalytic action is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgM - specific antibody in the sample. The results are read by a micro well reader compared in a parallel manner with calibrators and control [15, 16]. Index values higher than 1.00 are indicate the presence of IgM antibodies. Index values lower than 0.90 are indicating the absence of IgM antibodies. Index values between 0.90-1.00 are scored questionable.

***ELISA kit for the detection of IgG antibodies to Rubella virus** Purified rubella antigen is coated on the surface of micro wells. All sera were diluted (1:40), (100µl) of diluted sera, calibrators, and controls were added into the appropriate wells, and the rubella IgG specific

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antibody, if present, it binds to the antigen during incubation. After washing the wells with diluted wash buffer 5 times, to remove unbound samples. Antibody to human IgG conjugated with horseradish peroxidase (HRP)(100µl) is added and incubated at 37ºC for 30minutes; unbound conjugate is removed by a subsequent washing step. A solution of TMB (100µl) is then added to all micro wells. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG - specific antibody in the sample. The results are read by a micro well reader compared in a parallel manner with calibrators and control [15, 16]. Index values higher than 1.00 are indicate the presence of IgG antibodies. Index values lower than 0.90 are indicating the absence of IgG antibodies. Index values between 0.90-1.00 are scored questionable.

3.RESULTS

Table.(1): Show distribution of anti-rubella IgM titer in studied groups according to age

Note: PWA: pregnant women with abortion. **Control:** pregnant without abortion.

Sample	Age group (year)	IgM		Total	P-value
		-ve	+ve		
PWA	<25 count	19.0	5	24	0.35
	%	79.2%	20.8%	100.0%	P>0.05
	25-34 count	25	9	34	Non-
	%	73.5%	26.5%	100.0%	sig.
	>34 count	3	3	6	
	%	50.0%	50.0%	100.0%	
Total	count	47	17	64	
	%	73.4%	26.6%	100.0%	
Control	<25 count	9	0	9	
	%	100.0%	0.0%	100.0%	0.6
	25-34 count	13	1	14	P>0.05
	%	92.9%	7.1%	100.0%	Non-
	>34 count	3	0	3	sig.
	%	100.0%	0.0%	100.0%	
Total	count	25	1	26	
	%	96.2%	3.8%	100.0%	

Table.(2) shows the prevalence of anti-rubella IgGantibodies titer among pregnant women with abortion according to age groups, 37(57.8%) out of 64 pregnant women with the history

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of abortion has positive IgG antibody. The occurrence of anti-rubella IgG antibody is absent within control group. The occurrence of positive IgG antibody was found to be statistically non significant (p >0.05) when compared with control group.

Table.(2): Distribution of anti-rubella IgG titer in pregnant women with abortion according to age.

Sample	Age group (year)		IgG		Total	P-value
			-ve	+ve		
PWA	<25	count	7	17	24	0.1 P>0.05 Non-sig.
		%	29.2%	70.8%	100.0%	
	25-34	count	16	18	34	
		%	47.1%	52.9%	100.0%	
	>34	count	4	2	6	
		%	66.7%	33.3%	100.0%	
Total		count	27	37	64	
		%	42.2%	57.8%	100.0%	

Table.(3 &4) illustrates that the correlation between anti-rubella IgM , IgG,and the number of abortion are significant

Table.(3): Correlation between anti-rubella IgM & the number of abortion **Note:** Number of abortion: more than one abortion

Number of abortion	IgM	Number	P-value
	-ve	72	0.029 P<0.05 significant
	+ve	18	

Table.(4): Correlation between anti-rubella IgG& the number of abortion

Number of abortion	IgG	Number	P-value
	-ve	53	0.001 P<0.05 significant
	+ve	37	

Rubella is an infectious disease mainly affecting pregnant women &there fetus throughout the world, although the frequency of such infections varies from country to country &within a

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country[17].The Chi-square analysis revealed that the prevalence of IgG seropositivity decreased with increasing age .This results was nearly compatible with another studies who found that the seropositivity of IgG decreased with increasing age [18]. Another study that agree with the recent study that showed thesero-prevalence of IgM antibodies was higher among the women within the ages of 25-34 years[19].Another study done in Diyala-Iraq is nearly compatible with the recent results that show that the IgM-seropositivity in pregnant women with history of abortion8(26.7%) and 3(5%) in control group[20]. A studies in Baghdad-Iraq, the sero-prevalence of rubella virus in women with abortion 34.2%[21] , and in another study , a rubella virus was considered as an etiologic agent for abortion in Gaza, Palestine[22].In some countries socio-economic and demographic factors[23],dengue virus[24]& women age and their parity were suggested as cause of abortion[25].Another study done in Sudan may be agree with the current result, which show that the rubella-IgG antibodies was insignificant in differences among age group[26].In addition, another study was nearly compatible with current study which shows that there is a significant association between abortion & rubella infection[27].looking at the relationship between number of abortion and the prevalence of rubella, we observed that the prevalence of rubella significantly increased with the number of abortion($P<0.05$), this means that the higher antibody titer, the higher the probability of abortion, implying that those with higher rate of abortion had higher antibody titer. Rubella virus enters the fetus during the maternal viraemic phase through the placenta, the damage to the fetus seems to involve all germ layers & result from rapid death of some cells and persistent viral infection in others. Generally, rubella virus plays a significant role in the occurrence of abortion in the study population [28].Previous studies about etiologic factors for abortion, infectious agent such as Toxoplasma gondii ,Cytomegalovirus[29],and also immunologic factor such as anti-nuclear antibody[30]& Chlamydia Trachomatis were suggested as important causative agents for spontaneous abortion[31]. IgM antibody are present in people recently infected by rubella virus, if rubella-age-wise seropositivity to rubella was found to decrease with increasing age, possibly due to more frequent exposure of the younger age groups to rubella virus, with the waning of seropositivity with age[33].. Waning immunity is not important following natural infection with rubella but it has been documented that the antibody level declines over time in vaccinated persons[34].Though our study is limited to pregnant women, it is evident that rubella immunity is widely prevalent in pregnant women in our population, but a substantial

number still remains unprotected from this infection, especially in the older age groups. This emphasizes the need to formulate an effective rubella immunization programmed with administration of a second dose in adolescence, to help boost the declining immunity seen in the ≥ 30 year age group[18].

4.CONCLUSION

In the recent study there is no effect of age in the seroprevalence of rubella virus antibodies. Prenatal screening for anti-IgG &anti-IgM –rubella antibodies is an important tool to identify active infection and to provide obstetric management to avoid the risk of congenital rubella syndrome.

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