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Original Research Article

Serum Prolactin Levels and Toxoplasma Infection: A Prospective Observational Study

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Abstract

Aim: To study the of serum prolactin levels and *Toxoplasma* infection in humans. **Methods:** A prospective study was conducted in the Department of General Medicine, Patna Medical College and Hospital, Patna, Bihar, India for 1 year. A total of 343 blood samples were collected from individuals who had been referred for PRL measurement in medical diagnostic laboratories. Demographic characteristics such as sex, age, marital status, and current pregnancy status were recorded through questionnaires. ELISA was designed to detect anti-*Toxoplasma* IgG antibody in blood sera. **Results:** Of the total participants, 70% were women and 30% men. The highest frequency of participants 43% were found in the age group of 30–40years. Of 343 blood serum samples, 110 samples (32%) had anti-*Toxoplasma* IgG. Participants were divided into five age groups of below 19, 20–30, 30–40, 40–50, and above 50 years. According to the age of participants, the prevalence of anti- *Toxoplasma* IgG in 343 blood serum samples was as follows: <19 age group, (18.7%); 20–30, (29.8%); 30–40, (34.9%); 40–50 age group, (31.6%); and >50 age group, (38.5%). Of 240 serum samples of women, 68 (28.3%) had anti-*Toxoplasma* IgG while of 103 serum samples of men 42 (40.8%) had anti- *Toxoplasma* IgG antibody. In total, of 343 serum samples, 171 (49.8%) were considered as normal range of PRL, 16 (4.7%) and 156 (45.5%) samples were considered as hypoprolactinemia and hyperprolactinemia, respectively. **Conclusion:** The results of the current study confirmed the previous studies based on immuno regulatory role of PRL and indicated that high levels of PRL could be related to *T. gondii* sero- negativity in women.

Keywords: PRL, Toxoplasma, IgG

Introduction

Toxoplasmosis is a disease caused by a protozoan parasite. Human and a wide range of animals are its host. The infection has a worldwide distribution. Its estimated that one-third of human population are exposed to this parasite. Human may remain infected for life and will stay asymptomatic unless immunosuppression occurs.[1] The hormone levels in a certain situations could be altered and the dissimilar effects on the immune system may induce resistance or susceptibility to different parasite attacks. The sharp elevated sex steroids could worsen toxoplasmosis; mainly through suppressing host immune-endocrine network (IEN) and

progressing parasite replication.[2] Higher incidence of *Toxoplasma* encephalitis was recorded within AIDS-defining females than in males, this support that female hormones possibly predispose latent toxoplasmosis[3] and was confirmed to stimulate higher parasite load in guinea pigs. The actual dynamics stimulating latency are still unknown; however various stimuli were studied including hormonal factor.[4] In Baghdad, male total and free testosterone hormone recorded higher significant mean in both acute and chronic toxoplasmosis person than in control subject.[5] The levels of testosterone hormone were higher in both males and females with positive toxoplasmosis than control in Al-Yarmok Teaching Hospital[6] In Al-Mahaweel healthy center in north of Babylon province pregnant women with chronic *T. gondii* infection exhibited significant increases of testosterone serum levels and significant decreased of prolactin serum levels in all trimesters, a significant increase occurred to progesterone in seropositive IgG pregnant women when compared with those of the control group during third trimester.[7]Progesterone and estrogen hormones were measured in a group of *Toxoplasma* infected Iraqi pregnant women in Baghdad, the chronic infected women had higher hormone concentration than acute one.[8] Acute and chronic toxoplasmosis males record significantly higher concentrations in both total testosterone hormone (TTH) and free testosterone hormone (FTH) than in control group. The mean concentration of FSH revealed non-significant differences between diseased and un diseased control.[9] A direct relation between *Toxoplasma* infection, cortisol and testosterone increase were observed in men and women patients referred to Sina hospital, Tehran, stress and anxiety index also increased in men and women whereas depression index increased only in men.[10] indirect support for the assumption that testosterone may be implicated in the personality and behavioral differences between *Toxoplasma*-infected and *Toxoplasma*-free subjects.[11]

Materials and methods

A prospective study was conducted in the Department of General Medicine, Patna Medical College and Hospital, Patna, Bihar, India for 1 year, after taking the approval of the protocol review committee and institutional ethics committee. Men and women aged 15–60 years with no clinical complications participated in this cross-sectional study. A total of 343 blood samples were collected from individuals who had been referred for PRL measurement in medical diagnostic laboratories. Demographic characteristics such as sex, age, marital status, and current pregnancy status were recorded through questionnaires. Woman participants who were pregnant/nursing were excluded from the current study. Then, 3 mL of whole blood samples were collected from each of them; the sera were separated and stored at –20°C until use. After collecting samples, concentration of PRL was measured and the samples were divided into cases with high or low levels of PRL and comparison group with normal levels of PRL.

Serological tests

ELISA was designed to detect anti-*Toxoplasma* IgG antibody in blood sera. The cut-off values of ODs were calculated according to Hillyer et al.[12] The OD of each sample was compared with the cut-off and recorded as positive or negative result. The cut-off value with 95% CI was determined to be 0.45 for the detection of anti-*T. gondii* IgG.

Preparation of soluble antigens of T. gondii

Tachyzoites of *T. gondii*, RH strain was maintained in BALB/c mice with serial passages.[13] Tachyzoites that had been inoculated in peritoneal cavity of BALB/c mice were harvested by peritoneal washing with PBS (pH 7.2). The tachyzoites were washed two times with cold PBS, Sonicated, and centrifuged at 4°C,

14,000x*g* for 1 hour. Then, supernatant was collected as soluble antigen, and the protein concentration was determined by Bradford method.

Detection of anti-*Toxoplasma* IgG antibody using ELISA technique

Microtiter plates were coated with soluble antigens of *T. gondii*, RH strain. Sera were added in dilution of 1:100 in PBS followed by incubation and washing. Anti-human IgG conjugated with horseradish peroxidase (HRP; Dako Denmark A/S, Glostrup, Denmark) was added after incubation. After washing, chromogenic substrate ortho-phenyline-diamidine (OPD) was added and the reaction was stopped by adding sulphuric acid. The optical density was read and recorded by an automated ELISA reader at 490 nm.[14]

PRL assessment

Concentration of PRL was measured by Roche Elecsys 2010 analyzer, electrochemiluminescence (ECL) technology for all the collected sera according to the manufacturer's instructions. In the first step, 10 µL of the samples were incubated with a biotinylated monoclonal PRL-specific antibody. In the second step, a monoclonal PRL-specific antibody labeled with a ruthenium and streptavidin-coated microparticles were added to the mixture. The reaction mixture was aspirated to a measuring cell and the microparticles were magnetically captured on the surface of an electrode. Unbound substances were removed with ProCell/ProCellM. Chemiluminescence was measured by a photomultiplier and the concentration of PRL was determined via a calibration curve.[15] Interpretation of the PRL concentration was based on the manufacturer's recommendation as follows: normal range for men, 86–324 µIU/mL; and for non-pregnant women, 102–496 µIU/mL. Experiments were carried out in triplicate, and the mean was calculated for each sample

Statistical analyses

Data were analyzed by Statistical Package for Social Sciences software (version 25.0, IBM Corporation, Armonk, NY, USA).

Results

Of the total participants, 70% were women and 30% men. The highest frequency of participants 43% were found in the age group of 30–40years. Of 343 blood serum samples, 110 samples (32%) had anti-*Toxoplasma* IgG. Participants were divided into five age groups of below 19, 20–30, 30–40, 40–50, and above 50 years. According to the age of participants, the prevalence of anti- *Toxoplasma* IgG in 343 blood serum samples was as follows: <19 age group, (18.7%); 20–30, (29.8%); 30–40, (34.9%); 40–50 age group, (31.6%); and >50 age group, (38.5%) (Tables 1 and 2). Of 240 serum samples of women, 68 (28.3%) had anti-*Toxoplasma* IgG while of 103 serum samples of men 42 (40.8%) had anti- *Toxoplasma* IgG antibody (Tables 1 and 2). In total, of 343 serum samples, 171 (49.8%) were considered as normal range of PRL, 16 (4.7%) and 156 (45.5%) samples were considered as hypoprolactinemia and hyperprolactinemia, respectively. The detailed data of serum PRL levels according to the sex of participants are shown in Table 3

Table 1: Frequency of anti-*Toxoplasma* IgG antibody in 240 blood serum samples of women according to particular age groups by ELISA

	***Toxoplasma*-specific IgG**		
	Positive	**Negative**	**Total**
Age groups (years)	n (%)	n (%)	n (%)
Below 19	2 (28.6)	5 (71.4)	7 (100)
20–30	27 (27)	73 (73)	100 (100)
30–40	31 (29.2)	75 (70.8)	106 (100)
40–50	5 (23.8)	16 (76.2)	21 (100)
Above 50	3 (50)	3 (50)	6 (100)
Total	68 (28.3)	172 (71.7)	240 (100)

Table 2: Frequency of anti-*Toxoplasma* IgG antibody in 103 blood serum samples of men according to particular age groups by ELISA

	***Toxoplasma*-specific IgG**		
	Positive	**Negative**	**Total**
Age groups (years)	n (%)	n (%)	n (%)
Below 19	1 (11.1)	8 (88.9)	9 (100)
20–30	10 (41.7)	14 (58.3)	24 (100)
30–40	22 (47.8)	24 (52.2)	46 (100)
40–50	7 (41.2)	10 (58.8)	17 (100)
Above 50	2 (28.6)	5 (71.4)	7 (100)
Total	42 (40.8)	61 (59.2)	103 (100)

Table 3: Serum prolactin levels according to sex of the participants by Roche Elecsys 2010 analyzer

Sex	**Prolactin concentration (µIU/mL)**			
	Hypo	**Normal**	**Hyper**	**Total**
	n (%)	n (%)	n (%)	n (%)
Women	12 (5)	96 (40)	132 (55)	240 (100)
Men	4 (3.9)	75 (72.8)	24 (23.3)	103 (100)
Total	16 (4.7)	171 (49.8)	156 (45.5)	343 (100)

Table 4: Association of anti-*Toxoplasma gondii* IgG antibody and serum prolactin levels in 240 serum samples of women

Prolactin concentration (µIU/mL)	***Toxoplasma*-specific IgG**		**Total**	**$\chi 2$ (1 *df*)**	***P*-value**
	Positive	**Negative**			
	n (%)	n (%)	n (%)		
Hypo	4 (33.3)	8 (66.7)	12 (100)	0.045	1
Normal	35 (36.5)	61 (63.5)	96 (100)	–	–
Hyper	29 (30)	103 (70)	132 (100)	5.77	**0.016**
Total	68 (28.3)	172 (71.7)	240 (100)		

Table 5: Association of anti-*Toxoplasma gondii* IgG antibody and serum prolactin levels in 103 serum samples of men

Prolactin concentration (μIU/mL)	*Toxoplasma*-specific IgG		Total	□2 (1 *df*)	*P*-value
	Positive	Negative			
	n (%)	n (%)	n (%)		
Hypo	2 (50)	2 (50)	4 (100)	0.11	0.74
Normal	31 (41.3)	44 (58.7)	75 (100)	–	–
Hyper	9 (37.5)	15 (62.5)	24 (100)	0.11	1
Total	42 (40.8)	61 (59.2)	103 (100)		

Discussion

Complex hormonal regulations are necessary for specific immune responses to parasite antigens and effects on interleukins or interferon gamma.[16] Proliferation of lymphocytes in primary and secondary lymphoid organs depends on the interactions between PRL and growth hormone. PRL is a hormone secreted by the pituitary gland which is located below the cerebral cortex.[17] PRL is produced by the placenta uterus, B and T lymphocytes, and NK cells. B and T lymphocytes and macrophages have PRL receptors. PRL secretion is controlled by PRL inhibitory factors, and both men and women have low levels of this hormone in their blood.[18] The situation in which large amounts of PRL are in blood of men or non-pregnant women is called hyperprolactinemia that is fairly common in women.[19] Observed differences between men and women in the prevalence of many parasitic infections can indicate the potential role of sex hormones in the immunity against parasites.[20] One of the hormones that exhibits a wide range of biological activities, including immunomodulatory effects, is PRL. In this study, we have attempted to explain if there was an association between the level of PRL and the frequency of *T. gondii* infections among women and men. Preliminary data, comparing the prevalence of *T. gondii* infection in the population of patients with the PRL level below and above the normal with the population of those having normal PRL level, revealed lower seroprevalence in the group of men and women with hyperprolactinemia. However, differences of *Toxoplasma* seropositivity in women with high levels of PRL was statistically significant in comparison with the population of those having normal levels of PRL (P=0.016). In addition, in hyperprolactinemia women by increasing of PRL levels, the prevalence of *T. gondii* infection decreased. No *Toxoplasma* seropositivity was observed in five serum samples of participants with the highest concentration of PRL.

It has been proven that PRL deficiency in mice may increase the probability and severity of infections. Bromocriptine, the inhibitor of PRL secretion, is used in organ transplantation and autoimmune diseases to inhibit the immune system.[21] It is reported that human PRL has the ability to bind with live tachyzoites of *T. gondii*, RH and ME49 strains.[22] It was shown that PRL has the inhibitory effects on *Toxoplasma* proliferation in mononuclear cells of individuals with high PRL levels. Meli et al in 1996 reported the protective role of PRL against *salmonella typhimurium* in rat model and found that macrophage phagocytic activity and nitric oxide production increased in the rats that had received PRL.[23] Benedetto et al in 2001 showed that PRL can increase the production of interleukins 1 and 6 by microglial cells which stimulate anti-*Toxoplasma* function in the brain of infected mice.[24] Zhang et al in 2002 examined two patients with benign pituitary tumors and found *Toxoplasma* cyst among these tumor cells. They reported that multiplication of pituitary cells result in PRL production and anti-*Toxoplasma*

activation of microglial cells.[25] Moreover, the hypothesis on the protective role of PRL in protozoan infections is addition- ally supported by Gomez-Ochoa et al.[26] They concluded that lactating female hamsters that were infected with *Leishmania infantum* showed no symptom of infection compared with control group.[26] Li et al in 2015 showed that PRL-inducible protein (PIP) can impair Th1 immune response and increase susceptibility to *Leishmania major* in mice. PIP is a 14 kDa protein that is present in saliva of mice and upregulates by PRL, and it seems that this protein plays a role in host defense against pathogens.[27] In the study of Serrano et al in 2009 *Neospora* seropositive non- aborting cows had more PRL compared with non-infected ones.[28] Dzitko et al in 2010 suggested the in vitro effects of recombinant PRL on intracellular replication of *T. gondii*, BK strain. It seems that PRL has no direct cytotoxic effects on host cells or parasite, but it can probably bind to para- site surface protein and block its receptors.[29] In the study conducted by Dzitko et al in women with high PRL levels, *T. gondii* prevalence was lower than control group (33.9% vs 45.58%).[30] PRL receptors are located on the surface of B and T lymphocytes and macrophages and the production of cytokines such as TNF-α, IFNγ,, and IL-12 is induced by this hormone. The higher levels of TNF-α, IFNγ, and IL-12 in hyperprolactinemia patients may be the reason for protecting these individuals against toxoplasmosis. At the last stage of our analysis, the seroprevalence of toxoplasmosis in women was 28.3% while this value in men reaches to 40.8% (*P*=0.038), confirming earlier observations carried out on several parasitic diseases. Similar results reported a higher prevalence and intensity of infections for men than for women in the case of protozoan parasites such as *Entamoeba histolytica*, *Leishmania donovani*, *Leishmania braziliensis*, and *Plasmodium falciparum*.[31–34] The overall anti-*Toxoplasma* IgG prevalence was 32% in this study. The prevalence of toxoplasmosis in the general population of this area was 45.5% according to the study by Keshavarz et al in 1998.[35] The seroprevalence of toxoplasmosis among women and men was also estimated in relation to the age of the patients. The highest toxoplasmosis seroprevalence for men and women was found in 30–40 and >50 years age group, respectively (Tables 1 and 2). These data were in accordance with the range of general seropositivity expected for Iranian general population.[8]

Conclusion

The results of the current study confirmed the previous studies based on immunoregulatory role of PRL and indicated that high levels of PRL could be related to *T. gondii* sero- negativity in women

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