Original Research Article

Correlation between *Toxoplasma gondii* and Thyroid Function Hormone Levels in Sera of Patients Attending Private Clinics and Laboratories in Kirkuk City

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<table>
<thead>
<tr>
<th>Abstract</th>
<th>Keywords</th>
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| The present study aims are to estimate *Toxoplasma* occurrence among peoples in Kirkuk city and to detect incidence of abnormal thyroid function among patients in general and in *Toxoplasma* positive sera. During 2013 to 2014, a total of 246 sera were drawn from patients attending into two private medical labs in Kirkuk city. The sera were tested for *Toxoplasma* antibodies (IgM and IgG) along with thyroid hormones *viz.* triiodothyronine (T3), thyroxine (T4) and thyrotropine (thyroid stimulating hormone-TSH). The overall rate of toxoplasmosis was 36.17% that contributed a high rate of *Toxoplasma* IgG antibodies (28.45%) compared to *Toxoplasma* IgM (7.72%; *p*<0.05). A high rate of *Toxoplasma* IgM antibodies (10.72%) was recorded among females while it was 4.46% for males (*p*<0.05). Relationship between *Toxoplasma* antibodies distribution with patient age was not significant at *p*<0.05. The overall rate of abnormal thyroid function hormones was 60.97% in 150 samples distributed in the following rates: 26.74, 21.46 and 10.56% for TSH, T4 and T3 hormones respectively (*p*<0.05). Relationship between thyroid hormones and patient gender was significant, as high rates TSH and T3 were recorded among females, while high rate of abnormal T4 was recorded in males. Relationship between patients’ age and abnormal thyroid hormones level was significant especially with T3 and T4 hormones. Frequency of antibodies in sera of patients with abnormal thyroid functions was high (15.85%) and significant, contributing to 13% *Toxoplasma* IgG and 2.84% *Toxoplasma* IgM antibodies. *Toxoplasma* rate in Kirkuk city is high especially among female with high occurrence of IgM. It is from wisdom to check patients of sub-acute thyroiditis for *Toxoplasma* antibodies to avoid damages causes by *Toxoplasma* infection. | Abortion
ELISA
Hypothyroidism
Sub-acute thyroiditis
Toxoplasmosis |
Introduction

Toxoplasmosis is the most widespread zoonosis and an important human disease particularly in children, where it can cause visual and neurological impairment and mental retardation (Adesiyun et al., 2007). It is able to infect, or be present in the highest number of host species, any warm-blooded animal may act as an intermediate host, and oocysts may be transported by invertebrates (Paul et al., 2001). Toxoplasmosis is increasing or spreading mostly with age, education, crowding, sanitary, habits, socio-economic, ethnic considerations, undercooked meats and animal contacts including cat the final host (Al-Muhaymen and Ahmed, 2012).

The earliest record of toxoplasmosis in Kirkuk city was in 1999 by (Al-Attar, 2000) who recorded 33.6% of toxoplasmosis among peoples and animals, followed by the continues work of Salman, in 1992, who observed Toxoplasma antibodies among peoples in Kirkuk city from 1992 to 2012, and he reported the overall toxoplasmosis rate of 31.35% with a high incidence rate of 63.80% in 2005 compared to 17.59% in 2012 (Salman, 2014a). Prevalence of toxoplasmosis was studied in different parts of the world where the high rate was recorded among pregnant women in France, ranging from 87% in 1984 to 81-85% in 1999 (Ljugstrom et al., 1995) and (Heybas et al., 2003). In Arab countries, toxoplasmosis rates were fluctuated, the high rate 58.4% was recorded in Tunisia by Desmonts and Couvrew (1984).

Thyroid gland is an essential gland in the body of human being that produces essential hormones regulated by the hypothalamic-pituitary-thyroid axis (Laposata, 2010). The main function of thyroid gland is to secrete thyroxin to regulate basal metabolic rate mostly this hormone acts through nuclear receptors that are transcribed by numerous genes and these genes regulate a number of critical physiological functions in development and metabolism (Boelaert and Franklyn, 2005).

Thyroid disease is associated with various metabolic abnormalities, due to the effects of thyroid hormones on nearly all major metabolic pathways. Thyroid hormones regulate the basal energy expenditure through their effect on protein, carbohydrate, and lipid metabolism. This might be a direct effect or an indirect effect by modification of other regulatory hormones such as insulin or catecholamine (Kim, 2008).

Male reproduction is adversely affected by both thyrotoxicosis and hypothyroidism. Erectile abnormalities have been reported (Singh et al., 2011). Thyrotoxicosis induces abnormalities in sperm motility, whereas hypothyroidism is associated with abnormalities in sperm morphology; the latter normalize when euthyroidism is reached. In females, thyrotoxicosis and hypothyroidism can cause menstrual disturbances. Thyrotoxicosis is associated mainly with hypo-menorrhea and polycystic ovaries, whereas hypothyroidism is associated mainly with oligo-menorrhea (Poppi et al., 2007). Thyroid dysfunction has also been linked to reduced fertility (Krassas et al., 2010). Absence of documented data about thyroid dysfunction in relation to common disease (toxoplasmosis) in Kirkuk city stimulated us to undertake this study to find correlation between Toxoplasma gondii infections and thyroid functions among peoples with dysfunction of thyroid.

Materials and methods

Study period and location

From 1st November 2013 to 30th June of 2014 a cross sectional study was carried on in Ibn-Nafies and Ibn-Haitham Private medical laboratories in Kirkuk city, Iraq.

Selection of patients and blood sampling

All patients with thyroid dysfunctions and with a combination of infertility + thyroid malfunctions who were referred to private laboratories by clinicians were selected for the study. A total of 246 patients (females-134; males-112) aged between 15 and 65 years and above with an average age of 38.1±11 years were enrolled for the present study. Before drawing blood samples, a special questionnaire was given to each patient and complete information was obtained. Using a sterile syringe, 5 ml of venous blood was drawn to sterile dry tube, kept for about 15 min to obtain clot, then sera has been separated using electrical centrifuge. The separated sera were immediately tested for toxoplasmosis and thyroid functionality test. In case of delay suspected, sera were kept in freeze at -20°C till to use.
Analytical procedures

The serological tests were done using IgM and IgG ELISA Bio-kits reagents manufactured by Barcelona-Spain to detect anti-IgM and anti-IgG specific for *T. gondii*, these procedures were applied according to manufacturer’s guidelines according to Al-Jubori (2005). Thyroid hormones [triiodothyronine (T3), thyroxine (T4) and thyrotropine (thyroid stimulating hormone-TSH)] were determined using mini-vidas machine which can measure hormones, antibodies and tumor markers by using an automated ELISA and fluorescent collectively called ELFA technique (Enzyme Linked Fluorescent Assay). Mini-vidas kits were purchased from BioMerieux company-France. Clear serum of each patient measuring 200 µl was injected in to Solid Phase Receptacle (SPR) containing separately one of the following T3, T4 or TSH antibody labeled with alkaline phosphatase (conjugate). The sample/conjugate mixture was cycled in and out by using special tips and the antigen bound to antibodies coated on the SPR and to the conjugate, forming a sandwich. The unbound components were eliminated during the washing steps. During the final detection step, the substrate (4-methyl-umbeliferyl phosphate) in and out of the SPR and the end product of conjugate catalysis was the hydrolysis of 4-methyl-umbeliferyl phosphate in to fluorescent product which was easily measured at wave length of 450 nm. The intensity of fluorescence was proportional to the concentration of antigen present in the sample. The results output was taken automatically by internal printer. Assessments of three hormones were applied according to instructions of manufacturer’s guidelines to users.

Interpretations of laboratory methods

*Toxoplasma* antibodies levels using ELISA technique were taken as the level of IgM or IgG below 0.9 IU/ml considered negative and from 0.9 to 0.99 IU/ml was equivocal limit and should be rechecked, while positive level was equal to 1.0 IU/ml or above. Thyroid hormone levels were taken in to accounts as normal T3 hormone level (0.9 to 2.3 mIU/L), T4 (60 to 120 mIU/L) and TSH (0.25 to 5.0 mIU/L).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences version 17 (SPSS Inc., Chicago, IL., USA). Descriptive statistics such as mean, standard deviation (SD) and one-way ANOVA were used to evaluate the significance (*p*-value) between study variables. The independent sample *t*-test of significance was used for the comparison between two groups. The lowest level of significance was chosen when the probability (*p*) was less than or equal to 0.05 (*p*≤0.05).

Results

The overall rate of toxoplasmosis during eight months of study in Kirkuk city was 36.17%, contributing 28.45% for *Toxoplasma* IgG antibodies followed by 7.72% for *Toxoplasma* IgM antibodies (*p*<0.05). Sero-positive *Toxoplasma* antibody distribution in relation to gender was not significant in general, except sera of females revealing high rate (10.44%) of *Toxoplasma* IgM compared to 4.46% in males (*p*<0.05; Table 1).

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. examined (%)</th>
<th>No. positive (%)</th>
<th>No. Negative (%)</th>
<th><em>Toxoplasma</em> IgM Positive No. exam (%)</th>
<th><em>Toxoplasma</em> IgG Positive No. exam (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>112 (45.52)</td>
<td>40 (35.72)</td>
<td>72 (64.28)</td>
<td>5 (4.46)</td>
<td>35 (31.26)</td>
</tr>
<tr>
<td>Female</td>
<td>134 (54.48)</td>
<td>49 (36.56)</td>
<td>85 (63.44)</td>
<td>14 (10.44)</td>
<td>35 (26.19)</td>
</tr>
<tr>
<td>Total</td>
<td>246 (100)</td>
<td>89 (36.17)</td>
<td>157 (63.83)</td>
<td>19 (7.72)</td>
<td>70 (28.45) *</td>
</tr>
</tbody>
</table>

(*p*<0.05)

Table 1. Positive and negative rates of *Toxoplasma gondii* IgM and IgG antibodies among peoples in relation to gender.

In fact, there is a strong relationship between the acquiescing of infectious agents and host age, but the results of the present study exert the contrary observations which were summarized in Table 2. The relationship between *Toxoplasma* antibodies distribution in the present study and patients age was not significant statistically at *p*<0.05 in spite of the difference between *Toxoplasma* IgG antibody rate (27.23%) and *Toxoplasma* IgM antibodies.

Considering the estimation of thyroid function test (T3, T4 and TSH), the same sera examined for toxoplasmosis were tested by mini-Vedas machine for thyroid function elements (three hormones). From the examination of a total of 246 sera, only 150 sera with overall rate of 60.96% were shown abnormal levels including 65 sera (26.74%), followed by 53 sera (21.54%) and 26 sera (10.56%) for TSH, T4 and T3 hormones respectively (p<0.05; Table 3).

Statistical interpretation of the results on thyroid hormones showed high abnormal rates of TSH (35.07%) in females; 16.07% in males; and 15.67% of abnormal T3 hormone in females controversy to 4.41% in males with a significant difference at p<0.05; while high abnormal T4 hormone level of 36.60% was recorded in sera of males and of 8.95% in females (p<0.05).

### Table 3. Thyroid function test parameters (T3, T4 and TSH) among patients in relation to gender.

<table>
<thead>
<tr>
<th>Sexes</th>
<th>No. examined (%)</th>
<th>T3</th>
<th>T4</th>
<th>TSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abnormal No. (%)</td>
<td>Normal No. (%)</td>
<td>Abnormal No. (%)</td>
<td>Normal No. (%)</td>
</tr>
<tr>
<td>Male</td>
<td>112 (45.52)</td>
<td>5 (4.41)</td>
<td>107 (95.59)</td>
<td>41 (36.60)*</td>
</tr>
<tr>
<td>Female</td>
<td>134 (54.48)</td>
<td>21 (15.67)</td>
<td>113 (84.33)</td>
<td>12 (8.95)</td>
</tr>
<tr>
<td>Total</td>
<td>246 (100)</td>
<td>26 (10.56)</td>
<td>220 (89.44)</td>
<td>53 (21.54)</td>
</tr>
</tbody>
</table>

* *, ** p<0.05 Normal level of TSH (0.15 to 4.5 mIU/L), T4 (60 to 120 mIU/L) and T3 (0.9 to 2.3 mIL/L)

Table 4 is showing distribution of T3, T4 and TSH hormones with reference to the age of patients. A high rate of abnormal T3 hormone level of 25.53% was recorded in sera of patients’ age ranging from 15 to 25 years; while 43.75% of abnormal T4 hormone level was recorded in old age patients (aging from 56 years and above). The relationship between patients age and abnormal TSH hormone was not significant at p<0.05.

### Table 4. Distribution of thyroid function test parameters in sera of patients according to age.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No. examined (%)</th>
<th>T3</th>
<th>T4</th>
<th>TSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abnormal (%)</td>
<td>Normal (%)</td>
<td>Abnormal (%)</td>
<td>Normal (%)</td>
</tr>
<tr>
<td>15 to 25</td>
<td>47 (19.10)</td>
<td>12 (25.53)*</td>
<td>35 (91.41)</td>
<td>14 (29.78)</td>
</tr>
<tr>
<td>26 to 35</td>
<td>71 (28.86)</td>
<td>5 (7.04)</td>
<td>66 (91.55)</td>
<td>9 (12.67)</td>
</tr>
<tr>
<td>36 to 45</td>
<td>60 (24.39)</td>
<td>11 (18.33)</td>
<td>49 (88.34)</td>
<td>7 (11.66)</td>
</tr>
<tr>
<td>46 to 55</td>
<td>52 (21.13)</td>
<td>4 (7.96)</td>
<td>48 (90.39)</td>
<td>16 (30.76)</td>
</tr>
<tr>
<td>56 and above</td>
<td>16 (6.58)</td>
<td>0 (Nil)</td>
<td>16 (100)</td>
<td>7 (43.75)*</td>
</tr>
<tr>
<td>Total</td>
<td>246 (100)</td>
<td>32 (13.00)</td>
<td>214 (87.00)</td>
<td>53 (21.54)</td>
</tr>
</tbody>
</table>

*p<0.05, F-test value=3.84
To detect relationship between *Toxoplasma* antibodies distribution and abnormal thyroid functions, 135 sera of abnormal thyroid hormones levels were checked for *Toxoplasma* antibodies and the results were tabulated in Table 5. The results showed 39 sera with a rate of 15.85% were positive for *Toxoplasma* antibodies, contributing 13% and 2.84% of *Toxoplasma* IgG and IgM respectively ($p<0.05$).

Table 5. Frequency of *Toxoplasma gondii* IgM and IgG antibodies in sera of patients with abnormal thyroid functions.

<table>
<thead>
<tr>
<th><em>Toxoplasma</em> antibodies</th>
<th>Total number abnormal (%)</th>
<th>IgM antibodies positive (%)</th>
<th>IgG antibodies positive (%)</th>
<th>Total <em>Toxoplasma</em> antibodies No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3</td>
<td>29 (23.57)</td>
<td>1 (3.44)</td>
<td>6 (20.68)</td>
<td>7 (24.13)</td>
</tr>
<tr>
<td>T4</td>
<td>55 (44.71)</td>
<td>3 (5.45)</td>
<td>14 (25.45)</td>
<td>17 (30.90)</td>
</tr>
<tr>
<td>TSH</td>
<td>39 (31.70)</td>
<td>3 (7.96)</td>
<td>12 (30.76)</td>
<td>15 (38.46)</td>
</tr>
<tr>
<td>Total</td>
<td>123 (50.00)</td>
<td>7 (5.69)</td>
<td>32 (26.01)</td>
<td>39 (31.70)</td>
</tr>
<tr>
<td>All total number</td>
<td></td>
<td>7 (2.84)</td>
<td>32 (13.00) *</td>
<td>39 (15.85) *</td>
</tr>
</tbody>
</table>
| examined = 246          |                           | *All rate of *Toxoplasma* IgM = 9.05%, while IgG = 27.23%; * $p<0.05$; t-test value 5.23.

**Discussion**

*T. gondii* is an example for tissue apicomplexan protozoa, where the sluggish trophozoites (bradyzoites) released from the cysts in parasitized tissues to form active or proliferative tachyzoites that dwells in the circulated blood and reach other organs including thyroid glands; this consequently affect thyroid function by cyst formation and tissue changes such as hyperplasia in the thyroid gland, forming the basis of importance of this study. On the other hand, this study is the first of its kind in Iraq and literatures concerning the study subject are few, therefore for these reasons, the following discussions were made based on scientific studies. The overall rate of *Toxoplasma* (36.17%) in the present study is high when considering the size of the samples (246 sera) and short duration (8 months). This high rate reflects the degree of environmental contamination with *Toxoplasma* infective stage, the oocysts.

The present study was carried out during critical and unstable condition countenance in Iraq and Kirkuk Province in particular, with in which the continuous interruption of electric power was one of the reasons affecting food storage and water supplies in addition to the lack of certified quality insecticides to eliminate the mechanical vectors. All these factors might have played an important role highlighting why the overall rate of toxoplasmosis in the present study was high. The overall rate of toxoplasmosis in the present study agree with that of the studies recorded in the same province by Al-Attar (2000), Aljubori (2005), Salman (2014b) respectively recorded the following rates: 33.6, 35.6 and 31.15%; while the current study disagree with the results 92.1, 48.9 and 38.56% of toxoplasmosis in the same province recorded by Noori (2013), Tewfik (2013) and Salman (2014c) respectively. Similarly, the present study is disagreeing with the study of Zemene et al. (2012) in Ethiopia (3.6%).

The variances in the rates might be attributed to several factors, such as size of samples, type of laboratory method, site of the study and type of the patients (infected and not infected with other infectious agents) (Salman, 2007d). *Toxoplasma* antibody types in this study in relation to patient gender especially 10.44 % of IgM is critical to women health and baby conceiving; also 4.46% of the same type of antibodies within males was high, with possible prognosis to blurry vision, cataract and to mental retardation (Felgr, 2013). Also the finding of high rate of *Toxoplasma* antibodies in females than in males, is in agreement with the report of Hodkova et al. (2007), as a result of physiological and anatomical differences between males and females enhancing the appearance of clinical features in infant after delivery or during pregnancy as abortion (Muhammad et al., 2010). The results in this regard were in agreement with those recorded by Othman (2004) and Al-Jubori (2005).

Concerning frequency of *Toxoplasma* antibodies according to the age group, in spite of statistical analysis that reveal non-significant; but, *Toxoplasma* IgG antibodies of 27.23% was high, showing the vitality of the study to Health and
Ecology concerns in Kirkuk city. Also the overall rate 9.05% of *Toxoplasma* IgM antibodies is critical to public health especially to women in the study, contributing more than 50% of study population. This finding is not compatible to those recorded in Nepal (Acharya et al., 2014), Kirkuk (Tawfik, 2013) and in Tikrit (Ahmed, 2008).

Abnormal thyroid function has multiple implications for public health. However, the magnitude of the problem is not entirely known, nor is the exact relationships to other health problems which are well delineated; the overall 60.97% of abnormal levels of three hormones considered in the study was very high because the size of the samples, 246 is not too large and not comprehensive to all populations of Kirkuk city. The reasons for this high rate may be attributed to the type of nutrition, poverty, poor hygienic condition and absence of health programs concerning endocrinology in general and thyroid in particular, in addition to genetic disorders (Al-Terihy et al., 2012).

The prevalence of an elevated serum TSH level of 24.7% in the study population with high frequency of 35.07% in sera of females is very beneficial to the gynecologist for detecting infertility. The proportion of subjects with an elevated TSH level was greater among women than men and increased with advancement of age (Canaris et al., 2000). The T4 rate greater than 120 mUI/L was higher in males (36.40%) than in females (8.95%) which is significant (especially their serum TSH below 0.01mUI/L). The main causes to hyperthyroidisms may be related to most of the patients (especially males) in the study are polycthemic and with cardiovascular problems; in such condition hyperthyroidism has profound effects on cardiovascular system, including reduced systemic vascular resistance due to relaxation of vascular smooth muscle cells, enhanced heart rate and cardiac output due to increase in cardiac diastolic relaxation, contractility and heart rate (Faber et al., 2001; Sengupta et al., 2013). Also altered lipid profile is a well-known manifestation of thyroid dysfunction. Both plasma LDL-C and HDL-C increase in hypothyroidism and decrease in hyperthyroidism has been reported by Matsubara et al. (2001). This suggestion requires further study to carry out on thyroid dysfunction and lipid profile to obtain accurate interpretation for high T4 levels in the present study.

Regarding high rate of T4 among patients aging from 56 years and above, this can be attributed to the use of total (T4) and not free (FT4) thyroxin levels in this study; the total T4 concentrations may have been slightly elevated because of increases in thyroid hormone binding proteins in patients who were receiving certain concomitant medications; for example, estrogens (Canaris et al., 2000). While high rate (25.53%) of abnormal T3 hormone level (either below 0.9 mUI/L or above than 2.3 mUI/L) among patients aging from 15 to 25 years requires two explanations: for patients whose T3 is below 0.9 mUI/L, considered under euthyroid because their TSH levels were within normal; while those with T3 low and T4 also low, can be grouped under hyperthyroidism; those with T3 levels above 2.3 mUI/L associated with high T4, and low TSH level below 0.01mUI/L, can be grouped under hypothyroidism; so this finding is very critical to study male population particularly with oligo-zoospermia (synthesis increases of testosterone) (Krassas et al., 2010) and female population with hyper-prolactinemia and menstrual abnormality (Poppe et al., 2007).

Correlation of overall rate of toxoplasmosis of 36.17% with overall of abnormal thyroid functions (15.85%) in the present study is essential to keep peoples healthy in Kirkuk city, because rates were high and there is a strong link between damage caused to thyroid gland by the *Toxoplasma* parasite. Being a tissue parasite, its multiplication and propagation in the thyroid gland actually causes tissue changes such as hyperplasia (Gillespie and Pearson, 2001). On the other hand high rate of *Toxoplasma* IgG antibodies among peoples with abnormal thyroid hormonal levels of 13% from overall rate of 27.23% *Toxoplasma* IgG antibodies in the study is critical; because not all IgG positive result means protection or sterile provoke against previous toxoplasmosis, so reactivation from latent toxoplasmosis may persist after or within 6 months of recovery from first initiation of toxoplasmosis (Salman, 2007d).

Reactivation or seroconversion from latency had severe damage on the tissue (including thyroid gland). Considering *Toxoplasma* IgM antibodies, overall rate of 2.85% from the total *Toxoplasma* IgM of 9.05% in the present study is high exposing the light on acute toxoplasmosis. In such patients, especially cysts formation in the cervical lymphatic
glands and thyroid gland may involve with highly parasitemia or due to rupture of the cyst during trauma, leading to the release of trophozoites with parasite disposal product that may reach hypothalamus and alter its stimulation; therefore disturbance in TSH secretion and abnormal outcomes of T3 and T4 productions is resulted; in females this event may lead to infertility or inhibit child conceiving (Ain et al., 1987; Ballabio et al., 1991).

**Conclusion and recommendations**

The prevalence of abnormal biochemical thyroid function reported here is substantial. *Toxoplasma* antibodies detection using ELISA technique among patients with thyroid dysfunction is useful. Young age females should be overlooked for *Toxoplasma* antibodies and checking thyroid function test prior to child conceiving or prior to marriage.

From the results of this preliminary study about some thyroid function tests in relation to toxoplasmosis, it is highly recommended to interested researchers to carry on further studies with large size of patients and to assess all elements in thyroid tests in order to obtain obvious explanation about the role of infectious agents in sub-acute thyrotoxicosis.

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