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## Milk Tumor Necrosis Factor Alpha and Interleukin-1Beta Among *Toxoplasma gondii*-Free and Infected Women

Alaa Tareq Shakir Al-Hassnawi\*

Kassim Abdulla Hamza Al-Morshidy

Nuha Yaarub Al-Harbi

Department of Biology, College of Science, University of Babylon, Babylon, Iraq.

\*Corresponding author: [alaatark79@yahoo.com](mailto:alaatark79@yahoo.com)\*, [kassimalmorshidy2013@gmail.com](mailto:kassimalmorshidy2013@gmail.com), [nuhayaerob@yahoo.com](mailto:nuhayaerob@yahoo.com)

\*ORCID ID: <https://orcid.org/0000-0003-2173-0128>\*, <https://orcid.org/0000-0003-4930-8674>, <https://orcid.org/0000-0002-7652-6979>

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### Abstract:

Pro-inflammatory cytokines play an important role in intercellular communications. In the last two decades, many cytokines have been identified in human milk. These cytokines are variable according to different conditions such as pathogenic infections which strongly stimulated the immune response. The present study aims to determine of IL1 $\beta$  and TNF- $\alpha$  in *Toxoplasma gondii*-free and infected women in an attempt to clarify the impacts of the infections on cytokines especially in mother's milk. The serum and milk sample were collected from 96 samples (48 for seropositive and 48 for seronegative). To confirm the *Toxoplasma gondii* infection; enzyme linked immunofluorescence assay (ELIFA) was used to detect anti-*Toxoplasma* IgG antibodies. TNF- $\alpha$  and IL1 $\beta$  levels were measured by ELISA technique. Regarding milk TNF-alpha, there were statistical differences in *Toxoplasma*-infected from *Toxoplasma*-free subjects, whereas no statistical variations in TNF- $\alpha$  were found between other studied groups. IL1 $\beta$  showed significant variation just between milk and serum in infected and non-infected subjects, whereas no variations were recorded in other groups. In conclusion, based on statistical analysis, present study showed good evidence that toxoplasmosis is one of the risk factors for increasing milk cytokine. Also, a significant increase in TNF- $\alpha$  and IL1 $\beta$  in mother's breast milk may indicate a key role for inducing or developing of inflammations in suckling. Thus, further investigations are needed to cover this area of study.

**Keywords:** Infected mother, IL1 $\beta$ , Postpartum, TNF- $\alpha$ , *Toxoplasma gondii*.

### Introduction:

In the last two decades, different cytokines have been identified in breast milk. Most of these cytokines come from epithelial cells of mammary tissues, local immune cells and the mother's circulation system<sup>1</sup>. Maternal breast milk cytokines have potential impacts on the newborn immune system. Milk is not only a source of feeding but also as a method of adapting infants to their extra-uterine environment<sup>2</sup>. Immune cells in the mother's body play an important role to prevent fetal rejection. As well-known, pregnancy is controlled by having different concentrations hormones, such as estrogen and progesterone<sup>3</sup>. In addition, cytokines perform an important role in the success of pregnancy<sup>4</sup>. Several studies showed that the factors associated with abortion are Th1 while Th2 which are the factors that help to sustain pregnancy<sup>5</sup>. Further

studies also indicate that normal pregnancy is related with an improved immune response to Th2 while abortion occurs to raise the immune response to Th1. IL-12 has a crucial role in fetal loss stimulates natural killer cells output and TNF- $\alpha$  by macrophages and T lymphocytes<sup>6</sup>. The network of cytokine mediator in milk has not been well-studied, especially in case of parasite infections. As well known the *T. gondii* is the most successful parasite with prevalence rate from 5 to 90 %<sup>7</sup>. *T. gondii* invade different tissues such as brain, heart, lung, lymphoid and recently it's found in breast-milk<sup>8</sup>. Many researchers have discussed the roles of cytokine networks for the development and progression of many diseases, and this production of cytokines is almost related to invasion of pathogens especially parasitic infections<sup>9</sup>.

Therefore, in the light of increasing inflammatory diseases, the effects of parasites invasions on cytokines variability may give us an indication of this relationship.

## Material and Methods:

### Subjects:

All exclusion criteria, such as family history, chronic and genetic disease, drinking and smoking were excluded in current study. Ninety-six enrolled women had been complete clinical data and bio-investigation for the subdivision into two groups, *Toxoplasma*-free group and, *Toxoplasma*-infected group.

### Blood and milk collection

Five mls of milk were collected from enrolled women after 4–6 weeks postpartum, then putted in sterilized tubes and stored in the refrigerator, whereas blood was transferred to the gel tube. Serum was separated from whole blood by centrifugation (4000 rpm for 5 min.) and then blood was stored in the refrigerator immediately at  $-20^{\circ}\text{C}$ .

### Determination of Toxoplasmosis:

Serum anti-toxoplasma IgG antibodies were identified by using immunofluorescence assay (IFA). Briefly, this assay principle combines an enzyme immunoassay method by immunocapture with final fluorescent detection (ELFA). The procedure of this assay was accomplished by manufacture commercial kit by Biomerieux Company (France).

### Measurement of TNF- $\alpha$ and IL1 $\beta$

The concentration of TNF- $\alpha$  and IL1 $\beta$  was measured by using Enzyme-Linked Immunosorbent Assay (ELISA) technique. Commercial ELISA kit from Elabscience Company was used for this purpose.

### The study protocol and ethics

The study protocol was approved by the local ethical committee in the college of science /

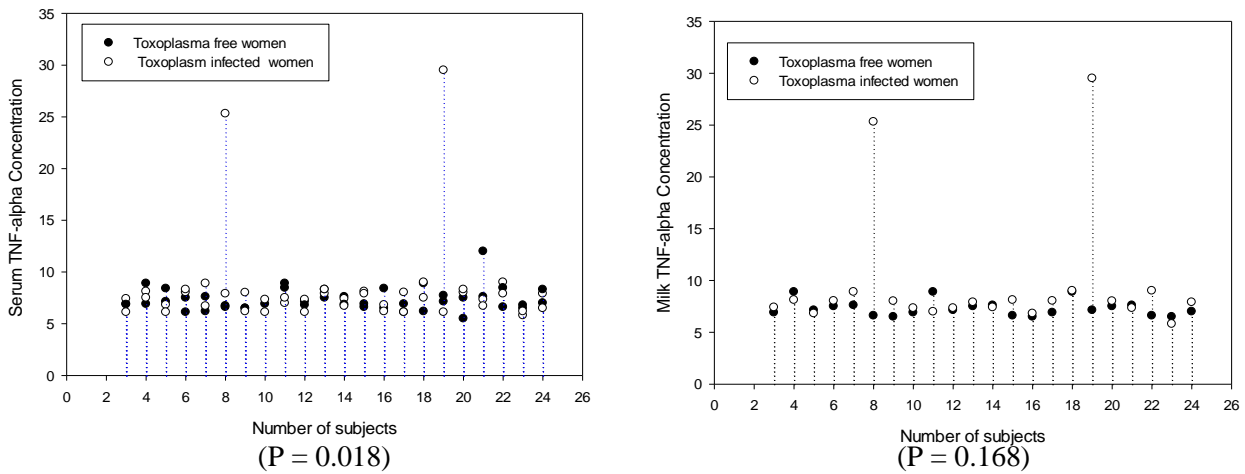
Babylon University, declaration Helsinki standards were adopted in the present study.

### Statistical Analysis

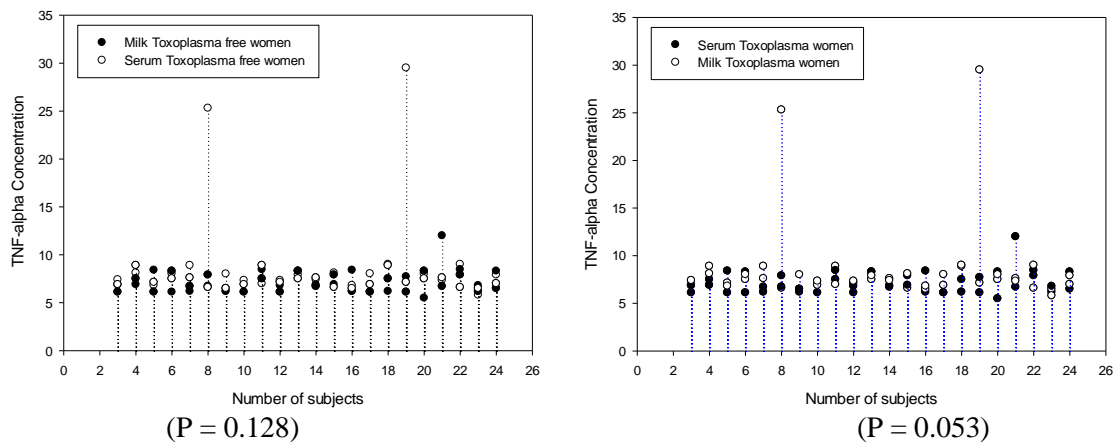
Statistical analyses were performed using a statistical software program (SPSS 10 Inc., Chicago, USA). T-test was used to search for differences regarding the nominal variables in our study. Variations were considered significant when  $P\text{-value} \leq 0.05$

### Results:

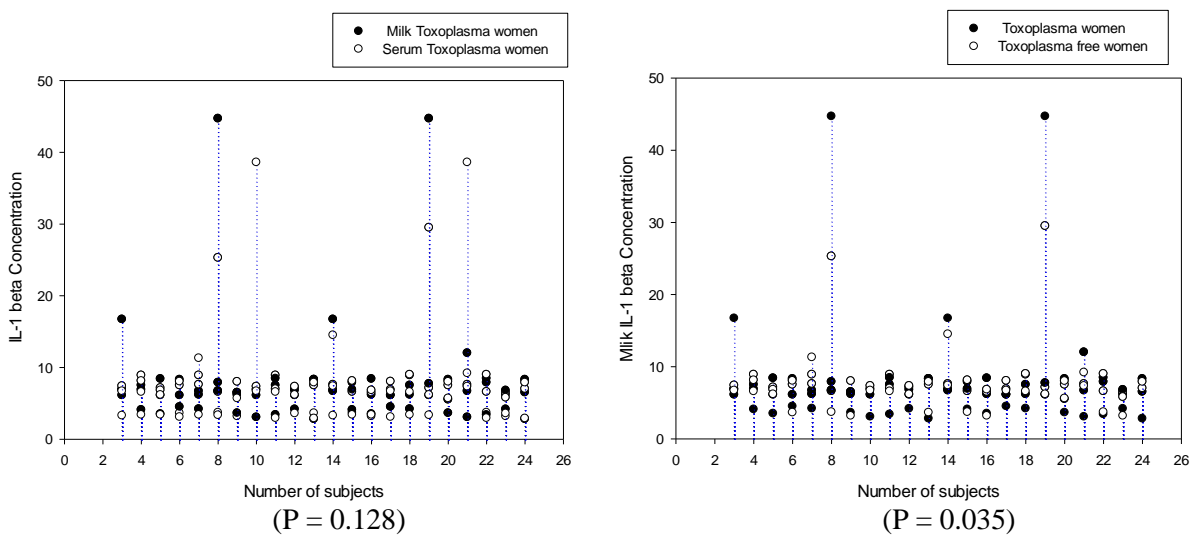
Results of milk TNF- $\alpha$  in *Toxoplasma gondii* in free and infected women are shown in Fig. 1. The differences in the median values between the two groups are greater than that would be expected by chance; thus statistically significant difference existed ( $P = 0.018$ ). TNF- $\alpha$  concentration was significantly higher in milk *Toxoplasma*-in the infected women as compared with non-infected subjects. No statistically significant difference was found between serum TNF- $\alpha$  levels in women with the *Toxoplasma* seropositivity group in comparison with those women who have seronegative (Fig. 1,  $P = 0.168$ ). Furthermore, TNF- $\alpha$  levels variations in the median values between milk and serum in seronegative subjects are not great enough to exclude the possibility ( $P = 0.128$ ). Finally, the TNF- $\alpha$  levels did not reach the significant variation between milk and serum in *Toxoplasma*-free and *Toxoplasma*-infected women Fig. 2. ( $P = 0.053$ ). IL1 $\beta$  showed significant variation between milk and serum in infected and non-infected subjects with values of 0.035 and 0.003 respectively (Figs. 3, 4). Also, our results showed no variations between IL-1beta in seropositive and seronegative subjects (Fig. 4,  $P = 0.008$ ). The data of IL-1beta levels in *Toxoplasma*-free and infected women showed no significant variation (Fig. 3,  $P\text{-value} = 0.128$ ). Personal correlations test showed significant variations between serum TNF- $\alpha$  and IL1 $\beta$  in seropositive subjects ( $P\text{-value} = 0.000267$ ). Also, a positive correlation is shown between milk TNF- $\alpha$  and IL1 $\beta$  in seropositive patients ( $P\text{-value} = 0.000293$ ).



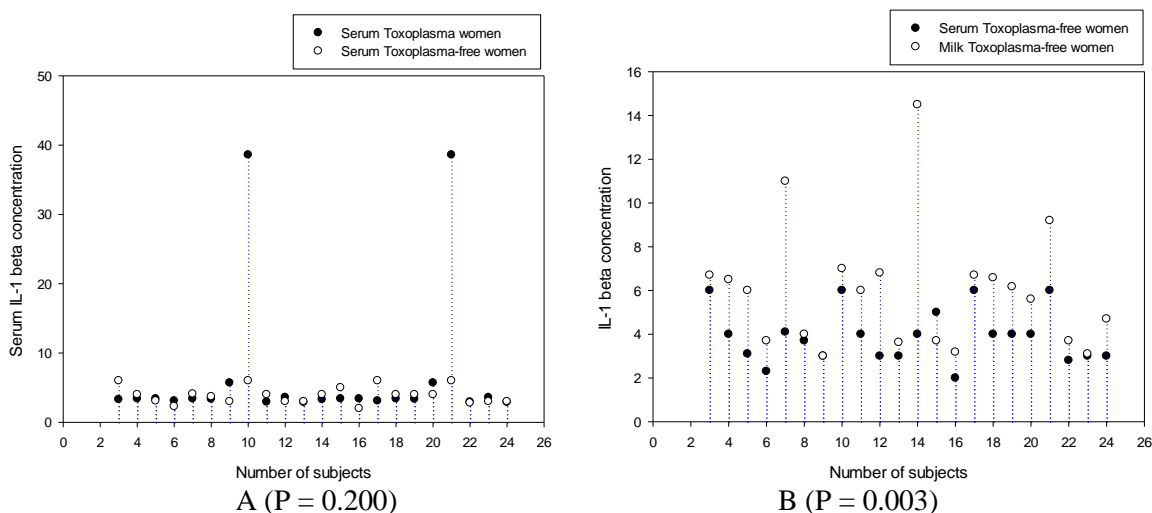
**Figure 1. Milk and serum TNF-alpha concentration (pg/ml) in *Toxoplasma gondii* free and infected women .**



**Figure 2. Milk and Serum TNF-alpha (pg/ml) variations among *Toxoplasma* infected and free women.**



**Figure 3. Milk and serum IL-1beta (pg/ml) variations among *Toxoplasma* -infected women.**



**Figure 4. Milk and serum (B) and serum (A) IL-1beta (pg/ml) variations among Toxoplasma-free women**

### Discussion:

In the present study two pro-inflammatory cytokines (TNF- $\alpha$  and IL1 $\beta$ ) were investigated in women during the first six weeks of lactation. It is well-known that IL1 $\beta$  and TNF- $\alpha$  are macrophage stimulating cytokines in which both mediates inflammation and shares many systemic responses, development, growth, hematopoiesis and cell signaling<sup>10,11</sup>. Data presented here showed significant differences in milk TNF- $\alpha$  level (Fig.1). Also, the same variations were found in milk and serum IL1 $\beta$  levels among *Toxoplasma*-free and infected women (Fig.4). Most of the inflammatory cytokines, such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8 and IFN- $\gamma$  are found at lower concentrations compared to anti-inflammatory cytokines<sup>12</sup>. Also, these concentrations decrease over lactation<sup>13</sup>.

However, during parasite infection the progress of pregnancy and mother's immune system response may have a different story<sup>14</sup>. Such responses may reflect strategies of host and parasites to maximize the fitness for each other. Also, it could be stated that milk is a communication tool be for mother-infant for protection against pathogens. Previous studies were suggested that the incidence of *T. gondii* leads to increase serum pro and anti-inflammatory cytokines levels<sup>15</sup>. Although, increasing the production of cytokines is associated with the protection against parasitic infections, yet this mechanism may be costly because the over cytokine productions induces another disease in mothers and infants<sup>16,17</sup>.

Furthermore, other studies suggest that abortion is always associated with increasing TNF- $\alpha$  level<sup>12</sup>. Therefore, the increase in cytokines productions may be related with parasite strategies to increase a chance of abortion. Our findings also show an increase in TNF- $\alpha$  concentrations in breast

milk of the infected mothers. These variations may be due to *T.gondii* infections that increase maternal injury or local cytokines production. Further, our results showed a positive significant correlation between TNF- $\alpha$  and IL-1 $\beta$  in the serum and milk of the infected mothers (Pearson correlations). This correlation confirms that the increasing levels of cytokines in the blood lead to the rise in milk. Cytokines increasing may be depicted the vital effects of toxoplasmosis in cytokine outcome and their effects on mother-infant pathological mediate. Also, cytokines outcome modulations may increase understanding the important role of pathogens in mother-infants regulation and development of immune response. Thus, further investigations are needed to cover this fruitful research area.

### Conclusion:

The importance of the current findings especially those related to increase pro-inflammatory cytokine in the toxoplasma-infected mother's milk makes the first step for shifting the understanding of the pivotal roles of cytokines, TNF- $\alpha$  and IL-1 $\beta$ , in pregnancy gaining or losing as well as the inductions or protections of many diseases in infants.

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### Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides,

the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.

- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Babylon.

#### Authors' contributions statement:

ATS, Al-hassnawi, designed the experiment and analysis the data. KAH, Al-Morshidy conducted the lab work. Al- NY, Harbi wrote and edited the manuscript. Editing, revision and proofreading were done by all researchers to improved the language of writing.

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## عامل النخر الوراثي وانترلوكين-1 بيتا في حليب النساء المصابات وغير المصابات بالمقوسات الجوندية

نهى يعرب محمد الحربي

قاسم عبدالله حمزه المرشدي

علاء طارق شاكر الحسنواوي

قسم علوم الحياة، كلية العلوم، جامعة بابل، بابل، العراق

### الخلاصة:

تلعب السيتوكينات المحفزة للالتهاب دوراً مهماً في التواصل بين الخلايا. ففي العقدين الأخيرين ، تم تحديد العديد من السيتوكينات في حليب الإنسان حيث تم العثور على السيتوكينات المثيرة للالتهابات في حليب الإنسان. تتنوع السيتوكينات تبعاً لظروف مختلفة مثل الالتهابات المسببة للأمراض التي تحفز بشدة الاستجابة المناعية. تهدف الدراسة الحالية إلى تحديد تراكيز  $TNF-\alpha$  و  $IL1\beta$  في النساء المصابات وغير المصابات بالمقوسات الجوندية في محاولة لتوضيح آثار العدوى على السيتوكينات وخاصة في حليب الأم. تم في الدراسة الحالية جمع عينات من مصال اللبن والحليب من 96 عينة (48 مصال موجب و 48 مصال سالب). لتأكيد الإصابة بطفيلي التوكسوبلازما تم استخدام تقنية الفلورة المناعية لهذا الغرض. تم قياس تركيز  $TNF-\alpha$  و  $IL1\beta$  بواسطة تقنية ELISA. فيما يتعلق بالحليب  $TNF-\alpha$  ، أظهرت نتائج الدراسة الحالية هناك فروق ذات دلالة إحصائية في النساء المصابات بالتوكسوبلازما وغير المصابات بها ، في حين لم يتم العثور على اختلافات إحصائية في  $TNF-\alpha$  بين مجموعات الدراسة. أظهر نتائج  $IL1\beta$  اختلافاً معنوياً فقط بين الحليب والمصل في الأشخاص المصابين وغير المصابين ، بينما لم يتم تسجيل أي اختلافات معنوية في مجموعات أخرى. الخلاصة وبناءً على التحليل الإحصائي ، أظهرت الدراسة الحالية دليلاً جيداً على أن داء المقوسات هو أحد عوامل الخطر لزيادة السيتوكينات في الحليب. إضافة لذلك قد تشير الزيادة الكبيرة في  $TNF-\alpha$  و  $IL1\beta$  في حليب الأم إلى دور رئيسي في إحداث أو تطور الالتهابات في الرضاعة. وبالتالي ، هناك حاجة إلى مزيد من الدراسات لهذا الغرض.

**الكلمات المفتاحية:** المرأة المصابة، عامل التنخر الورمي- الفاء، انترلوكين-1 بيتا، بعد الولادة، المقوسات الجوندية.