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Original Research Article

A Hospital-Based Assessment of the Possible Relation between Serum PRL Levels and Frequency of T. Gondii Infection in Humans

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Abstract

Aim: The present study was carried out to assess the possible relation between serum PRL levels and frequency of T. gondii infection in humans.

Material & Methods: A prospective study was conducted in the Department of General Medicine, Patna Medical College and Hospital, Patna, Bihar for the period of 6 months. Men and women aged 15–55 years with no clinical complications participated in this cross-sectional study. A total of 500 blood samples were collected from individuals who had been referred for PRL measurement.

Results: Of the total participants, 70% were women and 30% men. Of 500 blood serum samples, 162 samples (32.4%) 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 500 blood serum samples was as follows: <19 age group, (21.7%); 20–30, (32.43%); 30–40, (34.85%); 40–50 age group, (30.90%); and >50 age group, (42.10%). Of 350 serum samples of women, 68 (28.3%) had anti-Toxoplasma IgG while of 150 serum samples of men 57 (38%) had anti-Toxoplasma IgG antibody. In total, of 343 serum samples, 245 (49%) were considered as normal range of PRL, 20 (4%) and 235 (45%) samples were considered as hyperprolactinemia and hyperprolactinemia, respectively.

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.

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. It's estimated that one-third of human population are exposed to this parasite. [1] Toxoplasma gondii, the protozoan parasite distributed worldwide, is common among humans and a broad range of warm-blooded animals. [2] The main routes of

human infection are by the consumption of raw or undercooked meat containing tissue cysts and ingestion of oocysts via other food products, water, or vegetables. [3] Congenital infection can occur by vertical transmission of rapidly dividing T. gondii tachyzoites during pregnancy. Prenatal infection leads to an increased risk of spontaneous abortion, chorioretinitis, or serious neurodevelopmental disorders such as hydrocephaly and microcephaly. [4] Human may remain infected for life and will stay asymptomatic unless immunosuppression occurs.1 The hormone levels in a certain situation 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. [5]

Prolactin (PRL) hormone is secreted by pituitary gland which is located below the cerebral cortex. Low levels of this hormone are secreted in blood of female and male individuals and the secretion is under control by PRL inhibitory factors such as dopamine. [6]

Hyperprolactinemia is a situation in which large amounts of PRL exist in blood of men and pregnant women. The role of PRL has been proven in immune system as PRL receptors are located on the surface of B and T lymphocytes and macrophages and production of cytokines such as tumor necrosis factor alpha (TNF-α), interferon γ (IFNγ), and interleukin-12 (IL12) are induced by this hormone. [7] The inhibitory effects of PRL on proliferation of T. gondii in mononuclear cells of individuals with high levels of PRL have been shown previously. [8] The present study was carried out to assess the possible relation between serum PRL levels and frequency of T. gondii infection in humans.

Material & Methods

A prospective study was conducted in the Department of General Medicine, Patna Medical College and Hospital, Patna, Bihar for the period of 6 months. Men and women aged 15–55 years with no clinical complications participated in this cross-sectional study. A total of 500 blood samples were collected from individuals who had been referred for PRL measurement. 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. [9] 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.

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. [10]

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.11 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 22.0, IBM Corporation, Armonk, NY, USA). Data were analyzed using multiple univariate ANOVA and chi-squared test. Comparison of quantitative variants between two groups was assessed by Student's t-test. Data description was carried out by calculating frequencies and 95% CIs. Differences were considered as significant when $P \leq 0.05$.

Results

Table 1: Frequency of anti-Toxoplasma IgG antibody in 350 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	8 (29.62)	19 (70.37)	27
20–30	45 (30)	105 (70)	150
30–40	36 (30)	84 (70)	120
40–50	7 (23.34)	23 (76.66)	30
Above 50	11 (47.82)	3 (52.18)	23
Total	105 (30)	245 (70)	350 (100)

Table 2: Frequency of anti-Toxoplasma IgG antibody in 150 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	2 (10)	18 (90)	20
20–30	15 (42.85)	20 (57.15)	35
30–40	25 (45.45)	30 (54.54)	55
40–50	10 (40)	15 (60)	25
Above 50	5 (33.34)	10 (66.66)	15
Total	57 (38)	93 (62)	150 (100)

Of the total participants, 70% were women and 30% men. The highest frequency of participants was found in the age group of 30–40years. Of 500 blood serum samples, 162 samples (32.4%) 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 500 blood serum samples was as follows: <19 age group, (21.7%); 20–30, (32.43%); 30–40, (34.85%); 40–50 age group, (30.90%); and >50 age group, (42.10%). Of 350 serum samples of women, 68 (28.3%) had anti-Toxoplasma IgG while of 150 serum samples of men 57 (38%) had anti-Toxoplasma IgG antibody (Tables 1 and 2).

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	15 (4.28)	140 (40)	195 (55.71)	350
Men	5 (3.34)	105 (70)	40 (26.66)	150
Total	20 (4)	245 (49)	235 (45)	500 (100)

In total, of 343 serum samples, 245 (49%) were considered as normal range of PRL, 20 (4%) and 235 (45%) samples were considered as hypoprolactinemia and hyperprolactinemia, respectively.

Table 4: Association of anti-Toxoplasma gondii IgG antibody and serum prolactin levels in 350 serum samples of women

Prolactin concentration (µIU/mL)	*Toxoplasma*-specific IgG		Total	$\chi 2$ (1 *df*)	*P*-value
	Positive	Negative			
	n (%)	n (%)	n (%)		
Hypo	9 (30)	21 (70)	30	0.060	1
Normal	40 (35)	80 (65)	120	–	–
Hyper	56 (28)	144 (72)	200	5.55	0.012
Total	105 (30)	245 (70)	350(100)		

Table 5: Association of anti-Toxoplasma gondii IgG antibody and serum prolactin levels in 150 serum samples of men

Prolactin concentration(µIU/mL)	*Toxoplasma*-specific IgG		Total	$\chi 2$ (1 *df*)	*P*-value
	Positive	Negative			
	n (%)	n (%)	n (%)		
Hypo	5 (50)	5 (50)	10	0.11	0.72
Normal	40 (40)	60 (60)	100	–	–
Hyper	12 (30)	28 (70)	40	0.11	1
Total	57 (38)	93 (62)	150 (100)		

Discussion

Toxoplasma gondii, the protozoan parasite distributed worldwide, is common among humans and a broad range of warm-blooded animals. [11] The main routes of human infection are by the consumption of raw or undercooked meat containing tissue cysts and ingestion of oocysts via other food products, water, or vegetables. [12] Congenital infection can occur by vertical transmission of rapidly dividing T. gondii tachyzoites during pregnancy.3 Prenatal infection leads to an increased risk of spontaneous abortion, chorioretinitis, or serious neurodevelopmental disorders such as hydrocephaly and microcephaly. [13] Although T. gondii infection is benign in immunocompetent individuals, it is life threatening in congenital form and in

immunocompromised patients due to reactivation of the infection. [14]

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. [15] 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. [16] 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. [17] 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. [18]

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=0.012). 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. [19] It is reported that human PRL has the ability to bind with live tachyzoites of T. gondii, RH and ME49 strains. [20] 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. [21]

Moreover, the hypothesis on the protective role of PRL in protozoan infections is addition- ally supported by Gomez-Ochoa et al. [22] They concluded that lactating female hamsters that were infected with Leishmania infantum showed no symptom of infection compared with control group. 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. [23] In the study of Serrano et al in 2009 Neospora seropositive non-aborting cows had more PRL compared with non-infected ones. [24]

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%). [25] PRL receptors are located on the surface of B and T lymphocytes and macrophages and the production of cytokines such as TNF-α, IFN α, and IL-12 is induced by this hormone. The higher levels of TNF-α, IFNα, 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 30% while this value in men reaches to 38%, 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. [26-30]

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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