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Seroprevalence of *Toxoplasma gondii* IgG antibody in HIV/AIDS-infected individuals in Maputo, Mozambique

Soroprevalência de IgG anti-*Toxoplasma gondii* em pessoas infectadas pelo HIV/Aids em Maputo, Moçambique

ABSTRACT

OBJECTIVE: To analyze the prevalence of IgG antibodies to *Toxoplasma gondii* in patients infected with HIV/AIDS and the association of demographic and social variables.

METHODS: Descriptive cross-sectional study that included the analysis of sociodemographic data and laboratory findings of 200 patients infected with HIV/AIDS treated in a laboratory unit in Maputo, Mozambique, in 2010. Individual data for all participants were collected with a self-administered questionnaire. Plasma samples were tested for IgG testing of anti-*T. gondii* using hemagglutination for the analysis of antibodies.

RESULTS: The seroprevalence of IgG anti-*T. gondii* was 46.0% (95%CI 39.2;52.9), 39.3% (95%CI 29.5;50.0) in men and 50.9% (95%CI 41.9;59.8) in women, with no difference between sex (OR 1.30; 95%CI 0.95;1.77; p = 0.12). Ages ranged from 10 to 60 years, with a higher prevalence of infection in older age groups, but with no significant difference between them. Regularly consuming cattle meat (OR 1.74; 95%CI 1.04;2.89, p = 0.05), breeding cats/dogs (OR 6.18; 95%CI 3.60;10.62, p < 0.000) and having regular contact with soil (OR 3.38; 95%CI 2.19;5.21; p < 0.000) were significantly associated with risk of latent infection.

CONCLUSIONS: Toxoplasmosis is an infection with high prevalence in Mozambique. Cultural and behavioral aspects increase the risk. Toxoplasmosis can be responsible in our environment by the great burden of morbidity and mortality associated with meningoencephalic injuries in patients with HIV/AIDS.

DESCRIPTORS: Toxoplasmosis, epidemiology. AIDS-Related Opportunistic Infections. HIV Infections, epidemiology. Seroepidemiologic Studies.

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RESUMO

OBJETIVO: Avaliar a prevalência de anticorpos IgG anti-*Toxoplasma gondii* em pessoas infectadas pelo HIV/Aids e a associação de variáveis demográficas e sociais.

MÉTODOS: Estudo transversal que incluiu a análise de dados sociodemográficos e laboratoriais de 200 pessoas infectadas por HIV/Aids, atendidas em unidade laboratorial em 2010 na Província de Maputo, Moçambique. Os dados foram coletados por meio de questionário autopreenchido por todos os participantes. Para a análise de anticorpos, amostras de plasma coletadas foram confirmadas para testagem de IgG anti-*T. gondii* por hemaglutinação.

RESULTADOS: A soroprevalência de IgG anti-*T. gondii* foi de 46,0% (IC95% 39,2;52,9), 39,3% (IC95% 29,5;50,0) em homens e de 50,9% (IC95% 41,9;59,8) em mulheres, sem diferença entre sexo (OR 1.30; IC95% 0.95;1.77; p = 0.12). A idade variou de 10 a 60 anos, com maior prevalência de infecção em grupos etários mais idosos, mas sem haver diferença significativa entre eles. Ter consumo regular de carne de gado bovino (OR 1,74; IC95% 1,04;2,89, p = 0,05), possuir criação de gatos/cães (OR 6,18; IC95% 3,60;10,62, p < 0,000) e ter contato regular com a terra (OR 3,38; IC95% 2,19;5,21, p < 0,000) estiveram significativamente associados ao risco de infecção latente.

CONCLUSÕES: A infecção por toxoplasmose apresenta alta prevalência na população de Moçambique, cujo risco amplia-se pelos aspectos culturais e comportamentais. A toxoplasmose pode ser responsável pela grande carga de morbimortalidade associada a lesões meningoencefálicas em pessoas com HIV/Aids no país.

DESCRITORES: Toxoplasmose, epidemiologia. Infecções Oportunistas Relacionadas com a AIDS. Infecções por HIV, epidemiologia. Estudos Soroepidemiológicos.

INTRODUCTION

Infection with *Toxoplasma gondii* is common all over the world, making it one of the most common infections in humans.[13,18,21] Although the evolution of primary infection is usually with slight signs/symptoms, the majority of the human population infected remains asymptomatic.[3,13] The infection may cause an additional burden of morbidity and mortality in certain populations,[18] as in fetuses of pregnant women with acute infection[13] and people with compromised immunological response.[13,14,23]

Meningoencephalitis caused by reactivated toxoplasmosis is one of the most common neurological opportunistic infections in patients with HIV infection/AIDS and is typically observed in advanced stages of the disease.[16] The high burden of opportunistic infections with *T. gondii*, especially in immunocompromised individuals, mainly verified from 1980s, remains a significant public health problem in some countries, especially those that have failed to gain universal access to highly active antiretroviral therapy (HAART).[2,4]

The primary acquisition mode occurs mainly by ingestion of raw or undercooked meat of intermediate hosts containing viable tissue cysts, or by ingesting food and water contaminated with sporulated oocysts from the environment, existing and originating in cat faeces.[18] Close contact between humans and pets, especially dogs and cats, can generate situations of greater vulnerability to human infection.[13,21] There is also the possibility of transmission of tachyzoites in utero through the placenta or by ingestion of tachyzoites in non-pasteurized goat milk. *T. gondii* can also be transmitted by blood components, by solid organ transplants.[21]

Not all possible routes of infection are currently epidemiologically important, and sources of infection may vary greatly among different ethnic groups and geographical locations.[13,22] This happens especially in environments with characteristics of rural life, which increase the risk of infection.[8,11,13]

Its incidence has a direct relationship with the prevalence of anti-*T.gondii* in the general population or in populations with greater vulnerability.[1] The prevalence of latent infection with toxoplasmosis is between 30% and 60% in most countries, varying widely in different regions of the world.[5,10,11] Despite its importance, few studies on the prevalence have been conducted in sub-Saharan Africa.[7,9,10,20]

There are frequent reports of congenital toxoplasmosis or toxoplasmic meningoencephalitis in patients with AIDS in Mozambique, but the real situation is unknown, with a small number of publications.[19] The paucity of reliable information on risk factors, main means of transmission and of preventative measures used, is of concern not only for researchers,[9,18,22] but for society in general. Considering the scale of AIDS in Mozambique[6,12,16] and the vulnerability of HIV-infected people with toxoplasmosis, there is a clear need for further studies on this subject.[10,14]

This study aimed to analyze the prevalence of IgG antibodies to *Toxoplasma gondii* in patients infected by HIV/AIDS and the association of demographic and social variables in Maputo, Mozambique.

METHODS

Descriptive cross-sectional study with patients infected by HIV/AIDS, who were treated at a reference center in Maputo, Mozambique, in the second half of 2010.

The study was conducted in the Maputo Military Hospital (HMM) in the city of Maputo. This is a quaternary level health unit and a national reference between the military hospitals of Mozambique. The HMM, with four infirmaries and 220 bed capacity, provides medical assistance to the Defense and Security Forces and Veterans of the National Liberation Resistance, including members of their families. It has an emergency room, operating room services, physical therapy, radiology, ophthalmology, dentistry, and a clinical laboratory and a special examination room.

The Laboratory of HMM has a samples receiving unit, blood collection room, sections serology, microbiology/parasitology, hematology, biochemistry and molecular biology (especially designed to assist people living with HIV/AIDS).

The target population consisted of all patients infected by HIV, aged over 18 years old, male and female, who visited the laboratory of HMM, the serology section. Recruitment was carried out during routine follow-up and during initial visits. Patients who met the inclusion criteria and signed consent were recruited consecutively. A sample was drawn from this population and a cross-sectional survey was conducted. Data collection occurred between September and November 2010.

HIV diagnosis is available through voluntary counseling and testing (VCT) centers in Mozambique. Rapid tests are used to provide clients with an immediate result. HIV positive clients are referred to the Central Hospital for assessment and antiretroviral therapy (ART) is initiated. Based on the 2010 WHO staging[25] national treatment guidelines, initiation of ART is recommended to all adolescents and adults including pregnant women with HIV infection and CD4 counts of ≤ 350 cells/mm^3, regardless of the presence or absence of clinical symptoms. Those with severe or advanced clinical disease (including AIDS) should start ART irrespective of their CD4 T-cell count.

The definition of AIDS in Mozambique is the clinical diagnosis (presumptive or definitive diagnosis) of any stage 4 condition or CD4 T-cell count < 200 cells/mm^3 of blood in a HIV-infected adults.

Two hundred HIV/AIDS seropositive patients agreed to participate in the study. Self-administered questionnaires were used with information such as socioeconomic status, race/ethnicity, education level, marital status, profession, cohabitation or breeding of cats/dogs as pets, contact with the soil in gardens and unprotected plantations; consumption of beef or pig and type of water consumed. Questions were completed referring to patient records from HMM laboratory such as age, sex and address.

Blood sample were collected as part of the usual routine for evaluating the state of health of the patients and sample of the participants were subjected to serological assessment for identifying the presence of IgG anti-*T. gondii.* Approximately 2.5 ml of blood by venipuncture into vacutainer tube with sterile gel were collected. The serum was removed by centrifugation for analysis. Approximately 1 ml to 1.5 ml of serum was transferred to eppendorf tubes previously identified with the code assigned by the investigator during data collection, and subsequently frozen at -20ºC. This sample was maintained on average for ten days until the moment the test for determining the level of IgG anti-*T. gondii* was carried out in the microbiology laboratory, in the serology section of Maputo Centre Hospital.

The determination of IgG antibodies to *T. gondii* was performed by the serological agglutination visible method. The kit trade marked to "Atlas Medical-Toxo Latex Kit" (Lot: 81111304, William James House, Cowely Rod Cambridge, CB 4 4WX, UK), a latex agglutination test for the rapid qualitative detection and semi-quantitative serum antibodies to *T. gondii* was used. This commercial kit has a sensitivity of 96.1% and specificity of 89.6%. The technique of determination and interpretation of the results was performed according to the manufacturer's instructions and recommendations.

The samples were allowed to reach room temperature prior to testing. The reagent kits anti-*T. gondii* were

removed from the temperature change of 4°C-8°C and maintained also in a room temperature. The identification data were strictly checked prior to the execution of the test itself.

The presence of visible agglutination after the technique procedure is considered a positive result, i.e., the presence of IgG anti-*T. gondii* in the patient serum, equivalent to a concentration of antibodies equal or greater than 4 IU/ml, while its absence is negative result.

Tests were performed to define the presence of IgG anti-*T. gondii* of samples from the laboratory of Maputo Central Hospital and a private clinic in order to confirm the results and to guarantee the quality and validity of the reagents used. Ten randomized samples reagents and not reagent were tested with another kit test to determine anti-*T. gondii* (Armstrong) and all cases were confirmed.

Data were consolidated into a specific database and analyzed using the statistical package SPSS (Statistical Package for the Social Sciences) version 11.0. The analysis was based on descriptive statistics, with data presented into tables and graphs. The estimated prevalence of infection with *T. gondii* was performed with the confidence interval. For the univariate analysis, the outcome of latent infection by *T. gondii* was the dependent variable and the association between variables (gender, age, regular consumption of meat from cattle, breeding of cat/dog and regular contact with soil) was verified by chi-square (0.05 significance). The Fisher exact test was used for variables with expected frequency < 5. The estimates of odds ratios (OR) with 95% confidence intervals (CI) were calculated.

The study protocol was submitted to and approved by the Ethics Committee of the Institute of Health Sciences of Mozambique and the National Bioethics Committee of the Ministry of Health of Mozambique (Protocol nº TFCTBLAD036/10).

The execution of this work considered the ethical standards and all requirements of confidentiality. Participation in this study was voluntary and assured by the rendering and prior reading of the terms of consent. All study subjects signed the consent form that was encoded.

The researcher and the laboratory technicians involved in collecting blood samples ensured confidentiality both in the process of identifying samples for the study, as well as the results that were obtained later.

RESULTS

The majority of the individuals infected with HIV/AIDS were women (58.0%) aged between 31 and 40, with no evidence of regular consumption of cattle meat (76.0%), breeding cat/dog (83.0%) or regular contact with soil (73%) (Table).

The prevalence of IgG anti-*T.gondii* was of 46.0% (95%CI 39.2;52.9). Seropositivity was more frequent in women and in the 41 to 50 years old age group, although not statistically significant (respectively, OR = 1.30; 95%CI 0.95;1.77; p = 0.12 and OR = 2.33, 95%CI 0.84;6.44; p = 0.61). Seropositivity was higher in older age groups.

Table. Social and demographic characteristics of the 200 cases studied according existence of infection by *T. gondii*. Maputo, Mozambique, 2010.

Variable	Non-reactive *T. gondii*		Reactive *T. gondii*		Odds ratio	95%CI	p
	n	%	n	%			
Total	108	54.0	92	46.0	–	–	–
Gender							
Male	51	47.2	33	35.9	1	–	–
Female	57	52.8	59	64.1	1.60	0.91;2.83	p = 0.11
Age (years)							
10 to 20	7	6.5	2	2.2	1	–	–
21 to 30	26	24.1	22	23.9	2.06	0.70;6.12	p = 0.28
31 to 40	37	34.3	31	33.7	2.05	0.71;5.95	p = 0.29
41 to 50	26	24.1	28	30.4	2.33	0.84;6.44	p = 0.61
51 to 60	12	11.1	9	9.8	1.93	0.57;6.52	p = 0.42
Regular consumption of cattle meat							
No	121	79.6	31	64.6	1	–	–
Yes	31	20.4	17	35.4	1.74	1.04;2.89	p = 0.05
Breeding cat/dog							
No	151	90.9	15	44.1	1	–	–
Yes	15	9.1	19	55.9	6.18	3.60;10.62	p < 0.000
Regular contact with soil							
No	122	83.6	24	44.5	1	–	–
Yes	24	16.4	30	55.5	3.38	2.19;5.21	p < 0.000

Breeding cats was the situation most significantly associated with the presence of infection (OR = 6.18; 95%CI 3.60;10.62, p < 0.000), followed by having regular contact with soil (OR = 3, 38; 95%CI 2.19;5.21, p < 0.000) and regularly consuming cattle meat (OR = 1.74; 95%CI 1.04;2.89; p = 0.05) (Table).

DISCUSSION

The estimate of 46.0% prevalence of latent infection by *T. gondii* in the population infected by HIV/AIDS treated at a referral center in Maputo, Mozambique, is representative of the magnitude of the problem in the country.

The relevance of this study is strengthened by the lack of studies not only in Mozambique,[19] but in the African continent as a whole.[2,3,9,20,22,23]

The prevalence of latent infection by *T. gondii* found in this study was higher than most estimates in other studies conducted with HIV/AIDS positive individuals in many places around the world, particularly in neighboring countries belonging to sub-Saharan Africa. The estimates vary from 4.0% to 8.0% in Zambia, 5.0% to 17.0% in South Africa, 19.0% in Zimbabwe, 26.0% in Angola, 36.5% in Nigeria, 44.0% in Namibia, Uganda on 34.0% to 54.0%, 40.0% to 70.0% in Tanzania, 55.5% Kenya and 93.0% in Ethiopia.[1,5,7,10,20-23] In some countries in sub-Saharan Africa, particularly Mozambique, where the prevalence of HIV/AIDS is elevated, epidemiological information on toxoplasmosis in the whole population is limited because of the difficulties in carrying out laboratory investigations in network health services.[24]

A recent study evaluating the presence of active/recent infection by IgM anti-*T. gondii* revealed a prevalence of 31.3% in HIV pregnant patients at the Maputo Central Hospital.[19] This gives additional information to the results of this study about the load associated with toxoplasmosis in Mozambique.

Prevalence rates increase with age,[13,18,20] consistent with the more frequent finding in this study being in the 41 to 50 age group, where 51.8% of the 54 cases belonging to this group were reactive.

Despite the increased participation of women in this study and higher seropositivity in this population, there were no differences in relation to gender. Among the 116 women's samples tested, 29.5% were positive. This can probably be explained by the significant prevalence of HIV infection in this population and higher levels of seeking medical care in our reality.[6,12,16] This extends the vulnerability in this population, including the increased risk of vertical transmission not only of HIV, but also of *T. gondii*.[4,15]

Risk factors in this population reflect the data in the literature and the social, economic and cultural context of Mozambique.[19] Prevalence estimates of latent infections by *T. gondii* in HIV-infected patients showed a wide variation in the risk factors and ethnicity factors including classic factors such as those evaluated in this study, in terms of exposure to soil, animals and meat intake.[8,11,13,14,18,20,22] Findings of non-reactive tests for toxoplasmosis in studies addressing geographic regions where there are no cats, such as the Pacific Islands[25] are registered.

Throughout the history of the epidemic of HIV infection, toxoplasmosis has been one of the most significantly opportunistic infections detected in patients with AIDS.[4] Even in the era after the introduction of HAART, it still represents important public health problem, especially to vulnerable populations in developing and developed countries.[2]

This study has important implications, considering the possibility of reducing morbidity and mortality and increased survival in patients infected with HIV/AIDS with the adoption and development of HAART, both in adults and in children.[2,17] Different studies indicate changing clinical and epidemiological patterns related to neurotoxoplamosis[2,7,17] and its association with immune reconstitution inflammatory syndrome (IRIS).[2,15] Together, these findings extend the importance of adopting screening routines for *T. gondii* infection in all newly diagnosed cases of HIV infection, with follow-up serologic depending on the context of risk associated with each individual.

Limitations of this study include the sampling process, that may have undersized the real prevalence by tackling patients who have been followed in this reference unit in the country. Population which are highly vulnerable to both toxoplasmosis and HIV may not have been included in the analysis. Even with these restrictions, the prevalence is high and significant enough to indicate the magnitude of the problem.

The use of single test for assessment of IgG antibodies was unable to distinguish accurately from recent to chronic or reactivated infections in the studied population. It is appropriate to remark on the vulnerability of the health public services in Mozambique by finding no wide availability of specific reagents to detain anti-*T.gondii* in public clinical laboratories. This was made possible by this research, which reaffirms to the health authorities the social and economic relevance of investments in this sector.

It reinforces the positive impact of prophylaxis[2] to be established in HIV-infected individuals in Mozambique with advanced degree of immunodeficiency, given the high rates of morbidity and mortality associated. The importance of these findings is even

greater in a country with high prevalence and incidence of HIV infection and patterns of high endemicity for Toxoplasmosis,

It emphasizes the magnitude of toxoplasmosis in the HIV-infected population in Mozambique and the urgent need for additional clinical and epidemiological research. The prioritization of research and development to address the complex dynamics of transmission of toxoplasmosis and its impacts is urgent in Africa. Similarly, extending the programs oriented towards this population for the serological diagnosis and prevention of toxoplasmosis, including pregnant women, aiming to reduce the burden of morbidity and mortality associated with new infections in Mozambique should also be prioritized.

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