

The levels of IFN- γ , IL-12 And Testosterone Hormone in Persons with Asymptomatic Toxoplasmosis

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Summary:

Background: The primary goal of this study is to perceive the immunological differences in mean level of IFN- γ and IL-12 in persons who have positive results for anti-*Toxoplasma gondii* antibodies compared to persons who gave negative results for these antibodies. The secondary goal is to inspect the endocrine-immune interaction in these persons by detecting the effect of testosterone hormone level on cellular immune response namely, IL-12 & IFN- γ . This study also detect the effect of *Toxoplasma gondii* on the level of testosterone hormone in those persons compared with healthy control.

Objective: Investigate the relationship between *T. gondii* and serum IL-12, IFN- γ and testosterone hormone levels in asymptomatic persons with positive anti-*Toxoplasma* antibodies compared with healthy control and Study the endocrine-immune interaction in these persons.

Patients and methods: Seventy seven asymptomatic persons who have positive anti-*Toxoplasma* antibodies and 30 persons who have negative anti-*Toxoplasma* antibodies were included in this study and subjected to estimating the levels of IL-12, IFN- γ and testosterone hormone by ELISA technique.

Results: The levels of both IL-12 and IFN- γ were statistically higher in persons with positive anti-*Toxoplasma* antibodies than in control. The levels of testosterone hormone were higher in both males and females with positive toxoplasmosis than control. There was a positive association between increased level of testosterone and IL-12 level while there was no effectiveness of testosterone on IFN- γ level in persons with positive anti-*Toxoplasma* antibodies.

Conclusion: *Toxoplasma gondii* elicits cellular immune response detected by statistically important increased in IL-12 and IFN- γ level compared with control. Not only host hormone can affect responses to infection, but parasites can both enhance production and alter hormone concentration in their hosts.

Keyword: Toxoplasmosis, *Toxoplasma gondii*, parasites and sex hormones.

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Introduction:

Toxoplasma gondii is an obligate intracellular parasite which infect a wide variety of avian and number of mammals as intermediate hosts and number of cats' family as definitive hosts. (1) *Toxoplasma gondii* infection stimulates both cell mediated immunity (CMI) in addition to humoral immune response as antibody production, which includes IgM and IgG antibody. (2) Cell mediated immunity (CMI) is considered one of the most distinctive immunological features of *T. gondii* infection, CMI is elicited by the parasite, resulting in host protection against rapid tachyzoite growth and consequent pathologic changes (3). The parasite causes a very strong response of T-helper 1 (Th1) focused on Interferon gamma (IFN- γ), Tumor Necrotizing Factor alpha (TNF- α) and Interleukin 12 (IL-12), secreted by T-lymphocytes. This immune response limits the tissue extension of the parasite, and lead the parasite to convert to bradyzoite (4). The macrophage, T lymphocytes and natural killer (NK) cells on the one hand, and the cytokines, on the other are the major elements involved in immune response

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against *T. gondii*. Antibodies appear to play a minor role but remain essential means for preventing reinfection by the parasite (4) On the other hand, a hypothesis of endocrine-immune interaction is based on the fact that sex hormones, as example, testosterone, influence the immune system. The localization of sex hormones receptors in immune cells, including lymphocytes, macrophages, granulocytes, and mast cells illustrates that there are direct connections between the endocrine and immune systems and that endocrine factors can directly modulate the expression of target genes in immune cells. Thus, considerable research has focused on overcovering sex steroid hormone actions in the immune system and how these influence sex differences in healthy and disease conditions (5)

Patients and Methods:

A total of (107) serum sample were collected from persons referred to laboratory unit of Al-Yarmok Teaching Hospital for marriage testing profile between December 2009 and August 2010. They were divided into two groups: Persons who gave positive results for anti-*Toxoplasma* IgM or IgG antibodies and considered as patients group which include apparently

healthy females (A total of 40 blood samples were collected from females whose age ranged from 18-40 years) and apparently healthy males (a total of 37 blood samples were collected from males whose age ranged from 20-43 years), the second group included persons who gave negative results for anti-*Toxoplasma* IgM or IgG antibodies and considered as control group, which include (30) blood samples collected from apparently healthy individuals (15 male & 15 female) whose age range from (18-45) years. Persons included in this study were subjected to estimate the levels of IL-12, IFN- γ and testosterone by ELISA technique. *All the statistical analysis were done by using SPSS program (version-18) and Excel application. Descriptive and Inferential statistics were used in order to analyze and assess the results.

The incidence of IgM Ab in 77 persons with asymptomatic toxoplasmosis was higher in female than in male (table 1) with significant difference while IgG Ab did not reflect such difference. Table (2) shows the effectiveness of *T. gondii* on both of IL-12 and IFN- γ levels with statistically difference between persons with asymptomatic toxoplasmosis compared to healthy control. *Toxoplasma gondii* was effective on the level of testosterone hormone in both male and female with asymptomatic toxoplasmosis compared to healthy control as shown in (table 3). Table (4) shows the effectiveness of the increase in testosterone level on IFN- γ and IL-12 in persons with asymptomatic toxoplasmosis who gave high testosterone level compared with those with normal testosterone.

Results:

Table (1): Distribution of persons with asymptomatic Toxoplasmosis (male&female) according to IgM &IgG positive results.

Type	No.	%	Gender	No.	p-value	Within gender %	Within patients %
IgM positive persons	16	22.5%	Male	5	0.021 [S]	31.3%	7%
			Female	11		68.7%	15.5%
IgG positive persons	55	77.5%	Male	31	0.131 [NS]	56.4%	43.5%
			Female	24		43.6%	34%

Table (2): Mean serum IL-12(pg/ml) and IFN- γ (IU/ml) among asymptomatic Toxoplasmosis and healthy control .

Type	No.	IL-12 mean (pg/ml)	St.deviation	IFN- γ mean (IU/ml)	St.deviation
Asym.toxo. persons	77	155.3364	78.76441	2.4699	0.80806
control	30	122.1269	28.93851	1.5823	1.04126
		p-value= 0.002[HS]		p-value=0.008[HS]	

Table (3): Comparison between males &females with asymptomatic Toxoplasmosis and males &females of healthy control in mean levels of testosterone hormone (ng/ml).

Type	No.	Testosterone mean(ng/ml)	St.Deviation	P-value
Male asym.toxo.	37	8.0601	3.04751	0.000
Male control	15	4.1123	3.17078	[HS]
Female asym.toxo.	40	0.7213	0.35507	0.011
Female control	15	0.5249	0.18708	[S]

Table (4): Effect of testosterone hormone on IFN- γ &IL-12 levels in persons with asymptomatic Toxoplasmosis.

Type	No.	IFN- γ	St.deviation	IL-12	St.deviation
Asym. cases with high testosterone (male>10,female>0.95)	21	1.97	0.88925	186.9	84.5678
Asym.cases with normal testosterone	56	1.93	0.67577	147.96	11.7214
		P = 0.84 [NS]		P = 0.001 [HS]	

Discussion:

In current study the mean age of 77 patients was (30.9351), ranging from (18-43) years, while the mean age of 30 healthy control was (28.6667), ranging from (18-45) years, the difference between the two groups is statistically non significant and this is an important criteria for the accuracy of statistical analysis. Out of these 77 positive result (who were already diagnosed by ELISA as positive toxoplasmosis persons, 16(22.5%) gave positive result for anti-*Toxoplasma* IgM antibody, while 55(77.5%) gave positive result for anti-*Toxoplasma* IgG antibody (table 1), 6 patients

gave both IgM & IgG antibody positive results, this may be due to acute on chronic infection (6), so, we exclude these results from statistical analysis. In this study, the incidence of IgM antibody in female was higher than in male with significant difference (table 1). This result was agreed with Khalil (7), her results showed high incidence of IgM antibody in female compared to male. This could be explained by the fact that females in our countries are more closed to stray cats and deal with raw meat containing bradyzoites.. However, this result disagreed with Klien (8) who showed that male more susceptible than female to

many parasites, although Klein “suggest” that males are more resistant to some parasites than females and endocrine-immune interactions may underlie this sex reversal. In Iraq, closed results were obtained, Al-Sorchee (9) revealed that the percentage of anti-IgG antibody and anti-IgM antibody were (68.3%) and (31.7%) respectively. Other studies were done in Iraq by Abbas,(10) and in USA by Oliver *et al.*(11) showed that specific anti-*Toxoplasma* IgM antibodies in women were (60.2%),and (60.4%) respectively. Obviously these results were disagreed with this study. The explanation of this variability may be due to large sample size, geographical location, and the existence of different strains of *T. gondii* which play an important role in difference in infection rate. Within gender, male showed 31 (43.5%)positive result for IgG antibody and 5(7%) gave positive result for IgM antibody (table 1).These results were in-line with Jassam (12) her study were done on 100 male with schizophrenia they were also infected with *T.gondii*, (49%) of male patients showed positive IgG antibody, while (6%) showed positive results for IgM antibody. IL-12 is required for resistance to acute and chronic Toxoplasmosis due to its essential role in stimulating production of IFN- γ and facilitates formation of Th1 type response ,(15),(16) and activates lymphocyte cytotoxicity (15). This study showed high level of IL-12 in both male and female with asymptomatic toxoplasmosis in comparison with control group (table2). This may be due to the fact that the infection with *T. gondii* induce strong CMI characterize by a high Th1-cell response (16), (17). Our results are compatible with a study done by Kareem (18), her result revealed a significant difference between female infected with *T. gondii* and apparently healthy woman in IL-12 level. This study showed significant increase in the level of IFN- γ in asymptomatic Toxoplasmosis in comparison with control subjects (table2).This indicates that *T.gondii* is an opportunistic intracellular parasite which induces a highly strong type-1 cytokine response (19) such as IFN- γ and IL-2 (20) during initial infection as a result of early T-cell as well as NK cell activation (3). This result agrees with other studies which showed significant increase in the level of IFN- γ in persons infected with *T. gondii* compared with healthy control. On the other hand ,several lines of indirect evidence suggest that the subjects with latent infection of the coccidian parasite *Toxoplasma gondii* have a higher concentration of testosterone than uninfected controls (21). Here we searched for direct evidence of toxoplasmosis –associated differences in testosterone concentration among male and female patients with acute and latent Toxoplasmosis. This study showed that *Toxoplasma* –infected men and women have a higher concentration of testosterone than *Toxoplasma*-free controls with highly significant difference (table 3). The proximal mechanism of association between

testosterone and toxoplasmosis is not clear. As stressed in the articles of Flegr *et al.*(21), a case control study cannot decide whether *Toxoplasma* infection induce changes in testosterone concentration or whether low-testosterone and high-testosterone subjects differ in the probability of acquiring *Toxoplasma* infection or both . The increased level of testosterone in infected person could be explained by a positive association between testosterone and dopamine (22), (23), (24) that can increase in response to local inflammatory processes in the infected brain. Other study were done by Jassam (12) showed highly significant difference between *Toxoplasma*-infected men and controls which was compatible with this study. Several studies link sex differences in immune function with circulating steroid hormones, thus, the roles of sex steroids, including testosterone that showed the impact of sex –associated hormones on the cellular immune response (IFN- γ &IL-12) against *T. gondii*.This study showed that there was no significant difference in mean level of IFN- γ between asymptomatic toxoplasmosis with high testosterone (male>10), (female>0.95) and those with normal testosterone (table 5).In comparison with some previous studies in the world, this result was in-line with (25),(26),(27). They all believed that neither progesterone, nor testosterone altered or increased IFN- γ production. Table (4) also showed the increased level of IL-12 in asymptomatic cases with high testosterone compared to those with normal testosterone,This elevation of IL-12 was statically highly significant. This elevation could be explained as the testosterone stimulates monocyte IL-12 production. Indeed, physiological levels of testosterone increased IL-12 production by LPS-stimulated monocytes (27).

References:

1. Schreiber R, and Feldman H.(1980). Identification of the activator system for antibody to *Toxoplasma* as the classical complement pathway. *J. Infect. Dis. S;141(3):366-369.*
2. Phuangphet W.(2008).*Toxoplasmosis:Pathogenesis and immune response. Thammasat Medical Journals.(8):pp;487 [Abstract].*
3. Denkers, E.Y. and Gazzinelli, R.T. (1998). Regulation and function of T-cell-mediated immunity during *T.gondii* infection.*Clin Microbiol Rev;(11):569-588.*
4. Filisetti D and Candolfi E (2004). Immune response to *Toxoplasma gondii*. *Ann Ist Super Sanita.40(1):71-80.*
5. Klein SL.(2000). The effects of hormones on sex differences in infection:from genes to behavior. *Neurosci Biobehav Rev ; (24):627–638.*
6. Wilson , M.MC., McAuley, JB.(1999). *Toxoplasma in murrey, Manual or clinical*

microbiology .7th Ed.Washington.DC.ASM press 1374-1382.

7. Khalil, H.I.(2008). Some aspect in seroprevalence, diagnosis, and influence of sex hormones on immunity during human toxoplasmosis. Ph.D.Thesis. Collage of Medicine, AL-Mustansiriyah University.

8. Klein, SL.(2004).Hormonal and immunological mechanisms mediating sex-differences in parasitic infection.Parasite Immunology.(26):247-267.

9. Al-Sorchee, S.M.(2005). Immunological study on toxoplasmosis in women with a history of abortion. MSc. thesis,Collage of Education (Ibn AL-Haitham), Baghdad University.

10. Abbas , M.M.A.(2000).Seroepidemiological study with a history of abortion. MSc.Thesis ,College of Medicine,Al-Nahrain University.pp125.

11. Oliver, L ,Jose ,G. Naga , J. Tathmene ,M.Byron ,B. J. Kristen ,L ,and Remington, J.S.(2001).Confirmatory serological testing for acute toxoplasmosis and rate of induced abortion among women reported to have positive T. gondii IgM antibodies titers. AM. J.Obst.Gynecol.(2):184.

12. Jassam, F.S.(2010). Relationship between toxoplasmosis and testosterone hormone among schizophrenic patients in Baghdad. MSc. Thesis,College Council of Health and Medical Technology.pp81.

13. Gazzinelli ,R.T., Wysocka, M.,Hayashi,S.,Denkers, E.,Hieny, S.,Casper, P.,Trinehieri,G., and Sher,A.(1994).Parasite induced IL-12 stimulates early IFN- γ synthesis and resistance during acute infection with *Toxoplasma gondii*. J. Immunol.(153):2533.

14. Scharton,, Kersten ;T.M.;T.A. Wynn ; E.Y.Denkers; S.bala; L. Show; E. Grunvald; S.Hieny ;R.T. Gazzielli ; and A.sher; (1996). In the absence of endogenous IFN-gamma mice develop unimpaired IL-12 response to *Toxoplasma gondii* while fating to control acute infection .J. Immunol. (157):4045-4054(abstract).

15. Evering ,T.,Weiss,L.M.(2006). The immunology of parasite infections in immunocompromized hosts. Parasite Immunology;28(11):549-565.[pubMed].

16. Sher, A., Denkers, E.Y.,and Gazzinelli, R.T.(1995).Induction and regulation of host-cell-mediated immunity by *Toxoplasma gondii* .Ciba found. Symp.(195):95-109 [Medline].

17. Makhseed ,M., Raghupathy ,R. ,Azizieh ,F., Omu A., Al-Shamali ,E. ,and, Ashmani ,L.(2001).Th1 and Th2 cytokine profiles in recurrent abortions with successful pregnancy and with subsequent aborters .Department of Obstetrics &Gynecology and Microbiology Faculty of Medicine ,Kuwait University and Maternity Hospital,Kuwait.

18. Kareem ,S.S.(2008). Prevalence, serodiagnosis and some immunological aspects of toxoplasmosis among women in Baghdad province.MSc.thesis,Collage of Health and Medical Technology,Baghdad University.

19. Gavrilesco , L.C., Butcher, B.A.; Delrio, L.; Taylor, G.A.Denkers, E.Y.(2004).STAT is essential for antimicrobial effector function ,but dispensable for Gamma interferon production during *Toxoplasma gondii* infection . Infect. Immunol.(72):1257-1264.(abstract).

20. Abou-Bacar,A.,Pfaff,A.W.,Georges,S.,Letscher-BruV.et al.(2004). Role of NK cells and gamma interferon in transplacental passage of *Toxoplasma gondii* in a mouse model of primary infection. Infect. Immune;172(3):1397-1401.

21. Flegr,J., Lindova,J. , Kodym, P.(2008). Sex-dependent toxoplasmosis-associated differences in testosterone concentration in humans. National Reference Laboratory for Toxoplasmosis.pp427-431.

22. Szczycka ,M.S.; Zhou ,Q.Y. and Palmiter,R.D.(1998). Dopamine-stimulated sexual behavior is testosterone dependent mice. Behavioral Neuroscience.(122):1229-1235.

23. Hull E.M.,Muschamp J.W. and SatoS.(2004).Dopamine and serotonin:influences on male sexual behavior. Physiology and Behavior.(83):291-307.

24. Dominguez J.M. and Hull E.M.(2005).Dopamine, the medial preoptic area and male sexual behavior .Physiology and Behavior (86):356-368.

25. Piccinni MP, Giudizi MG, Biagiotti R, Beloni L, Giannarini L, Sampognaro S, Parronchi P, Manetti R, Annunziato F and Livi C (1995) Progesterone favors the development of human T helper cells producing Th2-type cytokines and promotes both IL-4 production and membrane CD30 expression in established Th1 cell clones. J Immunol (155): 128–133.

26. Giron-Gonzalez JA, Moral FJ, Elvira J, Garcia-Gil D, Guerrero F, Gavilan I and Escobar L (2000). Consistent production of a higher TH1:TH2 cytokine ratio by stimulated T cells in men compared with women. Eur J Endocrinol 143, 31–36.

27. Posma E, Moes H, Heineman MJ and Faas M. (2004) The effect of testosterone on cytokine production in the specific and non-specific immune response. Am J Reprod Immunol(52): 237–243.