Reactivation of *Toxoplasma gondii* Infection in Patients under Biological Treatment by Antitumor Necrosis Factor Alpha (anti-TNFα)

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Abstract

*Toxoplasma gondii* (*T. gondii*) is considered as a one of the highly resistant intracellular parasite mainly found in the central nervous system (CNS), however, it can persist in multiple tissues in the body. Moreover, *T. gondii* is causing infections among the patients under biological treatment for different autoimmune diseases. Thus, the aim of this study was to investigate the frequency of *T. gondii* infection among patients under biological treatment for different autoimmune diseases in Taif- Saudi Arabia. Fifty patients under biological treatment with anti-TNF-α for different autoimmune diseases and thirty healthy control were subjected to determination of anti *T. gondii* immunoglobulin M (IgM) antibody seropositivity and anti *T. gondii* immunoglobulin G (IgG) antibody seropositivity using commercially available enzyme-linked immuno sorbent assay kits (ELISA). The results showed that the seropositivity rate of anti *T. gondii* IgM antibodies and anti *T. gondii* IgG antibodies was found as 24% and 40% respectively and it indicates results are statistically significant. These statistically significant results support the association between *T. gondii* infection and the use of anti-TNF-α and suggest the usefulness of performing serological test for detection of previous infection by toxoplasma to avoid reactivation of the latent infection during treatment with anti-TNF-α.

Key words: *Toxoplasma gondii*, Anti-TNF-α, Systemic lupus erythematosus, Psoriasis, Chron’s disease.

1. Introduction

*Toxoplasma gondii* (*T. gondii*) is an obligate intracellular protozoan parasite capable of infecting wide variety of mammals, including humans. This protozoan is able to persist in multiple tissues where the latent stage of the
parasite is mainly found in the central nervous system. Although approximately 30% of the world’s population have *T. gondii* infection and harbor cysts in the brain, overt disease symptoms such as encephalitis are only evident during immune suppression [1].

An autoimmune disease is a condition arising from an abnormal immune response to a normal body part and the cause is generally unknown. Some autoimmune diseases such as lupus run in families and certain cases may be triggered by infections or other environmental factors. Some common diseases that are generally considered autoimmune include celiac disease, diabetes mellitus type 1, Graves’ disease, inflammatory bowel disease, multiple sclerosis, psoriasis, rheumatoid arthritis and systemic lupus erythematosus [2]. Globally autoimmune diseases affect about 3% of the population with a greater prevalence in women than men. They arise when a genetically predisposed individual with inadequate or non-functional immune regulatory mechanisms, has an immune response to an environmental pathogen [3-5].

The conventional treatment strategies to autoimmune diseases are not satisfactory [6]. The major focus of management is anti-inflammatory and immunosuppressive therapy to reduce the immune activation and thereby to reduce the inflammatory damage. Various cytokines and cells which participate in the ongoing inflammatory process are targeted. Single or combinations of immunosuppressive agents are used [7-8].

Understanding of cellular abnormalities during imbalanced pro and anti-inflammatory cytokine expression has led to the employment of new therapeutic regimens. These include soluble receptors, monoclonal antibodies and molecular mimetics that have been developed to enhance or gradually replace conventional immunosuppressive therapies [9-10]. New biologicals have been introduced, which target defined pathways of the adaptive immune response. Deregulation of TNF-α production characterizes many autoimmune responses. It is a pro-inflammatory cytokine which is elevated along with other cytokines during autoimmune lesions [11-12]. TNF-α appears to be more than a pro-inflammatory cytokine. It exhibits an immuno regulatory role that can alter the balance of T regulatory cells [13] and apart from orchestrating acute immunological responses; it re-establishes physiological homeostasis and immune regulation [14-15].

Despite its good results, anti-TNF-α therapy has a number of contraindications and side effects, especially when used in combination with classical immunosuppressive agents or corticosteroids [16]. It has been demonstrated to increase disease activity when administered to patients with opportunistic infections as toxoplasmosis and reactivation of latent tuberculosis [17]. A few cases of cerebral toxoplasmosis and toxoplasmic chorioretinitis have been reported in patients who were treated with anti-TNF agents either etanercept or infliximab [18-19]. The aim of this study was to evaluate the effect of TNF-α antagonist in reactivation of latent toxoplasmosis.

2. Experimental

2.1. Subjects and Methods

This study was conducted on patients under treatment of anti-TNF-α where samples were collected from different specialized hospitals, Taif, KSA. Fifty patients referred to the different departments were included (18 males and 32 females) and their age were between 18-45 years. Full clinical evaluation was done, blood samples were withdrawn and samples divided into two Groups such as Group(G1) (case): 50 patients who received biological treatment in the form of anti-TNF-α and Group (G2) (control): 30 normal healthy persons.
From all subjects included in this study, five milliliters of blood was taken under sterile conditions. Then, blood samples were centrifuged at 1000 rpm and the sera was separated and stored at -20°C until the determination of anti Toxoplasma IgG and IgM by a commercially available enzyme-linked immune sorbent assay (ELISA) kit (DRG® Toxoplasma IgM (TORCH) (EIA-1799), DRG International, Inc., USA) and (DRG® Toxoplasma IgG (TORCH) (EIA-1798), DRG International, Inc., USA). The test was performed in the Laboratory of Applied Medical Science College, Taif University following the manufacturer’s instructions. Serum samples were diluted in sample diluents at 1:100. Then, 100 μL of reference calibrator, positive control, negative control and diluted serum samples, was added to wells of microtiter plate coated with purified T. gondii RH strain antigen, incubated for 30 minutes at room temperature and followed by washing 5 times. Then, 100 μL of enzyme conjugate was added to each well, except the blank well and incubated for 30 min at room temperature. After washing, 100 μL of tetra methyl benzidine substrate was added to each well, including the blank well and incubated for 15 min at room temperature, followed by addition of 50 μL of the stop solution. The optical densities were read at 450 nm with a microwell reader. The mean of duplicated cut-off calibrator value (32 μ/mL), positive control, negative control and serum samples were calculated. T. gondii index of each determination was calculated by dividing the mean values of each sample by calibrator mean value. A sample was considered positive for IgM when a T. gondii index was equal or greater than 1.0 (>32 μ/mL), A negative reaction corresponds to T. gondii index less than 0.90 (<32 μ/mL), a positive reaction to T. gondii index of 1.00 or greater (>32 μ/mL), and an equivocal result to T. gondii Index between 0.91-0.99.

2.2. Statistical Analysis

Collected data were numbered, coded and introduced to a computer using the Statistical Package for Social Science for Windows version 22.0. The x² test was used to analyze the frequency of anti T. gondii IgG and IgM seropositivity in the studied groups to clarify statistically significant differences. A value of P<0.05 was considered statistically significant.

3. Results

In the present study, 15 (30%) cases had rheumatoid arthritis, 5 (10%) cases had systemic lupus erythematosus, 5 (10%) cases had psoriasis, 10 (20%) cases had multiple sclerosis and 15(30%) cases had Chon’s disease as in (Figure 1). It was found that the 12 (24%) of the 50 cases who received anti-TNF-α group (G1) and 2 (6.6%) of the 30 healthy volunteers (G2), were positive for anti T. gondii IgM antibody. The percentage of the anti T. gondii IgM antibody seropositivity in cases who received anti-TNF-α group G1 was significantly higher (P<0.05) than in the healthy volunteers group (Table 1). In addition, 20 (40%) of the 50 cases who received anti-TNF-α group (G1) and 3 (10%) of the 30 healthy volunteers (G2) were positive for anti T. gondii IgG antibody.
Figure 1. Total number of patients with different autoimmune diseases.

Table 1. Results of ELISA anti *T. gondii* IgM in study samples.

<table>
<thead>
<tr>
<th>Study Samples</th>
<th>Subjects (80)</th>
<th>ELISA Results</th>
<th>$X^2$</th>
<th><em>P</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Seropositive Subjects (%)</td>
<td>Seronegative Subjects (%)</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>50</td>
<td>12 (24%)</td>
<td>38 (76%)</td>
<td>3.78</td>
</tr>
<tr>
<td>G2</td>
<td>30</td>
<td>2 (6.6%)</td>
<td>28 (93.4%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Results of ELISA anti *T. gondii* IgG in study samples.

<table>
<thead>
<tr>
<th>Study samples</th>
<th>Subjects (80)</th>
<th>ELISA Results</th>
<th>$X^2$</th>
<th><em>P</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Seropositive Subjects (%)</td>
<td>Seronegative Subjects (%)</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>50</td>
<td>20 (40%)</td>
<td>30 (60%)</td>
<td>6.12</td>
</tr>
<tr>
<td>G2</td>
<td>30</td>
<td>3 (10%)</td>
<td>27 (90%)</td>
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4. Discussion

*Toxoplasma gondii* is a protozoan which causes chronic infection by forming cysts containing bradyzoites especially in the brain. The cysts persist in the host tissues for years without causing any local inflammatory reaction and controlled mainly by cellular immune mechanisms. However, if the balance between the host immune defenses and the parasite is disrupted, cyst rupture and renewed parasite proliferation may occur leading to clinical reactivation. Reactivation of toxoplasmosis was found to be a serious complication in patients receiving anti-TNF therapy [20].

TNF-α is a cytokine of inflammatory and immune response and produced by many cell populations; including macrophage, microglial cells and astrocytes in the CNS for the in-vivo control of *T. gondii* and survival of acute and chronic toxoplasmosis. These cytokines can subsequently activate CD8 T cytotoxic cells to turn into major cytotoxic effector cells for lysing tachyzoite-infected cells, limiting parasite dissemination during acute infection phase as well as inhibiting cyst formation during chronic infection [1].
TNF-α together with IL-6 can enhance proliferation and differentiation of B lymphocytes. It activates eosinophil cytotoxicity toward *T. gondii* protozoan and induces secretion of acute phase proteins via IL-6 production, resulting in inhibition of parasite replication. In addition, this cytokine plays a role in macrophage activation, differentiation and phagosome formation and also it is critical for the clearance of intracellular pathogens [21]. So, anti-TNF-α aggravates toxoplastic encephalitis. Radwan et al. [22] reported few cases of toxoplasmosis in patients receiving anti-TNF-α these studies in agreement with our results as showed in Table 1-2.

5. Ethical Consideration
The proposal of study was explained to the all participants, then, an informed consent was taken from them. The study was approved by the Research Ethical Committee of Applied Medical Science College, Taif University, Saudi Arabia.

6. Conflicts of Interest
The author(s) report(s) no conflict(s) of interest(s). The author along are responsible for content and writing of the paper.

7. Acknowledgments
NA

8. Conclusion
From this study, it was concluded that anti-TNF-α could have a role in reactivation of latent toxoplasmosis, so, serological screening for toxoplasmosis before prescribing anti-TNF-α drugs might offer a valuable aid for prevention of serious complication of toxoplasmosis reactivation.

9. References
Clinical Trials and Drug Interactions 2018; 1(1): 43-49