Sero-prevalence of Toxoplasma gondii infection in healthy human population in Kashmir Valley, India

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An IgG antibody prevalence of 24.2% (123/508) was found in the study population, while examination of specific IgA and IgM antibodies was negative. A significant increase in toxoplasmosis prevalence with increasing age was confirmed (p = 0.001). There were no significant differences between genders or between inhabitants of rural and urban areas (p = 0.079 and p = 0.291, respectively). An increased consumption of raw meat (40.7%) and raw vegetables or fruit (30.8%) was observed in serologically positive women. This difference between serologically positive and negative women was significant (p = 0.042). A significant difference between men and women eating raw vegetables and fruit was also found (p = 0.049). There was no difference in clinical symptoms between serologically positive and negative patients (p = 0.049). Lymphadenopathy with or without other symptoms (influenza, arthralgia, ocular symptom, dermatosis), 40.7%, was the most common clinical symptom in seropositive patients.

Toxoplasma gondii is a ubiquitous parasite of warm-blooded animals that causes one of the most common parasitic infections in humans (Tenter et al. 2000). The seroprevalence varies widely in different regions of the globe, measuring between 30% and 60% in most countries (Flegr et al. 2003). The prevalence changes according to social and cultural habits, geographic factors, climate, and transmission route, and it typically increases with age (Flegr et al. 2003, Cantos et al. 2000). It has been reported that the prevalence is higher in warm and humid areas (Flegr et al. 2003, Coelho et al. 2003). Toxoplasmosis is not a reportable disease and disease prevalence is based on regional studies.

Serological surveys indicate that about 80% of all primary infections are asymptomatic, due to the effectiveness of the immune system (Griffin & Williams, 1983). The tissue parasitism during the proliferative phase may occur without symptoms. It may lead to a transient illness characterized by lymphadenopathy, fever, fatigue, arthralgia, dermatosis, malaise, headache, and myalgia. Latent toxoplasmosis, i.e., the lifelong presence of cysts and anamnestic concentrations of anti-T. gondii antibodies in immunocompetent subjects, is considered asymptomatic and harmless. Toxoplasmosis is most dangerous to two populations: immunocompromised patients and foetuses whose mothers acquire acute infection during pregnancy (Pinard et al. 2003). Acute maternal infection during pregnancy can lead to transplacental transmission and subsequent infection in the foetus. Gestational age and the rate of transplacental transmission are in direct correlation, and an inverse relationship has been found between the development of clinical signs and the gestational age at which maternal seroconversion occurs (Pinard et al. 2003). Human infection generally occurs through the ingestion of raw or undercooked meat that contains cysts, through the ingestion of water or food contaminated with oocysts, or congenitally through trans-placental transmission from a mother infected during pregnancy.

The aim of this study was to evaluate the seroprevalence of toxoplasmosis in a non-endemic area among the healthy population in Kashmir (India). ELISA was used to detect the levels of IgG, IgA, and IgM antibodies. Epidemiological factors and clinical symptoms associated with seropositivity were investigated using questionnaires.

Material and methods

From January to October 2015, the prevalence of toxoplasmosis was prospectively monitored by examining serological levels of IgG, IgA, and IgM antibodies using the ELISA method in a population of 508 healthy individuals at the Department of Zoology, University of Kashmir. The study was approved by the hospital’s ethics committee, and informed consent was obtained from the patients.
There were 508 subjects in total: 436 adult blood donors and 72 children and adolescents. The healthy volunteers, i.e., blood donors, were consecutively recruited and examined between January and October 2015 at the SKIMS Soura- Srinagar. The patients were healthy persons, 19 years of age or older, who satisfied the following criteria: normal full blood count, normal blood pressure, no acute infection, normal alanine transaminase, negative HbsAg, negative anti-HCV antibodies, negative anti-HIV antibodies, and a negative TPHA test. The group of 72 children and adolescents was defined as healthy persons below 19 years of age coming for a routine annual check-up. Informed consent was obtained from their parents.

The subjects were further grouped according to gender, age, and place of residence. The study group consisted of 262 males aged 3–58 years and 246 females aged 5–56 years. The subjects tested were divided into 5-year age groups (Table 1). A total of 252 adult blood donors lived in an urban area and 184 adult blood donors lived in a rural area. All children and adolescents tested came from Kashmir. The gender of the individuals studied was only considered in the adult group.

Serum samples were examined by immunoenzyme assay (ELISA) to identify the presence of immunoglobulin IgG, IgM, and IgA. IgG antibodies were examined with a Euroimmun Anti-T. gondii ELISA (IgG) set. IgM and IgA antigens were detected with commercially available DS-EIA-Anti-Toxo-M-FAST strictly following the manufacturers instructions. Antibody levels were evaluated by following the instructions of the set manufacturers. Results were expressed in titers. A borderline (cut-off) titer was determined as 1:100 for all examined groups of antibodies. Control sera and calibration sera were not diluted. The absorbance of the samples examined was measured at 405/630 nm using a spectrometer manufactured by Behring (ELISA Processor, BEP II). During the test, sera were stored at +4 °C and then frozen at −20 °C for long-term storage.

Aiming to identify the presence of risk factors (working in a garden, eating raw meat and raw vegetables or fruit), the laboratory determination of toxoplasmosis was supplemented by epidemiological data gathered via questionnaires and by data on clinical symptomatology (lymphadenopathy, arthralgia, influenza, temperature, abortion, ocular symptom, dermatosis, somnolence, etc.) The seroprevalence of T. gondii antibodies in the population studied was evaluated by means of confidence intervals (CI) of relative frequencies for a selected level of probability (OR; odds ratio). The Chi Square test using contingency tables was chosen for the testing, and the software used was Epi Info version 6.

3. Results

We found the prevalence of IgG seropositivity in the study population to be 24.2% (123/508). The examination of specific IgA and IgM antibodies was negative. Moreover, we observed a significant difference in the prevalence of toxoplasmosis between the age groups (\( x^2 = 26.3; p = 0.001 \); Table 1). The prevalence of T. gondii infection among individuals in the age group below 19 years was lower than that in adults (\( p = 0.001 \)). The prevalence of IgG was 22.8% (56/246) in males and 30.0% (57/190) in females in the group of adult blood donors. The difference between gender groups was not significant (\( p = 0.079 \)). Prevalence in the subjects from urban areas and rural villages were 27.8% (70/252) and 23.4% (43/184), respectively, which was not statistically significant (\( p = 0.291; \ OR=1.26, \ 95\% \ CI=0.79–2.00; \ Table \ 2 \)). No correlation was found between risk factors and prevalence of IgG in the different age groups.

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Sex</th>
<th>IgG-positive patients (%)</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;19</td>
<td>Male (n =32)</td>
<td>10 (13.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female (n =40)</td>
<td>35 (20.7)</td>
<td>1.62 (0.73–3.57)</td>
<td>0.214</td>
</tr>
<tr>
<td>19–29</td>
<td>Male (n =48)</td>
<td>10 (12.7)</td>
<td>0.99 (0.32–2.53)</td>
<td>0.824</td>
</tr>
<tr>
<td></td>
<td>Female (n =31)</td>
<td>30 (34.2)</td>
<td>3.23 (1.41–7.56)</td>
<td>0.002</td>
</tr>
<tr>
<td>30–39</td>
<td>Male (n =48)</td>
<td>30 (38.9)</td>
<td>3.96 (1.65–9.67)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Place of residence</th>
<th>Sex</th>
<th>Number of patients</th>
<th>IgG-positive patients (%)</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban area</td>
<td>Male</td>
<td>116</td>
<td>26 (22.4)</td>
<td>0.6 (0.33–1.10)</td>
<td>0.079</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>136</td>
<td>44 (32.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural area</td>
<td>Male</td>
<td>128</td>
<td>30 (23.4)</td>
<td>1.01 (0.45–2.28)</td>
<td>0.974</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>56</td>
<td>13 (23.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Sex</th>
<th>IgG-positive patients (%)</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gardening/Contact with soil</td>
<td>Male</td>
<td>45 (28.7)</td>
<td>1.21 (0.65–2.24)</td>
<td>0.526</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>24 (25.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eating raw or undercooked meat</td>
<td>Male</td>
<td>30 (29.2)</td>
<td>0.60 (0.29–1.23)</td>
<td>0.129</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>22 (40.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eating raw vegetables and fruits</td>
<td>Male</td>
<td>33 (21.2)</td>
<td>0.60 (0.35–1.03)</td>
<td>0.049</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>32 (28.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

We observed an increased seropositivity in women that was related to the increased consumption of raw meat (40.7%) and raw vegetables or fruit (30.8%). The difference between men and women who ate raw vegetables and fruit was statistically significant (\( p = 0.049 \); Table 3). The difference in raw meat consumption between seropositive and seronegative women was statistically significant (\( p = 0.042; \ OR=0.50, \ 95\% \ CI=0.25–1.03) in seronegative women, the consumption of raw meat was 24% (32/133). We determined the presence of clinical markers in 27/123 (21.9%) of seropositive patients. There was no difference in clinical symptoms between seropositive and seronegative patients (\( p = 0.658; \ OR = 0.90, \ 95\% \ CI = 0.53–1.5) in clinical symptomatology, lymphadenopathy with or without other symptoms (influenza, arthralgia, ocular symptom, dermatosis) was dominant (40.7%). Five women reported having had previous abortions and eight patients arthralgies (Table 4).
The prevalence of toxoplasmosis in children and adolescents in our study group (13.9%) is comparable with the results of a study carried out in the United Arab Emirates (12.9%) (Abu-Zeid, 2002). The prevalence of toxoplasmosis can vary among different groups, and these differences have also been seen between rural and urban regions. Some studies determined a higher prevalence in rural regions (Stoll, 1975), while others did not show any difference between urban and rural inhabitants (Sousa, 1987). In our study group, we did not find any difference in the seroprevalence of toxoplasmosis between males and females, nor was any significant difference observed between urban and rural populations.

The prevalence of toxoplasmosis in the human population is associated with exposure to risk factors. An increased prevalence of toxoplasmosis is detected in people who are often in contact with soil, who eat raw or insufficiently heat-treated meat or raw vegetables, or who lack basic personal hygiene or have unhygienic food preparation (Tenter et al., 2000, Baril et al., 1996). In our study, we observed the potential impact of risk factors on the prevalence of toxoplasmosis. Based on our epidemiological data, we observed a higher frequency of raw meat consumption in serologically positive women (40.7%). The increased consumption of raw vegetables or fruit in seropositive women in comparison with seropositive men was also statistically significant (p = 0.049). Similarly, in a previous epidemiological study, an increased prevalence of acute toxoplasmosis was observed in women who ate raw meat more frequently (Cook et al., 2000). In clinical symptomatology of serologically positive patients, lymphadenopathy with or without other symptoms (influenza, arthralgia, ocular symptom, dermatitis) was dominant (40.7%).

We observed a decreasing trend in the prevalence of toxoplasmosis in the region examined that was related to qualitative changes in hygiene and dietary habits, as well as with improvements in food preservation quality or changes in nutrition.

The seroprevalence that we found correlates with the results of studies done in other countries with similar or dissimilar geographical, climatic, and socioeconomic conditions. In healthy populations in the Czech Republic and Scotland, prevalences of seropositivity of 32.1% and 27%, respectively, were found (Svobodova et al., 1998, Williams et al., 1981). On the other hand, an increased prevalence of toxoplasmosis was demonstrated in studies conducted in regions with increased risk factors. In Kenya, a hemagglutination test found antibodies in 54% of blood donors and a dye test found them in 42% of blood donors (Griffin & Williams, 1983). In Saudi Arabia (Yaneza & Kumari, 1994), seropositivity determined by an indirect hemagglutination test was 37.5% in blood donors, and in Brazil (Coelho et al., 2003), an EIA method found a prevalence of 79.9% in men and of 63.4% in women.

In accordance with other studies, we observed a correlation between increasing prevalence of antibodies against T. gondii and increasing age of blood donors (Bobic et al., 1998). Furthermore, we found a statistically significant difference between the groups of children and adolescents and adults (p = 0.001). The seroprevalence of toxoplasmosis in children and adolescents in our study group (13.9%) is comparable with the results of a study carried out in the United Arab Emirates (12.9%) (Abu-Zeid, 2002). The prevalence of toxoplasmosis can vary among different groups, and these differences have also been seen between rural and urban regions. Some studies determined a higher prevalence in rural regions (Stoll, 1975), while others did not show any difference between urban and rural inhabitants (Sousa, 1987). In our study group, we did not find any difference in the seroprevalence of toxoplasmosis between males and females, nor was any significant difference observed between urban and rural populations.

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We observed a decreasing trend in the prevalence of toxoplasmosis in the region examined that was related to qualitative changes in hygiene and dietary habits, as well as with increased quality in the preservation of food and changes in nutrition. Toxoplasmosis will remain a problem, mainly in risk groups such as pregnant women and immunocompromised patients. Improvement can only be attained by increasing prevention and reducing the risk factors.

5. Conclusion

- A health program should provide the public with information regarding this disease, its risk factors, and the negative influence it can have on the foetus and regarding prevention in risk groups. This could contribute significantly to an improvement in general public health.

- A program should be established to allow all pregnant women to be screened in their first trimester. This would identify individuals at risk and allow for timely treatment, where necessary.

- Measures should be implemented to prevent any possible spread of toxoplasmosis at institutions that individuals at risk are likely to visit. Furthermore, public health inspection agencies should check to see that restaurants and other food outlets comply with these measures.
Conflict of Interest

We declare that we have no conflict of interest.

Acknowledgment

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References