RISK FACTORS ASSOCIATED WITCH THE DEVELOPMENT OF ACUTE *Toxoplasma gondii* INFECTION IN POSITIVES FOR HIV/AIDS IN SOUTH OF BRAZIL: CASE-CONTROL STUDY

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ABSTRACT

This study aims to analyze the occurrence of acute *Toxoplasma gondii* infection in patients with HIV, relating it to clinical and epidemiological data. In order to achieve this, a case-control study was carried out in the proportion of chronically seropositive HIV-infected patients to *T. gondii* and HIV-positive patients with *T. gondii* acute infections. The analyzed variables were: demographic (age and sex) and clinical (toxoplasmosis diagnosis, laboratory results of CD4 $^{+}$T lymphocytes counts, the CD4/CD8 ratio, quantification of viral load, antiretroviral therapy and time of HIV infection). The risk factors for acute *T. gondii* infection in HIV patients after multiple logistic regression analysis were: low CD4 $^{+}$T lymphocyte levels (OR=6.65, 95% CI 3.88-11.41), low CD4/CD8 ratio (OR=8.03, 95% CI=1.69-38.14), irregular use of antiretrovirals (OR=2.83, 95% CI=2.34-12.73) and non-use of them (OR=1.13, 95% CI=0.63-2.01). After adjusting the analysis for the age variable, it was found that patients with CD4/CD8 ratio below 1 (one) are more likely to develop acute toxoplasmosis, as well as those with CD4 $^{+}$T lymphocytes below 350 cells/mm^3 of blood, as well as Those who use irregular antiretrovirals or do not use them, respectively, are more susceptible to the development of this opportunistic disease.

KEYWORDS: *Toxoplasma gondii*; HIV; epidemiology

1. INTROCTION

Toxoplasmosis is a worldwide occurring zoonosis and the protozoan *Toxoplasma gondii* is its etiological agent[1]. Infection with this parasite is highly prevalent in humans and animals[2]. It is estimated that more than half the world's human population is seropositive for *T. gondii*, however this varies widely according to geographic region[3,4]. Although this infection is self-limiting for almost all immunocompetent individuals, it can lead to serious problems for those who are immunocompromised. Protozoal infection may progress to opportunistic disease in HIV-infected individuals because of the immunosuppression, which is almost always due to reactivation of latent forms of the parasite[4, 5].

In immunosuppression caused by the HIV virus, the main type of cell affected is CD4 $^{+}$T lymphocytes. The virus causes these cells to have a progressive disruption and so the blood count suffers a decline[6]. The reduction in number of CD4 $^{+}$T lymphocytes leads to functional changes of CD8 $^{+}$T lymphocytes - because these cells require activation by CD4 $^{+}$T lymphocytes - this condition makes HIV-positive patients susceptible to opportunistic pathogens[7]. Thus, lymphocyte count is an important marker of immunodeficiency and it is used to evaluate the patient's immunological condition[8].

Clinical manifestations of toxoplasmosis may be related to host immunity[3]. The Central Nervous System (CNS) is the most typically encountered site for HIV/AIDS patients[3]. Thus, neurotoxoplasmosis is the most prevalent manifestation in these patients and it is among AIDS-defining diseases, usually developing when the levels of CD4 $^{+}$T lymphocytes in the blood are below 200 cells/mm^3[3,2,9,10]. Ocular toxoplasmosis is the second most frequent, which, although less prevalent in these individuals, it is an important manifestation because it is a treatable cause that can significantly affect the visual function[11,12,13,14].

The introduction of antiretroviral therapy (ART) since 1996 has had a significant impact on the progression of HIV infection and its correlation with other infections[15,16,17]. ART can effectively control HIV and significantly reduce morbidity and mortality because its proper use leads to suppression of viral replication, below the detection limit, and leads to an increase in CD4 $^{+}$T lymphocytes[18].

Routinely, the most commonly used laboratory diagnosis of toxoplasmosis is serological testing, which is based on IgG and IgM antibody tests[19,20,21].
In immunodepressed by HIV, can often occur a high increase in IgG class antibody levels may occur, suggesting exacerbation of infection[21,22,23]. Therefore, for the serological diagnosis of acute *T. gondii*

infection to be established, the presence of anti-*T.gondi* IgM antibodies and increased IgG antibody titers (≥ 1: 2048) or seroconversion are required[24,25].

This study aimed to analyze the occurrence of acute toxoplasmosis in patients with HIV virus, linking this disease with clinical and epidemiological data of the patients attended at the Specialized Care Service in the Medical School of the Federal University of Pelotas (UFPel).

2. MATERIAL AND METHODS

An analytical, case-control, epidemiological study was carried out at the Specialized Care Service (SCS) of the Medical School (FAMED) of UFPel, in Pelotas (RS), Brazil, from December 2015 to August 2016. Currently SCS serves around 4,413 HIV-positive patients, and it is considered a reference center for the treatment of HIV patients in the region. The records of all 4,413 patients seen in SCS were analyzed, since 1998 when this Service was created until August 2016. For each patient with acute *T. gondii* infection (case), another (control) was chosen randomly: chronically seropositive for *T. gondii*; so the number of clinical cases of acute *T. gondii* infection determined the sample size. Each group consisted of 161 patients, totaling 322 analyzes. The variables of interest included in this study were: demographic (sex and age) and clinical (diagnosis of toxoplasmosis, laboratory results of CD4 $^{+}$T lymphocytes count and CD4/CD8 ratio, viral load quantification, antiretroviral therapy use and time of infection by the HIV).

Initially, a descriptive analysis of the characteristics of the sample and the proportion of cases and controls, according to the categories of the exposure variables, was used for data analysis. Univariate analysis using logistic regression was performed in order to check for factors associated with manifestation of acute *T. gondii* infection. Next, a multivariate analysis was performed, also using logistic regression, aiming to determine the most relevant variables or the most strongly ones associated with the development of acute *T. gondii* infection in the studied risk group. The variable age presented p-value lower than 0.20 in the univariate analysis and, therefore, it was used for control. All analyzes were carried out using Stata 13 software. It was considered 0.05 for significance level. The protocol of this study was approved by the Human Research Ethics Committee of FAMED - UFPel (CAAE: 49907415.6.0000.5317). The exemption of the Informed Consent Form (ICF) was requested, which was accepted by the Research Ethics Committee, since it was impossible to obtain the ICF of all individuals included in the research.

3. RESULTS

Demographic and clinical data from 322 seropositive patients for *T. gondii* and HIV are shown in Table 1.

Age ranged from 15 to 75 years, 49% of clinical cases of acute *T. gondii* infection occurred among patients aged 30 to 39 years. Regarding gender, the distribution of the analyzed individuals was similar between men and women (179 and 143, respectively). Although acute *T. gondii* infection occurred more in males than in females, this difference was not statistically significant (p=0,251).

The levels of CD4 $^{+}$T lymphocytes from the analyzed patients varied between 3 and 1533 cells/mm^3 of blood. This variable was statistically significant (p=0.001) as 83.8% of the patients who developed acute *T. gondii* infection had indices below 350 cells/mm^3.

Table 1. Demographic and clinical variables and relation with acute *T. gondii* infection in HIV positive patients, attended by the SCS (FAMED-UFPel), in Pelotas, RS, Brazil, from 1998 to 2016.

Variables	n(%) cases*	n(%) control**	p-value
Sex			
Female	68 (42,2)	75 (46,6)	*0,251*
Male	93 (57,8)	86 (53,4)	
Age			
≤ 29	31 (19,3)	31 (19,3)	*<0,000*
30 a 39	79 (49,0)	42 (26,1)	
40 a 49	33 (20,5)	37 (23,0)	
≥ 50	18 (11,2)	51 (31,6)	
CD4			
<350	135 (83,8)	73 (45,3)	*<0,001*
>350	26 (16,2)	88 (54,7)	
Viral load			
<50	18 (11,2)	25 (15,5)	*0,326*
>50	143 (88,8)	136 (84,5)	
CD4/CD8 ratio			
<1	159 (98,8)	151 (93,8)	*0,035*
>1	2 (1,2)	10 (6,2)	
Time HIV			
≤ 5 anos	31 (19,3)	46 (28,6)	*0,230*
6 a 10 anos	40 (24,8)	35 (21,7)	
11 a 15 anos	46 (28,6)	45 (28,0)	
≥ 16	44 (27,3)	35 (21,7)	
Use HAART			
Regular	28 (17,4)	37 (23,0)	*0,013*
Irregular	32 (19,9)	14 (8,7)	
No use	101 (62,7)	110 (68,3)	

*cases - with manifestations of acute *T. gondii* infection
** control - without manifestations of acute *T. gondii* infection

The CD4/CD8 ratio ranged from 0.01 to 2.63 among all the patients participating in the study, being

lower than in those with manifestations of acute *T. gondii* infection (p=0,035).
The viral load ranged from <50 to 7,634,883 copies/ml. The time of HIV infection among the patients studied ranged from 1 to 29 years. It was verified that there was no statistically significant relation between these variables and the occurrence of acute *T. gondii* infection (p=0.326 and 0.230, respectively).

Regarding the use of antiretroviral therapy (ART), more than a half of the patients had not used it at the time of detecting seropositivity for *T. gondii*. Patients receiving regular antiretroviral therapy had a significantly lower chance of developing acute *T. gondii* infection (p=0,013).
The association between acute *T. gondii* infection according to each category of exposure showed that the variables: CD4 $^{+}$T lymphocyte level, CD4/CD8 ratio, and lack of antiretrovirals or the irregular use of them persisted as risk factors associated with acute *T. gondii* infection in HIV-immunosuppressed patients (Table 2).

Table 2. Association between acute T. gondii infection and possible risk factors in HIV-positive patients attended by SCS (FAMED-UFPel), in Pelotas, RS, Brazil, from 1998 to 2016.

Variables	Crude Odds ratio (OR)	95% CI
Sex	*0,432*	
Female	1,0	
Male	1,19	0,77 - 1,85
Age	*<0,001*	
≤ 29	2,83	1,36 - 5,89
30 a 39	5,33	2,77 - 10,25
40 a 49	2,53	1,24 - 5,16
≥ 50	1,0	1,0
CD4	*<0,001*	
<350	6,26	3,71 – 10,55
>350	1,0	
Viral load	*0,250*	
<50	1,0	
>50	1,46	0,7 - 2,80
CD4/CD8 ratio	*0,014*	
<1	5,27	1,14 - 24,42
>1	1,0	
Time HIV	*0,229*	
≤ 5 anos	1,0	
6 a 10 anos	1,70	0,89 - 3,23
11 a 15 anos	1,52	0,82 - 2,80
≥ 16	1,87	0,99 - 3,52
Use HAART	*0,011*	
Regular	1	
Irregular	3,02	1,36 - 6,70
No use	1,21	0,69 - 2,13

In the final model, the multiple logistic regression analysis adjusted for the age variable, presented in Table 3, revealed that patients with CD4/CD8 <1 ratio had a 8.03 higher chance of developing acute toxoplasmosis; those who presented CD4 $^{+}$T lymphocytes cells <350 cells/mm^3 of blood had 6.65 greater risks; those who used antiretrovirals irregularly or did not use them had 2.83 and 1.13 higher susceptibility, respectively.

Table 3. Risk factors for acute *T. gondii* infection in HIV positive patients: multiple logistic regression analysis in patients attended by SCS (FAMED-UFPel), in Pelotas, RS, Brazil, from 1998 to 2016.

Variables	OR adjusted*	95% CI
CD4	*<0,001*	
<350	6,65	3,88 - 11,41
>350	1,0	
CD4/CD8 ratio	*<0,001*	
<1	8,03	1,69 - 38,14
>1	1,0	
Use HAART	0,023	
Regular	1,0	
Irregular	2,83	1,25 - 6,40
No use	1,13	0,63 - 2,01

*adjusted for the age variable

4. DISCUSSION

Considering the immunodeficiency caused by HIV, a rapid and progressive decrease of CD4 $^{+}$T lymphocytes is observed over the years. The functionality of the immune system decreases when the levels of these cells becomes lower than 350 cells/mm^3, favoring the reactivation of opportunistic pathogens such as *T. gondii*[10]. This study confirmed the association between CD4 $^{+}$T lymphocyte counts and the prevalence of acute toxoplasmosis in this risk group, as it was found that a high percentage (83.8%) of patients developed acute *T. gondii* infection with CD4 $^{+}$T lymphocytes below 350 cells/mm^3 of blood. In a study carried out in São Paulo, the average of CD4 $^{+}$T lymphocytes found in patients with neurotoxoplasmosis and ocular toxoplasmosis was 256 cells/mm^3[3,26]. In the Czech Republic, it was found that patients with CD4 $^{+}$T lymphocytes below 300 cells/mm^3 were more likely to reactivate the infection[27]. In India, the average of CD4 $^{+}$T lymphocyte was 283.6 cells/mm^3 (ranged 43-504)[28]. All these averages are below 350 cells/mm^3, confirming what was found in this study.

Antiretroviral therapy is indicated for asymptomatic HIV-positive patients who have CD4 $^{+}$T lymphocyte levels ≤ 500 cells/mm^3. According to this current study, irregular use of antiretrovirals (OR=3.02, 95% CI=1.36-6.70) led to a higher risk of developing acute toxoplasmosis, in agreement with other studies that demonstrated that appropriate use of HAART may reduce this risk since the treatment suppresses viral replication to below detection limits and consequently leads to an increase in CD4 $^{+}$T lymphocyte levels[29,30,18,31]. The high percentage of non-use of ART in the control group is due to the fact that the data collected from the patients correspond to the initial moments of the HIV diagnosis before the therapy, considering that the anti-*T gondii* serological test is requested as part of the initial evaluation of the patient[32,10].

Although the frequency of acute *T. gondii* infection has decreased after the introduction of HAART, this pathogenesis can be a big problem in some patients: the need for high adherence to therapy, the discipline

required by it, its side effects, viral resistance and their toxicity cause some patients to give up treatment or to do it irregularly[10,7].

The HIV virus also has marked consequences on CD8 $^{+}$T lymphocytes. Among patients who developed acute toxoplasmosis in the present study, the CD4/CD8 ratio was a risk factor since 98.8% of the cases presented this ratio <1. The CD4/CD8 ratio, usually above 1, is gradually reduced during the clinical course of HIV infection, initially due to the increase in CD8 $^{+}$T lymphocytes, and then mainly due to the intense reduction of CD4 $^{+}$T lymphocytes, occurring inversion of the values[33,8].

5. CONCLUSION

This study allows us to conclude that individuals who are positive for HIV and seropositive for *T. gondii* are at higher risk of developing acute *T. gondii* infection when they present the following: low levels of CD4 $^{+}$T lymphocytes, low CD4/CD8 ratio and irregular use or non-use of antiretrovirals, suggesting that awareness campaigns on the correct use of HAART should be constant.

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