

Research Article



**Serological Study of CCL-2,CCL4, CXCL-5 in Aborted Women Infected With Toxoplasmosis in Basra Province**

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**KEY WORDS:**

Abortion  
CCL2  
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**Abstract:**

Toxoplasmosis is one of the main causes of miscarriage in pregnant women. The aim of this study is to investigate the chemokines concentration of CCL<sub>2</sub>, CCL4 and CXCL5 and the correlation with aborted women infected with toxoplasmosis and the relation of these chemokines with each other and with disease among an abortion. The total number of 166 serum samples (75 seropositive, 45 seronegative and 46 control), aged between 17-42 years who visited Basra Hospital for women and Children in Basra Province during the period between November 2022 to May 2023. This research is a case-control study on 160 serum samples from seropositive and negative aborted women and healthy control (women who have a normal delivery with, no history of abortion). The level of CCL2, CCL4 and CXCL5 were estimated in seropositive aborted cases compared with another groups by using ELISA method. The results show the mean concentration of CCL2 (79.8 pg/ml) was less than other groups, while CCL4 have concentration (173.1pg/ml) high in positive aborted women with significant than other groups. Further, the concentration of CXCL5 of seropositive aborted women was less than other groups with low statistically differ. Our study appear there were no statistically significant differences between all categories.

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**INTRODUCTION**

Toxoplasmosis, is a parasitic disease caused by *Toxoplasma gondii*, an apicomplexan. Infections with toxoplasmosis usually cause no obvious symptoms in adults. Occasionally, people may have a few weeks or

months of mild, flu-like illness such as muscle aches and tender lymph nodes (Robert-Gangneux and Darde<sup>[15]</sup>). A third of people worldwide are seropositive to the obligatory intracellular parasite *Toxoplasma gondii*, which causes toxoplasmosis. Abortion is a frequent

medical procedure. When done correctly, according to a method advised by the WHO, over the course of a pregnancy, and by a skilled practitioner, it is safe. Maternal fatalities and morbidities are primarily caused by unsafe abortion, which is also a preventable cause. It may result in issues with women's physical and mental health as well as social and financial pressures on communities and health systems (WHO<sup>[17]</sup>). Miscarriage may be caused by a number of causes, including infection, exposure to TORCH illnesses, hormonal abnormalities, STDs and others (Cleveland Clinic medical<sup>[12]</sup>).

Congenital toxoplasmosis, results from acute prime infection acquired via the mother through pregnancy. The incidence and severity of congenital toxoplasmosis differ with the trimester through which infection was attained. Because treatment of the mother may decrease the incidence of congenital cases, prompt and accurate diagnostic is important (Wonkam<sup>[16]</sup>).

Chemokines often help the immune system maintain homeostasis, inducible chemokines may also have a role, mostly in inflammatory events. Based on the quantity and spacing of the first two cysteine residues in a conserved cysteine structure (Griebel<sup>[5]</sup>). One of the inducible chemokines, thymus and activation-regulated chemokine (TCL17) is produced by endometrial gland cells during pregnancy. At the maternal-fetal interface, CXCL12 and its receptors CXCR4 are abundantly generated and play a regulatory function in mater no-fetal communication and immunological tolerance throughout the early stages of pregnancy. According to reports, CX3CL1 levels rise throughout the first trimester of pregnancy and in cases of term preeclampsia (Rasmak<sup>[14]</sup>). Hannan<sup>[7]</sup> demonstrated in two separate cohorts that women in the first trimester who are likely to miscarry have lower levels of the circulating first trimester Macrophage Inhibitory Cytokine-1 (MIC-1). Although encouraging, the diagnostic performance of measuring MIC-1 alone was insufficient for it to be a reliable miscarriage prediction test.

Present study aim to determine the CCL2, CCL4 and CXCL5 Chemokines in aborted women infected with toxoplasmosis compared with negative serological aborted and healthy women.

## MATERIAL AND METHODS

The study was conducted on 166 women (120 aborted women and 46 healthy control women) aged between 17-42 years who visited Basra Hospital for Women and Children in Basra Province during the period between November 2022-May 2023. Three ml of blood

was collected from radial vein from 160 participant (120 from aborted women and 40 from healthy control women) using disposable syringes was placed in gel tube for obtaining serum which used for measuring the concentration of CCL2, CCL4 and CXCL5 via ELISA method. The samples were kept at -20C for investigation.

## RESULTS AND DISCUSSIONS

**The LAT Agglutination Test:** Blood samples that used to examined the toxoplasmosis in aborted women in the current study show positive results 75 (62.5%) out of 120 aborted women, and related with age groups comparison between positive and negative results and control were shown in the table (1). The high positive rate (38.7%) was shown in age group (23-28) year than other groups.

**The Seropositive Results According to the Antibodies:** Table (1) show the rate of IgG antibodies was 59(78.6%) while IgM and the mixed IgG+IgM is 13 (17.3%) and 3(4%) respectively in aborted women infected with toxoplasmosis.

**ELISA Determination of CCL2, CCL4 and CXCL5:** The mean concentration of CCL2 were (79.8±41.7, 91.1±61.5 and 109.6±108.8) Pg/ml among positive and negative aborted women with toxoplasmosis and healthy control respectively with low difference (P= 0.6), same results found between positive and negative aborted women (P= 0.5) as shown in (Table 2).

The mean concentration of chemokines CCL4 in positive and negative aborted women and healthy control was (173.1±68.3, 171.4±61.9 and 141.3±131.1) respectively with show significant differences between positive aborted women and control (P=0.05).

The CXCL5 have low difference between the mean concentration of positive and negative aborted women and healthy control (246.9±128.7, 329.6±249.9 and 295.9±188.9) respectively with low differences between all categories (p=0.1).

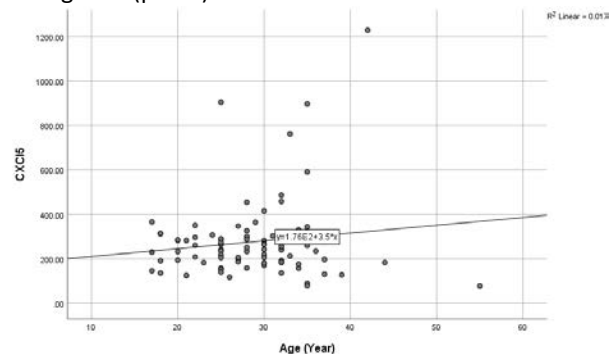


Fig. 1: The relationship between age of aborted women and CXCL5 (R2Linear = 0.017)

**Table 1: the distribution of antibodies in aborted women with toxoplasmosis**

Antibodies		IgG	IgM	IgG and IgM	Total
Seropositive	No.	59	13	3	75
	%	78.6	17.3	4	

**Table 2: Comparison of chemokines between aborted women (positive and negative results) and control**

Toxoplasmosis		CCI2	CCI4	CXCL5
Positive	N	50	50	50
	Mean	79.86	173.14	246.99
	Std. Deviation	41.75	68.30	128.74
Negative	N	20	20	20
	Mean	91.14	171.46	329.68
	Std. Deviation	61.52	61.99	249.98
Control	N	18	18	18
	Mean	109.67	141.32	295.99
	Std. Deviation	102.85	42.64	188.98
P-value*	All categories	0.66	0.12	0.14
P-value**	(Positive+ negative)	0.53	0.99	0.06
P-value***	(Positive+ control)	0.42	0.05	0.21
P-value****	(Negative+ control)	0.86	0.08	0.79

\* Kruskal-Wallis H Test \*\* Mann-Whitney U Test \*\*\* Mann-Whitney U Test \*\*\*\* Mann-Whitney U Test

**The Correlation Between Age of Aborted Women and Concentration of CXCL5:** Fig. (1) show significant difference between the age groups and the concentration of CXCL5 through the regression correlation as show in (fig. 1).

*Toxoplasma gondii* is a ubiquitous zoonotic parasite with an obligatory intracellular lifestyle. It relies on a specialized set of cytoskeletal and secretory organelles for host cell invasion (Delgado, et al., 2022). The intracellular protozoan infection *T. gondii* causes a strong and enduring immune response. (Denkers et al.<sup>[4]</sup>). Seroprevalence of *T. gondii* infection and related risk factors were studied in 75 positive aborted women in current study, from there was 59(78.6%) had IgG immunoglobulin, 13(17.3%) had IgM and three (4%) had together IgG and IgM. Similar study in Portugal 401 women of gestation age, from the 98 (24.4%) seropositive women, 92 (93.9%) had IgG, two (2.0%) had IgM and four (4.1%) others had both IgG and IgM (Lopes<sup>[10]</sup>).

The global and local studies estimates were done which included 250 cases with 723,655 prenatal women. The global (IgM) seroprevalence was 1.9%. In the regional, Eastern Mediterranean had the highest IgM seroprevalence (4.1%) and in Americas, the lowest (1.1%) with significantly difference between world health organization regions ( $p < 0.0001$ ). The global (IgG) seroprevalence was (32.9%). Among WHO countries, The Americas have the highest prevalence (45.2%) and Western Pacific was the lowest (11.2%) significantly difference between regions (Bignaet<sup>[11]</sup>).

Several studies about Chemokines through aborted women due to infected with toxoplasmosis had performed in last recent years. Therefore, chemokines are usually included in homeostasis of immune system. Chemokines may be also play important role in inflammatory routes and precarious regulators to the leukocyte migration. In addition, studies is need to shown that not all markers from the cytokine and

chemokines groups are similarly affected (Griebel<sup>[5]</sup>). In human researches in cases with toxoplasmosis an established infection case, no definite data had been gained so far. Most, of these researches were done out in South America (Denis<sup>[3]</sup>).

The immune response differentiated through the previous studies in *T. gondii* infection the patients, the factors related will always appears an influence. This show evident in various studies searching at cytokine response in chronically or acutely infected people (Marchioro et al.<sup>[4]</sup>). Pernaset<sup>[13]</sup> study comparing Colombian and USA pregnant women, showed a relatively minor impact of infection on the serum of cytokine levels in Colombia. While in French patients there were showed do not observed any mediator level of immune response.

The current reports have firm a series of physiological managers of CCL2, which functions in preserving normal recruitment of immune-cytes and angiogenesis. Though, abnormal levels of CCL2 had also been conveyed to be related with adverse gestation outcomes like as spontaneous abortion (Lin<sup>[9]</sup>).

In the current study, it was found that mean concentration of CCL2, protein levels were lower in fifty positive serological aborted women infected with toxoplasmosis (79.86) than 20 serological negative aborted women (91.14) and 18 control healthy women normal delivery (109.6). statistically was found the P value between all groups equal (0.6) that show not different in controls (healthy) and cases. These result findings notice do not preclude the probability, they still expected to play important roles (biological) in uterine, which they are created in abundance by several maternal endometrial cells, in both pregnant and non-pregnant tissue (Hannan et al.<sup>[6]</sup> and Jasper<sup>[8]</sup>). Similar study was revealed by (Hannan<sup>[7]</sup>) shown the Plasma levels of CCL2, CCL5 and CCL7 chemokines were no different in the aborted women compared to the controls.

In the present study, it was found that mean concentration of CCL4, protein levels were higher in fifty

positive serological aborted women infected with toxoplasmosis (173.14) than 20 serological negative aborted women (171.46) and 18 control healthy women normal delivery (141.32). Statistically was found significant difference ( $P=0.05$ ) between the positive cases and control, while show not different positive and negative cases ( $P=0.08$ ). similar studies were found more specifically at chronic and acute infections and looked significantly higher concentration of colony-stimulating factor 2 ((CSF2)), macrophage CSF, CXCL-11, transforming growth factor- $\beta$  ((TGF- $\beta$ )), CXCL9 and CXCL10 in the acute infection stage compared with the chronic stage (Marino<sup>[12]</sup> and Marchioroet<sup>[11]</sup>).

### CONCLUSIONS

The present study, show the mean of concentration of CXCL5 in positive serological aborted women was  $246.9 \pm 128.7$  were lower than negative serological aborted women with toxoplasmosis and health control ( $329.6 \pm 249.9$  and  $295.9 \pm 188.9$ ) respectively. Moreover, the median of the three groups respectively (234.2, 280.8 and 276.2). These results revealed with do not show statistically lower significant differ between infected aborted women with toxoplasmosis and non infected aborted and healthy control women. These results offered the study of dynamics between circulating immune cells and its local that should be explain the distinct location indices of *T. gondii* infection. These dynamic may be depended on the cute or chronic infection with the disease.

### REFERENCES

1. Bigna, J.J., J.N. Tochie, D.N. Tounouga, A.O. Bekolo and N.S. Ymele et al., 2020. Global, regional, and country seroprevalence of *Toxoplasma gondii* in pregnant women: A systematic review, modelling and meta-analysis. *Sci. Rep.*, 10: 1-10.
2. Denis, J., C. Gommenginger, T. Strehie, D. Filisetti, L. Beal, A.W. Pfaff and O. Villard, 2022. Dynamic immune profile in french toxoplasmosis patients. *J. Infect. Dis.*, 226: 1834-1841.
3. Denkers, E.Y., B.A. Butcher, L.D. Rio and S. Bennouna, 2004. Neutrophils, dendritic cells and toxoplasma. *Int. J. Parasitol.*, 34: 411-421.
4. Griebel, C.P., Halvorsen J, Golemon TB, Day AA. 2005. Management of spontaneous abortion. *Am. Fam. Physician.*, 72: 1243-1250.
5. Hannan, N.J. and L.A. Salamonsen, 2008. Cx3cl1 and ccl14 regulate extracellular matrix and adhesion molecules in the trophoblast: Potential roles in human embryo implantation1. *Biol. Reprod.*, 79: 58-65.
6. Hannan, N.J., K. Bambang, T.J. Kaitu'u-Lino, J.C.

- Konje and S. Tong, 2014. A bioplex analysis of cytokines and chemokines in first trimester maternal plasma to screen for predictors of miscarriage. *Plos one*, Vol. 9 .10.1371/journal.pone.0093320.
7. Jasper, M.J., K.P. Tremellen and S.A. Robertson, 2007. Reduced expression of il-6 and il-1a mrnas in secretory phase endometrium of women with recurrent miscarriage. *J. Reprod. Immunol.*, 73: 74-84.
8. Lin, Z., J.L. Shi, M. Chen, Z.M. Zheng, M.Q. Li and J. Shao, 2023. Ccl2: An important cytokine in normal and pathological pregnancies: A review. *Front. Immunol.*, Vol. 13 .10.3389/fimmu.2022.1053457.
9. Lopes, V.P., D.F. Stodden, M.M. Bianchi, J.A.R. Maia and L.P. Rodrigues, 2012. Correlation between bmi and motor coordination in children. *J. Sci. Med. Sport*, 15: 38-43.
10. Marchioro, A.A., C.M. Colli, C.Z. de Souza, S.S. da Silva and B.T. Tiyo et al., 2018. Analysis of cytokines ifn- $\gamma$ , tnf- $\alpha$ , tgf- $\beta$  and nitric oxide in amniotic fluid and serum of pregnant women with toxoplasmosis in southern Brazil. *Cytokine*, 106: 35-39.
11. Ana, P.M.P.M., L.I. dos Santos, P.M. Henriques, E. Roffe and D.V. Vasconcelos-Santos et al., 2020. Circulating inflammatory mediators as biomarkers of ocular toxoplasmosis in acute and in chronic infection. *J. Leukocyte Biol.*, 108: 1253-1264.
12. Pernas, L., R. Ramirez, T.H. Holmes, J.G. Montoya and J.C. Boothroyd, 2014. Immune profiling of pregnant toxoplasma-infected us and Colombia patients reveals surprising impacts of infection on peripheral blood cytokines. *J. Infect. Dis.*, 210: 923-931.
13. Roepke, E.R., V. Bruno, E. Nedstrand, R. Boij and C.P. Strid et al., 2019. Low-molecular-weight-heparin increases th1- and th17-associated chemokine levels during pregnancy in women with unexplained recurrent pregnancy loss: A randomised controlled trial. *Sci. Rep.*, Vol. 9 .10.1038/s41598-019-48799-6.
14. Robert, G., F. and M.L. Dardé, 2012. Epidemiology of and diagnostic strategies for toxoplasmosis. *Clin. Microbiol. Rev.*, 25: 264-296.