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ROLE OF *TOXOPLASMA GONDII* IN THYROIDITIS IN PREGNANT WOMEN

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РОЛЬ *TOXOPLASMA GONDII* ПРИ ТИРЕОИДИТЕ БЕРЕМЕННЫХ ЖЕНЩИН

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Abstract: Toxoplasmosis (acute and latent) is the most prevalent parasitic infection worldwide and can be associated with some problems in pregnant women. Thyroid diseases are the most common endocrine disorders secondary to diabetes among pregnant women. Previous studies proposed a relationship between latent Toxoplasmosis (LT) and autoimmune thyroiditis diseases (AITDs). This study intended to investigate the frequency and correlation between Toxoplasmosis and AITD in pregnant women. In this cross-sectional study, the statistical population included 1248 pregnant women at the gestational age of 9-16 weeks and in Tehran. The Toxoplasma IgM and IgG tests were assessed with enzyme-linked immunosorbent assay (ELISA). The diagnostic criteria for toxoplasmosis were abnormal IgG and IgM titers. In addition, FT4, TPO Ab, and TSH were evaluated using enzyme-linked fluorescence immunoassay (ELFA). TPO Ab was used to distinguish thyroid patients with autoimmune origin from those with other thyroiditis diseases. The analysis showed no significant relationship between keeping a house cat and acute toxoplasmosis. Acute and latent toxoplasmosis represented 3.4% and 29.6%, respectively. The frequency of thyroid diseases was 18.8% (hypothyroidism 15.8% versus hyperthyroidism 3%). The frequency of autoimmune thyroiditis diseases (AITDs) was 5.5%, and 27.9% of subjects with latent Toxoplasmosis (LT) had a thyroiditis disease, but 13.8% of pregnant women with LT had only AITD with a significant correlation ($p<0.001$). Results show that Toxoplasma IgG^+ can increase the risk of AITD by 10.39-fold and a higher TPO Ab titer in people with LT. It seems *Toxoplasma gondii* may cause thyroiditis in pregnant women likely because antigenic similarity of *Toxoplasma* and thyroperoxidase leads to cross-reactivity in the immune system, potentially causing AITD. It might be said that the high prevalence of LT among pregnant women may have a potential role in the stimulation of the immune system to the development of autoimmune diseases, such as AITD. So future studies could be conducted with a focus on discovering molecular similarities between thyroperoxidase and *Toxoplasma* antigens.

Keywords: latent toxoplasmosis, acute toxoplasmosis, autoimmune thyroiditis disease, hypothyroidism, hyperthyroidism

Резюме: Токсоплазмоз (острый и латентный) является наиболее распространенной паразитарной инфекцией во всем мире и может быть связан с некоторыми проблемами у беременных. Заболевания щитовидной железы являются наиболее частыми эндокринными нарушениями, вторичными по отношению к сахарному диабету, среди беременных женщин. Предыдущие исследования предполагали связь между латентным токсоплазмозом (ЛТ) и аутоиммунным тиреоидитом (АИТ). Целью данного исследования было изучить частоту и взаимосвязь токсоплазмоза с АИТ у беременных. В настоящем перекрестном исследовании статистическая популяция включала 1248 беременных женщин в гестационном возрасте 9-16 недель в Тегеране. Тесты на выявление IgM и IgG тел против Toxoplasma оценивали с помощью твердофазного иммуноферментного анализа (ELISA). Критериями диагностики токсоплазмоза были аномальные титры IgG и IgM. Кроме того, FT4, антитела к ТПО и ТТГ оценивали с помощью иммуноферментного флуоресцентного анализа (ELFA). Антитела к ТПО использовали для того, чтобы отличить пациентов с аутоиммунным заболеванием щитовидной железы от пациентов с другими формами тиреоидита. Анализ не обнаружил достоверной связи между содержанием домашней кошки и острым токсоплазмозом. У 3,4% и 29,6% женщин был острый и латентный токсоплазмоз, частота заболеваний щитовидной железы составила 18,8% (гипотиреоз: 15,8% против гипертиреоза: 3%). Частота заболеваний аутоиммунным тиреоидитом (АИТ) составила 5,5%, причем у 27,9% пациенток с латентным токсоплазмозом (ЛТ) обнаружен тиреоидит, а у 13,8% беременных с ЛТ имелось только АИТ с достоверной корреляцией ($p<0,001$). Результаты показывают, что наличие Toxoplasma IgG+ может увеличить риск АИТ в 10,39 раза и обуславливать более высокий титр

антител к ТРО у людей с ТП. По-видимому, Toxoplasma gondii может вызывать тиреоидит у беременных женщин, поскольку, вероятно, антигенное сходство токсоплазмы и тиреопероксидазы приводит к перекрестной реактивности иммунной системы и может вызывать АИТ. Можно сказать, что высокая распространенность ТП среди беременных может играть потенциальную роль в стимуляции иммунной системы к развитию аутоиммунных заболеваний, таких как АИТ. Таким образом, будущие исследования могут быть проведены с акцентом на обнаружение молекулярного сходства между тиреопероксидазой и антигенами токсоплазмы.

Ключевые слова: латентный токсоплазмоз, острый токсоплазмоз, аутоиммунный тиреоидит, гипотиреоз, гипертиреоз.

Introduction:

Toxoplasma gondii has a high prevalence, unlike other parasites, because of diverse transmission routes and the multiplicity of hosts in its lifecycle. Infection with this parasite is rarely associated with symptoms in healthy adults (1). However, people with a weakened immune system may become seriously ill, and pregnant women are at high risk of passing this parasite to their fetus through the placenta. Fetal complications depend on whether the parasite spreads at the early or late gestational age. The immune system causes Toxoplasma tachyzoites to convert into bradyzoites with the formation of tissue cysts, and the disease enters the latent phase or latent Toxoplasmosis (LT).

Changing the antigenic configuration is one of the escape mechanisms. The Ag-Ab complex will be separated from the tachyzoite surface and neutralized without damaging the parasite (2). Thus, *Toxoplasma* can be maintained in the body for years. It might reactivate or reinfect in some patients (3), or be a probable cause of autoimmune diseases (AIDs), such as lupus and multiple sclerosis (MS), in genetically susceptible people. Previous studies showed the possible role of *Toxoplasma* in the development of AITD (4, 5). The high prevalence of AITD has different aetiology (6, 7), and today many researchers have focused on the importance of AITD among the pregnant population.

Thyroid diseases are the most common endocrine disorders, secondary to diabetes, among pregnant women; they are associated with spontaneous abortion, impaired

fetal growth, preeclampsia, and preterm delivery (8). The importance of thyroid hormones for natural fetal growth is well established (9). Maternal thyroid dysfunction during the pregnancy, specifically in the first trimester, has a critical role in the neural development of the fetus since the fetal thyroid hormones are not produced until the 16-20 weeks (10).

Clinical diagnoses of normal euthyroid pregnant women and those with thyroid dysfunction have a significant overlap. Autoimmune thyroid disease during pregnancy is divided into four groups: asymptomatic autoimmune disease; primary hypothyroidism; Graves' hyperthyroid; and postpartum thyroid disease (11). Increased concentration of total serum T3 and T4 in natural pregnancy results from an increase in thyroxine-binding globulin (TBG); FT4 and TSH are the best predictors of thyroid function (2).

In this research, the frequency of acute and latent Toxoplasmosis and thyroid diseases among pregnant women at the gestational age of 9-16 weeks was studied to find any relationship between toxoplasmosis and thyroiditis and to examine the potential role of latent toxoplasmosis in late spontaneous abortions.

Materials and Methods:

The Research Ethical Review Committee approved this study, Code No: 95-01-30-26788. Based on the frequency of AITDs, the research population size was considered 1,248 pregnant women at the gestational age of 9-16 weeks. They were asked to complete the questionnaires that assess thyroid diseases, taking thyroid

medication, having a house cat, and intentional or unintentional abortions. Exclusion criteria for statistical analysis were: 1) a history of intentional abortion; 2) a history of taking thyroid medications; and 3) acute toxoplasmosis subjects. Serum samples were collected by standard procedures and kept frozen at − 20°C. The hemolytic and lipemic samples were replaced with re-sampling of women.

Serologic tests were carried out for Toxoplasmosis diagnosis in the Research Center of Iran University of Medical Sciences. Enzyme-linked immunosorbent assay (ELISA) with Virocell Kit (Spain) were used for the diagnosis of *Toxoplasma* IgG and *Toxoplasma* IgM (antibody capture). The diagnostic criteria for toxoplasmosis were abnormal IgG and IgM titers (Table 1). The enzyme-linked fluorescent assay (ELFA) with Biomerieux diagnostic kit (France) and VIDAS instrument was employed to analyze TSH (thyroid-stimulating hormone), TPO Ab (thyroperoxidase Ab), and FT_4 (free T4) for the diagnosis of autoimmune and subclinical thyroiditis. TPO Ab was used to distinguish thyroid patients with autoimmune origin from those with other thyroiditis diseases. Patients whose TPO Ab level was higher than 8 IU/ml, and who had abnormal TSH results at the same time, were placed in the $AITD^+$ group. Data were analyzed with SPSS 21 software and Mann-Whitney and Chi-square tests to determine the probable effect and relationship of Toxoplasmosis on TPO Ab, FT_4, TSH, and AITDs.

Results:

In this study, 1,248 pregnant women, with a mean age of 29 years (18-44), participated. Of them, 42 (3.37%) and 370 (29.64%) subjects had IgM and IgG seropositive Toxoplasmosis, respectively. Two hundred thirty-five women (18.8%) had thyroid disorders, of which 197 (15.8%) and 38 (3%) cases featured hypothyroidism and hyperthyroidism (Table 2); 39 subjects had a history of taking thyroid medications. The Chi-square test showed a significant correlation between Toxoplasma IgG and TSH (OR=2.84, $p<0.001$). The chi-square test and Mantel-Hansel test on Toxoplasma IgG and AITD indicated a meaningful relationship between these two variables (CI=95%, OR=9.281, $p<0.001$). These figures convey that the risk of AITD is 9.3-fold higher among people with Toxoplasma IgG^+ than Toxoplasma IgG^-. According to the results, 113 subjects (9.7%) had TPO Ab>8.0 IU/ml; the median titer of TPO Ab (190 IU/ml) was higher in Toxoplasma IgG^+ and $AITD^+$ women. The Mann-Whitney test showed a significant relationship between TPO Ab and Toxoplasma IgG^+, and TPO Ab was significantly higher in the Toxoplasma IgG^+ group.

In addition, no significant correlation was found between: age and AITD ($p=0.35$); or age and Toxoplasmosis ($p=0.42$). There were 52 subjects with abnormal TPO Ab titer placed into the $AITD^-$ group, indicating that abnormal TPO Ab titer does not necessarily lead to $AITD^+$, and 61 persons in the $AITD^+$ group were LT^+. There was a significant correlation between Toxoplasma IgG^+ and $AITD^+$ when TPO Ab was also positive ($p=0.046$). In this regard, Toxoplasma IgG^+ can increase the risk of AITD by 10.39-fold. On the other hand, there was a significant correlation

between these two variables concerning the relative risk of pregnancy complications.

According to the results, TPO Ab was higher than expected in 144 (11.5%) out of 1248 subjects. Further, 113 (9.7%) out of 1,167 issues (with AT and no history of thyroid medications) had abnormal TPO Ab. In other words, 54.1% of thyroid patients (235 patients) had a high TPO Ab level. The Chi-square test showed a significant correlation between Toxoplasma IgG and TPO Ab ($p<0.05$, Figure 1). Among subjects with LT, 27.9% had thyroid disease. Chi-square and Mantel-Haenszel tests showed a significant correlation between toxoplasmosis and thyroiditis ($p<0.001$).

The analysis showed no significant relationship between keeping a house cat and acute toxoplasmosis ($p=0.21$). However, the frequency of LT among those with a history of maintaining a house cat was significant ($p<0.001$). The Chi-square test showed no significant correlation between LT and unintentional abortion ($p=0.39$).

Discussion:

The prevalence values of clinical hypothyroidism, subclinical hypothyroidism, overt hyperthyroidism, and subclinical hyperthyroidism in the Saki et al. article were 2.4%, 11.3%, 1.2%, and 0.3%, (12), but in our study, they were 1.84%, 13.96%, 2.2%, and 0.8%, respectively. A relative increase in percentages in subclinical hypothyroidism, clinical hyperthyroidism, and subclinical hyperthyroidism could be seen, and these results could be an alarm for to thyroid

function diseases, especially hyperthyroidism. AITDs gradually weaken the function of the thyroid gland. However, with the compensatory rise of TSH levels, thyroid hormones maintain at an average level, so subclinical hypothyroidism patients display few signs and symptoms, which are harder to recognize.

FT4 level will drop, and TSH level will increase in subclinical hypothyroidism; this may increase the risk of pregnancy complications, such as placental abruption, preterm delivery, and low birth weight (13, 14). TSH levels higher than 10 μIU/ml in this stage would be called clinical hypothyroidism or overt hypothyroidism. Hyperthyroidism is relatively not prevalent (0.1-1%) during pregnancy (15-17). In the current study, the prevalence of clinical hyperthyroidism was 0.8%. There is no general guidance for treating thyroid disorders during pregnancy. Therefore, performing thyroid tests and checkups by a gynecologist, specifically in the first trimester, must be specially considered. In this study, 3.2% of subjects had a history of taking thyroid medications. Among 79 subjects with a history of thyroid disease, 37 cases had stopped, and 39 women continued the medicines. Of those with hypothyroidism or hyperthyroidism (aware of their problem), 45% had destroyed their medications. It would be better for women with a history of thyroid problems, specifically those on the verge of pregnancy, not to stop the medication arbitrarily.

According to Soldin et al., 12% of asymptotic healthy women and 1% of asymptotic healthy men are TPO Ab^+. The frequency of clinical hypothyroidism (9.21%) and subclinical hypothyroidism (3.7%) is higher in TPO Ab^+ than TPO

Ab$^-$ patients. The annual risk of clinical TPO Ab, accompanied by hypothyroidism, is almost 5-20% (18). Due to the binding effect of thyroid disorders on both fetus and mother, thyroid and TPO Ab screening should be included in trimester examinations for maternal health. The prevalence of postpartum thyroiditis directly correlates with TPO Ab titer, and is associated with an increased rate of clinical hypothyroidism within three to six months after delivery. Postpartum thyroid will appear in half of the women who become TPO Ab$^+$ in early pregnancy. Regarding the incidence of postpartum thyroid dysfunction, which is associated with several signs and postpartum depression symptoms, screening for postpartum diseases through the measurement of TPO Ab seems necessary (19).

In Kankova's retrospective article, the frequency of LT was not significant among AITD patients. It was mentioned a relationship between Toxoplasmosis and FT4, with a higher FT4 titer (p=0.033) (20). However, based on our results, it seems that LT may have a role in the incidence of AITD because our data was gathered within a specific time by considering similar and standard conditions for all samples. Eligible subjects were first selected and entered into the study after completing the questionnaire. Another point was patients aware of their thyroid disease, taking medications, and thus with normal FT4, as 26.5% of thyroid patients in our study got thyroid medicines. Despite having abnormal TSH results, their FT4 was average and excluded in this survey, which had not been done in Konkova's research.

In the same manner, the sera from 1591 women were tested for cytomegalovirus, Epstein—Barr virus, herpes simplex virus type 1, herpes simplex virus type 2, and Toxoplasma gondii. It was mentioned that prior infection with T. gondii was associated significantly with the elevation of TPOa Ab, whereas seropositivity for other infections was not (5). However, the relationship between LT and AITDs was not done. In another study, a multiplex array platform was performed for the detection of antibodies against *Toxoplasma gondii*, *Treponema pallidum*, *rubella virus*, *cytomegalovirus*, and *Epstein–Barr* virus in a large group of AITD patients and healthy controls. Antibody levels against *T. gondii* were significantly higher in AITD patients than in controls, suggesting that molecular mimicry of this protozoa may be involved in the initiation of AITD (21). Our results support the hypothesis that antigenic similarity of *Toxoplasma* and thyroperoxidase likely leads to cross-reactivity in the immune system and may cause AITD.

The initial screening test of Iranian pregnant women is the TORCH test, which includes a Toxoplasmosis diagnosis test. However, there is also a gap for thyroid screen tests in this program. Regarding the significant frequency of AITD in people with LT, gynaecologists propose prescribing thyroid tests in pregnant women. Although spontaneous abortion caused by congenital toxoplasmosis is well identified, the potential role of LT in abortion is still uncertain. However, some studies have reported a significant relationship between the frequency of IgG *Toxoplasma* antibody and abortion (22, 23), whereas some studies reported no correlation (24). The high frequency of intentional abortion has faded the role of

diseases in this process, and the only solution was to record the history of deliberate and unintentional abortions separately. Involuntary and spontaneous abortions are not associated with severe physical complications, whereas intentional abortion is related to maternal risks. It increases the risk of placenta previa (attachment of the placenta to the lower part of the womb). Therefore, those with a history of intentional abortion were excluded from the study, and no significant relationship between latent toxoplasmosis and unintentional abortion was seen. The causes of miscarriage and other pregnancy complications might be biological conditions or unmeasured common risk factors (25). Therefore, in the retrospective design of this study, it seems to be one of the study limitations.

Conclusion:

This study, along with previous studies, has shown higher TPO Ab titers in people with LT. It seems *Toxoplasma gondii* may cause thyroiditis in pregnant women likely because antigenic similarity of *Toxoplasma* and thyroperoxidase leads to cross-reactivity in the immune system perhaps leading to AITD. Thus, future studies could be conducted with a focus on discovering molecular similarities between thyroperoxidase and *Toxoplasma* antigens.

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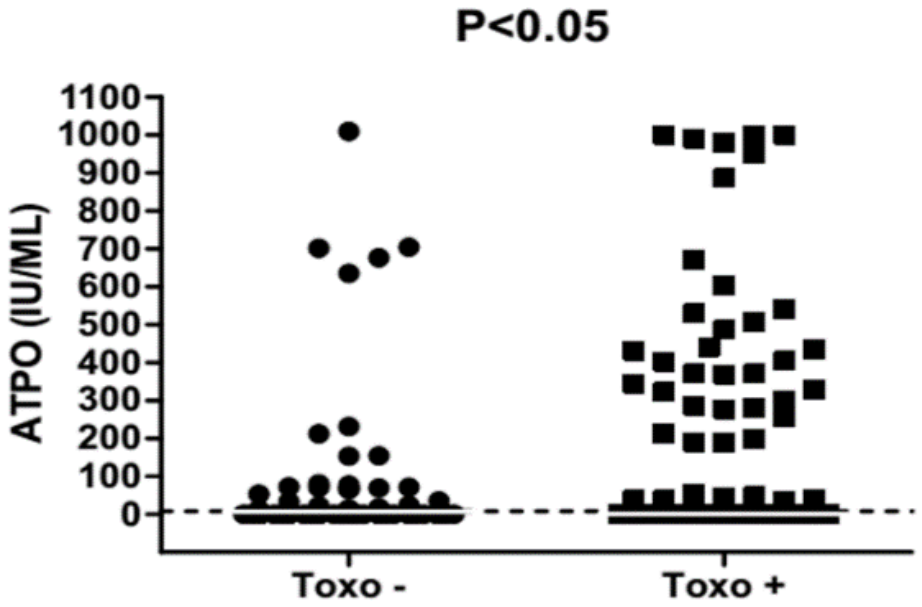
FIGURES

Figure 1. Association of latent toxoplasmosis and serum TPO Ab levels in pregnant women (9–16th gest. weeks).

Рис. 1. Связь латентного токсоплазмоза и уровня антител к ТРО в сыворотке крови у беременных (9–16-я гест. нед).



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TABLES

Table 1: Normal range tests.

Таблица 1. диапазон нормальных значений для использованных тест-систем

Test	Normal range	Method
Toxoplasma IgG	0-9 (IU/ML ME/мл) = negative негативный 9-11 (IU/ML) = suspicious Подозрение >11 (IU/ML) = positive Позитивный	ELISA
Toxoplasma IgM	0-9 (IU/ML) = negative 9-11 (IU/ML) = suspicious >11 (IU/ML) = positive	ELISA ИФА
TSH ТТГ	Normal KIT: 0.27-4.7 (μIU/ML) Стандартная тест-система (μМЕ/мл) (Trimester 1 триместр 1) 0.1-2.5 (μIU/ML) (Trimester 2) 0.2-3.0 (μIU/ML)	ELFA
TPO Ab ТПО Ат	< 8.0 (IU/ML)	ELFA
FT4	10.3-23 (pmol/L пмоль/л)	ELFA

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Table 2:

Hypothyroidism ***Гипотиреоз***	**15.8 %**	**Clinical hypothyroidism Клинический гипотиреоз**	**1.84 %**
		Subclinical hypothyroidism Субклинический гипотиреоз	13.96 %
Hyperthyroidism *Гипертиреоз*	3 %	Clinical hyperthyroidism Клинический гипертиреоз	2.2 %
		Subclinical hyperthyroidism Субклинический гипертиреоз	0.8 %

Prevalence of thyroid diseases

Распространенность заболеваний щитовидной железы

Table 3: Frequency and percentage of different variables among the research population, obtained from the questionnaire.

Keeping a house cat наличие домашней кошки	**25 (2.1%)**
His**tory of miscarriage** Выкидыши	371 (30.9%)
History of induced abortion Индуцированные аборты	97 (8.1%)
History of thyroid disease Заболевания щитовидной железы	76 (6.3%)
Use of medications for thyroid disease Лечение заболевания щитовидной железы	39 (32%)

Таблица 3. Частота различных показателей у обследованных лиц (на основании опросника)

TITLE PAGE_METADATA

Role of *Toxoplasma gondii* in Thyroiditis in Pregnant Women

Running Head: T. gondii in Thyroiditis in Pregnant Women

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