Relation Between Aerobic Bacteria, IFN-γ, TNF-α and Miscarriage in Sample of Iraqi Women

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Abstract

Pregnancy is one of the cases that lead to immune compression, therefore the women are exposed to different types of infections and may sequel to miscarriage. *Toxoplasma gondii* and urinary tract infection (UTI) are the most prevalent infectious agents in human and have a worldwide distribution. IFN-γ and TNF-α are pro inflammatory cytokine play important role in miscarriage. This study focuses on the determination, type of aerobic bacteria, concentration of IFN-γ and TNF-α among (75) abortive women and their relation with miscarriages. The results indicated that *S.aures* was the most prevalence bacteria isolated from aborted women infected with UTI or compensation with toxoplasmosis, and among the subjected women, the significant increases of IFN-γ concentration in serum across all groups involved in this study especially that infected with toxoplasmosis (74.84 ± 0.44 and 66.81 ± 0.74) pg/ml compared to control (25.60 ± 1.10) pg/ml whereas no significant alteration has occurred to TNF-α concentration in all groups (2.26 ± 0.05 , 3.32 ± 1.22 and 2.12 ± 0.06 ) ng/ml respectively compared to the control (2.11 ± 0.89) ng/ml, and when analyzed the results according to gestational age, all abortive women in different trimesters showed significant rises in the concentration of IFN-γ (51.58 ± 3.32, 48.95 ± 3.50 and 48.71 ± 4.84 ) pg/ml.

Keywords: *T. gondii*, IFN-γ, TNF-α, *S.aures*, UTI.

**العلاقة بين البكتريا الورمية، الانترفرون كاما، عامل الدخور الورمى والإجهاض في عينة من النساء**

**العلاقات**

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**الخلاصة**

بعد الحمل واحدة من الحالات التي تؤدي الى حدوث تغييرات في وظيفه الجهاز المناعي لذلك فإن النساء الحوامل ينتمون الى أنواع مختلفة من البكتيريا التي تؤدي الى فقدان الحمل. أن المؤسسات الكوبيدية وإخراج الجهاز الورمي شكل من الأورام الأكثر شيوعا في النساء الحوامل على المستوى العالمي. الانترفرون كاما وعامل النخر الورمى من الخلايا التائية التي لها دور كبير في حدوث الإجهاض. تهدف هذه الدراسة الى تحديد نوع البكتيريا الورمية، تركيز كل من الانترفرون كاما وعامل النخر الورمي وعمليته بدءاً من الإجهاض. اظهرت نتائج الدراسة ان البكتريا الورمية *S.aures* هي أكثر الأورام الورمية المزعجة من النساء المحبطة بخلختها انترفرون KAM وعامل الدخور الورمي وكذلك النساء المصابات بكل من إجهاض الجهاز الورمي المناعي.

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Introduction

Miscarriage is the spontaneous loss of a pregnancy between conception and 20 weeks into the pregnancy, which affects 15–25% of pregnant women [1,2]. The miscarriage until the 12th week of the pregnancy is termed, early pregnancy loss (EPL), which occurs in 15% of the cases [3,4] and the ones which occur between 12th and 20th weeks of the pregnancy are termed, late pregnancy loss (LPL) which has an incidence rate of 1–5%[5,6].

Evidence suggests that maternal genitourinary and intrauterine infections have been proposed as an etiology for complications in pregnancy[7,8]. Generally, 15% of early miscarriages and 66% of late miscarriages have been related to infections[9,10]. Some of infections were related to bacteria [11]. Other causes contributing in pregnancy loss and congenital complication are related to infection with Toxoplasma gondii, cytomegalovirus (CMV), rubella and herpes simplex virus (HSV) [12]. Toxoplasma gondii is an obligate intracellular parasite cause the disease toxoplasmosis which can infect a wide range of warm-blooded animals as man, birds, livestock and marine mammals. Congenital toxoplasmosis in pregnant women has a very severe complication for the fetus, since the infection may result in miscarriage mental retardation and intracranial calcifications in the newborn [13]. Other infectious disease may related with abortion is urinary tract infections (UTI), that consider one of the most prevalent infections and the significant cause of mortality and morbidity [14,15]. In pregnant women, the occurrence of asymptomatic bacteriuria was found to be 2% to 10% [16,17]. Pregnancy increases the succession from asymptomatic to symptomatic bacteriuria which can cause acute kidney disease and contribute to perinatal outcomes like postpartum hypertensive, prematurity and increased fetal mortality rates[18-20].

IFN-γ is a cytokine produced mainly by Th1 and NK cells, and consider the central cytokine that inducing anti-Toxoplasma effector mechanisms. These mechanisms include, the activation of host cell death upon infection, the acidification of the intravascular environment or the direct destruction of parasite vacuole (PV) [12,22]. TNF-α is a pro-inflammatory cytokine that inflict both innate and adaptive immune response and regulates cell proliferation, differentiation, and cell death[23] TNF-α mediates its protection against T. gondii by increasing the expression of nitric oxide (NO) [24]. The cytokines (TNF-α and IFN-γ) are abortogenic via alteration of 12 prothrombinase activity, and these cytokines are thought to increase uterine activity, either directly or by inducing prostaglandin production, unattraction of leukocytes, and tissue remodeling[25,26].

Material and Methods:

The present study was conducted in Baghdad, through a period of 3/2017 – 6/2018. The study was carried out on 75 aborted Iraqi women and 25 others apparently healthy pregnant women represented as a control group. Blood and urine samples were collected from each aborted and pregnant woman. The subjects were divided into three groups (subjects suffered from toxoplasmosis only, others suffered from UTI only, and those were suffered from toxoplasmosis and UTI).

Blood collection: By using sterile gel tube (Afco-dispo / Jordan), 5 ml of venous blood sample were collected from each patient and control subject. After centrifugation at 3000 rpm for 5 minutes, the serum was collected and kept at -20°C for further immunological tests.

Microbial study: Midstream urine samples were collected in a sterile container, and characterized the bacterial isolate using standard microbiology techniques [27]

Immunological tests: The levels of IFN-γ and TNF-α were assessed by using Enzyme linked Immunosorbert Assay (ELISA) technique and according to the instructions of company, the concentration of IFN-γ and TNF-α are defined.
Statistical Analysis
The Statistical analysis system- SPS -21 program was used to study the effect of different factors in the parameters. The chi-square test was used to significant compare between percentage and least significant difference –LSD test (ANOVA) was used to significant compare between means.

Result and discussion

The prevalence of bacterial isolates in the different group

The results showed that *S. aureus* was the most prevalent bacteria among abortive women infected with UTI or in combination with toxoplasmosis, and Chi-Square ($\chi^2$) analyses recorded significant differences (P<0.01) for this bacterium isolate than other types of aerobic bacteria which represented 10(40%) and 19 (76%) respectively, followed by *E.coli* 6(24%) and 4 (16%), while *K. pneumonia* was 4 (16%) and 1 (4%). Whereas, *Streptococcus* spp. represented 4(16%) and 1 (4%) for each group (Table-1)

<table>
<thead>
<tr>
<th>Groups</th>
<th><em>S.aureus</em></th>
<th><em>E.coli</em></th>
<th><em>K. pneumoniae</em></th>
<th>Strepto spp.</th>
<th><em>S.epidermids</em></th>
<th>Chi-Square ($\chi^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected with a urinary tract infection</td>
<td>10 (40%)</td>
<td>6 (24%)</td>
<td>4 (16%)</td>
<td>4 (16%)</td>
<td>1 (4%)</td>
<td>9.54 **</td>
</tr>
<tr>
<td>Infected with toxoplasmosis and urinary tract infection</td>
<td>19 (76%)</td>
<td>4 (16%)</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
<td>0 (0%)</td>
<td>13.60 **</td>
</tr>
<tr>
<td>Chi-Square ($\chi^2$)</td>
<td>8.73 **</td>
<td>4.38 *</td>
<td>4.69 *</td>
<td>4.9**</td>
<td>1.27 NS</td>
<td>----</td>
</tr>
</tbody>
</table>

* (P<0.05), ** (P<0.01), NS: Non-Significant.

These results were determined the importance of UTI throughout pregnancy period because bacteriuria that progresses to pyelonephritis during pregnancy is associated with severe complication for both the child and mother, including premature birth (PTB), maternal sepsis and perinatal death. Even without progression to pyelonephritis, bladder infection during pregnancy is associated with increased risk of maternal hypertension, anemia, amnionitis, and premature labor [28]. In contrast to some studies, *S.aureus* was recorded highly prevalence than *E. coli*, and these results disagreed with [29,30] who mentioned that most bacterial organisms which cause this disease included *E.coli, K. pneumonia, Proteus* spp. *Streptococcus* Group B and *Pseudomonas aeruginsosa*. In addition, our results disagree with [31] who reported that *E.coli* was the most bacterial strains isolated from the urine of toxoplasma positive women.

The present finding and most reported documents emphasized the risk effects of bacterial UTI during pregnancy. The susceptibility of pregnant women to UTI may be related to alteration in urine chemical composition with elevated glucose levels which lead to promote bacterial growth [32]. Furthermore, [33] reported an increase Preeclampsia infection in women with any UTI during pregnancy versus those without UTI. On the other hand, an important implication of these findings is that, although *S. aureus* is considered one of the normal bacterial flora in urogenital region, it may cause UTI especially when immune-compromised state such as pregnancy and that may be attributed to immunological alteration during pregnancy, which increases the risk of UTI [34]

Estimation of IFN-γ and TNF-α conc. in all groups

The results in (Table-2) showed a significant increase in the conc. of IFN-γ in all abortive women, especially that related with toxoplasmosis (74.84 ± 0.44, 33.26 ± 0.89 and 66.81 ± 0.74) pg/ml compared to control (25.60 ± 1.10) pg/ml.
Simultaneously the recent finding confirmed the result mentioned by Ashkar et al.,(2000) [35] who testified that NK cell in uterine derived IFN-γ and lead to modify the genes expression in the uterine vasculature and stroma which causes instability of vessel and facilitates pregnancy-induced remodeling of decidua arteries, that may lead to abortion squeal. Moreover, T. gondii infection was increased and conserved subsequently caspases 8 and 3, and the trophoblasts cell apoptosis that co-cultured with NK in vitro [36]. They supposed that the reason may be related to IFN-γ level that associated confidently with the apoptosis of trophoblasts. Also [37] reported that, IFN-γ consider the most important cytokines induce early abortion through infection of T. gondii. IFNγ are very toxic and suppress the proliferation of human trophoblast cells [38, 39]. IFNγ toxicity due to production of Nitric oxide (NO) free radicals by immune cells [40]. Nitric oxide has been implicated as an apoptotic activator during T. gondii infection which leads to placental trophoblast cells apoptosis and embryo death [41].

Furthermore, IFNγ induces apoptosis by the stimulus of Fas expression and increases trophoblast sensitivity to Fas-mediated apoptosis [39,42]. Apoptosis is initiated when Fas is expressed on the maternal lymphocyte surface and contacts with Fas-L on placental cells [43]. After Fas-Fas L interaction occurs, a series of caspases are activated that will eventually degrade cellular DNA resulting in cell death [44]. This could explain the possible mechanism of abortion due to toxoplasma infection. On the other hand, the result in (Table-2) showed no significant alteration was occurred to TNF-α conc. in all groups (2.26 ± 0.05, 3.32 ± 1.22 and 2.11 ± 0.06) respectively, compared to the control (2.12 ± 0.89) in spite of slight rises in the group of abortive women infected with UTI. This result approximately agreed with Coyle,(1993) [45] finding who mentioned that highly increasing of TNF-α was observed in abortive women infected with UTI which may be related to gestational infection, such as bacterial vaginitis ,UTI, Streptococci group B, and Staphylococcus spp. This has been associated with spontaneous miscarriage because TNF-α mediates pathophysiologic alteration associated with exposure to LPS by triggering the acute phase response leading to teratogenicity and fetotoxicity.

In addition, the present finding has disagreed with Chang et al.,(1990) [46] who mentioned that, IFN-γ induced the stimulation of TNF-α and the anti-parasitic effect provided by IFN-γ seemed to be dependent partly on the stimulation of TNF-α. They reported that TNF-α and IL-1 may play a central role in modulating the immune defense against parasite infection. Therefore, we can suggest according to the recent finding, there is no relationship between IFN-γ and increasing of TNF-α concentration. Sher et al., (1993) [47] confirmed the recent finding throughout their results on splenic adherent cells which produce low levels of TNF-α in response to the parasite. Nevertheless, TNF-α alone is not sufficient to stimulate NK cells to eradicate the parasite, and when analyzed the results according to gestational age (Table-3), all abortive women showed significant rises in the concentration of IFN-γ. The results obtained in this study are broadly consistent with. Zhang et al.,(2015)[36] who mentioned that the concentration of IFN-γ were increased at <24 hour following toxoplasma infection.
It is well known that IFNγ plays a major role in a resistance against Toxoplasma infection. Marshal and Denker (1998) [48] found that T. gondii is a strong inducer of type-1 cytokine and IFNγ, probably reflecting the beneficial effect, in keeping the host alive during infection. There is a possibility that strong cytokines action stimulated in the early stage of infection will induce abortion during this stage of disease[49,50] and this may interpret the result of the present study in which the serum level of IFNγ was strongly elevated throughout different stages of infection. According to Filiscetti and Candolf (2004) [51] stimulation of IFNγ in mice increases the macrophages activity and CD8+ lymphocyte cytotoxicity. Increased production of IFNγ is strongly associated with parasite virulence and increased apoptosis consequently. IL12 and IFNγ both are confused in the protection against toxoplasma infection, however, it will increase the probability of miscarriage throughout stimulate uterine activity, directly or via an increase in prostaglandin stimulation, tissue remodeling and leukocytes attraction. Reducing the inflammatory infiltrate or inhibiting cytokines production in these cells might be effective in the treatment of premature labor, in the same pathway, these cytokines may cause miscarriage [26]

### References


### Table 3- IFN-γ and TNF-α conc. in all groups according to gestational age

<table>
<thead>
<tr>
<th>Gestational age</th>
<th>INF-γ conc. (pg/ml)</th>
<th>TNF-α conc. (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>51.58 ± 3.32 A</td>
<td>3.13 ± 0.77 A</td>
</tr>
<tr>
<td>Second</td>
<td>48.95 ± 3.50 A</td>
<td>1.89 ± 0.08 A</td>
</tr>
<tr>
<td>Third</td>
<td>48.71 ± 4.84 A</td>
<td>1.68 ± 0.09 A</td>
</tr>
<tr>
<td>Control</td>
<td>25.60 ± 1.10 B</td>
<td>2.11 ± 0.89 A</td>
</tr>
<tr>
<td>LSD value</td>
<td>11.590 NS</td>
<td>1.991 NS</td>
</tr>
<tr>
<td>P-value</td>
<td>0.813</td>
<td>0.219</td>
</tr>
</tbody>
</table>

NS: Non-Significant.

Means having with the different letters in same column differed significantly.


