Seroprevalence of Rubella virus and cytomegalovirus infections in infertile women in Baghdad city

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Abstract:

Infertility in women ranked the eighth highest serious global disability and thus the need for health care is conventional to the rights of personal with disability. Infectious agents can impair reproduction as well as many important human vital functions. Cytomegalovirus (CMV) and Rubella viruses are the commonest viral infections in prenatal period and they are the leading causes for congenital infections that may ends with fetal death and spontaneous abortion. This study was constructed on infertile women attendingAbdull-Majeed private hospital in Baghdad of the period from 6/2014 to 11/2015. Eighty-five infertile women aged from 18 -40 years tested for the presence of recent or past infection with cytomegalovirus or rubella virus. Another forty-two pregnant women with no history of abortion were included as normal control. For the purpose of comparison, cases were distributed on two age groups. Both groups were tested for the presence of anti-CMV and anti-rubella antibodies, IgM and IgG by using enzyme linked immunosorbent assay (ELISA) (mono bind Microplate reader awareness, US). About 54.1% of infertile women found to be infected with CMV compared to 60.2% of normal control, while 34.1% of infertile women found to be infected with Rubella virus compared to 42.8% of normal control. Absence of anti-rubella IgG (indicator of past infection or vaccination) in 69.4% of infertile women compared to 62.9% of normal control.

الإنتشار المصلي للإصابة بفايروسات المضخم للخلايا والحميراء لدى النساء العقيمات في مدينة بغداد Keywords: Cytomegalovirus, Rubella virus, Infertility

الخلاصة:

يصنف العقم في النساء بالمرتبة الثامنة بين أنواع الإعاقة الخطيرة على المستوى العالمي والذي يستوجب حق الشمول بالرعاية الصحية اللازمة للأشخاص المعاقين. تؤدي الإصابة بالعوامل المعدية إلى تعطيل وظائف بشرية مهمة مختلفة ومنها التكاثر. إصابة الأمهات بفايروسات الحميراء والفايرس المضخم للخلايا هي أكثر الإصابات الفايروسية شيوعا أثناء فترة الحمل وهي من الأسباب الرئيسية للإصابات الخلقية والتي قد تنتهى بوفاة الجنين والإسقاط التلقائي .

أجريت هذه الدرّاسة على نساء عقيمات من مراجعي مستشفى عبد المجيّد حسين الخاص في بغداد للفترّة من 2014/6 لغاية (2015/11 خمس وثمانون امرأة تراوحت أعمارهن بين 18 إلى 40 سنة تم الكشف عن وجود إصابة قديمة أو حديثة لديهن بفايروسات المضخم للخلايا والحميراء. شملت التجربة اثنان وأربعون امرأة أخريات من الحوامل اللواتي ليس لديهن حالات إسقاط سابقة كمجموعة ضابطة. لغرض المقارنة تم توزيع النساء إلى فئتين عمريتين. كلا المجموعتين خضعتا حالات إلى 10 سنة الى والمعن من الحوامل اللواتي ليس لديهن حالات إسقاط سابقة كمجموعة ضابطة. لغرض المقارنة تم توزيع النساء إلى فئتين عمريتين. كلا المجموعتين خضعتا لفحوص الكشف عن وجود الأضداد من نوع IgM,IgG المضادة لفايروسات المضخم للخلايا والحميراء. وجد أن ما من الحوامل اللواتي والمعتاد المعاد من الحوام المقارنة م توزيع النساء الى فئتين عمريتين. كلا المجموعتين خضعتا مالات إلى الحميراء. من المقارنة م توزيع النساء إلى فئتين مى الحوامل اللواتي ليس لديهن حضعتا حالات إسقاط سابقة كمجموعة ضابطة. لغرض المقارنة تم توزيع النساء إلى فئتين معريتين. كلا المجموعتين خضعتا ولمحوص الكشف عن وجود الأضداد من نوع 19,190 من المصادة لفايروسات المضخم للخلايا والحميراء. وجد أن المحاد من المعاد المالالي المصادة المالية المناء المعاد المالية المالية من معموعة السيطرة بينما وجد أن 19,50% من مجموعة السيطرة بينما وجد أن 19,50% من المعاد المالية المصادة السيطرة بينما وجد أن من النساء العقيمات مصابات بفايرس المصاحم الخلايا مقابل 60,20% من مجموعة السيطرة .

أوجدت الدراسة غياب الضد IgG المضاد لفايرس الحميراء (و هو مؤشر على حصول إصابة سابقة أو لقاح) في 69,4% من النساء العقيمات مقابل 62,9% من النساء في مجموعة السيطرة.

Introduction:

Infertility is a disease of the reproductive system may be clinically defined by "the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse."(1). The comparative report No. 9 of the world health organization (WHO) about infertility in developing countries defined infertility on the demographic bases as " Inability of those of reproductive age (15-49 years) to become or remain pregnant within five years of exposer to pregnancy "(2). Mascarenhaset al added another concept as he defined infertility as "Inability to become pregnant with a live birth, within five years of exposer based upon a union status. consistent lack of contraceptive non-lactating use. and maintaining a desire for a child" (3).

Among population under the age of 60, infertility in women was ranked the eighth highest serious global disability (4) and thus the need for conventional health care falls under the rights of persons with disability. Infertility classified into two categories, primary infertility in which a woman is unable to ever bear a child. either due to the inability to become pregnant or the inability to carry pregnancy to a live birth without ever having had a live birth and secondary infertility in which a woman is unable to bear a child, either due to the inability to become a pregnant or the inability to carry a pregnancy to a birth following either previous live pregnancy or a previous ability to carry a pregnancy to a live birth (3).

Infectious agents can impair different important vital functions. Viruses, as well as many other microorganisms, are able to interfere with the reproductive function in both sexes (5). Certain viruses suspected to be involved in development to infertility. Recent studies have shown that the presence of Human immunodificeinsy virus(HIV), Hepatitis B virus(HBV) or Hepatitis C virus(HCV) in semen impairs sperm parameters. Informations about semen infections with human Papillomaviruses (HPV), Herpesviruses (HSV), Cytomegalovirus (HCMV), and adeno-associated virus (AAV) are controversial. However, recent studies suggest that HPV semen infections has a negative influence on sperm parameters, fertilization, and the abortion rate (6).

Cytomegalovirus (CMV) and Rubella viruses are the commonest viral infections in prenatalperiod and they are the leading causes for congenital infections with a permanent hearing and vision loss and neurological impairment (7-9).These viruses infect women with mild or no apparent symptoms. However, infection during early pregnancy results in serious abnormalities to the fetus that may ends with fetal death and spontaneous abortion (9).

Initial antibody response consists of IgM antibody that do not persist, in general, longer than 6 weeks after illness. This antibody is replace by IgG that persists longer (for life in Rubella infection). However,to confirm a recent infection, either a rise in the antibody titer between two serum samples taken at least 10 days apart or specific IgMin a single sample must be detected (9).

Aim of the study: Investigating the seroprevalence of anti-CMV and antirubella antibodies IgM and IgG in infertile women in Baghdad against healthy pregnant women.

Materials:

Eighty-five infertile women (primary or secondary) aged between 18 to 40 years old were attending Abdul-Majeed private hospital in Alkarrada, Baghdad from the period 6/2014 to 11/2015. Another fortytwo healthy pregnant women with no history of abortion were included as control group..

Methods:

About five milliliters of blood were obtained from each woman under study. Blood were left to clot then centrifuged at 10000 rpm for 5 minutes. Sera were collected then tested by enzyme linked immunosorbent assay(ELISA) (mono bind Microplate reader awareness, US) kit for the presence of anti-CMV antibodies (IgM, IgG) and anti-Rubella antibodies (IgM, IgG). Cut off values had recorded and positive results were determined as shown in table (1).

Results and discussion:

Determination of positive and negative samples for the presence of specific antibodies (IgM and IgG) for CMV and rubella was done by ELISA system depending on the cut-off values of standard positive and negative controls as shown in table (1).Equivocal CMV IgM or IgG results may occur during acute infection or may be due to non-specific binding reactions (12), thus, Samples showed equivocal titers were considered negative for the presence of specific antibody.

 Table (1): Cut-off values of optical density of specific anti-CMV and anti-rubella IgM and IgG antibodies.

Agent	Virus specific Abs	Abs Titer (iu/ml)							
		negative		Positive					
CMV	IgM	<0.9	0.9	>0.9					
	IgG	<1.2	1.2	>1.2					
Rubella	IgM	<0.9	0.9	>0.9					
	IgG	<15	15	>15					

Indication of antibody presence (IgM or IgG) relates to the stage of the disease, i.e. either recent or past infection. The presence of IgM antibody (alone or with IgG) indicates acute or recent infection, while the presence of IgG antibody indicates past infection or vaccinated state (just in rubella since there is no vaccine for CMV yet).

For the purpose of comparison and analysis, studied groups (infertile and pregnant healthy women) were distributed on two age groups as shown in table 2.

 Table (2): Distribution of women studied on age groups.

condition	Age grou	Total	
	18-29	30-40	
Infertile	36	49	85
Healthy	18	24	42

Distribution of anti-CMV and anti-Rubella IgM and IgG among infertile women is explained in the table (3).

One case showed recent Rubella (IgM) infection and past CMV (IgG), whereas seven cases were positive for anti-CMV and anti-rubella IgG. Another seven cases showed anti-CMV IgM and IgG together with no rubella marker. Twenty-four women were positive for anti-CMV IgG only. Nineteen women were positive for anti-rubella IgG only. One woman was positive for anti-rubella IgM and IgG with no evidence for CMV infection. Eighteen out of eighty-five (21.2%) women were negative for both markers of CMV and rubella infections.

		A	Anti-CM	IV antibodies	
Anti-		IgM	IgG	IgM+IgG	negative
Rubella	IgM	0	1	0	0
antibodies	IgG	0	7	0	19
	IgM+IgG	0	0	0	1
	negative	7	23	7	18

 Table (3): Distribution of anti-CMV and anti-rubella IgM and IgG antibodies among infertile women studied.

Distribution of age groups on the specific anti-CMV and anti-rubella markers showed that anti-CMV IgM exists in 27.8% of infertile women under 30 years of age compared to 11.1% of healthy ones, . studies other showed different rates, Tumaet al(2013) reported 63% (13), Albaiatiet al(2014) reported 8-17% (14), while women over 30 years showed 8.2% and 45.8% for infertile and healthy women , respectively. Albaiatiet al(2014) reported 9-12% (14);Tumaet al(2013)reported 21% (13) for this group.

Total percentage of anti-CMV IgM existence was 16.5% in infertile women compared to 30.9% of healthy ones.Other studies reported different results, Saleh*et al*(2015)recorded 72% (15), 21% by (16), 87% by (17), 90.4% in Anbar (18), 60% in Thiqar (19), 60.2% in waset (20).

For anti-CMV IgG, 38.9% of infertile women under 30 years of age have this marker compared to 33.3% of the healthiest of the same age group. Albaiati*et* al(2014)reported 75-84% (14). On the other hand, 36.7% of infertile women over 30 years were positive for this antibody compared to 58.3% of healthy ones of the same age group. Albaiati*et* al(2014)recorded 82-91% (14). Total percentage of anti-CMV IgG in infertiles was 37.6% compared to 47.6% of normal, while it was 84%, 100%, and 55.5% cited by (14, 17, 20) respectively.

of Rubella Existence markers (IgMandIgG) in studied groups was as follows: 2.8% of infertile women under 30 years were positive for anti-rubella IgM compared to 11.1% of healthy women, a close percentage (9.4%), reported by Abdullaet al(2009) (21). on the other hand, 6.1% of infertile women over 30 years were positive for this marker whereas none of healthy women were positive of the same group. Abdulla*et* age al(2009) recorded 2.9% for this group (21). Total anti-rubella IgM was 4.7% in this study. Jasimet al(2011)in Waset reported 62.3% (20).

Anti-rubella IgG: In this study, 19.9% of women under 30 years were positive for this marker, while 92% was reported in Baquba (22). On the other hand, 38.8% of women over 30 years were IgG positive compared to 45.8% of healthy women of the same age group while 85.7% was recorded in Baquba (22). Total anti-rubella IgG was 30.6% compared to 38.1% of healthy control while it was 91.6% in Baquba (22) and 54.3% in Waset (20). Results shown in table-4.

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	Infection	Specific			I	Age grou	p (ye	ars)		Total	
	type	Ab			18-2	9	30-40				
condition				No	(%)	(%)	No	(%)	(%)	No	(%)
					of	of age		of	of		
					total	group		total	age		
									group		
infertile		IgM	(+)	10	11.8	27.8	4	4.7	8.2	14	16.5
	CMV		(-)	26	30.6	72.2	45	52.9	91.8	71	83.5
		IgG	(+)	14	16.5	38.9	18	21.2	36.7	32	37.6
			(-)	22	25.9	61.1	31	36.4	63.3	53	62.2
		IgM	(+)	1	1.2	2.8	3	3.5	6.1	4	4.7
	Rubella		(-)	35	41.2	97.2	46	54.1	93.9	81	95.3
		IgG	(+)	7	8.2	19.9	19	22.3	38.8	26	30.6
			(-)	29	34.1	34.1	30	35.3	61.2	54	69.4
normal		IaM	(+)	2	4.8	11.1	11	26.2	45.8	13	30.9
	CMV	IgM	(-)	16	38	88.9	13	30.9	54.2	29	69.1
		IaC	(+)	6	14.3	33.3	14	33.3	58.3	20	47.6
		IgG	(-)	12	28.5	66.7	10	23.8	41.7	22	52.4
	Rubella –	IaM	(+)	2	4.8	11.1	0	0	0	2	4.8
		IgM	(-)	16	38	88.9	24	57.1	100	40	95.2
	Kubella	IaC	(+)	5	11.9	27.8	11	26.2	45.8	16	38.1
		IgG	(-)	13	30.9	72.2	13	30.9	54.2	26	62.9

Table (4): Distribution of age groups on specific anti-CMV and anti-rubella IgM andIgG antibodies in infertile and normal women.

According to the markers indication, we can concluded that 22% of infertile females under 30 years have had recent CMV infection and 33.3% of this group had past CMV, while 8.2% of women over 30 years had recent CMV and 26.5% had past infections (Table 5). Also detection for rubella revealed that infertile group under 30 years did not exhibits any recent infection while 13.9% had past (or vaccinated) cases. On the other hand, 2%

of women over 30 years had recent rubella infection and 28.6% of this group had past infection (or vaccinated). 2.8% of women under 30 years had mixed recent infection (CMV and rubella together) and 5.5% of the group had past CMV and rubella infections. No female over 30 years reported to have recent mixed infection while 10.2% of the group found to have past CMV and rubella infections, results are shown in table 5.

condition	Type of	stage		To	Total			
	infection		(years)					
			18	-29	30-40			
			No.	(%)	No.	(%)	No.	(%)
Infertile	CMV	Recent	8	22	4	8.2	12	14.1
No.=85		Past	12	33.3	13	26.5	25	29.4
	Rubella	Recent	0	0	1	2	1	1.1
		past	5	13.9	14	28.6	19	22.3
	mixed	Recent CMV	1	2.8	0	0	1	1.1
		Recent rubella						
		Recent CMV	0	0	0	0	0	0
		Past rubella						
		Past CMV	0	0	1	2	1	1.1
		Recent rubella						
		Past CMV	2	5.5	5	10.2	7	8.2
		Past rubella						
Normal	CMV	Recent	0	0	8	33.3	8	19
No.= 42		Past	6	33.3	5	20.8	11	26.2
	Rubella	Recent	0	0	0	0	0	0
		past	5	27.8	6	25	11	26.2
	Mixed	Recent CMV	2	11.1	0	0	2	4.7
		Recent rubella						
		Recent CMV	0	0	3	12.5	3	7.1
		Past rubella						
		Past CMV	0	0	0	0	0	0
		Recent rubella						
		Past CMV	0	0	2	8.3	2	4.7
		Past rubella						

Table (5): Distribution of study groups on the infection type (single, mixed) and the
stage (recent, past).

In table (6) which describes diseases under study as separate cases, 25% of infertile women under 30% years had recent CMV infection compared to 11.1% of healthy women at the same stage of the disease, while 38.9% of this group had past CMV infection compared to 33.3% of healthy women. Infertile women over 30 years showed that 8.1% of them had recent CMV and 38.8% had past CMV infections compared to 45.8% of healthy women had recent CMV and 29.2% had past CMV infections.Total percentage of CMV infection (recent and past) in infertile women was 54.1% compared to 60.2% of healthy females found to be infected.

Detection for rubella indicates that 2.8% of infertile women under 30 years of age had

recent infection compared to 11.1% of healthy ones, while 19.4% of them had past rubella compared to 27.8% of healthy ones of the same age group. On the other hand, 4% of infertile women over 30 years had recent infection and 38.8% of them had past rubella attack. In contrast, no recent rubella infection was reported for healthy women of the same age group while 45.8% of them had past rubella infections (Table 6).

Total percentage of rubella infections (recent and past) in infertile women was 34.1% compared to 42.8% of healthy women for the same conditions.

Condition	Infection type	stage	Age	group (ye	ars)	Total			
		_	18-29		30-40				
			No.	× /		(%) of	No.	(%) of	(%)
				age group		age group		the stage	of total
Infertile	CMV	Recent	9	25	4	8.1	13	15.3	54.1
No = 85		Past	14	38.9	19	38.8	33	38.8	
	Rubella	Recent	1	2.8	2	4	3	3.5	34.1
		Past	7	19.4	19	38.8	26	30.6	
Healthy	CMV	Recent	2	11.1	11	45.8	13	30.1	60.2
No = 42		Past	6	33.3	7	29.2	13	30.1	
	Rubella	Recent	2	11.1	0	0	2	4.7	42.8
		Past	5	27.8	11	45.8	16	38.1	

Table (6): Distribution of the study groups according to type of infection and the stage of the disease.

It is noteworthy to mention that most researchers whom studied the presence of CMV and rubella markers have treated with IgM and IgG antibodies separately, as they neglect the presence of IgM and IgG together in one's patient sample. It is well known that acute or recent viral infection associate with the presence of anti-virus IgM and low avidity IgG and these markers last for few months (about four months in CMV infection), then decline to be replaced by high avidity IgG that lasts for years or life according to the type of viral infection.

The presence of anti-virus IgG alone may refer to a past infection or a vaccination process (the later in case of rubella since there is no vaccine against CMV), so, the presence of IgM and IgG together indicates a recent infection and may exclude a past infection. Thus, there are fifty-one out of the eighty-five (60%) infertile women did not get CMV infection and at least fiftyeight (68%) did not infected or vaccinated against rubella virus infection.

Total CMV infections:

Thigar (19). In other countries was 96.6% in Tunisia (23), 63.3% in Saudi Arabia (24), 55.3% in India (25), 97% in Brazil (26).

On the other hand, 60.2% of healthy pregnant women with no history of previous abortion were positive for CMV in this study. Other studies showed 100% (17), 70% (12), 36% (27), 66% (20), 13.4% by Aljoburi(2013) (although he used ELFA technique) (28), 31.7% in Kerkuk (29). In other countries was 100% in Turkey (30), 84% in Malaysia (31).

Total Rubella infections:

In this study, nearly 34.1% of infertile women were positive for rubella infection, since the presence of 65.9% of women, who were negative for anti-rubella IgG, may exclude effective vaccination processes. Other studies showed 27.9% by (15), 20.6% (32), 34.2% (33), 58.3% in Waset (20), 91.8% in Baquba (22), 78.3% in Babylon (34), 16% in Mosul (35). In other countries was 6.7% in Qatar (36), 34.7% in Sudan (37), 24% in Srilanka (38), 21.1% in India (39) and 91.2% in Iran (40).

Healthy pregnant women in this study showed 42.8% positivity for rubella infection. This percentage was close to In this study, 54.1% of infertile women were Jasimet al(2011) who reported 37.7% in positive for CMV (recent and past infections). Waset (20) but far of others as 86% in Other studies were 72.09% (15), 100% (17), 58% Baquba (22), 10.1% by Aljoburi(2013) (20), 21% (16), 90.4% in Anbar (18), 60% in (28), 68.4% in Basra (41), 19.4% in Kerkuk (29), 86.5% in Turkey (30) and 82% in Iran (40).

In this study, a slight rise in CMV and Rubella existence were found in healthy pregnant women than in infertile ones. This result is similar to (20). Other researches revealed close results (17, 20). This rise in the percentages of CMV and Rubella existence in healthy pregnant women may be due to the differences in the educational level, geographical factors, in addition to the nutritional, hormonal, physiological and immunological status of the studied cases.Differences in results with other researches may due to variations in experiment design, methods used, cultural and geographical variations of study subjects.

Conclusion:

First: The present study revealed that the mere existence of CMV or rubella virus infections in women do not necessarily leads to infertility since close percentages were found in pregnant women as normal controlmay accuse another factors thatplaying critical roles in association with these viruses in development to infertility.Second: High percentage of women seronegative for anti-rubella IgG (an indicator for anti-rubella vaccination) necessitates demand the forwomen vaccination especially those at the age of child bearing to prevent undesirable outcomes like abortion or congenital infections.

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