Seroprevalence of *Toxoplasma gondii* in people at an increased risk of HIV infection – a pilot study

Małgorzata Smoguła, Marta Pawłowska, Roland Wesołowski, Karolina Szewczyk-Golec, Celestyna Mila-Kierzenkowska

Department of Biology and Medical Biochemistry, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, Toruń, Poland

**Abstract**

**Introduction:** The observation of the epidemiology of toxoplasmosis may prevent the development of a severe form of the disease in HIV patients.

**Aim:** The aim of the study was to evaluate the seroprevalence of *Toxoplasma gondii* in the population of the Kuyavian-Pomeranian Voivodeship at high risk of contracting HIV.

**Material and methods:** Blood serum samples of 43 patients of the Consulting and Diagnostic AIDS Center were tested for the presence of anti-HIV-1/HIV-2 antibodies and p24 antigen, and for the presence of anti-toxoplasma IgM and IgG antibodies.

**Results:** Anti-toxoplasma IgG antibody prevalence of 53.5% (23/43) was found in the study population, while the examination of specific IgM antibodies was negative. A high IgG antibody avidity index was obtained in 18 (94.7%) seropositive samples. Thirty (69.77%) of the samples were female, and 13 (30.23%) were male. Among men, HIV was detected in 1 (7.69%), and IgG antibodies against *T. gondii* in 7 (53.85%) samples. IgG antibodies against *T. gondii* were found in 16 (53.33%) women. The HIV-positive individual was 24 years old. The presence of antibodies against *T. gondii* in the IgG class was found in people of different ages (in women aged 38.44 ±13.00 years old and in men aged 29.29 ±10.86 years old). The risky situation that could cause HIV infection is in most cases sexual contacts (79.07%).

**Conclusions:** High seroprevalence of *T. gondii* was found among the studied subjects at a high risk of HIV infection. Further research is required on a larger study group.

**Keywords:** antibodies, HIV, seroprevalence, *Toxoplasma gondii*

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**Streszczenie**

**Wstęp:** Obserwacja epidemiologii toksoplazmy może zapobiec rozwojowi ciężkiej postaci choroby u pacjentów z HIV.

**Cel:** Celem pracy była ocena seroprewalencji *Toxoplasma gondii* w populacji województwa kujawsko-pomorskiego o wysokim ryzyku zakażenia wirusem HIV.

**Materiał i metody:** Przebadano 43 próbki surowicy krwi pobrane od pacjentów Punktu Konsultacyjno-Diagnostycznego Krajowego Centrum ds. AIDS w kierunku obecności przeciwciał anty-HIV-1/HIV-2 i antygenu p24 oraz przeciwciał w klasie IgM i IgG przeciwo toksoplazmie.

**Wyciąg:** W wybranych przypadkach pozycja HIV była 24 lat. Rzeczą ryzyka, która mogła spowodować infekcję HIV, jest w wielu przypadkach seksualne kontakty (79,07%).

**Wniosek:** Wysoka seroprewalencja *T. gondii* była zauważona w badanych osobach na podstawie wysokiego ryzyka infekcji HIV. Dalsze badania wymagane są na większej populacji.

**Słowa kluczowe:** antyciała, HIV, seroprewalencja, *Toxoplasma gondii*
INTRODUCTION

Toxoplasma gondii is a protozoan that causes toxoplasmosis, a zoonotic disease in humans. Chronic infection with this parasite affects approximately one-third of the human population worldwide [1]. T. gondii can be transmitted to humans via different ways, including consuming raw or undercooked meat, ingesting cat shed oocysts via contaminated soil, food or water, or congenitally by transplacental transmission of tachyzoites [2]. T. gondii infection can also occur through blood transfusion or organ transplantation [3]. The diagnosis of toxoplasmosis is most commonly made by the detection of specific immunoglobulins, including immunoglobulins M (IgM) and immunoglobulins G (IgG) [4]. IgM antibodies are detectable about one week after the infection and remain high for several months or years, while the presence of IgG antibodies suggests the occurrence of infection, but does not provide any information about the timing of the infection [1]. Thus, an anti-toxoplasma IgG avidity assay is used for improved estimation of infection acquisition. Low IgG avidity usually specifies the first few months of the infection, whereas high avidity specifies nonprimary infections [5].

Toxoplasmosis is usually benign or asymptomatic in immunocompetent subjects, but it can have severe consequences if it occurs in immunodeficient subjects or fetuses [6]. In HIV-positive individuals, it causes severe opportunistic infections, which is of major public health concern as it results in physical and psychological disabilities [7]. T. gondii may cause progressive and recurring necrotizing retinochoroiditis and is the most common cause of infectious uveitis worldwide [8]. Ocular and cerebral toxoplasmosis is a particular problem in AIDS patients and may even be an AIDS-defining condition [9]. Hence, an early diagnosis of T. gondii...
infection is important in all HIV-positive individuals to prevent complications of central nervous system (CNS) toxoplasmosis.

The prevalence of *Toxoplasma gondii* infection in HIV patients is approximately 35.8% and in high-income countries it is approximately 26.3%. The clinical presentation of cerebral toxoplasmosis is nonspecific. In the course of toxoplasmosis, rapidly progressive and diffuse encephalitis, ventricular inflammation, and symptoms resembling a cerebrovascular accident may occur. The most common symptoms include headache, fever, seizures, focal neurological defects, palsy of the cranial nerves, visual disturbances, confusion, and psychomotor or behavioral changes. With delayed treatment, it can develop into dementia, coma, and death [10].

In Poland, within the framework of the National AIDS Center, there are many Consulting and Diagnostic Centers, where HIV tests can be performed free of charge, anonymously and without a referral, combined with post-test counseling. The tests are preceded by an interview with a certified HIV/AIDS adviser, who explains the possible results, helps to estimate the risk, and answers any questions [11]. Although much is known about the HIV/AIDS epidemic in Poland, data concerning toxoplasmosis prevalence among those patients is limited.

**AIM**

The aim of the study was to detect anti-toxoplasma IgM and IgG antibodies in blood samples of people from the population of the Kuyavian-Pomeranian Voivodeship, tested for HIV presence. To the best of our knowledge, this is one of the few studies to estimate the prevalence of *Toxoplasma gondii* infection in people at high risk of contracting HIV in Poland.

**MATERIAL AND METHODS**

Forty-three blood serum samples were tested for the presence of both anti-HIV-1/HIV-2 antibodies and p24 antigen, as well as for the presence of anti-toxoplasma IgM and IgG antibodies. The samples were taken from people (women aged 37.53 ±12.35 years old and men aged 33.46 ±12.02 years old) reporting to the HIV Consulting and Diagnostic Center at the Provincial Sanitary-Epidemiological Station in Bydgoszcz, Poland. Figure 1 presents the enrollment scheme study group selection. Among the studied samples, 30 (69.77%) were taken from females and 13 (30.23%) from males. Blood serum samples were separated after centrifugation of blood samples (collected for a clot). Laboratory diagnostics was performed using the ELFA technique (Enzyme Linked Fluorescent Assay) on the VIDAS instrument by bioMérieux (France). The commercial tests, including VIDAS HIV DUO Quick kit (HIV6), VIDAS TOXO IgM kit (TXM), and VIDAS TOXO IgG II kit (TXG), were used. After the initial detection of anti-toxoplasma IgG, an avidity index was determined with the use of VIDAS TOXO IgG AVIDITY (TXGA) kit.

Each kit includes SPR pipettes and test strips. An SPR (Solid Phase Receptacle) pipette serves as a solid phase as well as a pipetting device during the assay. The avidity determination
uses a double strip consisting of a reference strip and a test strip. All determination steps are carried out automatically by the device. The reaction medium is repeatedly introduced and discharged from the SPR pipette. The fluorescence of the product obtained is measured at a wavelength of 450 nm. The intensity of the fluorescence is proportional to the concentration of antibody and/or antigen present in the sample. At the end of the determination, the results are automatically calculated by the device against a standard and then printed. In the case of avidity determination, the ratio between the amount of high avidity antibodies (test strip) and the total amount of antibodies (reference strip) is a factor that indicates the avidity of the antibodies in the test sample. Avidity samples were tested for the presence of IgG antibodies and found positive (titer ≥ 8 IU/ml). Samples with antibody concentrations greater than 300 IU/ml were re-tested after prior dilution to bring the titer to 15 IU/ml.

There is a Microsoft Excel spreadsheet for data analysis. The performed tests were qualitative research. The statistical analysis was performed using Statistica 12.0 software (StatSoft, Inc.). The Mann-Whitney test was used to detect significant differences (P<0.05) in three types of parameters.

**RESULTS**

Patients of Consulting and Diagnostic Centers do the tests anonymously. Only a short questionnaire is completed, including: password, year of birth, gender, province, possible route of HIV infection and sexual orientation.

Among 43 serum samples tested, the presence of anti-HIV-1/ HIV-2 antibodies and p24 antigen was found only in 1 (2.33%) sample. According to the completed questionnaires, sexual contact was a risk situation which could result in HIV infection in 34 (79.07%) cases, while 9 (20.93%) subjects indicated occupational exposure. No positive result for anti-toxoplasma IgM antibodies was obtained, while a diagnostic level of anti-toxoplasma IgG antibodies was observed in 23 (53.49%) serum samples. There was one questionable result for the presence of IgM antibodies and one questionable result for the presence of IgG antibodies (according to the cut-off values and the interpretation of the test manufacturer’s results for questionable samples’ index: < 0.55 and < 0.65, respectively). The number of negative, positive and questionable results obtained for the studied parameters is presented in figure 2. The avidity of IgG antibodies was assessed in all 23 seropositive samples. A high IgG antibody avidity index was obtained in 21 (91.3%) samples, while the avidity index was low in 2 (8.7%) samples.

The positive HIV test result was detected in one person aged 24 years old, who had also anti-toxoplasma IgG antibodies found. The presence of anti-toxoplasma IgG and IgM antibodies was indicated in women aged 38.44 ±13.00 years and in men aged 29.29 ±10.86 years. Considering sex, HIV was detected in one sample (7.69%) among men and none among women. Anti-toxoplasma IgG antibodies were found in 7 samples obtained from males and 16 samples obtained from females. The questionable results for the detection of IgM and IgG antibodies concerned 2 women. Table I shows the percentage of positive results by sexes.

The risky situation that could cause HIV infection is in most cases sexual contacts (79.07%). In turn, the occupational exposure concerned 9 people. The study participants included people with different sexual orientation: hetero – 32 (74.42%) people, bisex – 7 (16.28%) people, msm – 4 (9.30%) people. The HIV virus was detected in a group of bisexual people, while the presence of IgG antibodies against *T. gondii* was detected in people of different sexual orientation.

<table>
<thead>
<tr>
<th>SEX</th>
<th>PARAMETER</th>
<th>NEGATIVE RESULTS</th>
<th>POSITIVE RESULTS</th>
<th>QUESTIONABLE RESULTS</th>
<th>PERCENTAGE OF POSITIVE RESULTS [%]</th>
</tr>
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<td>Female (n = 30)</td>
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<tr>
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<td>anti-toxoplasma IgG</td>
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<td>16</td>
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<tr>
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</tr>
<tr>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>anti-toxoplasma IgG</td>
<td>6</td>
<td>7</td>
<td>0</td>
<td>53.85</td>
</tr>
</tbody>
</table>

Table I. The percentage of the detection of anti-HIV-1/HIV-2 antibodies and p24 antigen, as well as anti-toxoplasma IgM and IgG depending on sex in the tested patients.
DISCUSSION

Toxoplasmosis has historically been considered one of the most important opportunistic infections detected in HIV/AIDS patients. It has been estimated that approximately 30% of AIDS patients who have anti-toxoplasma antibodies will develop CNS toxoplasmosis, which is the most common cause of expansive brain lesions and has high morbidity and mortality in subjects with advanced immunosuppression [12]. Thus, Toxoplasma screening programs should be recommended for all newly diagnosed HIV-positive patients.

In the present study, the appearance of anti-toxoplasma IgM and IgG antibodies in blood serum was investigated in the selected population of the Kuyavian-Pomeranian Voivodeship, tested for HIV infection because of the increased risk of contracting HIV. However, only IgG antibodies of high avidity were detected in the studied individuals (53.49%), which proves the past toxoplasmosis and the absence of active Toxoplasma infections. Moreover, only one HIV-positive result was obtained and this individual also had anti-toxoplasma IgG antibodies found. According to the present study, up to 50% of seropositive individuals with the presence of anti-toxoplasma IgG antibodies could be reported in the Polish population. The anti-toxoplasma IgG antibodies of high avidity, found in the studied subjects, are in low (so-called environmental) concentration and may persist practically until the end of the individual life [13]. The estimated seroprevalence in Poland (up to 50%) is in accordance with many reports from other countries [7]. In the studies by Pawełczyk et al. of 152 HIV-infected patients tested for T. gondii-specific IgM and IgG, 6 (3.9%) and 50 (32.9) were positive, respectively. Of 168 serum samples from blood donors, 1 (0.6%) and 49 (29.2%) were positive for IgM and IgG, respectively. In HIV-infected patients, low avidity index (<45%) was observed in 3 cases, equivocal avidity (45-60%) in 4, while in the remaining 43 samples, the avidity values were high (>63.2%).

Across the world, the prevalence rate of Toxoplasma infection among HIV-positive individuals has been found to vary from 3% to 97% [16]. In the studies in the Ethiopian population, the prevalence of toxoplasmosis for HIV/AIDS patients was 60% [17], while in Brazil 80%, with a history of neurotoxoplasmosis in 4.8% and of ocular toxoplasmosis in 1.6% of the patients [18]. In contrast, the seroprevalence of toxoplasmosis among HIV/AIDS patients from southern Iran was only 18.2% with 10.4% and 89.6% of subjects with and without Toxoplasma encephalitis, respectively [19].

Considering the age of the individuals, no relationship between age and toxoplasma infection was revealed in the present study, as anti-toxoplasma IgGs were found in women aged 38.44 ± 13.00 years old and in men aged 29.29 ± 10.86 years old. Those results are in contrary to the studies of Wilking et al. [20], who demonstrated an increase in seroprevalence from 20% in the 18-29 age group to 77% in the 70-79 age group in the German population. The highest prevalence in the third and fourth decade of life was also shown by other authors [7]. The higher prevalence rate found in older people may be due to an increased risk of exposure to infection with age [7]. Elsheikha et al. [21] imply the particular importance of prophylaxis to reduce the risk of serious disease caused by toxoplasma in immunocompromised people. Eating of raw or undercooked meat and drinking of untreated water must be avoided. Vegetables should be washed thoroughly before eating because they may have been contaminated with cat feces. During gardening and any other contact with soil or sand, which may be contaminated with cat feces, protective gloves should be worn and hands should be washed with soap and water after the contact [6]. Moreover, a great number of authors suggest that all HIV-infected individuals should be tested for baseline IgG antibodies and counseled regarding exposure to Toxoplasma gondii infections [16-19].

CONCLUSIONS

Concluding, in the present study, the high prevalence of T. gondii infection was found in the group of people at high risk of contracting HIV from the Kuyavian-Pomeranian Voivodeship in Poland. However, the research was conducted on a small study group, so the data may be underestimated. It should be noted that it is a pilot study and further research is planned on a larger study group. Continuing the research and observation of the epidemiology of toxoplasmosis seem to be extremely important, because an early detection of the parasite infection in HIV/AIDS patients may prevent the development of a severe form of the disease.

REFERENCES


