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Serological Cross Reaction among Some Causative Agents of Women Abortions (Toxoplasma gondii & Cytomegalo Virus & Rubella Virus), with the Incidence of Hepatitis Virus (B &C)

Yahya Gargies Salman
College of Medicine / Kirkuk University

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ABSTRACT:-

Primary maternal infection with infectious agents of abortions in women is frequently associated with transplacental transmission to the fetus. This study was conducted to test the utility ELISA assay to detect recent infection with Toxoplasma (TOXO), Rubella virus (RV), Cytomegalovirus (CMV), the incidence of viral hepatitis B (HBS) & C (HCV) in addition the determination of cross reactivity among serological tests. Three hundred forty three women with high risk of pregnancy (who had previous recurrent abortion & congenital abnormalities) & 60 normal pregnancies (control) were tested for the presence of the tests that described in above. The all rate of infectious agents was 22.15 % that divided into 8.88%, 8.02% & 4.84 % for RV, CMV, & Toxo antibodies respectively. Igm Ab of RV(31.5%) was higher than CMV(17.6%) & Toxo(3.39%) P<0.05.While IgG Ab shows the reveres as 17% for Toxo, followed by 6.75% & 7.89 % for RV& CMV respectively P<0.05.Relationship between distribution of Toxo Ab concerning age was not significant P>0.05. incidence of HCV shows equality rates 2.63% within sero-positive Toxo,RV&CMV, while HBS shows high occurrence in sero-positive for RV (5.26%) than in sero-positive for Toxo & CMV(1.31%)P<0.05. Relationship between frequency of abortions & stillbirths& infectious agents was significant P<0.05. In addition .relationship between women occupations & frequency of sero-positive reveals significance P<0.05 especially in sera of homemakers (housewives) than other women occupations. Control groups were negative for viral hepatitis &Toxo, except few cases>0.05.

Key words: Abortions, Stillbirth, Totoplasma, Cytomegalovirus, Rubella, Hepatitis Viruses B & C.

التفاعلات التصالبية المصلية بين بعض العوامل المسببة لإجهاض النساء(المقوسة الكوندية ،الحمة المضخم للخلايا،وحمة الحصبة الألمانية) مع نسبة حدوث حمة التهاب الكبد نمط (ب، س)

يحيى جرجيس سلمان

المستخلص:-

خمج الأم الأولي بمسببات عوامل الإجهاض في النساء كثيرة الحدوث ويصاحبها نقل المسبب عبر المشيمة.أجريت الدراسة لاختبار استعمال المقايسة المناعية(أليزا)لتحديد الخمج بالمقوسة الكوندية، الحمة المضخم للخلايا، حمة الحصبة الألمانية ونسبة تكرار أو حدوث حمة التهاب الكبد نمط ب، و نمط سي فضلا عن معرفة التفاعل التصالبي للمصول الموجبة بين العوامل المسببة.لهذا الغرض تم فحص 343 مصل من النساء الأكثر تعرضا(اللواتي لهن إجهاض متكرر، عيوب خلقية) و 60 نساء ذات الحمل الطبيعي(مجموعة ضبطة) كانت نسبة الخمج الكلية 22.15 % والتي توزعت إلى 8.88% و8.02% و4.84% لحمة الحصبة الألمانية، حمة المضخم للخلايا و المقوسة الكوندية على التوالي P<0.5 . نسبة الغلوبيولين المناعي أي جي أم لحمة الحصبة 31.5%كانت أعلى من حمة المضخم للخلايا 17.6% و المقوسة 3.39%، P<0.05. بينما نسب الغلوبيولين المناعي أي جي جي أظهر العكس 17% للمقوسة تبعها 6.75%و 7.89%لحمة المضخم للخلايا وحمة الحصبة على التوالي P<0.05. العلاقة بين توزيع الأجسام المضادة للمقوسات والعمر كان غير معنويا P>0.05 . . نسبة حدوث حمة التهاب الكبد نمط سي أ ظهرت نسب متساوية في المصول الموجبة للعوامل الممرضة الثلاثة 2.63%وأعلى من حدوثها بالنسبة لحمة ألتهاب الكبد نمط ب والتي ظهرت بنسبة 5.26 % لحمة الحصبة أعلى من المصول الموجبة للمقوسة وحمة المضخم للخلايا 1.31% P<0.05 . العلاقة بين تكرار الإجهاض ،ولادة أطفال ميتين والعوامل المسببة كانت معنوية P<0.05. العلاقة بين وظيفة النساء وتكرار حدوث العوامل المسببة ظهرت بشكل معنوي وعالي وبخاصة في مصول ربات البيوت مقارنة بغيرهن. مصول المجموعة الضابطة كانت خالية من العوامل الممرضة ما عدا بعض الحالات P<0.05.

INTRODUCTION:-

The causes of spontaneous abortions in women relies to different factors: Physiological, organic and infectious agents, excluding two formers, the later factor had value among women specially when they acquire the infection during pregnancy, which is frequently associate with the transmission of infectious agent to fetus (1).Transplacental passage will damage or injure fetus, leading to miscarriage, stillbirth or congenital abnormalities, depending on stage of gestation when the infection occurs (2).The common infectious agents includes: protozoan parasite Toxoplasma gondii(TOXO), & viruses like Rubella virus(RV) , Human cytomegalovirus (HCMV) , & other infectious agents. Toxoplasmosisis caused by Toxoplsama *gondii*, an-obligate intracellular protozoan parasite, It is a zoonotic disease at least 500 million people worldwide have the antibodies against parasite (3).Clinically toxoplasmosis is divided into: Acquired asymptomatic toxoplasmosis, self limited & congenital toxoplasmosis which is widely to be associated with symptoms causing congenital abnormalities among women specially after conception , with squally of microcephaly, encephalitis, chorio-retinitis , deafness & mental retardation (4).Current diagnosis of toxoplasmosis relies either on serologic detection of specific anti-toxoplasma immunoglobulin , culture of amniotic fluid, fetal blood or on other non specific indicators of infection (5,6,&7). Cytomegalovirus (CMV) are part of Herpesviridae family, the form that infects human cytomegalovirus (HCMV) is also known as human herpesvirus 5 (HHV5) (8).Infection with CMV is common rates are approximately 40% in developed areas, & approach 100%in undeveloped countries (9). Infection may be a symptomatic, or may include: mononucleosis-like symptoms with prolonged fever & mild hepatitis. Infected individual may shed virus intermittently, without any detectable sign or symptoms, CMV can be shed in urine, saliva, blood, tears, semen breast milk(10).Women who are infected with CMV for the first time during pregnancy may transmit the virus to their unborn child, these infants may develop a generalized infection with symptoms ranging from enlargement of the liver, spleen to fatal illness & in untreated infants ,it will for developing complications such as hearing loss, vision impairment & mental retardation (11 & 12). Infection with Rubella virus are usually mild, often presenting with maculopapular rash of the head & trunk, lymph-adenopathy ,a fleeting fever & arthritis (13 & 14).However squally result from maternal infection during the first trimester of pregnancy with 90 % risk of fetal damage ,if the infection occurs in the third month (15 & 16) .Teratogenic infection in utro include: Ocular defects (Cataracts, retinopathy & glaucoma), partial or complete cochlear deafness& mental retardation associated with microcephly or encephalitis (17). In adulthood, affected individuals have been reported to have increased levels of diabetes, osteoporosis & thyroid disorders (18). Relation ship between congenital abnormalities & hepatitis viruses' type (HBV &HCV) was not previously found, although the similarities in risk between CMV, RV & hepatitis viruses may cause liver cirrhoses & hepatocellular carcinoma (19). Sexual transmission & mother to infant transmission of HCV were previously not found to be important.& people who received more frequent during injection had a higher rate of HCV infection (20&21).sero-prevalence study among women in Spain(22) shows that, the rate of Toxoplasma gondii antibody is lower & the HBV &HCV antibody rate is around 0.4 %. In France (23) found the following rates 53.6 % of pregnant women had toxoplasma antibody, 181(85.8) had RV & CMV rate was (97.2 %). In Africa (BurkinaFuso) sero prevalence study reveals that Toxo/HBV was 0.048 versus 0.015 & Toxo/HCV was 0.014 versus 0.08 among pregnant women (24`). No previous study in regard of cross reaction among (TOXO, CMV,RV) in sera of pregnant women , so this study was conducted to show the prevalence of TOXO, CMV, RV,HBV & HCV antibodies (Ab) in sera of pregnant women in Kirkuk province with an attempt to detect serological cross reaction among infectious agents.

MATERIALS AND METHODS:-

Patient selection: From 1st June 2006 to 30th April 2007 a total of 343 serum samples were collected from pregnant women whom they considered at high risk group (Abnormal pregnancy outcomes, recurrent abortions & possible exposure to sources of Toxoplasma, CMV, RV, HBS&HCV), Also 60 serum samples (as control) were collected from healthy women with history of a normal pregnancy& were selected from a consecutive sample of women attending private gynecologic clinics for routine checking. All women were interviewed using a structured questionnaire, which included (name, age, occupation, number of pregnancy, and number of abortions, still birth, previous infections, vegetable & meat contact, drinking un pasteurized milk contact with soil, cats or other domestic animals nearby).

Serological tests: After separation of clear sera (non hemolyzed) , all were tested for the presence of specific IgM &IgG antibodies to CMV,RV (25&26) , while for toxoplasma antibodies sera were tested using Direct agglutination test (DAT),semi-quantitative micro-titration test (SQMT) using kits purchased from Biokit Company/ Spain(27), & by ELISA apparatus using both IgM & IgG kit purchased from Bio-Check,INC(28). Lateral immuno-chromatography test was used for detecting HBs& HCV antibodies in sera. (Acon laboratories INC, USA)(29).

Statistical analysis: Data were analyzed with Chi-Square & Fisher exact test to evaluate the possible differences between study groups. A P value < 0.05 was considered significant.

RESULTS:-

From a total 343 sera examinations using ELISA technique, the all rate of infectious agents was 22.15 % ,which distributed into following rates:- 8.88% for RV followed by 8.02 % for CMV & 4.84 % for TOXO antibodies, while negative rates were 16.04 %,12,06 % & 47.87 % for CMV,RV & Toxo antibodies respectively<0.05 ,see table {1}. Distribution of IgM & IgG antibodies of three different infectious agents was clarified in Table {2}, through which's IgM only 3(3.94%) of the 184 high risk cases tested for specific Igm Ab for toxoplasma, while IgG Ab was found in 17(17.10 %). & both IgM+ IgG was found in one sample with the rate 1.31 %.High titers were compared with none in the control group (titer <1.0 IU /ml). Rubella IgM Ab was found in 24 sera with the rate(31.57 %),while RV IgG Ab was found in 5(6.57 %) P<0.05, & both Igm+IgG Ab positive recorded in 2(2.63 %).Pregnant women posing CMV IgM was in 13(17.10 %) compared to 6(7.89 %) for CMV IgG Ab, while CMV IgM + IgG Ab were found in 12 sera with the rate (15.78 %) P <0.05 Relationship between distribution of sero-positive toxoplasma antibodies & age was shown in table {3}, through which's the following rates were obtained: 23.33 %, 22.85 % & 18.18 % for age groups (26-----35 years),(18---- 25years)& (36- 41 years& above) respectively P>0.05. Regarding Hepatitis viruses HBS& HCV antibodies distribution among sera positive For TOXO,CMV & RV, only 4 sera were positive HBS Ab from total 31 sero-positive for RV with the rate 5.26 %, while sero-positive TOXO & CMV was seen as 1.31% for each respectively. In regard HCV distribution in sera positive for TOXO,CMV &RV the rate of HCV was 2.63% for each equally , P>0.05 . table{4} Frequency of abortions & stillbirth in women tested for toxoplasma antibodies is clarified in table {5}, from 184 women, 183 had previous abortions with the 99.45%, this rate included 39.13 %, 35.32 %, 21.19 % & 3.80 % as double, single triple & tetrad abortions respectively. While stillbirths were found in 171 with the rate 92.93% that divided into single stillbirth, double triple &tetrad still births as in the following rates 46.19%, 25 % , 7.6%,7.6%respectively.Considering women with abortions & still births only 75 women with the rate 40.76 % shows this mixed pattern. The result of the presented study shows that, the relationship between women occupations & acquiring infections (TOXO, CMV, & RV) was significant P<0.05,specially the sera of housewives, from total examining of 261 sera of housewives, 60 sera were positive for infectious agents which distributed as 25(7.28%),23(6.7%) &12(3.49%) for RV,CMV& TOXO respectively. These rates were significant

P<0.05, when it were compared to other women occupations in the study Table {6}.

DISCUSSION:-

Precise knowledge of about infectious agents causing habitual abortion or congenital abnormalities required risk assessment of infections as basis for cousling, prevention & treatment (5&30). The rate of infection among women acquiring the infection during pregnancy in Iraq is not well known, the causes to that, may be attributed to several factors such as: laboratory methods, size of sampling(number of women per study), economic cause (the test is expensive) low level of sanitation, low encourage by physician(gynecologist) to refer patient for laboratory investigations. The all rate 22.15 % of infections with three infectious agents is high, specially the rates of RV&CMV (8.88% &8.02%) respectively, while the rate of TOXO Ab 4.84% is low when it was compared to that recorded in the same province by (31 & 32), whom they recorded the following rates (52.32 % & 33.14 %) respectively. Also with that recorded in Africa by (24). Interpretation of high rate of sero-positive CMV required understanding the fact, that CMV has the capability to persist in its human host indefinitely as latent infection in several glands & the kidneys (33). In addition, CMV has ability of escaping host defenses specially, when CMV establish latency in host cells allowing the virus to persist without triggering immune responses, in addition the virus has ability to encodes protein that enable it down regulate the MHC & to inhibit NK cell killing (23) .the result of the present study is disagree with that recorded in India by (34) whom they record 15.98 % of seropositive CMV, the difference may be due to very large score of sampling 1918 samples. Although the rate of sero-positive of RV in the present study is high 8.88% comparing to rates of CMV & TOXO, but it is very low when it was compared to that recorded in Australia by (35), whom they recorded the ranged rate (84.2% to 96.5 %) from testing 220 blood samples using mini-vides kit. Toxoplasma sero-positive cases classified as 3.39 % positive IgM Ab, which is lower than 17.0% for IgG Ab, these rates means that, it is necessary to follow up a positive IgM result as IgM can not cross the placenta to provide protection to the fetus. It should be followed for rising titer of antibodies or confirmed by IgG Ab avidity test, through which's, if IgM positive & the avidity is low, this suggests, but does not confirm the recent infection.(36 & 37). While IgG Ab rate 17.0% required repeating the test after 3---4 weeks for watching IgG Ab level, via which's , if IgM Ab is negative & IgG positive, this suggest infected women for over one year & if both IgG & IgM positive this suggest possible infection with in the previous 12 months (38). Women posing RV sero-positive antibodies IgM 31.5 % higher than IgGAb 6.75 %) can be explained by sero- conversion or a significant rise in the titer of IgG Ab is good evidence of recent RV infection & may still be detected even if the individual presents after the symptoms have subsided (14).While if IgG Ab level is high at presentation & a rise titer can not be demonstrated, the presences of RV IgM is usually used to determine acute infection. Therefore, an accurate RV IgM assay is critical to the diagnose of acute RV infection (39). Regarding CMV rates 17.6 & 7.89 % for IgM & IgG Ab respectively, this can reflects that, both primary CMV & reactivation of the dormant virus can cause serious disease leading cause of congenital virus infection in humans & is a primary cause of morbidity in immuno-compromised individuals (2, 8&12). The prevalence of specific antibodies to Toxoplasma gondii is directly proportional to the population age, indicating that the infection is acquired throughout life (40) ,in spite of the relationship in the present study is not significant P>0.05 between women age & distribution of three infectious agents, but the rates are high & should take in consider & suggest that, women at fertilizing ages(15 years to above than 45 years) in kirkuk province were on risk & they were more exposure for getting infectious agents. The result of the present study is in agreement with that recorded by (40) & disagree with those recorded by (31&32) in Iraq & with (41) in Jordan & with (42) in Brazil. The all rates 7.89 % for HBS & HCV within total positive rate 22.15 % for CMV, RV & TOXO is very high, this suggests that hepatitis

viruses HBS & HCV had role in causing serious illness & damage to fetus of infected pregnant women specially, during RV infection 5.26%. Considering the incidence of HCV & HBs Ab 2.63 & 1.31% among women sero-positive for toxoplasma, these rates were low, when it was compared to that recorded by (24) whom they record, the following rates 5.4 % & 9.8 % for HBS& HCV respectively. In addition, the same author in separate study in 2005 recorded HCV infection as the rate 3.3 % with in the sera tested for Toxoplasma & HIV (43). The result in table-5 reflects the degree of injury that caused by toxoplasma infection to women, specially abortions & also stillbirths 86.41 %, this suggest that women in the study were acutely infected or have reactivation of toxoplasma gondii during pregnancy(i.e., because of immuno-suppression)can transmit the organism transplacentally. The risk of congenital; disease is lowest (10—25%) when maternal infection occurs during first trimester & highest (60---90%) when maternal infection occurs during third trimester, this can explain why stillbirths in the present study was high (5). However congenital disease is more sever when infection is acquired in the first trimester(44).The result in present study is in agreement with that recorded in the same province in 2000 & in 2005 by(31&32) respectively. Considering relationship between women occupations & frequency of ser- positive TOXO,RV,& CMV, the high rate occurrence in housewives ,reflects poor hygienic condition & low level of sanitation at women, because most of them arte not educated , in addition ,they were more exposure to infectious agents during submitting house services(contact with meat & vegetables & with carriers).

Table (1) Sero- Positive & Negative Rates of Toxoplasma Rubella & Cytomegalovirus among Women in Kirkuk Province

Tests	T. No. Exam.	%	No. +ve	% +ve	No. -ve	% -ve
TOXO	184	52.72	17	4.84	167	47.85
RV	75	24.06	31	8.88	44	12.6
CMV	84	21.48	28	8.02	56	16.04
Total	343	100	76	22.15	267	78.85

$P < 0.05$

Table (2) Distribution of Sero-Positive Antibodies of Toxoplasma, Rubella & Cytomegalovirus as IgM, IgG & IgM + IgG Antibodies

			Tests	T. No. Exam.	No. +ve	% +ve	IgM Ab.	IgG Ab.	IgG + IgM + IgG Abs
				No. +ve	% +ve	No. +ve	% +ve	No. +ve	% +ve
Toxo	184	17	4.84	3	3.39	13	17.0	1	1.31
Rv	75	31	8.88	24	31.5	5	6.75	2	2.63
CMV	84	28	8.02	13	17.6	6	7.89	9	11.84
Total	343	76	22.1	40	52.6	24	31.5	12	15.88

$P < 0.05$

Table (3) Relationship between Distributions of Sero-Positive Toxoplasma Antibodies & Age ($P > 0.05$)

Age Groups /years	T. No. Exam	%	T. No. +ve	% +ve	TOXO Sero +ve	
					No. +ve	% +ve
18...25	160	46.64	35	21.87	8	22.85
26...35	134	39.06	30	22.38	7	23.33
35...41 above	49	14.04	11	22.44	2	18.18
Total	343	100	76	22.15	17	22.36

Table (4) Distribution of Hepatitis B Virus (HBS), Hepatitis C (HCV) among Sero-Positive for Toxoplasmosis (Toxo), Rubella (RV) & Cytomegalovirus (CMV) & in Control Group

Tests	No. +ve	% +ve	HBS		HCV	
			No. +ve	% +ve	No. +ve	+ve %
Tox	17	4.48	1	1.31	2	2.63
RV	31	8.88	4	5.26	2	2.63
CMV	28	8.02	1	1.31	2	2.63
Total	76	22.15	6	7.89	6	7.89

Total serum number examined =343 P > 0.05

Table (5) Frequency of Abortions & Stillbirths in Women Tested for Sero-Toxoplasmosis

Pregnancy lost	Numbers	Percentages %
A-Abortions		
Single	65	35.32
Double	72	39.13
Triple	39	21.19
Tetrad & above	7	3.80
Total	173	99.45.*
B- Stillbirths		
Single	85	46.19
Double	46	25
Triple	14	7.6
Tetrad & above	14	7.6
Total	159	86.41
c- abortions& stillbirths	75	40.76

* P < 0.05

Table (6) Relationship between Women Occupations & Frequency of Sero-Positivity of Toxoplasmosis, Rubella & Cytomegalovirus

occupation	T. No. Exam.	%	T. No. +ve	% +ve	RV	CMV		TOXO	
				No. +ve	% +ve	No. +ve	% +ve	No. +ve	% +ve
Housewives	216	76.09	60	17.7	25	7.28	23	6.7	12	3.49 *
Teacher	38	11.07	8	2.33	4	1.16	2	0.38	2	0.85
Officers	38	10.78	8	2.33	2	0.85	3	0.38	3	0 87
Total	343	100	76	22.1	31	9.03	28	8.81	17	4.95

$P < 0.05$

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