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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.

العلاقه بين البكتريا الهوائيه ، الانترفرون كاما ،عامل النخر الورمي والاجهاض في عينه من النساء العراقيات

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الخلاصة

يعد الحمل واحدا من الحالات التي تؤدي الى حدوث تغيرات في وظيفه الجهاز المناعي،لذلك فالنساء الحوامل يكن عرضة لأنواع مختلفه من الاصابات التي تودي الى فقدان الحمل . ان المقوسات الكونيديه واخماج الجهاز البولي تعد من الامراض الاكثر شيوعا في النساء الحوامل على المستوى العالمي. الانترفرون كاما وعامل النخر الورمي من الحركيات الخلويه التي لها دور كبير في حدوث الاجهاض. تهدف هذه الدراسه الى تحديد نوع البكتريا الهوائيه ، تركيز كل من الانترفرون كاما وعامل النخر الورمي وعلاقته بحدوث الاجهاض . اظهرت نتائج الدراسه ان بكتريا *S.aures* هي اكثر الانواع البكتيريه المعزوله من النساء المجهظات المصابات باخماج الجهاز البولي وكذالك النساء المصابات بكل من اخماج الجهاز البولي

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والمقوسات الكونيديه ،وان هناك زياده معنويه في تركيز الانترفرون كاما في مصل مجاميع الدراسه خاصه لدى النساء المصابات بالمقوسات الكونيديه حيث بلغت(74.84± 0.44 ، 33.26 ± 0.89 و 66.81± 0.74) بيكومول/مللتر على التوالي بالمقارنة بمجموعة السيطرة (25.60± 1.10) ومن ناحية اخرى لم يظهر تركيز TNF–α اي تغاير معنوي لجميع المجاميع (2.26± 0.05 ، 3.32± 1.22 و 2.12± 0.06) نانوغرام / مللترعلى التوالي بالمقارنةبمجموعه السيطرة (2.11± 0.89) نانوغرام / مللتر وعند تحليل النتائج اعتمادا على فترة الحمل ، اظهرت النتائج ان جميع النساء المجهضات اظهرن زيادة في تركيز IFN– γ (51.58± 3.32، 48.95± 3.50 و 48.71± 4.84) بيكومول/مللتر

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 intravacuolar environment or the direct destruction of parasite vacule (PV) [12,22]. TNF-α is a pro-inflammatory cytokine that inflect 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, un attraction 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 ºC 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 Immunosorbent 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 (χ^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)

Table1- Number and percentage of bacteria isolated from abortive women infected with UTI alone and infected with UTI plus toxoplasmosis

Groups	*S.aureus*	*E.coli*	*K. pneumoniae*	Strepto spp.	*S.epidermids*	Chi-Square (χ^2)
Infected with a urinary tract infection	10 (40%)	6 (24%)	4 (16%)	4 (16%)	1 (4%)	9.54 **
Infected with toxoplasmosis and urinary tract infection	19 (76%)	4 (16%)	1 (4%)	1 (4%)	0 (0%)	13.60 **
Chi-Square (χ^2)	8.73 **	4.38 *	4.69 *	4.9**	1.27 NS	----
* (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 aeruginosa*. 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.

Table 2- IFN - γ and TNF-α conc. in all groups

The Group	Mean ± SE	
	IFN - γ conc. (pg/ml)	TNF-α conc. (ng/ml)
Infected with toxoplasmosis	74.84 ± 0.44 A	2.26 ± 0.05 A
Infected with urinary tract infection	33.26 ± 0.89 C	3.32 ± 1.22 A
Infected with toxoplasmosis and urinary tract infection	66.81 ± 0.74 B	2.12 ± 0.06 A
Control	25.60 ± 1.10 D	2.11 ± 0.89 A
LSD value	2.333 **	2.127 NS
P-value	0.0001	0.619
**(P<0.01), NS: Non-Significant. Means having with the different letters in same column differed significantly.		

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 instable 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 trophoplast 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 stimulate 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.

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

Gestational age	Mean ± SE	
	INF- γ conc. (pg/ml)	TNF-α conc. (ng/ml)
First	51.58 ± 3.32 A	3.13 ± 0.77 A
Second	48.95 ± 3.50 A	1.89 ± 0.08 A
Third	48.71 ± 4.84 A	1.68 ± 0.09 A
Control	25.60 ± 1.10 B	2.11 ± 0.89 A
LSD value	11.590 NS	1.991 NS
P-value	0.813	0.219
NS: Non-Significant. Means having with the different letters in same column differed significantly.		

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-1cytokine 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

1. Zhou, X., Yu, Y., Tao, J. and Yu. L. **2014**. Production of LYZL6, a novel human c-type lysozyme, in recombinant Pichia pastoris employing high cell density fed-batch fermentation. *J. Biosci. Bioeng*. **118**: 420–425.
2. Wang, Y., Lv, Y., Wang, L., Gong, C., Sun, J., Chen, X., Chen ,Y., Yang, L., Zhang, Y. and Yang, X. **2015**. MicroRNA in decidua: A new approach to assess the maintenance of pregnancy. *Fertil Steril*. **103**: 980–989.
3. Ventura, W., Koide, K., Hori, K., Yotsumoto, J., Sekizawa, A., Saito, H. and Okai, T. **2013**. Placental expression of microRNA-17 and −19b is down-regulated in early pregnancy loss. *Eur J Obstet Gynecol. Reprod. Biol*. **169**: 28–32.
4. Tang, L., Gao, C., Gao, L., Cui, Y. and Liu, J. **2016**. Expression profile of micro-RNAs and functional annotation analysis of their targets in human chorionic villi from early recurrent miscarriage. *Gene*. **576**: 366–371.
5. Michels, T.C. and Tiu, A.Y. **2007**. Second trimester pregnancy loss. *Am. Fam. Physician.* **76**: 1341–1346.
6. Larsen, E.C., Christiansen,O.B., Kolte, A.M. and Macklon ,N.**2013**.New insights into mechanisms behind miscarriage. *BMC Med*. **11**: 154.
7. Nigro, G., Mazzocco, M. and Mattia, E. **2011**. Role of the infections in recurrent spon-taneous abortion. *J. Matern .Fetal. Neonatal Med*. **24**: 983-9
8. Petit, E., Abergel, A. and Dedet, B. **2012**. The role of infection in preterm birth. *J. of Obstet. Gynecol. and Reprod. Biol*(Paris) Feb;**41**: 14-25
9. Srinivas, S.K., Ma, Y., Sammel, M.D., Chou, D., McGrath, C. and Parry, S. **2006**. Placental inflammation and viral infection are implicated in second trimester pregnancy loss. *Am. J. Obstet. Gynecol*. **195**: 797–802
10. Baud, D., Regan, L. and Greub, G. **2008**. Emerging role of Chlamydia and Chlamydia-like organisms in adverse pregnancy outcomes. *Curr. Opin. Infect. Dis* **21**: 70–76.

11. Allanson,B.,Jennings,B.,Jacques,A.,Charles,A.K.,Keil,A.D.andDickinson,J.E **2010.**Infection and fetal loss in the mid-second trimester of pregnancy. *Aust N Z J. Obstet. Gynaecol*. **50**: 221-225
12. Looker, K. J., Magaret, A.S., Turner, K.M., Vickerman, P., Gottlieb, S. L. and Newman, L.M. **2015**. Global estimates of prevalent and incident herpes simplex virus type 2 infections in 2012. *PLoS One*. **10**: 114989
13. Peyron, F., Mc Leod, R., Ajzenberg, D.,Contopoulos-Ioannidis, D., Kieffer, F. and Mandelbrot, L. **2017**.Congenital Toxoplasmosis in France and the United States: one parasite, two diverging approaches. *PLoS Negl. Trop. Dis*.**11**: 0005222.
14. Raksha, R. H., Srinivasa, R.S. and Macaden. **2003**. Occurence and characterisation of uropathogenic Escherichia coli in urinary tract infections. *Indian J. Med. Micribiol.* **21**: 102-107.
15. Shruthi, N., Ravikumar and Ravish, k. **2012.** Phenotypic study of virulence factors in Escherichia coli isolated from Antenatal cases, catheterised patients, and faecal flora. *J. Clin. Diagn. Res*. **6**: 1699- 1703.
16. Kerure, S. B., Surpur, R., Sagarad, S. and Hegadi, S. **2013**. Asymptomatic bacteriuria among pregnant women. *Int. J. Reproduction, Contraception, Obstet and Gynecol*. **2**: 213-216.
17. Rajshekhar, D., Kerure, and Umashanker. **2013**. Prevalence of Asymptomatic bacteriuria among pregnant women in a tertiary care hospital. *Int. J. Sci. Res. Publications***. 3**: 1-4.
18. Graham, J.C. and Galloway. A. **2001**. The laboratory diagnosis of urinary tract infection. *J. Clin. Pathol*. **54**: 911-919.
19. Raul, R. **2003**. Asymptomatic bacteriuria. Clinical significance and management. Int. *J. Antimicrobial agents*. **22**: 45-47.
20. Manju, S.R. **2014**. Prevalence of asymptomatic bacteriuria and its antibacterial susceptibility pattern among pregnant women attending the antenatal clinic at Kanpur, India. *J. Clin. Diagn. Res*. **8**: DC01-DC03.
21. Clough, B. and Fricke,l E.M. **2017**. The Toxoplasma Parasitophorous vacuole: an evolving host-parasite frontier Trends. *Parasitol*. **33**: 473-488
22. Krishnamurthy, E.K., Konstantinou, L.H., Young, D.A., Gold, J.P.J. and Saei, j. **2017**.The human immune response to Toxoplasma: autophagy versus cell death .*PLoS Pathog*. **13**: 1006176
23. Qidwai, T. and Khan, F. **2011**. Tumour necrosis factor gene polymorphism and disease prevalence. *Scandinavian J of immune*. **74**: 522-547.
24. Yap, G.S., Scharton-Kersten, T., Charest, H. and Sher, A. **1998**. Decreased resistance of TNF receptor p55- and p75-deficient mice to chronic toxoplasmosis despite normal activation of inducible nitric oxide synthase in vivo. *J. of immunol*. **160**: 1340–1345.
25. Clark, D.A., Ding, J., Yu, G., Levy, G.A. and Gorczynski, R.M. **2001**. Fg 12 prothrombinase expression in mouse trophoblast and decidua triggers abortion but may be countered by OX-2. *Mol. Hum. Reprod*. **7**: 185-194
26. Young A, Thomson AJ, Ledingham M, Jordan F, Greer IA, Norman JE**. 2002**. Immunolocalization of proinflammatory cytokines in myometrium, cervix and fatal membranes during human parturition at term. *Biol Reprod*. **66**(2): 445-9.
27. Zaria, L.T., Raufu, I.A. and Mohammed, H.S. **2010**. Isolation and antibiotic sensitivity of *Escherichia coli* from pregnant and nonpregnant women attending the university of Maiduguri Teaching Hospital (UMTH), Maiduguri. *Nigeria. Int. J. of Biomed and Health Sci.* **6**: 159-64.
28. Schnarr, J. and Smaill, F. **2008**. Asymptomatic bacteriuria and symptomatic urinary tract infections in pregnancy. *Eur J Clin Invest*. **38**: 50-7.
29. Emamghorashi, F., Mahmoodi, N., Tagarod, Z. and Heydari, S.T. **2012**. Maternal urinary tract infection as a risk factor for neonatal urinary tract infection. *Iran J Kidney Dis.* **6***:* 178–80.
30. Giraldo, P.C., Araújo, E.D., Junior, J.E., Amaral, R.L.G.D., Passos, M.R.L. and Gonçalves, A.K. **2012**. The Prevalence of Urogenital Infections in Pregnant Women Experiencing Preterm and Full-Term Labor. *Infect Dis Obstetrics Gynecol*. 1–4.
31. AL-Aaraji, M., Hussein, J. H. and Sarhan. A. A. **2017**. Microbial infections of urinary tract in females with Toxoplasmosis. *Int. J. Pharm. Sci. Rev. Res*. **40**: 235-240
32. Jubaida, N., Kawsar, N.M., Elora, N., Rahimgir, M., Shapla, N.R. and Al-Muid, S.M.A. **2013**. Prevalence of asymptomatic bacteriuria in pregnant women. *JAFMC Bangladesh.* **9**: 64-9.
33. Minassian, C., Thomas, S.L., Williams, D.J., Campbell, O. and Smeeth, L.**2013**. Acute maternal infection and risk of pre-eclampsia: a population-based case-control study. *PLoS One*. **8**: 73047

34. Smart, E.A. and Easter, G.N. **2015**. Prevalence of asymtomatic bacteriuria among pregnant women attending antenatal in Port Harcourt Township, Nigeria and antibiogram of isolated bacteria. *Am J of Biomedical* Sci. **7**: 125- 33.
35. Ashkar, A. A., Di Santo, J. P. and Croy B. A. **2000**.Interferon γ contributes to initiation of uterine vascular modification, decidual integrity, and uterine natural killer cell maturation during normal murine pregnancy. *J Exp Med*. **192**: 259–270.
36. Zhang, L., Zhao, M. and Fang, J**. 2015**. Interferon gamma is involved in apoptosis of trophoblast cells at the maternal–fetal interface following *Toxoplasma gondii* infection. *Int. J. of Infect. Dis*. **30**: 10-16
37. Abou-Bakar,A.,Pfaff,A.W.,Letscher-Bru,V.,Filisetti,D.,RajapakseR.,Antoni,E., Villard,O., Kelein,J.P. and Candolfi, **2004.** Role of gamma interferon and T cells in congenital *Toxoplasma* transmission. **26**: 315-318.
38. Berkowitz, R., Hill, J.A., Kurtz, C.B. and Anderson, D.J. **1988.** Effects of products of activated leukocytes on growth of malignant trophoblast cells in vitro .*Am J Obstet Gynecol* **.151**: 199-203.
39. Yui, J., Garcia-Lioret, M., Wegmann, T.G. and Guilbert, L.J. **1994**. Cytotoxicity of tumor necrosis factor-alpha and gamma- interferon against primary human trophoblasts. *Placenta*, **15**(8): 819-835.
40. Gagioti, S., Scavone, C. and Bevilacqua, E. **2000**. Participation of the mouse implanting trophoblast in nitric oxide production during pregnancy. *Biol Reprod*, **62**: 260-268
41. Clark, D.A., Chaouat, G., Arck, P.C., Mittruecker, H.W. and Levy, G.A. **1998.** Cytokine-ependent abortion in CBA X DBA/2 mice is mediated by the procoagulant fgl 2 prothombinase. *J Immunol,* **160**: 545-549.
42. Neale**,** D., Demasio, K., Illuzi, J., Mor, G. and Romero, R**. 2003.** Maternal serum of women with pre-eclampsia reduces trophoblast cell viability: evidence for an increased sensitivity to Fas-mediated apoptosis. *J Matern Fetal Neonatal Med*. **13**: 39-44
43. Hashimoto, K., Komine. F., Hayashi, M. and Ohkura, T. **2002**. Plasma soluble Fas changes during early pregnancy and miscarriage. *Clin. Chimica Acta*. **323**:157-160
44. Jerzak, M. and Bischof, P**. 2002.** Apoptosis in the first trimester human placenta: the role in maintaining immune privilege at the maternal-foetal interface and in the trophoblast remodeling. *J Obst Gyn Reprod Biol*. **100**: 138-142.
45. Coyle, P., Philcox, J.C. and Rofe, A.M. **1993**. Corticosterne enhances the zinc and interleukin-6-mediated induction of metallo-thionein in cultured rat hepatocytes. *J. Nutr.*, **123**: 1464–1470.
46. Chang, H. R., Grau, G. E. and Pechère, J. C. **1990**. Role of TNF and IL-1 in infections with *Toxoplasma gondii*. *Immunol***. 69**: 33–37.
47. Sher, A., Oswald, I. P., Hieny, S. and Gazzinelli. R. T. **1993.** *Toxoplasma gondii* induces a T-independent IFN-gamma response in natural killer cells that requires both adherent accessory cells and tumor necrosis factor-alpha. *J Immunol*. **150**: 3982-3989
48. Marshal, A. J. and Denker, E.Y. **1998.** *Toxoplasma gondii* trigger granulocyte-dependent cytokine-mediated lethal shockin D-galactose amine sensitized mice.*Infect Immun*. **66**: 1325-1333.
49. Raghupathy, R. **1997**. TH1-type immunity is incompatible with successful pregnancy. *Immunol Today,* **18**: 478-482.
50. Roberts, C.W., Walkker, W. and Alexander, J. **2001**. Sex mediate hormones and immunity to protozoan parasite. *Clin Microbiol,* **14**: 476-488.
51. Filiscetti, D. and Candolf, E. **2004**. Immune response to *Toxplasma gondii* . *Ann 1st Super Sanita,* **40**(1): 71 -80