TORCH Screening Test in Pregnant Women of Kirkuk City

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ABSTRACT

Accepted 22/5/2016 In the course of pregnancy, the developing fetus might have some infections that can be transmit to him transplacentally from his mother. Early identification and treatment of these infections in a neonates is essential. Prenatal caution comprehends a range of tests, involving a TORCH screen test.

From the beginning of July till the end of December 2014. 500 blood samples were collected for TORCH screening from pregnant women whom attended virology section in Azadi Teaching Hospital. The TORCH screening was performed using ELISA technique.

The result of TORCH screening tests was not differed significantly between year 2013 and 2014, but there was significant differences between the frequency of the microorganisms included in TORCH screening, the highest was for Cytomegalovirus and Rubella with rates of 29.2, 39.1 % and 30.3, 41.8 % for each of the two viruses respectively. Lower frequency was observed for Toxoplasma with rate of 9.5, 8.9 % followed by Herpes simplex virus with a rate of only 1.1, 2.6 %. High rate of mixed infection were between Cytomegalovirus and Rubella. The frequency of chronic infections were significantly higher comparing with acute and subacute cases. The rate of infections was not effected by patients age.

Cytomegalovirus, Rubella and Toxoplasma are prevalent among pregnant women in Kirkuk city and probably they are the causative agents of abortion and infertility found among them, therefore it's better for pregnant woman or those planning to become pregnant to be tested for TORCH infections, and vaccinated against Rubella ,Cytomegalovirus, Herpes simplex virus and Toxoplasma to grantee her health as well as her baby.

Keywords: TORCH screening, Pregnant women, Kirkuk city.

الخلاصة

خلال مرحلة الحمل، الجنين النامي قد يتعرض إلى بعض الإمر اض الذي من الممكن أن ينتقل من الأم عبر المشيمة. التشخيص و العلاج المبكر لهذه الأمراض في الجنين ضروري. العناية بالأم الحامل يشمل عدد من الفحوص من ضمنها فحص التورج. بدءا من شهر حزيران و إلى نهاية كانون الأول لسنة 2014 جمعت 500 عينة دم من النساء الحوامل المراجعين لمستشفى أزادي التعليمي لغرض فحص التورج. الفحص تم إجرائه باستخدام تقنية الإليز ا.

نتائج فحص التورج لم يختلف معنويا للسنتين 2013 و 2014. و لكن كان هناك اختلاف معنوي ما بين المسببات المرضية في فحص التورج، التردد الأعلى كان لفيروس CMV و Rubella و بنسبة 39.1%، 29.2% و 41.8%، 30.3% لكل من الفايروسين على التوالي، تردد اقل كان لطفيلي Toxoplasma بنسبة 8.9% و 9.5% و تبع بفيروس HS بنسبة 2.6% و 1.1% فقط. النسبة الأعلى للإصابات المشتركة كان لفيروس CMV و Rubella . الإصابات الكامنة تردد بنسبة أعلى معنويا من الإصابات الحادة و تحت الحادة. نسب الإصابات لم يتأثر بعمر المريض. CMV و Rubella و Toxoplasma منتشر بين نساء الحوامل في مدينة كركوك و هناك احتمالية لكون هذه الكائنات هي المسبب لحالة الإجهاض و عدم الإنجاب بين النساء. لذا يكون من الأفضل للنساء الحوامل أو اللواتي في نيتهن الإنجاب أن يخضعن لاختبار التورج. و يأخذن اللقاحات اللاز مة لضمان صحتهن و صحة أطفالهن.

INTRODUCTION

Some microbial infections can be spread from a woman to her developing fetus during pregnancy. Early identification and treatment of these infections in a newborn child is important. A variety of tests achieved as antenatal care, including a TORCH screen test [1]. TORCH test is often includes Toxoplasm gondii, Rubella virus, Cytomegalo virus, and Herpes simplex virus. This test is generally done on newborns, but its ordered, if a woman shows signs for any of these diseases especially in the period of pregnancy [2]. The test itself screens for serum antibodies to the diseases, and can provide information as to whether never been exposed to the infection, an individual has been recently infected, or has had a past infection [3]. These diseases can transmit transplacentally to the developing fetus causing abortion or congenital defects. Neonates infested with one of these infections may be born with cataracts, mental retardation, deafness, jaundice, low platelet levels, seizures, or heart defects [4].

<u>Toxoplasm</u> *a* is a parasite that usually enters the body through the mouth. Undercooked meat consumption, raw foods, and exposure to cat litter or cat feces are possible sources of this parasite. Congenital transmission can led to abortion, stillbirth, or born of defected child [5]. *Rubella*, also known as German measles, is a virus that causes a rash but it can cause serious birth defects if a fetus is exposed to the virus [6].

Cytomegalo virus (CMV), is part of the herpes virus group. The virus cause flu-like symptoms in adults, while hearing loss, <u>epilepsy</u>, and mental retardation can be resulted in a developing fetus,.[7]. *Herpes Simplex* on the other hand is usually transmitted from a mother to her fetus in the uterus or in the birth canal at delivery and sever nervous system symptoms can appear on the baby [8]. TORCH infections had given a great attention by researchers from different regions, a rate of 71.82% for TORCH had been recorded among women with bad obstetric history (BOH), 90 (38.98%) samples were positive for T. gondii antibodies, and 40 (34.18%) were positive for HSV (Herpes Simplex Virus) in private gynecological clinic and laboratory, and a significant difference between women with BOH and women with normal pregnancy had appeared among 24.2% of HSV 2 seropositive samples, in women of Kirkuk province respectively [9, 10, 11]. In Waset governorate, from 300 samples examined, 162 have a history of spontaneous abortion [12]. Among fifty infertile women who referred to the Infertility Clinic of Kammal Al-Sammaree Hospital, 52% of the them had complained from primary infertility and (48%) from secondary infertility [13]. In Baghdad, among 178 pregnant and nonpregnant women 29 had infected with CMV and 21 had infected with Rubella [14]. In TORCH screening test, Toxoplasma was 36.36%, rubella 20.45%, Cytomegalovirus 29.55% and herpes simplex virus was13.64% in a study in gynecology wards in Hospital and out clinic from Babylon, Karbala and Baghdad city [15].

The aim of this study was to compare between the prevalence of each pathogen included in TORCH test and detect their relation as causative agent of abortion among pregnant women in Kirkuk city because abortion and infertility nowadays are representing a big serious problem among women in the city.

MATERIALS AND METHODS

Patients selection: This study was carried out in Azadi Teaching Hospital in Kirkuk, from July to December 2014, a total of 500 women patients whom attended virology section were chosen for this study. A complete information include (age, number of abortion, residence, number of healthy or abnormal children) from all patients were recorded. The comparison data between the two years were collected from the hospital registries.

Blood collection: venous blood was taken carefully and centrifuged to separate clear serum, the sera were stored in tubes and were kept at $(-20^{\circ}C)$ till used.

Type of kits used

Enzyme Immunoassay test kit for *Toxoplasma* gondii IgM and IgG, Rubella virus IgM and IgG, Cytomegalovirus IgM and IgG and Herpes simplex virus IgM and IgG all kits from Bio check/USA company.

Principle of the tests

Diluted patient serum with specific antibody is added to microwells coated with purified microorganisms antigens, incubated to allow reaction, washing the wells will remove unbound sample, a conjugated of horseradish peroxidase (HRP) is added. washing again removes unbound conjugate. A solution of TMB reagent is then added. The enzymatic reaction is stopped. The intensity of the color is read by a microwell reader compared in a parallel manner with calibrator and control.

Procedure: In order to investigate for serum antibody against these microorganisms, kits (Bio Check, Inc. USA) of IgG and IgM Enzyme Linked Immnosorband assay test were used for this purpose. The steps for detection were performed according to the kits instructions provided with each kit. The performance procedure was the same for all kits used.

Results interpretation:

Negative (*Toxoplasma, Rubella*, CMV and *Herpes*)

IgG and IgM Index less than 0.90 are negative for IgG and IgM antibody to (*Toxoplasma*, *Rubella*, CMV and *Herpes*).

Equivocal (*Toxoplasma*, *Rubella*, CMV and *Herpes*)

IgG and IgM Index between 0.91-0.99 are equivocal. Sample should be retested.

Positive (*Toxoplasma, Rubella, CMV* and *Herpes*)

IgG and IgM Index of 1.0 or greater are positive for IgG and IgM antibody to (*Toxoplasma, Rubella, CMV* and *Herpes*).

Statistical analysis

Statistical analyses were performed using Chisquare and T test to compare categorical variables. A p-value less than 0.05 or 0.01 were considered significant.

RESULTS

As it clear in Table 1, the prevalence of TORCH infection in year 2013, was not differed significantly from that of year 2014. In both years *Rubella* and *CMV* had high frequency rates followed by *Toxoplasma*, the lowest was for *Herpes*.

Vear	No. of	Number of +ve samples in single infection									
I Cal	patients	Т	%	R	%	C	%	Н	%		
2014	1524	136	8.9	462	30.3	446	29.2	17	1.1		
2013	1856	177	9.5	776	41.8	727	39.1	50	2.6		
T T test values Evaluated t value = 2.4 , t value of P< $0.05 = 2.35$ (non significant)											
		Ν	Number of -	⊦ve samples	in mixed i	nfection					
		T + R	%	T + C	%		C + R %				
2014	1524	12	0.7	46	3.0		56	3.6			
2013	1856	41	2.2	33	1.8		67	3.6			
T tes	t values		Evaluated	d t value = 1	.3, t value of	of $P < 0.05 = 2$.92 (non signi	ficant)			

Table 1: TORCH positive tests frequency in years 2013, 2014

T= Toxoplasma, R= Rubella, C= Cytomegalovirus, H= Herpes simplex virus.

Among 500 serum samples examined, as shown in Table 2, 221 samples were positive

for TORCH screening with rate of 44%, a 213 samples (43%) were for single infection and 8 samples (2%) were for mixed infection.

Table 2: TORCH positive tests frequency from July to September 2014

	No. of			
Total No.	+ve	No. of -	+ve	Total
Total No.	samples	ve	samples	+ve
examineu	in single	samples	in mixed	No.
	infection		infection	
500	213	279	8	221
%	43	56	2	44

As indicated in Table 3, approximately all positive samples were chronic cases, with IgG frequency rates of 98.8, 90.5, 72.7% for each of *Rubella*, CMV and *Toxoplasma* respectively, whereas high rate of acute cases were for *Toxoplasma* with a frequency rate of 18.2 %. On the other hand a number of CMV and *Toxoplasma* positive samples had both IgM and IgG antibodies with rates of 9.5, 9.1 % for each microorganisms respectively.

Table 3: IgM and IgG frequency in TORCH tests.

	No. of	IgM	IgG	IgG+Ig
TORCH		(acute	(chroni	M (sub
microorganis ms	samples)	c)	acute)
		+ve	+ve	+ve
	/0	%	%	%
Toxoplasma	33	6	24	3
	15.5	18.2	72.7	9.1
				1
Duballa	84	0	83	
кидена	39.4	0	98.8	
				1.2
Cytomegalovi	95	0	86	9
<i>Cytomegutovi</i>			90.5	
1 43	44.6	0	70.5	9.5
Herpes	1	1	0	0

simplex virus	0.5	100	0	0				
Total	213	7 3.3	193 90.6	13 6.1				
χ2 value	Evaluated $\chi 2$ value = 23.8, $\chi 2$ value o P< 0.01 =16.8 (significant)							

Although no significant differences had appeared between ages but all tested microorganisms were more prevalent among 15-28 years old female, followed by 29-38 and lowest prevalent was among bigger ages (39-48) as indicated in Table 4.

Table 4: TORCH infection frequency according to age groups.

Ag e in vea	No. of exami ned	Toxopla sma	Rube lla	Cytomegalo virus	Herp es simpl ex				
rs	sample s	+ve %	+ve %	+ve %	+ve %				
15-	112	17	44	52	1				
28	112	15.2	39.2	46.4	0.9				
29-	60	9	29	31	0				
38	09	13	42	44.9	0				
39-	22	7	11	12	0				
48	32	21.8	34.4	37.5	0				
Tot	212	33	84	95	1				
al	213	15.5	39.4	44.6	0.46				
χ2 value		Evaluated $\chi 2$ value = 0.07, $\chi 2$ value of P< 0.05 =3.84 (none significant)							

A assenting result to Table 4 is Table 5, which reveals that TORCH infection is more frequent in women who married for short period of time (1-12 years) comparing with those who had been married for long period of time (13-24).

Table 5: TORCH infection	frequency	in females b	v duration	of their marriage
	nequency	III Iomaios c	y adiation	or mon marinage

Duration of	No. of	Toxoplasma		Rubella		Cytomegalovirus		Herpes simplex		
marriage	examined samples	+ve	%	+ve	%	+ve	%	+ve	%	
>1	16	1	6.2	4	25	6	37.5	0	0	
1-12 years	113	23	20.4	61	54	69	61	1	0.9	
13-24 years	84	9	10.7	19	22.6	20	23.8	0	0	
Total	213	33	15.5	84	39.4	95	44.6	1	0.46	
χ2 value	Evaluated χ^2 value = 19.2, χ^2 value of P< 0.01 = 16.8 (significant)									

On one hand *Rubella* and *Cytomegalovirus* had been found to be more associated with

infertility, Table 5. While *Cytomegalovirus* and *Toxoplasma* infections were more associated

with defected child, but on the other hand women who were seropositive against TORCH

microorganisms had healthy or healthy and defected children Table 6.

	Table 0. Token integration to child state.										
Child state	No. of	Toxoplasma		Rub	ella	Cytomegalovirus		Herpes			
	examined samples	+ve	%	+ve	%	+ve	%	+ve	%		
No child	70	8	11.4	28	40	33	47	1	1.4		
Healthy	114	15	13.1	44	39	49	43	0	0		
Healthy+ defected	21	7	33	12	57	13	62	0	0		
Defected	8	3	38	0	0	5	63	0	0		
Total	213	33	15.5	84	39.4	95	44.6	1	0.46		
χ^2 value	Eva	Evaluated $\chi 2$ value = 36.7, $\chi 2$ value of P< 0.01 = 16.8 (significant)									

Table 6: TORCH infection frequency in relation to child state.

All the pathogens included in TORCH test were found to be implicated with abortion and still birth, but *Cytomegalovirus* and *Rubella* were implicated at first degree, while *Toxoplasma* was implicated at second degree Table 7.

No. of	No. of	Тохор	Toxoplasma		oella	Cytomeg	galovirus	Herpes simplex virus
abortions	samples	+ve	%	+ve	%	+ve	%	+ve %
No abortion	80	10	12	27	34	31	39	0 0
1-2 times	100	18	18	46	46	52	52	1
3-4times	29	5	17	9	31	11	38	0 0
Total aborted	129	23	19	55	42	63	49	1 0.8
Still birth	4	1	25	2	50	1	25	0 0
Total	213	33	15.5	84	39.4	95	44.6	1 0.46
χ2 value		Evaluated 2	χ^2 value = 3	5.1, χ2 v	alue of	P< 0.01 = 21.7	(significant)	

Table 7: TORCH infection frequency in relation to abortion

All microorganisms were prevalent in both city center and environs. No significant differences had appeared between the microorganisms prevalence in city center and environs. But in the city center some areas were tended to be more infected than other area, see Table 8.

Table 8: TORCH infection frequency in different areas of Kirkuk city.

Residential area	No.of +ve	Toxoplasma		Rubella		Cytomegalovirus		Herpes	
Kesidenilai area	samples	+ve	%	+ve	%	+ve	%	+ve	%
Tariq Baghdad	8	2	25	3	37.5	3	37.5	0	0
Panjaali	16	1	6.2	7	43.75	8	50	0	0
Shorijh	14	2	14.3	6	42.85	6	42.85	0	0
Raheemawa	10	0	0	4	40	6	60	0	0

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Wasti	9	1	11.1	4	44.44	4	44.44	0	0
Haiaal skari	15	1	6.7	7	46.66	7	46.66	0	0
Qadisiyah	11	1	9	5	45.45	5	45.45	0	0
Almas	8	1	12.5	3	37.5	4	50	0	0
Shoraw	6	1	16.7	2	33.33	3	50	0	0
Musala	7	1	14.3	3	42.85	3	42.85	0	0
Domiz	4	0	0	2	50	2	50	0	0
Azadi	7	1	14.3	3	42.85	3	42.85	0	0
Suqalhasir	2	0	0	0	0	1	50	1	100
Failak	6	2	33.3	2	33.3	2	33.3	0	0
Teseen	1	0	0	1	100	0	0	0	0
Amalshabi	2	0	0	1	50	1	50	0	0
Hai al-neda	1	0	0	0	0	1	100	0	0
Hai al-asser	3	1	33.3	1	33.33	1	33.33	0	0
1 huzairan	2	0	0	1	50	1	50	0	0
Hai alwehda	5	0	0	2	40	3	60	0	0
Al-huria	11	3	27.3	4	36.36	4	36.36	0	0
Eskan	2	0	0	1	50	1	50	0	0
Hai al-asra	3	1	33.3	1	33.33	1	33.33	0	0
Haialkaram	2	1	50	0	0	1	50	0	0
Total	155	20	12.9	63	40.64	71	45.80		1
Environs	58	13	22.4	21	36.2	24	41.38	0	0
Total	213	33	15.5	84	39.44	95	44.6	1	0.46

DISCUSSION

Some maternal infections play a critical role in abortion and fetus losing, early or later infancy sickness can contribute to neonatal mortality [16]. Infection with any of TORCH diseases during pregnancy may reach the placenta and the fetus and can led to miscarriage, stillbirth, or a birth of a child with serious defects [17]. In our study no significant differences was noted between the pathogens prevalence in both 2013 and 2014 years, this may be attributed to that, the initial stage in most of these infections are asymptomatic and hard to detect clinically and infected individual can hold the pathogen for long period of time without any symptoms [5-8]. CMV and Rubella were the most prevalent pathogen among pregnant followed by Toxoplasma, the lowest rate was for HSV in current study, this will agree with results from other studies, how found (18, 25, 20, 16%) [2], (20.6, 69.9, 79.1, 3.1%) [18], (33, 71, 95, 25%) [19] respectively for Toxoplasma, Rubella, CMV, HSV in each study, this may due to that CMV and Rubella can transmit easily, Rubella virus can spread through respiratory tract, CMV virus is spread through body secretions (urine, saliva, vaginal secretions, semen and breast milk) while herpes simplex type is transmitted through oral secretions or sores on the skin and Toxoplasma by mean of foods [5-8]. Not alike to our result

is, *Toxoplasma*- 54.8%, CMV- 60.2%, *Rubella*-62.3%, and HSV -73.9% [12] or *Toxoplasma* 36.36%, rubella 20.45%, cytomegalovirus 29.55% and herpes simplex virus 13.64% [15]. This results fluctuation may because of that different regions have different endemicity rates of pathogens.

The overall prevalence of IgM/IgG antibody in current study was 3.3%, 90.6% respectively for each type of antibody. Approximately all studies [1-15] had referred to same results. The explanation to this is that these infections are asymptomatic in most of cases, thus only IgG will be present in patients serum, since IgM is the antibody responsible for acute stage of infection then decline or disappear after initial infection.

The sero-prevalence of *Toxoplasma* IgM/IgG among pregnant women in our study was 18%, 24 % respectively for each type of antibody. Sadik, *et al.* [2] and Turbadkar, *et al.* [20] have reported an incidence of 6% /18 % and 10.5%/42.1% respectively for IgM, IgG antibodies in each studies. Janak, *et al.* [21] reported overall IgM antibody positivity of 8.3% in 60 cases of BOH women. Studies have proved that toxoplasmosis in pregnant women is ether because of new infection or flaring up of persistence of encysted forms in chronic infection, that may rupture during placentation leading to fetus infection in different semesters of pregnancy [2, 20, 21].

Rubella IgM/IgG rates were 0%/98.8 % respectively, in the present study. while other workers reported rates ranging from 3-17% /18-57.7 % [9, 12, 14]. *Rubella* is a mild viral illness in children but can occasionally infect adults. WHO estimates that, worldwide, more than 1 lakh (equal to one hundred thousand) children are born with congenital *Rubella* syndrome each year, most of them in developing countries. Nearly 50% of *Rubella* infection is subclinical.[22].

The present study also showed sero-positivity rate of 0%/90.5% for CMV IgM/IgG in pregnant women. Other studies have reported results somehow similar to our records, 5-8/24-80 % rates had been recorded in women with BOH [9,12,14]. The transmission of CMV infection to fetus occurs in 40% of the cases with primary infection and results in the delivery of 10-15% symptomatic and 85-90% asymptomatic congenitally infected newborns [22]. Very low incidence (0.5%) of HSV infection was detected in this investigation, Turbakar, et al. [20] and Janak, et al. [21] reported a low seropositivity rate of HSV IgM as 3.6 and 3.3% respectively for each research's study. HSV in asymptomatic women with recurrent infection during pregnancy was found to be 0.6-3% previously [20]. The role of CMV and Rubella virus and T. gondii infection on abortion, stillbirth or defected children is well proved here. HSV were found to have low influence than the other pathogens this because of the very low prevalence of HSV, (among 123 positive samples only one sample was positive for HSV).

Most of positive cases in current research were appeared among women of 1-12 years duration of marriage, on one hand this may because these years is child bearing age, which is after that the rate of pregnancy will decline among women, on the other hand most of these infections are transmitted sexually or by oral and body fluid [6-8]. Our result also indicated that all the pathogens were implicated in abortion, stillbirth, defected child or healthy one. Identical results had been observed by others [1, 2, 9, 12]. High number of aborted women had 1-2 or 3-4 times abortion, most of other studies had revealed similar results [1, 2, 4, 9, 12, 15]. This probably due to that after initial infection and abortion the patients may had taken antiviral or anti-toxoplasmal drugs, or due to acquired immunity to these infections which may reduce the abortion rates.

Primary infection with TORCH complex in pregnant women can lead to adverse outcome, these infections are asymptomatic in adults and are difficult to diagnose clinically, Thus, these tests are better to performed before or as soon as pregnancy is diagnosed to determine the mother's exposure to these pathogens and the necessary precautions be taken.

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