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Determination of Toxoplasmosis Disease Activity by Estimation of IL-6 Cytokine

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Abstract

Toxoplasma gondii (Nicolle and Manceaux) infects all warm-blooded animals, including humans. Early diagnosis and determining the infective stage are critical for treating immunosuppressed individuals and pregnant women with toxoplasmosis. This parasite modulates pro- and anti-inflammatory responses to regulate parasite multiplication and host survival. The aim of this study was to investigate the probability of using IL-6 as a marker of toxoplasmosis disease activity (acute and chronic) in different groups of women (miscarriage, pregnant and single) and estimate the relationship between infection and gestational age and history of abortion in miscarriage and pregnant women. The most abortion were occurred at the first trimester in chronic infected miscarriage women also, the most abortion were occurred at the first trimester in acute infected pregnant women. The result showed that acute infected miscarriage women with previously abortion scored high significant percentage of infection in comparison to non- abortifacient, chronic infected one and pregnant women. IL-6 was significantly higher in acute infected miscarriage women in comparison to single and pregnant acute one but chronic infected pregnant women characterized by the lowest level of this cytokine in comparison to all studied groups.

Keywords: toxoplasmosis, IL-6, miscarriage, pregnant.

تحديد نشاط داء المقوسات الكوندية بواسطه تقدير مستوى الانترلوكين 6

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الخلاصة

طفيلي *Toxoplasma* تصيب كل الحيوانات ذوات الدم الحار من ضمنها الانسان .التشخيص المبكر وتحديد الطور المعدي يكون ذو اهميه بالغه في علاج افراد الاشخاص ذوي المناعه المنخفضه والحوامل المصابات بداء المقوسات الكوندية. هذا الطفيلي يتوسط الاستجابه البادئه والمضادة للالتهابات لتنظيم تكاثر الطفيلي وبقاء المضيف. الهدف من هذه الدراسه هو للكشف من امكانيه استخدام انترلوكين 6 كدلاله لنشاط داء المقوسات الكونديه (الحاد والمزمن) في مجاميع مختلفه من النساء (مجهضات ، حوامل وغير متزوجات) وتقدير العلاقة بين الاصابه ومدة الحمل و تاريخ الاجهاض في النساء المجهضات والحوامل. اغلب الاجهاضات حصلت في الاشهر الاولى من الحمل في النساء المجهضات ذوات الاصابه المزمنه ، كذلك اغلب الاجهاضات حصلت في الاشهر الاولى من الحمل في النساء الحوامل ذوات الاصابه الحادة. اظهرت النتائج انه النساء المجهضات ذوات الاصابه الحادة والمجهضة سابقا سجلت اعلى نسبه اصابه للمرض مقارنة بالنساء المجهضات ذوات الاصابه المزمنه غير المجهضات سابقا والنساء الحوامل. وبينت النتائج ان مستوى انترلوكين 6 كان

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عاليا معنويا في النساء المجهضات ذوات الاصابه الحادة مقارنة مع الغير متزوجات والحوامل ذوات الاصابه الحادة لكن النساء الحوامل ذوات الاصابه المزمنه تميزت بمستوى اقل من هذه ا مقارنة مع كل المجاميع المدروسه.

Introduction

Toxoplasma gondii is an important zoonotic intracellular protozoan parasite, which can affect all warm-blooded mammals and birds throughout the world, including humans [1]. It is transmitted by ingestion of tissue cysts from undercooked or raw meat, consumption of food or drinking water contaminated with oocysts, or ingestion of oocysts from the environment by accident. Nearly one third of the global human population has been infected with *T. gondii*, however, infection in healthy individuals is usually asymptomatic and only a small percentage of exposed people have obvious clinical symptoms [2].

Furthermore, if a primary infection with *T. gondii* occurs during pregnancy .Despite the mechanisms in place in the intestine to control *T. gondii*, a number of parasites are still able to migrate across the epithelium via complex processes and disseminate to other cells, tissues and organs. *T. gondii* can invade and survive quite well inside cells such as dendritic cells (DCs) and macrophages, and takes

Serologic testing is often the first step in diagnosis, using IgG and IgM antibodies. The diagnostic challenge is differentiating between a primary and a chronic infection, and results of IgG and IgM testing can often be difficult to interpret. For this reason, it is important to consult with an expert in this area when confirming the diagnosis [3].

Consequently, studies on immunity to *T. gondii* have focused on the role of T cells in resistance to this organism, however, it is now recognized that *Toxoplasma* induces a strong innate immune response that provides a mechanism of resistance during acute infection and influence subsequent adaptive response [4].

Toxoplasmosis is normally asymptomatic in healthy individuals because control of this pathogen result from the host's ability to mount a robust cell-mediated immune response which is dominated by production of interferon-gamma (INF-γ) by NK cells during the earliest stages of infection, and parasite specific cluster of differentiation 4(CD4+) and cluster of differentiation 8(CD8+) T cells thereafter [5,6]. The clinical significance of these event is illustrated by patients with primary or acquired defect in T cell function,in whom a failure to control parasite replication result in overt disease [7].

Historically, macrophages have been considered important effectors of resistance during toxoplasmosis in large part because of early work that established their ability to kill parasites [8] and produce chemokines and cytokines like IL-12 and Tumor Necrosis Factor- alpha (TNF-α), which are critical for the production of INF-γ[9,10]. In the last decade, studies using macrophages and dendritic cells (DCs) have significantly advanced the understanding of the parasite- derived factors and host signaling pathways that are involved in the innate recognition of *Toxoplasma* [11].

Interleukin 6 (IL-6) is produced by a large number of cells, including monocyte macrophages, endothelial cells, and fibroblasts, myelomatous and neoplastic cells. It is main mediator responsible for hepatocytic production of acute inflammatory phase proteins; it exerts a synergetic action with IL-1, TNF- α and glucocorticoids. IL-6 is therefore a pyrogenic factor and a remarkable stress marker. IL-6 increases the cytotoxic activity of Natural killer (NK) cells and later induces differentiation of BL into antibody secreting cells and differentiation of cytotoxic TL. In murine toxoplasmosis, a gradual increase in serum IL-6 is correlated with clinical signs [6].

Materials and methods

A total of three hundred-sixty five women (116 miscarriages, 108 pregnant and 141 singles) were enrolled in this study. They were attended to different hospitals in Baghdad province (Fatima al-Zahraa hospital for women and children, Al-Elwiya Hospital, Baghdad Medical city) during the period from October-2013 till the end of January 2014.

Serological technique: Detection of parasite antibodies was achieved by using (LAT) kit (spectrum-Germany), ELISA kit (human-Germany) for IgG and IgM and ELISA kit (CUSABIO –China) for IL-6. These tests were done according to manufactures instruction.

Statistical analysis: The Statistical Analysis System- SAS (2012) was used to effect of different factors in study parameters. Chi-square test was used to significant compare between percentage & least significant difference –LSD test was used to significant compare between means in this study.

Results and discussion

Out of total 366 blood samples of three different groups of women (miscarriage, pregnant and single) included in this study. 152 samples revealed seropositive of toxoplasmosis by Latex (LAT) giving an incidental rate of 41.53%.The infection with *T.gondii* distributed in different women groups by LAT test as follow: 57/117 (48.71%) miscarriage, 45/108 (41.66%) pregnant and 50/141 (35.46%) in single women. Figure -1.

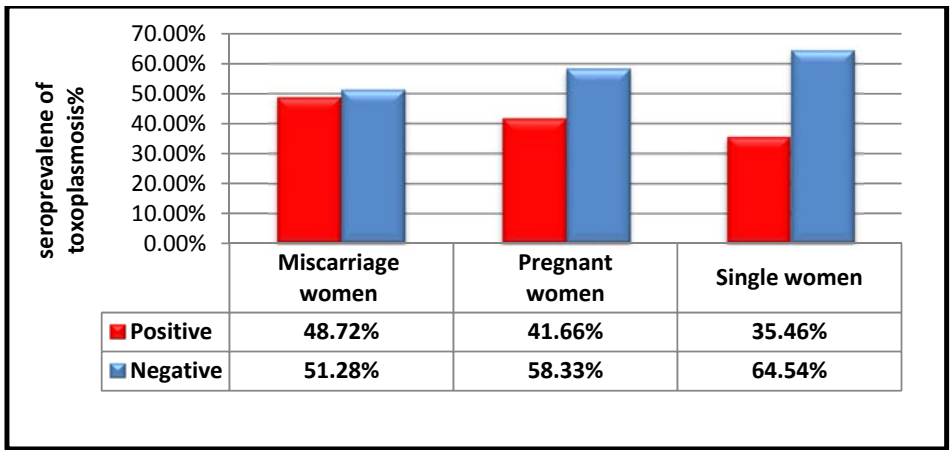

Figure 1- Seroprevalence of toxoplasmosis in different groups of women by LAT test

These differences in comparison to many other studies results may be due to that seroprevalence estimated for human population varies greatly among different geographical areas within one country, and among ethnic group living in the same area [12], or may be attributed to several other factors including cultural level, nutritional habits, age or rural and urban area [13].

Serodiagnosis of *T.gondii* in different groups of women by ELISA IgG test

All positive and negative samples in LAT subjected to another more specific test ELISA, Two tests of ELISA used, the first one is ELISA IgG. The results revealed different percentages of seroprevalence of toxoplasmosis by this test, Miscarriage women also characterized by higher percentage of anti-toxoplasmosis IgG antibodies 62/117(52.991%),while the percentage was 43/141(30.496%) in single women, and pregnant women with the lowest 30/108 (27.777%). The total positive percentage of toxoplasmosis by ELISA IgG was135/366 (36.885%) Figure -2.

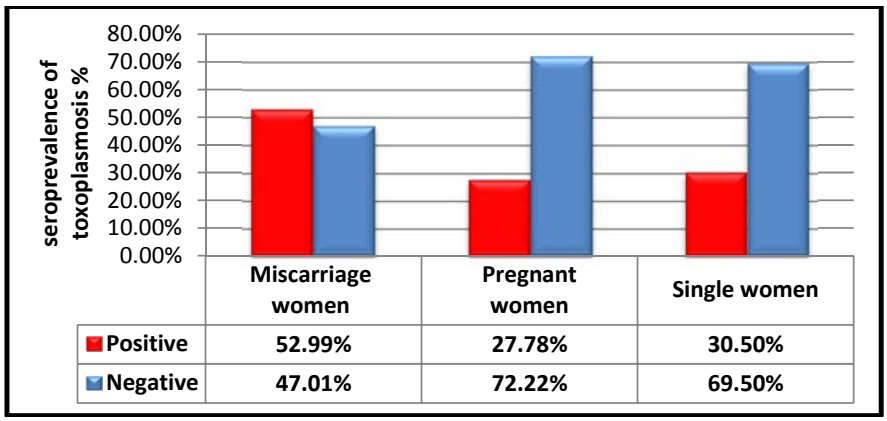

Figure 2- Seroprevalence of toxoplasmosis in different groups of women by ELISA IgG test.

These different results may be happened due to used the same technique with different sensitivity [14] or due to using kits supplied or purchase from companies with low level of quality, in addition to geographical location play an important role in differences in the seroprevalence rate of toxoplasmosis [15]. Other reasons also play a role such as decreasing in health consciousness, increasing of free streets cats and accumulation of trash in different places in the Baghdad province, as well as seropositivity is

higher in hot and humid area [16,17].The variability also may be due to nutrition, habitat, economic state [18].

The most frequent age group of infection with *T.gondii* was 23-28 year in both miscarriage and pregnant women, and it represents 45.161%, 40% respectively. While the most frequent age group for a single woman was 17-22 years, and represent 46.511% of the total number of this group (Table -1). This can be explained by that the number of pregnant women at this age group / year was higher than the others or may be they exposure to the parasite in the past more than others groups by cat contacts or soil exposure and IgG will persist throughout the life of the patients [19].

Table 1- Distribution of toxoplasmosis according to age group/year in studied groups by ELISA IgG test

Studied group		**Age group / year**			**Total**	**Chi-square- χ^2**
		17-22	**23-28**	**29-35**		
Miscarriage women (+ve)	No.	22	28	12	62	8.43 **
	%	35.48	45.16	19.35	100%	
Miscarriage women (-ve)	No.	20	19	16	55	2.07 NS
	%	36.36	34.55	29.09	100%	
pregnant women (+ve)	No.	10	12	8	30	5.27 *
	%	33.33	40.00	26.67	100%	
Pregnant women (-ve)	No.	40	20	18	78	9.14 **
	%	51.28	25.64	23.08	100%	
Single women (+ve)	No.	20	14	9	43	8.77 **
	%	46.51	32.56	20.93	100%	
Single women (-ve)	No.	45	30	23	98	8.39 **
	%	45.92	30.61	23.47	100%	
Total	No.	151	129	86	366	8.42 **
	%	41.26	35.25	23.49	100%	
Chi-square- χ^2	---	8.93 **	8.37 **	5.028 *	---	**---**

Serodiagnosis of *T. gondii* from different groups of women by ELISA IgM test

The second test was ELISA IgM , The results revealed lower percentage of acute toxoplasmosis in different studied groups 25/366(6.830%), miscarriage women showed high percentage of acute toxoplasmosis14/117(11.965%) in comparison to other groups single7/141(4.964%) and pregnant 4/108(3.703) (Table -2).

Table 2- Seroprevalence of toxoplasmosis in different groups of women by ELISA IgM test.

Studied group		**ELISA IgM (+ve)**	**ELISA IgM (-ve)**	**Total**	**Chi-square- χ^2**
Miscarriage women	No.	14	103	117	14.38 **
	%	11.97	88.03	100 %	
Pregnant women	No.	4	104	108	15.07 **
	%	3.70	96.30	100%	
Single women	No.	7	134	141	14.89 **
	%	4.96	95.04	100%	
Total	No.	25	341	366	14.64 **
	%	6.83	93.17	100%	
Chi-square- χ^2	---	2.048 NS	2.048 NS	----	----

In acute stage of *T.gondii* infection IgM antibodies estimation appears early in the course of infection, while IgG antibodies appears too late about three weeks later of IgM appearance and reaches peak level within six months to one year and remains in high level for long duration[20,21].

Diagnosis of *T.gondii* infection which is based on the clinical appearance and serology is not easy. The detection of specific IgM antibodies is the most common method used to determine the time of primary infection and it is crucial for the clinical management of pregnant women [22].

In the current study the presence of IgM class antibodies against toxoplasmosis was interpreted as diagnosis of the acute form of the disease. However, low levels of IgM antibodies are able to persist for many months, even years after acute infection.

this result was in agreement with Al-Obeady [23] who found the percentage of toxoplasmosis among aborted women was 12% , and higher than Al-Rawi [24]who found the percentage of toxoplasmosis was (4.16%c xassewws454),but lower than Kareem [25] who found the percentage of infection among aborted women 44% by using ELISA technique.These variable results may be due to the differences in the type of specimens used by each researcher or due to differences in sensitivity and specificity of the used technique, different laboratory methods in addition to number of samples, time of sampling, play an important role in difference IgM seroprevalence rate of toxoplasmosis [26, 17].

In relation to the age groups, the group of 17-22 year characterized by the highest percentage of acute toxoplasmosis in all studied groups and miscarriage women with the most higher percentage 9/14(62.28%) in comparison to other groups, pregnant and single women (50%)and (42.857%) respectively. (Table -3)

Table 3- Distribution of toxoplasmosis in studied group according to age group/year by ELISA IgM test

Studied group		Age group / year			Total	Chi-square- χ^2
		17-22	23-28	29-35		
Miscarriage women (+ve)	No.	9	4	1	14	10.41 **
	%	64.28	28.57	7.14	100%	
Miscarriage women (-ve)	No.	55	26	22	103	9.96 **
	%	53.40	25.24	21.36	100%	
Pregnant women (+ve)	No.	2	1	1	4	9.74 **
	%	50.00	25.00	25.00	100%	
Pregnant women (-ve)	No.	60	24	20	104	10.05 **
	%	57.69	23.08	19.23	100%	
Single women (+ve)	No.	3	2	2	7	8.53 **
	%	42.86	28.57	28.57	100%	
Single women (-ve)	No.	56	42	36	134	8.25 **
	%	41.79	31.34	26.87	100%	
Total	No.	185	99	82	366	9.85 **
	%	50.55	27.05	22.40	100%	
Chi-square- χ^2	---	8.69 **	2.017 NS	9.62 **	---	---

Serodiagnosis of *T. gondii* in miscarriage and pregnant women by ELISA IgM and IgG tests according to gestational age and history of abortion

(Tables -4) illustrated that the most of abortions were occurred at the first trimester in miscarriages women infected with toxoplasmosis which represented 8/14(57.42%) by ELISA IgM, this percentage was higher non-significant than the percentage in chronic infected women 28/62(45.161%) at the same trimester. While, chronic infected women at the second trimester (4-7 month) characterized by significantly ($P \leq 0.01$) higher percentage of abortion 24/62(38.709%) in comparison to acute infected one 5/14(35.741%) at the same trimester

At the end of pregnancy (third trimester 7-9 month) an interesting results appeared, acute infected women revealed lower percentage of abortion 1/14(7.142%) in comparison to chronic infected women 10/62(16.129%).

These results were accepted according to the fact that showed the rate of clinical pregnancy loss is known to decrease with gestational age from 25% at 5-6 weeks to 2% after 14 weeks [27]. Early studies revealed that the congenital infection essentially occurred only in infants born to women who acquired their primary infection during gestation. Women infected before gestation were not at risk to transmit the parasite to their fetus, unless they were severely immunocompromised [28]. It also became obvious that the primary infection in pregnant women often occurs without clinical manifestations and without a history of the most common epidemiologic risk factors (e.g., ingestion of undercooked or raw meat, or unrecognized ingestion of material contaminated with oocyts) [29].

Table 4- Distribution of infected miscarriage women with toxoplasmosis according to gestational age by ELISA IgM and ELISA IgG tests.

Studied Groups		Gestational age by ELISA IgM test			Total	Gestational age by ELISA IgG test			Total
		1-3 month	4-7 month	7-9 month		1-3 month	4-7 month	7-9 month	
Miscarriage Women (+ ve)	No.	8	5	1	14	28	24	10	62
	%	57.42	35.74	7.14	100%	45.16	38.71	16.13	100%
Miscarriage Women (- ve)	No.	45	30	28	103	23	11	21	55
	%	43.69	29.13	27.18	100%	41.82	20.00	38.18	100%
Total	No.	53	35	29	117	51	35	31	117
	%	45.29	29.91	24.78	100%	43.59	29.91	26.50	100%
Chi-square- χ^2	--	0.548 NS	2.409 NS	1.783 NS	---	0.674 NS	6.943 **	8.025 **	---

(Table -5) showed that the most infected pregnant women with *T.gondii* were occurred at the first trimester in both disease activity acute and chronic phase at the same percentage 50 %. While, the second trimester the chronic phase of infection revealed high significant increase ($P \leq 0.05$) of infected pregnant women 9/30(30%) than those in acute phase 1/4(25%). At the end of pregnancy (third trimester 7-9 month) the results showed that the percentage of infected pregnant women was 1/4(25%) in acute phase which was higher non-significant ($P \geq 0.05$) than chronic infected pregnant women 6/30(20%) at the same trimester.

Table 5- Distribution of infected pregnant women with toxoplasmosis according to gestational age by ELISA IgM and ELISA IgG tests.

Studied Groups		Gestational age by ELISA IgM test			Total	Gestational age by ELISA IgG test			Total
		1-3 month	4-7 month	7-9 month		1-3 month	4-7 month	7-9 month	
Pregnant Women (+ ve)	No.	2	1	1	4	15	9	6	30
	%	50.00	25.00	25.00	100%	50.00	30.00	20.00	100%
Pregnant Women (- ve)	No.	60	25	19	104	47	17	14	78
	%	57.69	24.04	18.27	100%	60.26	21.79	17.95	100%
Total	No.	62	26	20	108	62	26	20	108
	%	57.41	24.07	18.52	100%	57.41	24.07	18.52	100%
Chi-square- χ^2	--	2.68 NS	0.783 NS	2.74 NS	---	4.92 *	4.58 *	0.944 NS	---

The ability of sex- and pregnancy-associated hormones to influence the severity of *T. gondii* infection is of particular public health interest due to the ability of this parasite to cause congenital disease if infection occurs during pregnancy. There is currently considerable evidence that steroid hormones affect the course of toxoplasmosis in humans [30, 31].

At the first trimester the level of sex hormones (Estrogens and progesterone) are low with a little T-helper (Th2) bias. In this case the chance of transmission of the parasite to the fetus is low, while the chance of abortion is high. Conversely, in the second or third trimester, when there is a strong Th2 bias is unlikely to induce abortion with a high chance in congenital transmission [30].

According to history of abortion the result showed that out of 14 miscarriage women acute infected with *T.gondii* only two 2/14 (14.285%) not previously aborted while, all the other twelve

12/14(85.714%) had previously abortion. When the results compared with control group (miscarriage women without toxoplasmosis) they showed that the percentage of acute infected women had significantly P≤0.01 higher percentage (85.714%) of previously abortion than the control group (24.271%). This may related to the infection with *T.gondii* itself especially when previous study suggested its role in the causation of abortions [32] and serious health problems in the fetus if the parasites are transmitted [i.e., congenital toxoplasmosis] and cause severe sequelae in the infant including stillbirth or death shortly after birth, mental retardation, blindness, and epilepsy [33,34].
While, the chronic infected women with *T.gondii* the results revealed that the history of abortion in miscarriage women had significant differences P≤0.01 between both groups (previously aborted and not abortifacients) (51.612%) and (48.387%) respectively (Table -6).

On the other hand acute infected women with previously abortion had significantly higher percentage P≤0.01. (85.714%) than chronic infected women with previously abortion (51.612%). This may relate to early infection with *Toxoplasma* because acute primary maternal toxoplasmosis if acquired during the first trimester of pregnancy can cause significant morbidity and mortality in developing fetuses [35]. The principal modes of transmission are from mother-to-fetus, through food or water contaminated with cat faeces or by eating undercooked meat of infected animals [36, 35].

Table 6- Distribution of toxoplasmosis according to history of abortion in miscarriage women by ELISA IgM and ELISA IgG tests.

Studied Groups		History of abortion by ELISA IgM test		Total	History of abortion by ELISA IgG tes		Total
		Previously aborted	Not abotifacients		Previously aborted	Not abotifacients	
Miscarriage Women (+ ve)	No.	12	2	14	32	30	62
	%	85.71	14.29	100%	51.61	48.39	100%
Miscarriage Women (- ve)	No.	25	78	103	5	50	55
	%	24.27	75.73	100%	9.09	90.91	100%
Total	No.	37	80	117	37	80	117
	%	31.62	68.38	100%	31.62	68.38	100%
Chi-square- χ^2	--	10.28 **	10.28 **	---	9.85 **	9.85 **	---

While the pregnant women in acute phase of infection, the percentage of the women who had history of abortion in compatible to the percentage of not abortifacients women 2/4 (50%), while the women at chronic phase of infection and previously aborted were 20/30 (66.666%) which represented significantly higher P≤0.01 than not abortifacients women 10/30 (33.333%), (Table 7), The explanation of this result may be due to the probability that those women became pregnant within a short period after the first miscarriage while the cell mediated immunity is still active which may led to the second miscarriage. Another cause of miscarriage is due to the false diagnosis and the treatment given by the physicians which was not the right medicine of infection [24].

Table 7- Distribution of toxoplasmosis according to history of abortion in pregnant women by ELISA IgM and ELISA IgG tests.

Studied Groups		History of abortion by ELISA IgM test		Total	History of abortion by ELISA IgG tes		Total
		Previously aborted	Not abotifacients		Previously aborted	Not abotifacients	
Pregnant Women(+ ve)	No.	2	2	4	20	10	30
	%	50.00	50.00	100%	66.67	33.33	100%
Pregnant Women(- ve)	No.	30	74	104	12	66	78
	%	28.85	71.15	100%	15.38	84.62	100%
Total	No.	32	76	108	32	76	108
	%	29.63	70.37	100%	29.63	70.37	100%
Chi-square- χ^2	--	8.52 **	8.52 **	---	10.75 **	10.75 **	---

Level of (IL-6) in serum of different groups of women

Interleukin IL-6 is proinflammatory cytokine that produced at the site of inflammation and plays a key role in the acute phase response as defined by a variety of clinical and biological features such as the production of acute phase proteins [37].

The current results showed that acute infected miscarriages women with toxoplasmosis distincted with significant increase ($P \leq 0.05$) mean level of IL-6 (177.31±12.53 Pg/ml) in comparison to chronic infected miscarriage women (119.36 ± 14.07 Pg/ml), and both infected women revealed significant increased ($P \leq 0.01$) IL-6 in comparison to miscarriages women without toxoplasmosis (98.41 ± 9.63 Pg/ml), as well as the results obtained that infected women with toxoplasmosis (acute and chronic) characterized by highly significant increase ($P \leq 0.01$) of IL-6 level in comparison to non-miscarriage without toxoplasmosis (86.06 ± 4.85 Pg/ml), (Table -8).

Table 8- Mean levels evaluation of serum IL-6(Pg/ml) in miscarriage women in chronic and acute phase of toxoplasmosis.

Studied Groups	Level of IL-6 in Acute phase(Pg/ml)		Level of IL-6 in Chronic phase(Pg/ml)		Comparison of sig.	
	No.	Mean ± SE	No.	Mean ± SE	P-value	Sig.
Miscarriage women with *Toxoplasma*	14	177.31±12.53 a	62	119.36 ± 14.07 b	0.637	*
Miscarriage without *Toxoplasm*	30	98.41 ± 9.63 c	30	98.41 ± 9.63 c	0.803	NS
Non miscarriage without *Toxoplasma*	30	86.06 ± 4.85 d	30	86.06 ± 4.85 d	1.00	NS
Total	74	---	122	---	---	---
P-value	---	0.0027	--	0.0029		
Sig.	---	**	--	**	---	---

These results indicated that the parasite itself may play a role in an increasing the level of IL-6 in infected women than in un-infected one, this result considered normal especially when this cytokine elevated significantly during acute infection. (38 - 41].

The increased serum levels of this cytokine and interferon-γ (INF-γ) were found to correlate with the severity of infection .So the current increased of this cytokine in early infected women reflect the severity of inflammation by toxoplasmosis parasite (tachyzoites).Several cytokines, particularly IL-6, stimulate the production of acute phase proteins in response to varied stimuli. The patterns of cytokine production and the acute phase response differ in different inflammatory conditions. Acute phase changes reflect the presence and intensity of inflammation, and they have long been used as a clinical guide to diagnosis and management [37].

IL-6 may be used as an indicator for disease activity especially when this cytokine increased significantly in acute infection more than chronic infection. The anti-inflammatory mechanisms associated with the control of immune response, elicited to maintain system homeostasis, involves the release of anti-inflammatory cytokine (IL-4, IL-10, and IL-13) [42]. Particular attention is focused on IL-10 which inhibits the release of proinflammatory cytokine, such as TNF-α, IL-1, IL-6 and IL-8 by activated macrophages [43,44].This fact explain the reason of IL-6 dampen in chronic infection with toxoplasmosis.In toxoplasmosis, the *in vivo* administration of an anti-IL- 10 monoclonal antibody to Severe combined immunodeficiency (SCID) mice delays the death of the animals following *Toxoplasma* infection [45].*In vitro*, recombinant IL-10 has immunosuppressant properties on the proliferation of spleen cells taken from mice infected with *T. gondii* [46].And inhibits the capacity of murine macrophages, activated by IFN-γ, to destroy *T. gondii* [47].

In the acute phase of infection the result of IL-6 level in miscarriage women (177.31±12.53 Pg/ml) was significantly ($P \leq 0.05$) higher than acute infected pregnant (145.76±16.82 Pg/ml), But at chronic phase of infection the results of miscarriage women was (119.36 ± 14.07 Pg/ml) higher significantly ($P \leq 0.01$) than pregnant women (48.41 ± 2.79 Pg/ml). The cytokines (like TNF-α and IFN-γ) are abortogenic via up regulation of fg 12 prothrombinase activity [48]. It is widely accepted that term as well as preterm labor is associated with elevated uterine production of proinflammatory cytokines, IL-1β, IL-6, IL-8 and TNF-α [49]. These cytokines are thought to stimulate uterine activity, either directly

or via an increase in prostaglandin production, attraction of leukocytes, tissue remodeling, and it's may cause abortion [50].

While, pregnant women infected with acute toxoplasmosis showed twofold higher significant (P≤ 0.01) level of IL-6 (145.76±16.82 Pg/ml) as compared to non pregnant without *T.gondii* (77.27 ± 4.59 Pg/ml) and pregnant without toxoplasmosis (86.06± 4.85Pg/ml) (Table -9).

This result may be happened due to that the most of studied pregnant infected women were at the first trimester and those low sex hormone (Estrogen and progesterone) and high IL-6 and vise verse in second and third trimester [51].

Table 9- Mean levels evaluation of serum IL-6(Pg/ml) in pregnant women in acute and chronic phase of toxoplasmosis.

Studied Groups	Level of IL-6 in Acute phase (Pg/ml)		Level of IL-6 in Chronic phase(Pg/ml)		Comparison of sig.	
	No.	Mean ± SE	No.	Mean ± SE	P-value	Sig.
Pregnant women with *Toxoplasma*	4	145.76±16.82 a	30	48.41 ± 2.79 b	0.0048	**
Pregnant without *Toxoplasma*	30	86.06± 4.85 c	30	86.06± 4.85 c	1.00	NS
Non pregnant without *Toxoplasma*	30	77.27 ± 4.59 d	30	77.27 ± 4.59 d	1.00	NS
Total	64	---	90	---	---	---
P-value	---	0.0038	---	0.294	---	---
Sig.	---	**	---	*	---	---

The major function of IL-6 is the involvement in the immune response through the action on lymphocytes B. It is a mediator responsible for the production of acute phase proteins and increased cytotoxic activity of NK cells. IL-6 is an early and sensitive, although nonspecific, marker of inflammatory states [52].

On the other hand the level of IL-6 in chronic infected pregnant women was low significantly (P ≥0.01) (48.41± 2.79 Pg/ml) in comparison to acute infected women and other studied groups. (Table -9), [50] found the level of IL-10 to be fivefold higher in the course of toxoplasmosis than in healthy controls. Therefore, that could have expected a decrease in IL-6 production, which was however not found. IL-10 plays an essential role in the inflammatory response during acute *T. gondii* infection, since it inhibits the cellular-type immune response (IL-12, TNF-α) and inflammatory response (IL-6) [53, 54]. IL-10 counteracts the harmful effects of the inflammatory response which is based on the increased production of TNF-α, IFN-γ, and NO associated with intestinal multiplication of *T.gondii*. IL-10 is able to deactivate macrophages, induce IFN- γ by *T.gondii*, and facilitate intracellular parasite survival. IL-10 induces immunosuppression during *T.gondii* invasion, which is beneficial both for the host and the parasite [55].

This study also showed that the level of IL-6 cytokine in single women infected with toxoplasmosis in acute phase of infection (118.98 ± 15.68Pg/ml) was non-significantly (P≥ 0.05) higher than chronic phase (106.65 ± 17.91Pg/ml) and both disease activity were significantly higher (P≤0.05) in comparison to non-infected women (86.06 ± 4.85Pg/ml) (Table -10) this increase may related to the burden of *Toxoplasma* parasite itself that led to occurrence of inflammation and thus increase the inflammatory cytokine.

Table 10- Mean levels evaluation of serum IL-6(Pg/ml) in single women in acute and chronic phase of toxoplasmosis

Studied Groupsw	Level of IL-6 in Acute phase (Pg/ml)		Level of IL-6 in Chronic phase (Pg/ml)		Comparison of sig.	
	No.	Mean ± SE	No.	Mean ± SE	P-value	Sig.
Single women with *Toxoplasma*	43	118.98 ± 15.68a	7	106.65 ± 17.91a	0.629	NS
Single without *Toxoplasm*	30	86.06 ± 4.85 b	30	86.06 ± 4.85b	1.00	NS
Total	73	---	37	---	---	---
P-value	---	0.0427	---	0.0497	---	---
Sig.	---	*	---	*	---	---

An outlook for the present data, In the acute phase of infection the result of IL-6 level in miscarriage women (177.31± 12.53 Pg/ml) was higher significantly (P≤ 0.05) than acute infected pregnant (145.76 ± 16.82 Pg/ml), But at chronic phase of infection the results showed an interesting high level of IL-6 in miscarriage women was (119.36 ± 14.07 Pg/ml) in comparison to pregnant women (48.41 ± 2.79 Pg/ml) and this elevation highly significant (P≤0.01). And all infected women showed significant increased levels of IL-6 in comparison to non- infected one. This increase may relate to the presence of *Toxoplasma* parasite itself that led to occurrence of inflammation, so this cytokine may consider a marker of *Toxoplasma* parasite infection. This result coincides with previous work that suggested IL-6 is a marker of inflammatory states [50].

However, the high different range of IL-6 between acute and chronic toxoplasmosis in pregnant women may enable to use this cytokine as indicator of disease activity (acute and chronic) in those women group of women.

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