Detection of Anti-rubella Virus IgM and IgG in Aborative Pregnant Women in Al-Qadisiya Governorate.

-*Ja'afar K. Nama (Ph.D.).
-Yuniss A. Al-Khfaji (Ph.D.), Dept. of Microbiology College of Dentist Medicine Babylon University,Iraq.
-*SAIF J. Yasir(M.Sc.)
*Dept. of Medical microbiology College of Medicine, University of Kufa,Iraq.

الملخص

الدراسة الحالية إلى تحديد الحالة المناعية ضد فايروس الحصبة الألمانية للنساء الحوامل المجهضات من خلال تشخيص الذين عين من الأجسام المضادة الـ (IgM وIgG)في أمصالهن. الدراسة تضمنت جمع عينات دم وريدي من ثلاثمائة امرأة مجهضة واللاتي تراوحت أعمارهن من (15-35)سنة في محافظة القادسية المناعي الأنزيمي الممتز تم استخدامه لتحديد الاستجابة المناعية ضد فايروس الحصبة الألمانية .فحص الاليزا اظهر إصابات جديدة وكانت (8%) نتيجة موجبة. فحص الأجسام المضادة لفيروس الحصبة الألمانية نوع IgG أعطى نسبة (70%) نتيجة موجبة.

Abstract

The present study aimed to detect the levels of IgM and IgG immunoglobulins in their sera. The study included a collection of venous blood samples from three hundred women underwent abortion ranging in age from (15-35) years from Al-Qadisiya governorate.

Enzyme- Elinked immunosorbant assay (ELISA), was used to determine the immunological response against rubella virus in our the samples. ELISA test reflected a new infections which was (8 %) positive. Anti-rubella IgG antibodies ELISA test revealed (70%) positive result.

Aims of the Study

The study aims to fulfill the following :

1. Studying the anti-rubella IgG and IgM antibodies in the pregnant women who underwent abortion.

2. Evaluation of immunological response among vaccinated and non-vaccinated women who underwent abortion . In addition, the study aims to shed a light on the success of the ongoing vaccination program in Al-Qadisiya Governorates.

Introduction

Rubella (German Measles) is an infectious, generally mild viral disease. The severity of the effect of rubella virus on the fetus depends largely on the time of gestation at which infection occurs. Up to 85% infants are infected in the first trimester of pregnancy (CDC,1992– 1994).

Rubella is of public health importance because rubella infection acquired during early pregnancy often results in fetal anomalies 'congenital rubella syndrome' (Immunise Australia Program, 2000). However, Rubella has almost been eradicated by immunization programs in many developed countries, but outbreaks amongst the unvaccinated still occur (Miller, 1991 & Reef *et al.* , 2002). Feature of rubella include signs of upper respiratory tract infection, mild fever and rash that typically starts on the face and then progresses down the body. Swelling of lymph nodes, particularly around the jaw and ears, is a common noticed. Congenital rubella syndrome (CRS) is a major complication of rubella that is of public health interest and continues to represent a problem worldwide in spite of the effective vaccination program that was introduced in 1969 (Reef *et al.*, 2002 ; Sadighi *et al.* , 2005). In spite of the vaccination programs, rubella continues to be endemic in many parts of the world, and therefore, cases of CRS continue to occur (Reef *et al.*, 2002). The incidence of CRS depends on the number of susceptible pregnant women, the circulation of rubella virus, and the coverage of rubella vaccination. (Atreya *et al.*, 2004 and Sadighi *et al.* , 2005) Estimates that 10–25% of nonimmunized women of child-bearing age are susceptible to rubella infection. The introduction of rubella vaccination has strongly reduced the incidence of CRS in the United States, and other developed countries (Hahne *et al.*, 2005). A prolonged virus excretion many months or years after birth is one of the main characteristics differentiating CRS from a natural rubella infection (Lee & Bowden , 2000 ; Menson & Lyall , 2005).

In Iraq ,WHO data showed that the reported cases of rubella virus infections in Iraq were: in 2005 reported 99 cases, 2004 were 383 cases and 2003, 2000 reported 612 cases but in 2002, 2001 and 1990 there is no reported cases.

Materials and Methods

Sample size and Study design:The samples in this study included three hundred serum samples obtained from women aged 16-43 years with abortion in Al-Qadisiya Governorate. In order to detect serum IgG and IgM level against rubella virus.These samples were obtained from Maternity and Children Hospitals in Al-Qadisiya .The period of sampling was between July 2005 to April 2006.

Sampling procedures and processing:In cases of women with abortion 5 ml of blood was obtained each time. All blood samples were subjected

to centrifugation at 3000 rpm for 10 minutes; the serum was removed then stored at -70°C for further study.

Serologic studies:Rubella virus-specific IgM antibodies were detected by indirect enzyme-linked immunosorbant assay (ELISA; Biokit,S.A.Lisca d Amunt. Barcelona-Spain), Hemagglutination and Hemagglutination inhibition test. All of the Methods were carried out according to the manufacturer's instructions.

Statistical Analysis:

Statistical analysis was performed using Chi-square testes according to (Daniel, 1988). * P < 0.05 mean significant ,P >0.05 non signicant.

Results

Anti-rubella IgG antibodies ELISA test revealed 210 (70%) positive result samples whereas the remaining and 90 (30%) samples gave negative anti-rubella ELISA test. Collected data of aborted women case history revealed that 162 (76%) of the 210 (79%) positive and 51 (24%) negative anti-rubella IgG ELISA antibodies test samples were obtained from vaccinated women and the remainder 48 (55%) positive and 39 (45%) negative anti-rubella IgG ELISA test samples were from non-vaccinated women. The study included the detection of anti-rubella IgM ELISA antibodies. ELISA test for detection of anti-rubella IgM antibodies of samples revealed that a total of 24 (8%) positive IgM samples were detected (2 vaccinated + 16 non vaccinated positive IgG anti-rubella samples and one vaccinated + 5 non vaccinated negative IgG anti-rubella samples).

It was shown that the age group (15-19) years was the highest group in regards to the number of vaccinated women (79%), while the lowest age group was that of (30-35) years which was about 12 (60%) in both Governorates, while the over all vaccinated women with abortion in all age groups is 213(71%).(Table 1)

It was found that , the majority of pregnant women with abortion are IgG seropositive and the range of IgG positively (71%-84.2%) in Al-Qadisiya Governorate, their no significant differences (P› 0.05) between the groups of aborted women in relation to their gestational ages.(Table 2).

In case of IgM seropositivety, it was found that seropositivity (8%) which showed the highest IgM seropositive in 3rd month of gestation (10.7%).

In order to estimate the efficacy of rubella vaccination program. It was found that most of those who were previously vaccinated gave IgG seropositive (87%) and the group which showed highest IgG seropositivity after vaccination was the youngest age group (15 – 19) and (20–24) years.

Regarding the IgG seropositivity in non-vaccinated women in our study, it was shown that those with IgG positive serum were lower than those with IgG negative 39 versus 48. (Table 3).

The incidence of IgM seropositivity among those pregnant women who underwent abortion who were IgG seropositive and IgG seronegative was also studied. It was found that the rate of IgM positive sera in those who were IgG positive are very few 6/210 (2.9%). While the IgM seropositivity rate among those who were IgG negative shown to be more or relatively higher especially. They were 18/72 (20%). (Table 4).

The IgM seropositivity among vaccinated and non vaccinated pregnant women who underwent abortion was also studied. It was shown that the incidence of IgM positivity was very little among those who were vaccinated in the Governorate. 3/210 (1.4 %), while the incidence of IgM seropositivity among non-vaccinated women differed , 21/66(24 %). (Table5).

Table 1: Incidence of anti-rubella IgG and IgM seropositivity in pregnant women who underwent abortion

Gestational age (month)	IgG		Total	IgM		Total
	+ve *	-ve		+ve	-ve *	
1	32 (84.2%)	6 (15.8%)	38	1 (2.6%)	37 (97.4%)	38
2	50 (71.7%)	31 (35.3%)	81	7 (8.6%)	74 (81.4%)	81
3	66 (71%)	27 (29%)	93	10 (10.7%)	83 (89.3%)	93
4	43 (67%)	21 (33%)	64	4 (6.2%)	60 (93.8%)	64
5	19 (79%)	5 (21%)	24	2 (8.3%)	22 (91.7%)	24
Total	210 (70%)	90 (30%)	300	24 (8%)	276 (92%)	300

*** P < 0.05**

Table 2: Relationship between anti-rubella IgG seropositivity and the history of vaccination against rubella virus

Age group (year)	sample	Vaccinated		Total	Non vaccinated		Total
		IgG * +	IgG -		IgG +	IgG * -	
15-19	71	40 (78%)	11 (22%)	51	8 (40%)	12 (60%)	20
20-24	100	61 (87%)	9 (13%)	70	17 (57%)	13 (43%)	30
25-29	87	44 (71%)	18 (29%)	62	10 (40%)	15 (60%)	25
30-35	42	17 (57%)	13 (43%)	30	4 (34%)	8 (66%)	12
Total	300	162 (76%)	51 (24%)	213	39 (45%)	48 (55%)	87

*** P < 0.05**

Table 3 : Incidence of anti-rubella IgM seropositivity among pregnant women who underwent abortion in relation to anti-rubella IgG result

Age group (years)	sample	IgG +		Total	IgG -		Total
		IgM +	IgM * –		IgM +	IgM * -	
15-19	71	1 (1.9%)	51 (98.1%)	52	3 (15.7%)	16 (84.3%)	19
20-24	100	2 (2.7%)	72 (97.3%)	74	5 (19%)	21 (81%)	26
25-29	87	2 (3.6%)	57 (96.4%)	59	7 (25%)	21 (75%)	28
30-35	42	1 (4%)	24 (96%)	25	3 (17.5%)	14 (82.5%)	17
Total	300	6 (2.9%)	204 (97.1%)	210	18 (20%)	72 (80%)	90

*** P < 0.05**

Table 4: The rate of anti-rubella IgM seropositivity among vaccinated and non vaccinated pregnant women who underwent abortion against rubella virus

Age	Samples	Vaccinated	Total	Non vaccinated	Total

group (years)	No.						
		IgM +	IgM * –		IgM +	IgM * -	
15 - 19	71	---	51 (100%)	51	4 (20%)	16 (80%)	20
20 - 24	100	1 (1.5%)	69 (98.5%)	70	6 (20%)	24 (80%)	30
25 - 29	87	2 (3.2%)	60 (96.8%)	62	7 (28%)	18 (72%)	25
30 - 35	42	---	30 (100%)	30	4 (33.4%)	8 (66.6%)	12
Total	300	3 (1.4%)	210 (98.6%)	213	21 (24%)	66 (76%)	87

*** P < 0.05**

Discussion

Prevalence of vaccinated and non- vaccinated pregnant women with abortion in relation to their age was studied in (table 1) and it was found that the youngest age group (15–19) years was the group that had high positive history of vaccination against rubella in regards to other age groups. The explanation of this finding could be attributed to the large scale use of vaccination programs in the last years in Iraq which were adopted by the health authorities and supported by WHO programs (WHO position paper, 2000). The incidence of IgG and IgM seropositivity was studied in aborted women in relation to the gestational age at which abortion occurred. It was found that there was no significant differences (P › 0.05) between the groups with abortion in relation to other gestational age when IgG seropositivity is taken.

Although there were some IgM positive women who had IgG positive serum at the same time (Tang *et al*., 2003) , the majority of IgM positive women with abortion were IgG negative, and this reflects the incidence of the new seroconversion (a new infection with rubella virus) while those who showed IgG positive sera with negative IgM, reflects those vaccinated or previously infected individuals, which constituted the majority of the studied populations at the governorate. These results are in agreement with what was found by (Atreya *et al*., 2004 ; Hahne *et al*., 2005) who stated that the majority of IgG positive women ,whether pregnant or not, had a positive history of previous vaccination, while those with IgG and IgM positive at the same time either they were previously vaccinated or had re-infection because of a low IgG titer , or because they were newly infected with rubella virus in a period of not less than 6 weeks (Tang *et al*., 2003) .

The relationship between IgG seropositivity and history of vaccination with rubella vaccine was studied in (table 3). It was found that most of those who were previously vaccinated gave IgG positivity

(76%) in Governorate and the group which showed highest IgG seropositivity after vaccination was the youngest age (20 – 24) years. In regards to the IgG seropositivity in non-vaccinated women in this study, it was shown that those with IgG positive serum were lower than those with IgG negative 39 versus 48. It was shown from (tables 3) that the immunity states (IgG) level for rubella virus after vaccination decline over time, to below the productive level, as it was shown the highest level of IgG was found in the youngest age group (20 – 24) years in comparison to other age groups. This could be explained by the effect of multiple factors like diseases, drugs, malnutrition, to which the mother could be exposed during her life, and it agreed with other studies conducted by (Broadbent *et al*., 1980; Al-Muslih *et al*., 1988; Yaseen, 1992; Aboudy *et al*., 2000). A pregnant women with no or low immunity needs to be vaccinated immediately after delivery and antibody status checked after 3 months. It important that vaccination shoud be given in the three months following administration of immunoglobulin. National Health and Medical Research, 1997 reported that a pregnant women has had contact with an illness that might be rubella, clinically should be encouraged to check immune states and look for evidence of acquired infection. (Table 4) shows that women with abortion who gave IgM positive test were usually of IgG negative sera 18/90 (20%) while those who were IgG positive , showed only lower incidence of IgM positive sera 6/210 (2.9%). These results reflected the highest risk of rubella virus infections, as those who were IgG positive, are less susceptible to infection in contrast to those who were IgG negative, in which they have more susceptible to rubella infection. These results are similar to those which were found by (Miller *et al*.,1982 & Cooper,1985) in which similar figures were reported in other developing countries such as Pakistan (23% of pregnant women were IgG negative (Azmi *et al*., 1987) Brazil and Chile (20)% were IgG negative. And among IgG negative women there was 15-20% chance of infection (Dowdle *et al*.,1970 & Bhaskaram *et al.*, 1991). The incidence of IgM positive pregnant women (Table5) was studied in relation to the past history of vaccination against rubella infection. It was shown that those who were vaccinated previously had very little chance of getting IgM positive serum during pregnancy 1.4%. While those who were non vaccinated had more chance of getting IgM seropositivity 24%. The differences were significant (P › 0.05).

These results were suspected because those who were previously vaccinated had a persistent, life–long IgG positive serum against rubella vaccines. Similar results were found by (Miller, 1991& Lutwick , 1997) , who stated that vaccination or infection with a virus confers a life – long immunity, and those who were infected after those two incidences either had a failure of vaccination or the serum vanished or decreased by the effect of many factors like time, energy response ,mis

recording, cold change, disease and drugs (Pullen *et al*., 1986 ; Yaseen , 1992; Bottigur & Jensen , 1997) . The reason for the continuing occurrences of such cases is that a small proportion of pregnant women is still susceptible to rubella either because they have not been offered or have refused vaccine prior to pregnant,and they have failed seroconvert after vaccination or had a frailer vaccination (Rager-Zisman *et al.*, 2003).

Australian Bureau of Statistics, 1996 stated that infections encountered are more likely to be reinfections, generally seen in those with low post vaccination antibody titers . Atreya *et al*.,2004 estimated that 10-25% of non- immunized women of child bearing age are susceptible to rubella infection.

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