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ORIGINAL ARTICLE

Role of *Cytomegalovirus* and *Toxoplasma gondii* in women abortion and congenital anomalies in Kirkuk city using ELISA

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ABSTRACT

Key words:

Toxoplasma, Cytomegalovirus, Abortion, Spiramycin, Acyclovir and BOH

Background: *Bad obstetric history (BOH) due to Cytomegalovirus (CMV) and Toxoplasma gondii among women with abortion and previous congenital anomalies were watched in Kirkuk city-Iraq.* ***Objective:*** *To achieve this aim a total of 770 serum samples were collected from women attending Ibn-Nafies medical private lab, who complaining from single or more of abortions. Sera were collected, separated and examined by using ELISA machine and using both IgM and IgG kits for both infectious agents. The second aim was to study the impact of 200 mg twice daily and 3million unit twice daily were administrated by some positive women against Cytomegalovirus and Toxoplasma gondii respectively .Positive cases were watched for rising titer .****Methodology:*** *7 positive women for Cytomegalovirus and 11 women positive for toxoplasmosis were agreed to continue these trials which extends for 3 months involve 3 trials of retesting both infectious agents by ELISA.* ***Results:*** *The overall positive rate for toxoplasmosis and Cytomegalovirus antibodies was 71.94% in 554 sera. This rate was divided into 49.87 % for Cytomegalovirus antibodies and 22.07 % for toxoplasmosis, P<0.05. Primary infection rates contributed 7.79 % and 6.10 % from the overall rate; whereas IgG antibodies positive 36.23 % and 13.89 % were found for Cytomegalovirus and toxoplasmosis respectively, p< 0.05. Moreover 47 (6. 10%) of the tested sera exert both IgM and IgG antibodies with high frequency 45(5.84 %) for CMV compare to 2(0.25%) for Toxoplasma gondii ; p<0.05 . Equivocal antibody levels (from 0.9 to 0.99 IU/ml) were recorded in 61 sera 7.92 %. Therelationship between both infectious agents' distributions with seasons was not significant. The impacts of acyclovir and spiramycin administrations exert the following results, for CMV-IgMmean antibody (1.18, 0.88 and 0.64 IU/ml) was obtained for first, second and third month respectively. While the same patients' sera reveal increasing CMV-IgG antibody titers from 1.37 in the first month to 3.20 IU/ml in the third month. Only sera belongs to patient number 7 remains 0.9 IU/ml equivocal. A Cure rate for CMV-IgM was 85.71 %. While all sera show increasing of CMV-IgG Abs titers (100 %) of cures. Spiramycin influences on toxoplasmosis showmean IgM antibodies rates: 1.28, 0.99 and 0.47 IU/ml for first, second and third months respectively p<0.05. Watching rising titer also determined as of IgM antibodies; only 7 sera reveal rising titer of one fold increase while the rest 4 sera no reveal any increase in spite of their IgM antibodies were declining below than 1.0 IU/ml (negative). The following total means of IgG antibodies 1.03, 1.23 and 1.90 IU/ml were recorded for first, second and third months respectively.* ***Conclusions:*** *CMV and toxoplasmosis rates among women with BOH were high. For getting a sure about the real role of spiramycin and acyclovir employee against Toxoplasma gondii and CMV respectively; further studies using a large number of patients and other drug doses are demanded, because the obtained results in the current study was primary.*

INTRODUCTION

Bad obstetric history (BOH) among women in Iraq particularly in Kirkuk Province was high[1]. It causes prenatal and perinatal infections falling under the designation of TORCH agents[2], which involve *Toxoplasma gondii*, Rubella virus, cytomegalovirus (CMV), and herpes simplex virus (HSV),[3]. Bad obstetric history (BOH) implies previous unfavorable fetal outcome in terms of two or more consecutive spontaneous abortion, history of intrauterine fetal death, intrauterine growth retardation, still births, early neonatal death and/or congenital anomalies [4].

Toxoplasmosis is the most widespread zoonosis and an important human disease particularly in children where it can cause visual and neurological impairment

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and mental retardation [5].The parasite is belongs to apicomplexa protozoan parasite called *Toxoplasma gondii* that parasitized a wide range of host, any warm-blooded animals by the different means; more common by ingestion of the oocysts through soil or water [6]. *Toxoplasma gondii* infection can cause severe neurologic-ocular disease in the fetus during human pregnancy. Humans acquire their infections from ingestion of oocyst contaminated soil and water, from tissue cysts in undercooked meat by transplantation, blood transfusion, laboratory accidents or congenitally [7].

Most people infected after birth are asymptomatic, some may develop fever, malaise and lymphadenopathy. Congenital toxoplasmosis often results in debilitating ocular disease, causing among other manifestations, retino-choroiditis and anterior uveitis

Congenital[8]. Historically, women demonstrating exposure to *T.gondii* prior to pregnancy through serology were considered safe from future infection and risk to the fetus. However, apparent recrudescent infections during pregnancy can occur in immune-competent mothers, although few cases have been reported[9].An attention for toxoplasmosis was towered after economic sanction against Iraq, when a high rate of women sera show high rate 55.3 % of toxoplasma antibodies[10],and the followed studies most of concern with serological diagnosis [11,12,13,14,15].

Cytomegalovirus is the most common congenital infection in the UK affecting around 3 per 1000 births [16] and can cause neurological impairment such as hearing loss[17]. In utero transmission of CMV can occur following primary maternal infection during pregnancy but can also occur in women with natural immunity, either because they reactivate latent virus or become re-infected with a different strain[18]. CMV can also be transmitted prenatally from mother to child where it is usually asymptomatic, except in premature babies[19]. Postnatally, CMV can be transmitted from mother to child through breastfeeding and close contact. In developed countries like the UK where breastfeeding is less prevalent and of shorter duration than in developing countries, child to child transmission of CMV is common in day care and similar settings. Early childhood infection with CMV is usually asymptomatic or causes only mild, flu-like symptoms. Uninfected adults in regular contact with young children are at risk of CMV infection, of particular significance for female childcare workers of childbearing age[20]. The risk of intrauterine transmission of cytomegalovirus (CMV) during pregnancy is much greater for women who contract primary CMV infection after conception than for women with evidence of infection (circulating CMV antibodies) before conception. Thus, laboratory tests that aid in the identification of recent primary CMV infection are important tools for managing the care of pregnant women suspected of having been exposed to CMV. CMV IgM detection is a sensitive marker of primary CMV infection, but its specificity is poor because CMV IgM is also produced during viral reactivation and persists following primary infection in some individuals [21]. Incidence of CMV in the world was fluctuated, the following rates were recorded: In Japan an earliest study was done on watching CMV-IgG antibodies among women 18 years old, they found that the primary record rate 93.2 % was declined to 66.7 % from 1980 to 1998 using complement fixation test; they conclude that age and parity of pregnant women associated with the immune status; 35.6% of recent young prematures were susceptible [22] .

In Palestine in a serological study only women 6 % sera reveal CMV-IgM positive [23], In Turkey TORCH agents among women was studied, they found high rate of CMV-IgG 94.9 % versus to 0.4 % of women sera bearing CMV-IgM antibodies[24]. In the same country; Uyar et al, reported high rate of seropositive CMV 97 % also from which only 1 % of women sera showed primary CMV –IgM[25]. In Ireland a retrospective study was performed on 572 women with in 3 consequence years for watching rising titer of CMV-IgM antibodies; they found only 37 women has CMV-IgM antibodies [26]. In Iraq, particularly in Kirkuk Province studies about CMV are very rare except, the aspirate study was carried out on 343 women with abortion and congenital anomalies using ELISA technique by [27], who found 24.35 % of CMV sera positive from which CMV-IgM contribute 17.6 % versus to 6.75 % for CMV-IgG sera positive .

Spiramycin is a macrolide antimicrobial agent that is very active against gram positive bacteria and increasing resistance has been recorded against *Streptococcus pneumoniae*.[28].Spiramycin also has activity against some gram-negative bacteria and even on *Chlamydia, Legionella pneumophilia* [29].Cross-resistance between spiramycin and erythromycin has been reported [30]. After the finding of Pyrimethamine (classical anti-apicomplexian drug) toxicity for bone marrow spiramycin as less teratgenic and has been found to be safe in the pregnant woman so it has been used alternatively during pregnancy and congenital toxoplasmosis [31].Also it reduces the transmission of toxoplasmosis from the pregnant woman to the fetus ; however, it will not affect the severity of disease in an already infected fetus[32].

The mechanism of action of spiramycin is not clear, however it is thought to reversibly bind to the 50 S subunit of bacterial ribosomes, resulting in blockage of the trans-peptidation or translocation reactions, inhibiting protein synthesis and subsequent cell growth[33]. It is primarily bacteriostatic, but may be bactericidal against more sensitive strains when used in high concentrations. Spiramycin also accumulates in high concentrations in the bacterial cell. Unlike

erythromycin, spiramycin does not produce gastrointestinal motility stimulation [29].

Spiramycin is highly concentrated in tissues, such as the lungs, bronchi, tonsils, sinuses, and female pelvic tissues. These high tissue concentrations persist long after serum concentrations have fallen to low levels. Peak concentrations in the saliva are 1.3 to 4.8 times greater than those found in the serum. Spiramycin crosses the placenta and is distributed into breast milk; however, fetal blood concentrations are only 50% of the maternal serum concentrations. Concentrations in the placenta are up to 5 times higher than the corresponding serum concentration. High concentrations are also found in the bile, polymorph nuclear leukocytes, and macrophages. Biliary concentrations are 15 to 40 times higher than the serum concentration. Spiramycin does not cross the blood-brain barrier [34] .

Despite the lack of virus-specified thymidine kinase activity, human cytomegalovirus may be sensitive to acyclovir in vitro at concentrations between 10 and 25 mg/l. The inhibitory effect of acyclovir can be further increased by the presence of small amounts of human alpha or beta interferon. Twenty-one allogeneic marrow graft recipients with biopsy-proven cytomegalovirus pneumonia were treated with either high doses of acyclovir (eight patients) or the combination of acyclovir and human alpha (leukocyte) interferon (13 patients). Acyclovir doses of 400 to 1200 mg/m2/dose and interferon doses of 2 to 40X10(4) units/kg/day were used [35] .

To exposure the light on the incidences of Toxoplasmosis and *CMV* as causes for women abortion and congenital anomalies outcomes and to evaluate role of spiramycin against *Toxoplasma gondii* and acyclovir against *CMV, so* this study was conducted to achieve the aims.

METHDOLOGY

Patients:

Mixed cross sectional and retrospective study were carried on in Ibn-Nafies private medical lab-Kirkuk Province-Iraq. For detecting *Toxoplasma gondii* and *CMV* in sera 770 of women who choosed and referred by obstetric and gynecologists in private clinics in Kirkuk Medical street. For each woman 5 ml of venous blood was drawn and sera were extracted in other tube, kept in deep freeze till to processing using both IgM and IgG ELISA kits. Women ages ranged from 18 to 50 years .majority of women has one or more than one abortion and congenital anomalies pregnancy outcomes.

Methods:

For assessing the role of spiramycin in treatment of toxoplasmosis, 11 *Toxoplasma* sero-positive women were enrolled in the spiramycin trials for three consecutive months using 3million unit twice per day (No specific drug company used for purchasing the drug). At the end of each month, *Toxoplasma gondii* both antibodies were checked using ELISA machine.

On the other hand only 7 sero-positive for CMV were agreed to participate in acyclovir employee against CMV infection. Twice daily 400 mg of the drug was administrated by each women for three months same. At the end of each trial (one month) , new samples of sera were drawn from women to determine level of CMV in their sera.

For both acyclovir and spiramycin treatment IgM antibodies were watched for declining below 1 IU/ml as negative value while for IgG antibodies were watched for rising two folds or more folds titer increases.

For each of *Toxoplasma gondii* and CMV positive 10 women refused to take any treatment against toxoplasmosis or CMV were agreed to participate the trials for three months as control group. Prior to blood sampling a special questionnaire was completed containing all information regarding the study and their signature for participation in the experiments.

Interpretations of laboratory methods

Toxoplasma antibodies levels using ELISA technique were taken in consider as the level of IgM or IgG below 0.9 IU/ml considered negative and from 0.9 to 0.99IU/ml is equivocal limit and should be rechecked ,while positive level is equal to 1.0 IU/ml or above.

Statistical Analysis

The statistical analysis was performed using statistical analysis system (SPSS); version 16. (SPSS Inc. Chicago IL. USA). Frequency and percentage were used with qualitative data. Z-test and Chi-square were used to compare frequencies.

RESULTS

From a total of 770 sera Elisa testing only 554(71.94 %) was positive for toxoplasmosis and *Cytomegalovirus* antibodies. This rate was divided into 384 (49.87 %) for *Cytomegalovirus* antibodies followed by 170(22.07%) for *Toxoplasma gondii* antibodies, P<00.5. Primary infections were correlated to IgM antibodies for both infectious agents with no significant differences between them via which the following rates 6.10% and 7.79 % were recorded for toxoplasmosis and cytomegalovirus respectively. On the other hand the total rate for both infectious agents 13.89 % when it was compared to IgG 51.94% ;statistically showed significance p<0.05. Regarding CMV IgG antibodies rate 36.23% was higher than 15.71 % for toxoplasmosis, p<0.05;table -1 .Moreover 47 (6.10 %) of the tested sera exert both IgM and IgG antibodies with high frequency 45(5.84%) for CMV compare to 2(0.25%) for *Toxoplasma gondii*; p<0.05 .

Table 1: Percentages of *Toxoplasma gondii* and *Cytomegalovirus* antibodies rates in sera of women with abortions

Types of infections	*IgM antibodies No and % +ve*	*IgG antibodies No and % +ve*	*IgM and IgG Antibodies***	*Total antibodies****
Toxoplasma gondii	6.10) (47	121(15.71)	2 (0.25)	170 (22.07)
Cytomegalovirus	60 (7.79)	279 (36.23)	45 (5.84)	384 (49.87)
Total	107 (13.89)	400 (51.94) *	47 (6.10)	554 (71.94).

*,**,*** P<0.05 Total number examined : 770 sera.

Equivocal Elisa results (from 0.90 to 0.99 IU/ml) for both infectious agents were arranged in the table-2 which contribute 61 (7.92%) for both infectious agents; that involve 33(4.27%) and 28 (3.62 %) for CMV ant *Toxoplasma gondii*,p >0.05. Statisatically the differences between equivocal antibodies rates of both agents were not significant ; P>0.05.

Table 2: Frequency of equivocal antibody values(0.9 to 0.99 IU) number and percentages in sera of women with abortion.

Types of infections	*IgM antibody No and % ±ve*	*IgG antibodies No and % ±ve*	*Total antibodies No and % +ve*
Toxoplasma gondii	12 (1.55)	16 (2.07)	28 (3.62)
Cytomegalovirus	16 (2.07)	17 (2.20)	33(4.27)
Total	28 (3.62)	33 (4.27)	61 (7.92)

Table-3 was clarifying variations in the incidences of both infectious agents for both types of antibodies through which high rate 167(21.68%) was recorded during winter compare to 86(11.16%) in autumn, p<0.05. Statistically CMV IgG antibodies rate during winter 89 (11.55%) was significant and higher than CMV IgM antibody rate 29 (3.76%). Controversy to *Toxoplasma gondii* antibodies that show no differences in there occurrence; P>0.05 .

Table 3: Seasonal distribution of *Toxplasma gondii* and *Cytomegalovirus* among women. *Toxoplasma gondii* *Cytomegaloviru*

Types of infections	*IgM antibodies No and % +ve*	*IgG antibodies No and % +ve*	*IgM antibodies No and % +ve*	*IgG antibodies No and % +ve*	*Total*
Autumn	14 (1.81)	17 (2.20)	4 (0.51)	51 (6.62)	86 (11.16)
Winter	19 (2.46)	30 (3.89)	29 (3.76)	89 (11.55)	167(21.68)
Spring	1 (0.12)	39 (5.06)	16 (2.07)	67 (8.70)	123 (15.97)
Summer	13 (1.68)	35 (4.54)	11 (1.42)	72 (9.35)	131 (17.01)
Total	47(6.10)	121(15.71)	60(7.79)	279 (36.23)	507 (65.84)

Total number examined 770 sera

The influence of safety macrolide spiramycin was accessed on rising IgG antibodies titer and decreasing level of IgM by administration of 3 million unit twice daily in 11 seropositive cases through the current study. This experiment was extended for 3 consecutive months within which the following mean IgM antibodies rate were obtained: 1.28, 0.99 and 0.47 IU/ml for first, second and third months respectively p<0.05. Watching rising titer also determined as of IgM antibodies; only 7 sera reveal rising titer of one fold increase while the rest 4 sera no reveal any increase in spite of their IgM antibodies were decline below than 1.0 IU/ml (negative). The following total means of IgG antibodies 1.03, 1.23 and 1.90 IU/ml were recorded for first, second and third months respectively table(4).

Table 4: *Toxoplasma gondii* antibodies levels during patients follow up through treatment by spiramycin drug 3million unit twice daily.

Patient numbers	*IgM in the first trial*	*IgM in the second trial*	*IgM in the* third trial*	*IgG in the First trial*	*IgG in the Second trial*	*IgG in the Third trial*
1	1.35	0.68	0.31	1.15	1.22	4.10
2	1.47	1.14	0.47	1.10	1.35	2.23
3	0.87	1.12	0.77	1.14	1.18	2.14
4	1.34	0.92	0.72	1.04	1.43	1.67
5	0.99	0.87	0.34	1.68	2.56	3.34
6	1.35	0.89	0.28	1.10	1.22	2.32
7	1.90	1.58	0.54	0.55	0.68	0.78
8	1.27	1.0	0.8	0.78	0.97	0.88
9	0.95	0.66	0.47	0.43	0.56	0.72
10	0.94	0.55	0.52	1.13	1.58	1.92
11	1.74	1.54	0.53	0.77	0.83	0.89
Total mean	1.28	0.99	0.47	1.03	1.23	1.90

*P<0.05

The impact of Acyclovir drug administration against seven CMV positive cases was applied by 200 mg of Acyclovir orally twice daily for three months during each month both IgM and IgG of CMV antibodies were tested by using Elisa subsequently. The results were arranged in table-4; via which the following results for CMV antibody were recorded (1.18, 0.88 and 0.64 IU/ml) for first, second and third month respectively. Only sera belongs to patient number 7 remains 0.9 IU/ml equivocal. Cure rate was 85.71%. Whereas CMV IgG antibodies rates show two fold increases in titers from initial test and all sera show 100% of cures as the following rates were recorded 1.37, 1.76 and 3.20 IU/ml for first ,second and third month respectively.

Table 5: Cytomegalovirus antibodies levels during patients follow up through treatment by acyclovir drug 200 mg twice daily.

Patient numbers	*IgM in the first trial*	*IgM in the second trial*	*IgM in the Third trial*	*IgG in the First trial*	*IgG in the Second trial*	*IgG in the Third trial*
1	1.26	1.12	0.79	1.03	1.62	4.48
2	1.82	1.22	0.83	1.54	2.42	4.46
3	0.92	0.54	0.49	1.43	1.63	2.36
4	1.43	1.15	0.47	1.58	1.74	3.21
5	0.99	0.53	0.60	1.20	1.29	1.62
6	0.97	0.70	0.44	1.58	1.78	1.81
7	0.93	0.90	0.90	1.26	1.84	4.49
Total means	1.18	0.88	O.64*	1.37	1.76	3.20 **

*,** P<0.05 .

The sera of 10 women positive for toxoplasmosis and the same number for cytomegalovirus positive when lifted without treatment, the following means of both antibodies were watched in consecutive 3 months .For *Toxoplasma gondii* 1.32 IU/ml, 1.38 IU/ml and 0.98 IU/ml were recorded parallel to 1.35IU/ml, 1.67IU/ml and 1.97 IU/ml for IgG antibodies respectively for first, second and third months. While for *Cytomegalovirus* the following means 0.99IU/ml , 1.22 IU/ml and 1.09 IU/ml were obtained in first ,second and third months respectively. On the other hand *Cytomegalovirus* IgG Abs means reveal 1.65 IU/ml, 2.13IU/ml and 2.46 IU/ml for the first, second and three months respectively also.

Table 6: Total means of both IgM and IgG antibody levels of *Toxoplasma gondii* and *Cytomegalovirus* in sera of women positive lifted without treatment.

Types of infections	*IgM Abs In the first month*	*IgM Abs In the second month*	*IgM Abs In the Third month*	*IgG Abs In the First month*	*IgG Abs In the Second month*	*IgG Abs In the third month*
Toxoplasma gondii total means	1.32	1.38	0.98	1.35	1.67	1.97
Cytomegalovirus total means	0.99	1.22	1.09	1.65	2.13	2.46

DISCUSSION

The overall rate of *Toxoplasma* rate 22.07 % in the present study is high .This high rate can reflects the degree of the environmental contamination with *Toxoplasma* infective stage the oocysts, because the present study was carried out during the critical and unstable condition facing Iraq and Kirkuk Province particularly, with in which the electric power was continuously interrupted that affect food storing and lead to problems in water supplies; in addition to the lack of insecticides and its good quality to kill the mechanical vectors , all these factors may had role in highlighting why the overall rate of toxoplasmosis in the present study was high. The overall rate of toxoplasmosis in the present study was not agree with that recorded in the same province by [10,12,14], whom they record the following rates: 33.6%, 35.6% and31.15 % .Also it was disagreed with those recorded [36,3,15] , whom they record 92.1% 48.9% and 38.56 % of toxoplasmosis in the same province respectively. These findings of the regional prevalence of *T. gondii* seropostivity was higher than those detected by other studies including Egypt by [37,38]. In Turkey by [39,28]. In Malaysia [40], India by [41] In Saudi Arabia by [42]. In Mali [43] and in northeast Thailand by[44]. Also it was very lower when it was compared with 83.6 % in Ethiopia recorded by [45]. The variances in the rates might be attribute to several factors, such as size of samples, type of laboratory method, site of the study and type of the patient(infected not infected with other infectious agents),(Salman, 2007d).

Regarding the all rate of CMV-IgG Abs 49.87 %, this rate was higher than 34.92 % recorded in the same province by [1]. The result of the present study is disagree with that recorded in India by[46], whom they record 15.98 % of seropositive CMV. The difference may be due to very large score of sampling 1918 samples. Interpretation of high rate of sero-positive CMV 34.92 % required understanding the fact, that CMV has the capability to persist in its human host indefinitely as latent infection in several glands and the kidneys [47]. 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 and to inhibit NK cell killing.

Interestingly the rate of both infectious agents regarding IgM and IgG antibodies distribution was critical to women's health, because the rate of women with primary or recent toxoplasmosis and CMV 107(13.89%) was very high. This finding may interpret why the abortion is more common in this governorate. It was signaling to vertical transmission particularly, when some women may get infected in the third period of gestation (Congenital toxoplasmosis or CMV). Moreover, it was suggested that pregnancy may reactivate the latent virus leading to further reproductive wastages. This finding was lower than those recorded in Iraq by [1]. The difference might be due to large number of samples 770 in current study versus to 252 the previous study, recent events after 2014 that destruct the infra base ,displaced peoples who migrate from neighbor Provinces to Kirkuk Province give rise big crowding and increasing level of contamination by direct contact specially for CMV, active transporting the tachyzoites of *Toxoplasma gondii* through blood donation, consumption of un properly meat because continuous electric power interruption in this Province. All of these factors collectively in addition to the poverty, low immune status due malnutrition may explain the high rate BOH due to CMV and toxoplasmosis in the current study.

The advantages of ELISA test are in exerting equivocal levels of testing antibodies, which depends on the designed cutoff for each test. Therefore 61(7.92 %) of equivocal positive for toxoplasmosis and CMV was very significant for laboratory doctor and gynecologist, because this (0.99 to 0.99 IU/ml) was silent and mostly not found in other diagnostic test. This benefit increases the flexibility and efficacy of ELISA test in detecting infectious agents including TORCH agents. Considering the IgM Abs for both agents was vital to exclude primary infection when the IgM of the same patient will be watched within 2-3 weeks, if the IgM increased therefore this case is typical of recent or primary infection. Contrary equivocal or low IgM Abs so it means residual IgM (false IgM) or the patient get cured. On the other hand, this watching should be parallel to IgG because when the latter increased one or two folds and IgM declined this mean protection against previous infection, because it has been known that CMV infection subside with producing lifelong immunity as the Toxoplasma gondii infection produce sterile immunity [3,12,13,14].

Seasonal variations in the distribution of *Toxoplasma gondii* and *CMV*, particularly high incidence 21.68 % during winter compared to 11.16 % during theautumn months can be explained by the fact the that summer and autumn weather are very hot in Kirkuk city and most of the infective stage of *Toxoplasma gondii* will destruct. While the climate in winter was rainy and fine. The second suggestion to high rate in winter might the patients get an infection during the autumn, complete incubation period and the disease appear. This finding was close and agreed those recorded by[10,14,11]. Moreover CMV IgM (3.76 %) in winter lower than IgG (11.5%) in the same season might be related to patients visiting clinics more than other season. In general, high rate of both infectious agents'

frequencies might be attributed to high rate of environmental contamination and poor hygienic condition in this Province.

Literatures about using retrospective study in the treatment of toxoplasmosis by spiramycin are rare, therefore the followings can be taken in consider in interpreting the results. It was very obvious from the result in a table (4). That spiramycin had excellent impact in rising titer of *Toxoplasma*-IgM Abs in sera of all the 11 women participate the experiment as the mean IgM in the first month 1.28 IU/ml get decline to 0.99 IU/ml in the second month (6 sera equivocal and 5 sera positive) and lastly the mean level was reached to 0.47% (negative Toxo-IgM Abs).The real mechanism of spiramycin as antibacterial macrolide against protozoan parasite was not clear ,but it may be thought that spiramycin in the animal cells including *Toxoplasma gondii* will bind to the 50S subunit of parasite ribosomes, resulting the blockage of the trans-peptidation or translocation reactions , inhibiting protein synthesis and subsequent cell death as the spiramycin reveal this type of mode action in most of gram positive bacteria [48,49]. Another suggested explanation is based on the usage of high dose 6 million Units in current study via which the high concentrated spiramycin on the parasitophorous vacuole inclosing *Toxoplasma* trophozoites (Bradyzoites) within the cysts may interfere with some enzymes on the wall leading for pore formation and subsequently death of the trophozoites [50]. Moreover the cure rate might be related to time as it was known that the IgM and IgA antibodies persists 3 to 6 months in sera and converted into IgG antibody classes [51].

Considering the *Toxoplasma* IgG Abs (1.03, 1.23 and 1.90 IU/ml) within which, only 7 women sera gets rising titers while 4 sera reveal failure and not raised . This finding refers to good immunization from previous toxoplasmosis in 7 women while the rest may face reactivation or sero-conversion and showing recurrent toxoplasmosis that might be associated with miscarriages or congenital anomalies in next gestation [1].

Regarding Administration of 200 mg twice per day orally by 7 volunteer women the level of CMV-IgM Abs 1.18 in the first trial was declined into 0.88 and 0.64 IU/ml in next second and third months. This finding means complete blocking CMV shedding in spite of one serum remain equivocal. The action of blocking might be attributed to drug damage on thymidine kinase in CMV that leads to mutation in the infected cell with CMV, subsequently, will damage the double strand DNA of CMV leads to death of the infected cell and eventually the CMV [52]. Although it has been found that acyclovir and its derivatives show any side effect such as thrombocytopenia, leucopenia and depress bone marrow, but patients enrolling the study not show any kinds of side effects ,This might be related to usage of moderate dose of the acyclovir. On the other hand good level of rising titers of IgG Abs may reflect good status of women immune system and good responses to the drug and prescribed dose [53]. Furthermore the sera belongs to patient number 1 ,2 and 7 reveal 4 fold of IgG increases this may highlight that they get long life immunity against previous CMV infection.

Considering control group, it was clear that without any anti-toxoplasmosis or CMV prescription women are risk particularly, for sero-conversion because the difference in the level of IgM for both agents were positive and persist high. Meanwhile CMV IgG show one fold of increase that will evoke lifelong immunity produced against previous CMV infection. Contrary to CMV-IgG; Toxo-IgG Abs shows no significant increase this, may refer to partial or incomplete immunization or protection .

In conclusion: The rate of CMV and toxoplasmosis among women in child bearing age in Kirkuk province was high. ELISA was high efficacy method for demonstrating the infectious agents of BOH particularly the equivocal levels of IgM and IgG Abs. In spite of good results were obtained by prescribing 6 million units of spiramycin and 400 mg of acyclovir daily for a period of 3 months for women with toxoplasmosis and CMV infection, but these results were preliminary to be confirmed by using large number of women and adding drug monitoring by serum examination to prove the net efficacy of the used drugs in the current study.

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