Original article

Increased serum levels of soluble tumor necrosis factor receptor-2 (sTNFR2) in patients with active toxoplasmic retinochoroiditis

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ABSTRACT

This study aimed to investigate the serum levels of the cytokine TNF-α and its soluble receptors (sTNFR1 and sTNFR2) in patients with toxoplasmosis retinochoroiditis (TR) and controls. 37 patients with TR and 30 subjects with positive serology for toxoplasmosis but without history and signs of uveitis were included in this study. Serum concentrations of TNF-α, sTNFR1, and sTNFR2 were determined by ELISA. Serum concentrations of TNF-α and sTNFR1 were similar in controls (mean ± SD median values; 56.57 ± 141.96 and 504.37 ± 163.87, respectively) and TR patients (mean ± SD values, 121.62 ± 217.56 and 511.15 ± 189.30, respectively). Serum concentrations of sTNFR2 were higher in the uveitis group when compared to the control group (respectively, mean ± SD values, 1734.84 ± 379.32 and 1442.75 ± 309.47; p=0.002). There was no association between the serum levels of the molecules and the time of first symptoms, severity of vitreous haze, size or localization of active lesions, levels of visual acuity, and presence of vasculitis. These results suggest that TR is associated with changes in the circulating levels of inflammatory biomarkers, but they are not correlated with local/ocular signs.

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Introduction

Toxoplasmosis is a common infection worldwide, but the disease is often asymptomatic in immunocompetent individuals. It is one of the most common causes of posterior uveitis in both immunocompetent and immunocompromised individuals.¹

Toxoplasmic retinochoroiditis (TR) is the most common identifiable cause of posterior uveitis in many parts of the world, especially in Brazil.²

Few infected subjects develop ocular lesions, and it is uncertain why this happens. The intensity of the damage to the retina and choroid depends on the virulence of the infection and the associated inflammatory reaction.³,⁴
response to antibiotic therapy in combination with cortico-
steroids and the clinical presentation vary significantly, with
some patients presenting one episode of mild inflammation,
and others having multiple recurrences of severe uveitis lead-
ing to loss of sight. The immune response is likely to play a
role in determining the evolution of disease and possibly in
the response to conventional therapy.5,6

TNF-α is a cytokine secreted in response to injury or
infection by different cell types, and it coordinates early pro-
inflammatory signals during innate immune response and is
responsible for many of the associated systemic effects.7–9
TNF-α exerts its major effects by binding to two high-affinity
cell surface receptors, TNFR1 (p55) and TNFR2 (p75).10 The
extracellular portions of these receptors may constitute sol-
uble forms (sTNFR1 and sTNFR2) and can be measured in
the circulation.8 Since the soluble forms of TNF receptors can
compete with the cell-associated TNF-receptors for TNF, it was
suggested that they may function as inhibitors of TNF activ-
ity. Conversely, it has been shown that low concentrations
of sTNFRs stabilize the activity of TNF-α by gradual cytokine
release.11 Therefore sTNFrs may play a role as modulators of
the biological activity of TNF-α. Therefore, measurement of
circulating levels of the two sTNFRs may be useful to deter-
mine the overall production of TNF-α, and may be considered
more reliable markers of inflammatory activity than TNF-α
concentration itself.8,12,13 Since the production of sTNFRs is
led by TNF-α, their concentrations may reflect TNF-α activ-
ity.

Many studies have documented that TNF-α is important
for controlling resistance to acute14,15 and chronic16,17 T. gondii
infection. Experimental studies suggest that the TNF-α/TNFrs
pathway may be beneficial for host protection against T.
gondii.18,19 Immunity to T. gondii depends on TNFR1-mediated
immune reactions,20 and experimental studies have demon-
strated the crucial role of TNF-α for controlling intracerebral
toxoplasmosis.16 In an animal model of ocular toxoplasmosis,
treatment with anti-TNF-α antibodies resulted in worsening
of ocular lesions, with the development of retinal necrosis
and marked inflammation of the vitreous and underlying
choroid.21

The aim of the present study was to evaluate serum lev-
els of TNF-α and sTNFRs in TR patients compared to controls.
It was also investigated whether their levels were associated
with the clinical parameters of TR.

Material and methods

Subjects

The study protocol adhered to the Declaration of Helsinki, and
was approved by the local institutional review board. Patients
were informed verbally and in writing of the potential benefits
and risks of the study, and all patients signed an informed
consent.

Adult subjects were recruited from the Uveitis Section of
the Department of Ophthalmology of the Universidade Federal
de Minas Gerais - Brazil. 37 patients with acute TR were
invited to participate in this study (16 males, 21 females,
mean age [± standard deviation, SD] 28.16 ± 8.83 years). No
subject enrolled in this study presented autoimmune or sys-
temic infectious disease, or was under treatment for TR at
the moment of the first evaluation. The diagnosis of TR was
based on positive serological test for T. gondii (IgG) and typi-
cal ocular lesions in the retina (i.e. gray-white focus of retinal
necrosis next to a pigmented retinal scar or in the other
eye).1,2,22

All patients underwent a detailed ocular examination,
including best-corrected visual acuity, applanation tonometry
for intraocular pressure, slit lamp examination, fundus exam-
ination with 78-D lens, and indirect ophthalmoscope. The
number and location of retinochoroidal lesions were doc-
umented for all patients by careful fundus drawings or
photographs. The intensity of the inflammatory process was
evaluated by the size of active retinochoroiditis lesions (graded
by optic disc diameter [DD]), the presence of retinal vasculi-
tis, and vitreous haze. Visual acuity (VA) was measured and
expressed as the logarithm of the minimum angle of resolu-
tion (logMAR).

For comparison, 30 age- (mean age 30.37 ± 6.46 years; p =
0.095; Mann-Whitney’s U test) and gender-matched (13 males,
17 females; p = 0.994) adult subjects with no evidence of
active ocular disease and with positive serological test (IgG
antibody) for T. gondii were recruited for the control group.
None had a history of uveitis, and each underwent a com-
plete ocular examination (identical to the uveitis group) to
rule out the presence of retinal scars suggestive of previous
TR.

Blood sampling and biochemical measurements

In all subjects, blood was collected aseptically after the ocu-
lar examination. Patients were with active disease and had not
yet received any treatment. Serum was prepared and stored at
-70 °C. Serum levels were tested according to the procedures
supplied by the manufacturer, using sandwich enzyme-linked
immunosorbent assays (ELISA) kits for TNF-α, sTNFR1, and
sTNFR2 (DuoSet, R&D Systems – Minneapolis, Minn., USA),
as routinely performed in this laboratory. All samples were
assayed in duplicate. The values are presented in pg/mL and
represent the mean result of the two measures. The variance
between measures was under 10%.

Statistical analysis

Statistical analysis and the graphs were performed using the
Statistical Package for Social Sciences (SPSS) for Windows
(16.0; SPSS, Inc., Chicago) and GraphPad Prism 4.00, respec-
tively. All variables were tested for normality of distribution
by means of the Kolmogorov-Smirnov test. Only the vari-
able age showed normal distribution. Non-parametric analysis
was performed using the Mann-Whitney’s U test for compar-
ison of unpaired data from two groups and the Wilcoxon
test for comparison of paired data. Differences between three
groups were evaluated using the Kruskall-Wallis test. Correla-
tion analysis between age and cytokine levels was performed
using Spearman’s correlation coefficient. Pearson’s test was
used for comparison of categorical data (e.g. gender). Staisti-
cal significance was set at p < 0.05.
Results

Thirty-seven patients and 30 healthy age- and gender-matched controls were included in this study. There was significantly diminished visual acuity (VA – LogMAR) in the TR group: means of 0.97 and 0.45, in the TR and control groups, respectively (p = 0.001). The main demographic and clinical features of patients with TR are presented in Table 1.

There were no significant differences in serum concentrations of TNF-α and sTNFR1 between TR patients and controls. In contrast, significantly increased levels of sTNFR2 were found in TR patients (Table 2).

There was no correlation among TNF-α, TNFR1, and TNFR2 even when controlling for TR. Correlation analyses also failed to establish any relationship between serum levels of the molecules and the time of first symptoms, severity of vitreous haze, size or localization of active lesions, levels of visual acuity, and presence of vasculitis.

Discussion

There is a great controversy regarding which factors might be responsible for the occurrence or recurrence of TR. Among the proposed factors are the strains of parasites, hormonal changes, and the production of some molecules that participate in the immune system, such as cytokines.1,2

A recent study suggested that the chemokine CXCL8 can participate in the inflammatory process of TR and may be a useful marker for patient follow-up.23 It has also been suggested that polymorphisms of the genes responsible for the production of cytokines could predispose the occurrence and/or recurrence of TR. A study in humans found that the genotypes related to a low production of IL-10 may be associated with the occurrence of TR.24 Another study suggested that genotypes related with a high production of IL-1α may be associated with TR recurrence.25

To the best of the authors’ knowledge, this is the first study to assess the serum levels of TNF-α and sTNFRs in patients with TR and to correlate these levels with clinical parameters. The present results demonstrated increased levels of sTNFR2 in serum of patients with TR in comparison with controls, but no differences in TNF-α and sTNFR1 levels were found. The meaning of this change in circulating levels of sTNFR2 in TR is uncertain. This result may represent either systemic activation of inflammatory cascades or endocrine signaling at distance.

The protective role of cytokines such as TNF-α in TR has been demonstrated in animal models.21 In humans, few studies have investigated circulating levels of TNF-α, sTNFR1, and sTNFR2 in infectious uveitis. Takase et al.26 did not find any significant difference between the serum and aqueous humor levels of TNF-α in patients with anterior herpes simplex uveitis, acute retinal necrosis, and Vogt-Koyanagi-Harada uveitis. Yamamoto et al.27 analyzed blood samples of patients with acquired ocular toxoplasmosis and congenital ocular disease. They found higher levels of TNF-α in patients with acquired disease when compared with control subjects, but the difference was not statistically significant. Lahmar et al. (2009) analyzed the levels of TNF-α among other cytokines in aqueous humor of patients with TR and another infectious uveitis, finding similar levels of TNF-α in both conditions.28

The levels of cytokines in aqueous humor did not correlate with any demographic or clinical variables (including degree of uveal inflammation and etiology of the infection, i.e., congenital or primary acquired). Interestingly, the titers of TNF-α were significantly higher in aqueous humor in comparison with serum, suggesting that the cytokine is cleared more rapidly in this compartment.28 These findings of unaltered serum levels of TNF-α in infectious uveitis are in agreement with the present study. However, significantly increased serum levels of sTNFR2 in patients with TR were demonstrated in comparison with that of controls. As previous studies have suggested that sTNFR1 and sTNFR2 are the natural homeostatic regulators of the TNF-α activity,8,12,29 and seem to be reliable markers of TNF-α activity,8,12,13, the present finding of elevated sTNFR2 suggests the involvement of TNF-α in human TR.

The distinct behavior of sTNFR1 and sTNFR2 in TR is not unique and has been reported in other infectious diseases.8,12

Table 1 – Clinical and demographic characteristics of patients with toxoplasmic retinochoroiditis (TR) and controls.

<table>
<thead>
<tr>
<th></th>
<th>TR (37)</th>
<th>Controls (30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Female</td>
<td>21</td>
<td>17</td>
</tr>
<tr>
<td>Mean age (years ± SD)</td>
<td>28.16 (8.83)</td>
<td>30.37 (6.46)</td>
</tr>
<tr>
<td>Visual acuity (LogMAR) mean (SD)</td>
<td>0.97 (0.86)</td>
<td>0.45 (0.74)</td>
</tr>
<tr>
<td>Time of first symptoms (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ seven days</td>
<td>43.24</td>
<td>—</td>
</tr>
<tr>
<td>&gt; seven days</td>
<td>56.76</td>
<td>—</td>
</tr>
<tr>
<td>Eye affected (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right eye</td>
<td>54.05 (n = 20)</td>
<td>—</td>
</tr>
<tr>
<td>Left eye</td>
<td>45.94 (n = 17)</td>
<td>—</td>
</tr>
<tr>
<td>Both eyes</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Size of lesion (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1 DD</td>
<td>32.43</td>
<td>—</td>
</tr>
<tr>
<td>1-2 DD</td>
<td>37.84</td>
<td>—</td>
</tr>
<tr>
<td>2-4 DD</td>
<td>27.02</td>
<td>—</td>
</tr>
<tr>
<td>&gt;4 DD</td>
<td>2.7</td>
<td>—</td>
</tr>
<tr>
<td>Vasculitis (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>58.33</td>
<td>—</td>
</tr>
<tr>
<td>No</td>
<td>41.66</td>
<td>—</td>
</tr>
<tr>
<td>Lesion location (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zone 1</td>
<td>37.84</td>
<td>—</td>
</tr>
<tr>
<td>Zone 2</td>
<td>48.65</td>
<td>—</td>
</tr>
<tr>
<td>Zone 3</td>
<td>13.51</td>
<td>—</td>
</tr>
<tr>
<td>Vitreous haze</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>13.51</td>
<td>—</td>
</tr>
<tr>
<td>1+</td>
<td>48.65</td>
<td>—</td>
</tr>
<tr>
<td>2+</td>
<td>24.32</td>
<td>—</td>
</tr>
<tr>
<td>3+</td>
<td>10.81</td>
<td>—</td>
</tr>
<tr>
<td>4+</td>
<td>2.70</td>
<td>—</td>
</tr>
</tbody>
</table>

* One patient with 4+4+ of vitreous haze was not considered in the analysis.

DD, optic disc diameter.
Interestingly, a previous experimental study highlighted the role of TNFR1, but not TNFR2, in murine toxoplasmosis. Indeed TNFR1 and TNFR2 have different mechanisms of regulation. Inflammatory stimuli such as TNF-α and IL-1β increase the expression of TNFR2 through transcriptional activation, whereas TNFR1 is more commonly down-regulated associated with changes in the circulating levels of inflammatory biomarkers (sTNFR2), but they are not correlated with local/ocular signs. Additional studies involving repeated measurements with the same patients may elucidate a potential role for serum levels of sTNFRs as biomarkers of development and progression of TR.

**Conflict of interest**

All authors declare to have no conflict of interest.

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**REFERENCES**


