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Original Research

To determine the correlation between serum prolactin levels and Toxoplasma infection

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ABSTRACT:

Aim: To determine the correlation between serum prolactin levels and Toxoplasma infection. **Methods:** The Department of General Medicine performed a prospective research. A total of 200 blood samples were taken from people who had been sent to medical diagnostic labs for PRL assessment. The ELISA test was developed to detect anti-Toxoplasma IgG antibodies in blood serum. **Results:** The overall number of participants was 140 (70 percent) women and 60 (30 percent) males. Anti-Toxoplasma IgG antibodies were found in 46 (32.86 percent) of 140 female serum samples and 24 (40 percent) of male serum samples. In total, 102 (51%) of 200 blood samples were determined to be in the normal range of PRL, whereas 10 (5%) and 88 (44%) were considered to be in the hypoprolactinemia and hyperprolactinemia, respectively. In this research, the total anti-Toxoplasma IgG prevalence was 35%. Toxoplasmosis seroprevalence in women was 32.86 percent, while it reached 40 percent in males. **Conclusion:** The present study's findings corroborated earlier research based on PRL's immunoregulatory function, indicating that high levels of PRL may be associated with T. gondii sero-negative women.

Keywords: Serum prolactin, Toxoplasma infection

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INTRODUCTION

Toxoplasmosis is a parasitic infection caused by a protozoan parasite. Its hosts include humans and a variety of animals. The illness is found all over the globe. It is believed that one-third of the human population is infected with this parasite. Humans may be infected for life and remain asymptomatic until immunosuppression is used. [1] Hormone levels may be changed in particular settings, and the disparate impacts on the immune system may result in resistance or vulnerability to certain parasite infections. The significant increase in sex hormones may aggravate toxoplasmosis, mostly by inhibiting the host immune-endocrine network (IEN) and advancing parasite reproduction. [2] Females with AIDS had a greater prevalence of Toxoplasma encephalitis than men, indicating that female hormones may predispose to latent toxoplasmosis[3] and were verified to promote higher parasite load in guinea pigs. The true dynamics of latency are unclear; nevertheless, several triggers, including hormone factors, have been explored. [4] Male total and free testosterone hormone levels were significantly greater in acute and chronic toxoplasmosis patients in Baghdad than in controls. [5] In Al-Yarmok Teaching Hospital, testosterone levels were higher in both men and females with positive toxoplasmosis than in controls. [6] Pregnant women with chronic T. gondii infection exhibited significant increases in testosterone serum levels and significant decreases in prolactin serum levels in all trimesters at the Al-Mahaweel healthy centre in the north of Babylon province, with a significant increase in progesterone in seropositive IgG pregnant women when compared to the control group during the third trimester.[7] Progesterone and oestrogen hormone levels were examined in a group of Toxoplasma infected Iraqi pregnant women in Baghdad; chronic infected individuals had greater hormone levels than acute infected women. Males with acute and chronic toxoplasmosis had considerably greater levels of total testosterone hormone (TTH) and free testosterone hormone (FTH) than the control group. The mean FSH concentration exhibited no statistically

significant differences between sick and uninfected controls. [8,9] In men and women sent to Sina hospital in Tehran, a direct relationship between Toxoplasma infection, cortisol and testosterone rise was identified. Stress and anxiety indexes rose in both men and women, while depression index increased only in males. A research on students from the Faculty of Sciences at Charles University in Prague gave [10] indirect evidence for the hypothesis that testosterone may be involved in the personality and behavioural differences between Toxoplasma-infected and Toxoplasma-free people. [11]

MATERIALS AND PROCEDURES

After receiving clearance from the protocol review committee and the institutional ethics committee, a prospective research was undertaken at the Department of General Medicine. This cross-sectional research included men and women aged 16 to 58 with no clinical problems. A total of 200 blood samples were taken from people who had been sent to medical diagnostic labs for PRL assessment. Questionnaires were used to collect demographic information such as gender, age, marital status, and current pregnancy status. Women who were pregnant or breastfeeding were not allowed to participate in this research. The sera were separated and kept at -20°C until usage, after which 3 mL of whole blood samples were obtained from each of them. After collecting samples, PRL concentrations were analysed, and the samples were classified into cases with high or low PRL levels, and a reference group with normal PRL levels.

SEROLOGICAL TESTS

The ELISA test was developed to detect anti-Toxoplasma IgG antibodies in blood serum. The OD cutoff values were obtained using Hillyer et al. [12] The OD of each sample was compared to the cutoff and the result was reported as positive or negative. For the detection of anti-T. gondii IgG, the cutoff value with 95 percent CI was established to be 0.45.

PREPARATION OF SOLUBLE ANTIGENS OF T. GONDII

T. gondii RH strain tachyzoites were maintained in BALB/c mice by repeated passages. Thirteen Tachyzoites injected in the peritoneal cavity of BALB/c mice were collected by peritoneal washing with PBS (pH 7.2). The tachyzoites were washed twice with cold PBS, sonified, then centrifuged for 1 hour at 4°C, 14,000xg. Supernatant was then collected as soluble antigen, and protein content was measured using the Bradford technique.[13]

DETECTION OF ANTI-TOXOPLASMA IGG ANTIBODY USING ELISA TECHNIQUE

Soluble antigens of T. gondii, RH strain, were coated on microtiter plates. Sera were added to PBS at a 1:100 dilution, followed by incubation and washing. After incubation, anti-human IgG coupled with horseradish peroxidase (HRP; Dako Denmark A/S, Glostrup, Denmark) was added. Following washing, the chromogenic substrate ortho-phenyline-diamidine (OPD) was added, and the reaction was terminated with sulfuric acid. An automated ELISA reader was used to measure and record the optical density at 490 nm. [14]

PRL ASSESSMENT

For all collected sera, the concentration of PRL was determined using the Roche Elecsys 2010 analyzer using electrochemiluminescence (ECL) technology according to the manufacturer's instructions. The samples were treated with a biotinylated monoclonal PRL-specific antibody in the first phase. The combination was then treated with a monoclonal PRL-specific antibody tagged with ruthenium and streptavidin-coated microparticles in the second stage. The reaction mixture was sucked into a measurement cell, and the microparticles were collected magnetically on the surface of an electrode. ProCell/ProCellM was used to remove unbound chemicals. A photomultiplier was used to quantify chemiluminescence, and a calibration curve was used to calculate PRL concentration. 15 The following was the manufacturer's guideline for interpreting PRL concentration: The typical range for males is 86-324 IU/mL, while the usual range for non-pregnant women is 102-496 IU/mL. Experiments were carried out in triplicate, and the mean for each sample was computed.

STATISTICAL ANALYSES

Data were analyzed by Statistical Package for Social Sciences software (version 23.0, IBM Corporation, Armonk, NY, USA).

RESULTS

Of the total participants, 140(70%) were women and 60(30%) men.

Table 1 Gender distribution

Sex	Number	Percentage
Male	60	30
Female	140	70

According to the age of participants, patients was as follows: <25 age group, (20%); 25–35, (30%); 35–45, (47%); 45–55 age group, (31.6%); and >50 age group, (38.5%)

Tables 2: Age distribution

Age groups (years)	Number	Percentage
below 25	28	14
25-35	36	18
35-45	94	47
45-55	24	12
Above 55	18	9

The highest frequency of participants 47% were found in the age group of 35-45years. Of 140 serum samples of women, 46 (32.86%) had anti-*Toxoplasma* IgG while of 60 serum samples of men 24 (40%) had anti-*Toxoplasma* IgG antibody (Tables 3).

Table 3 Prevalence of anti- *Toxoplasma* IgG antibody in men and women

	***Toxoplasma*-specific IgG**			
Gender	**Positive**	**Negative**	**Total=N**	**%**
Male	24(40%)	36(60%)	60	30
Female	46(32.86%)	94(67.14%)	140	70
	70(35%)	130(65%)	200	100

In total, of 200 serum samples, 102 (51%) were considered as normal range of PRL, 10 (5%) and 88(44%) samples were considered as hypoprolactinemia and hyperprolac- tinemia, respectively. The detailed data of serum PRL levels according to the sex of participants are shown in Table 4

Table 4 Serum prolactin levels according to sex of the participants

Gender	**Prolactin concentration (µIU/mL)**			
	Hypo	**Normal**	**Hyper**	**Total**
	n (%)	n (%)	n (%)	n (%)
Women	8 (5.71)	57 (40.71)	75 (53.57)	140
Men	2 (3.33)	45 (75)	13 (21.67)	60
Total	10(5)	102 (51)	88 (44)	200

Table 5 Association of anti-*Toxoplasma gondii* IgG antibody and serum prolactin levels in 200 serum samples

Prolactin concentration (µIU/mL)	***Toxoplasma*-specific IgG**		**Total**	**$\chi 2$ (1 *df*)**	***P*-value**
	Positive	**Negative**			
	n (%)	n (%)	n (%)		
Hypo	4 (40)	6 (60)	10	0.041	1
Normal	42 (41.18)	60 (58.82)	102	–	–
Hyper	24 (27.27)	64 (72.73)	88	5.66	**0.019**
Total	70	130	200		

DISCUSSION

Specific immune responses to parasite antigens and effects on interleukins or interferon gamma need complex hormonal control. [16] The interactions between PRL and growth hormone influence lymphocyte proliferation in primary and secondary lymphoid organs. The pituitary gland, which is positioned underneath the cerebral cortex, secretes PRL. [17] The placenta, uterus, B and T lymphocytes, and NK cells all make PRL. PRL receptors are found on B and T cells as well as macrophages. PRL inhibitory factors regulate PRL secretion, and both men and women have low amounts of this hormone in their blood. [18] Hyperprolactinemia, which is very frequent in women, is characterised by high levels of PRL in the blood of males or non-pregnant women. [19] The observed variations in the incidence of numerous parasitic diseases between men and women may point to the possible function of sex hormones in parasite immunity. [20] PRL is a hormone that has a variety of biological actions, including immunomodulatory effects. We sought to understand if there was a link between PRL levels and the prevalence of T. gondii infections in men and women in this research. Preliminary findings comparing the prevalence of T. gondii infection in individuals with PRL levels below and above normal with the population with normal PRL levels found a decreased seroprevalence in men and women with hyperprolactinemia. However, variations in Toxoplasma seropositivity between patients with high levels of PRL and those with normal levels of PRL were statistically significant (P=0.019). Furthermore, boosting PRL levels in hyperprolactinemia women reduced the frequency of T. gondii infection. Toxoplasma seropositivity was not detected in five serum samples from patients with the highest PRL levels.

PRL deficiency in mice has been shown to increase the likelihood and severity of infections. Bromocriptine, a PRL secretion inhibitor, is used to suppress the immune system in organ transplantation

and autoimmune illnesses. [21] It has been shown that human PRL can bind to live tachyzoites of T. gondii, RH, and ME49 strains. [22] PRL has been found to decrease Toxoplasma growth in mononuclear cells from persons with high PRL levels. Meli et al., 1996, reported on the protective function of PRL against Salmonella typhimurium in a rat model and discovered that PRL boosted macrophage phagocytic activity and nitric oxide generation in the rats. [23] In 2001, Benedetto et colleagues discovered that PRL may promote anti-Toxoplasma activity in the brains of infected mice by increasing the production of interleukins 1 and 6. [24] In 2002, Zhang et colleagues evaluated two individuals with benign pituitary tumours and discovered Toxoplasma cysts among the tumour cells. They discovered that pituitary cell multiplication results in PRL synthesis and anti-Toxoplasma activation of microglial cells. [25] Furthermore, Gomez-Ochoa et al. support the concept that PRL has a protective function in protozoan infections. [26] They found that breastfeeding female hamsters infected with Leishmania infantum had no signs of infection when compared to the control group. [26] In 2015, Li et al discovered that PRL-inducible protein (PIP) may impair Th1 immune response and make mice more susceptible to Leishmania major. PIP is a 14 kDa protein found in mouse saliva that is upregulated by PRL and seems to have a function in host defence against infections. [27] Serrano et al2009 .'s research found that Neospora seropositive non-aborting cows had higher PRL than non-infected ones. [28] Dzitko et al. (2010) proposed the in vitro effects of recombinant PRL on T. gondii, BK strain intracellular replication. PRL seems to have no direct cytotoxic effects on host cells or parasites, although it may bind to para-site surface proteins and inhibit their receptors. [29] T. gondii prevalence was lower in women with high PRL levels in a research done by Dzitko et al than in the control group (33.9 percent vs 45.58 percent). [30] PRL receptors are located on the surface of B and T lymphocytes and macrophages and the production of cytokines such as TNF-□, IFN□,, and IL-12 is induced by this hormone. The higher levels of TNF-□, IFN□, and IL-12 in hyperprolactinemia patients may be the reason for protecting these individuals against toxoplasmosis. At the end of our study, the seroprevalence of toxoplasmosis in women was 32.86 percent, whereas it was 40 percent in males (P=0.042), supporting previous findings on various parasitic infections. In the case of protozoan parasites such as Entamoeba histolytica, Leishmania donovani, Leishmania braziliensis, and Plasmodium falciparum, similar findings revealed a greater incidence and severity of infections in males than in women. [31] In this research, the total anti-Toxoplasma IgG prevalence was 35%. According to a 1998 research by Keshavarz et al, the prevalence of toxoplasmosis in the general population in this region was 45.5 percent. [32] The seroprevalence of toxoplasmosis among men and women was also calculated in relation to the patients' ages.

CONCLUSION

The present study's findings corroborated prior research based on PRL's immunoregulatory function, indicating that high levels of PRL may be associated with T. gondii sero-negative women.

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