

CD4 T-Lymphocytes Count in HIV-*Toxoplasma gondii* Co-Infected Pregnant Women Undergoing a Prevention of Mother-to-Child Transmission Program

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Abstract

Toxoplasma gondii (*T. gondii*) is a parasite responsible of toxoplasmosis, a disease often asymptomatic but with serious consequences in pregnant women and immunocompromised subjects. **Objective:** This study aimed to investigate the impact of *T. gondii* infection on CD4+ T lymphocytes count in HIV-infected pregnant women. **Methods:** This was a cross-sectional study of pregnant women co-infected by HIV and *T. gondii*. The study was conducted from January to July 2016 at the Prevention of Mother-to-Child Transmission of HIV (PMTCT) sites in the Health District of Lacs in Togo. Diagnosis of HIV was performed by immuno-chromatographic methods with Determine TM HIV-1/2 and immuno-filtration with Tri-Dot HIV-1 and 2 kits. Presence of anti-toxoplasmic IgG and IgM antibodies was established via enzyme immunoassay using ELISA-BIOREX® kit. Flow cytometry was used to count CD4+ T lymphocytes. **Results:** Our study found that of the 4599 pregnant women, 111 (2.41%) were HIV-positive. Among them, 109 (98.20%) were infected by HIV-1 and 2 (1.98%) by HIV-2. Antibodies against *T. gondii* were detected in 5.36% (IgM), 25% (IgG) and 3.57% (both IgM and IgG) of HIV 56 infected women. There was no significant difference between CD4 cell count in HIV (+)/*T. gondii* IgM (-)/IgG (-) infected pregnant women (378.8 ± 222.8 cell/μl) compared to HIV (+)/*T. gondii*/IgM (+) (457.3 ± 183.3 cell/μl), HIV (+)/*T. gondii* IgG (+) (419.4 ± 287.3 cell/μl) and HIV (+)/*T. gondii* IgM/IgG (+) (480.5 ± 252.4 cell/μl). **Conclusion:** This study showed that intracellular parasite *T. gondii* did not alter CD4+ T lymphocytes count in HIV/*T. gondii* co-infected pregnant women.

Keywords

HIV, *Toxoplasma gondii*, Co-Infection, CD4+ T Lymphocytes Count

1. Introduction

Human immunodeficiency virus (HIV) infection is a global health problem and affects all countries in the world [1]. Globally, about 2.3 million adolescents and young women are living with HIV in 2015, representing 60% of the total population of young people (aged 15 - 24 years) living with HIV [2]. However, HIV infection among women varies considerably across regions, with the highest prevalence occurring in sub-Saharan Africa. In this region, 56% of new HIV infections occurred in women, and this was even higher among young women aged 15 - 24 years, accounting for 66% of new infections [2]. In Togo, the prevalence of HIV is 2.5% in the sexually active population aged 15 - 49 years [2]. However, this prevalence is higher in women (3.1%) than in men (1.7%).

On the one hand, mother-to-child transmission of HIV is responsible for the majority of HIV infections in children under 15 years of age. Two million children are living with HIV, and of every 10 infected children under 15 years of age, 9 live in sub-Saharan Africa [1]. Mother-to-child transmission of HIV can occur during pregnancy (*in utero*), childbirth, or breastfeeding. This risk of transmission depends on a number of factors including amniotic fluid infections, vaginal and mammary infections [3], immuno-viral factors such as maternal viral load and CD4+ T lymphocyte rate at delivery [4].

As a result, since 1981 to date, more than 20 million people have died of AIDS leaving behind widows and orphans [1]. The onset of HIV/AIDS has seen an upsurge in opportunistic infections. Most AIDS-related deaths are linked to opportunistic infections that may be parasitic, bacterial, fungal and viral; due to the immune depression caused by HIV in AIDS patients. Toxoplasmosis is one of the opportunistic parasitic infections commonly found among AIDS patients. It is classically benign in the immunocompetent but formidable subject in the immunocompromised subject by its cerebral complications and especially in the pregnant woman by its serious fetal complications [5].

Previous studies on the continent have established that the prevalence of *T. gondii* antibodies among pregnant women varies greatly from country to country. It is 60% among women of childbearing age in Côte d'Ivoire [6], 54% among people living with HIV in Uganda [7]; 28.5% among HIV-infected pregnant women versus 20.20% among non-infected women in Burkina Faso [8]; 75.4% in Nigerian pregnant women with occurrence of stillbirths and congenital malformations [9]; 58.4% in Tunisia [10]; 34.1% from pregnant women in Sudan [11]; 40.2% in Senegal, at Dakar [12]; and 53.6% in Benin [13]. In Togo, it is however not clear the prevalence of toxoplasmosis and HIV co-infections due to the limited number of studies in the previous years. The present study was carried out

in order to investigate the prevalence of HIV/*Toxoplasma gondii* co-infection in pregnant women and to evaluate their immuno-hematological profile.

2. Material and Methods

It was a cross-sectional study, which took place from January to July 2016 in the Health District of the Lacs, south-eastern part of Togo.

In total, 4599 pregnant women were enrolled in the study at the first trimester of the pregnancy. Antibodies against *T. gondii* screening and CD4 T lymphocytes count were performed in 56 HIV-infected pregnant women following a PMTCT program. HIV serological screening was performed according to the algorithm for the detection of HIV infection in Togo. Alere Determine TM HIV 1/2 (Alere Medical, Chiba, Japan) was used as a presumptive test and Tri-Dot HIV 1 and 2 (Okhla Industrial Area, New Delhi, India) were used as a differential test when the first was positive. ImmunoComb II HIV1 and 2 Bispot (ImmunoComb II HIV-1 & 2 Bispot, Orgenics, Yavne, Israel) were used as a confirmatory assay.

The enzyme immunoassay method was used for the detection of Immunoglobulin M (IgM) and Immunoglobulin G (IgG) with the Biorex Diagnostics Limited kit (Antrim Technology Park, United Kingdom).

Flow cytometry using the Cyflow Partec II machine was used for the counting of CD4+ T lymphocytes with the SYSMEX CD4% easy count kit (Sysmex Partec GmbH, Görlitz, Germany).

Statistical analysis: The data were analyzed on the PRISM 5 software (Graph-Pad Software, Inc., La Jolla, USA) and Excel 2010. For statistical comparisons, the Kruskal-Wallis test was used and tests were considered statistically significant when $p < 0.05$.

3. Results

3.1. Prevalence of HIV among the Pregnant Women

The prevalence of HIV among the pregnant women was 2.41% (111/4599). Of these HIV positive individuals, 98.20% (109/111) were HIV-1 infection and 1.98% (2/111) were HIV-2.

3.2. Population Characteristics

Among the 111 HIV positive women, 56 undergoing a PMTCT program were tested for *T. gondii* antibodies and included in this study. The socio-demographic data of 56 pregnant women who have been tested for *T. gondii* antibodies are presented in **Table 1**. The participants were aged from 20 to 43 years with an average of 32.29 ± 5.27 years. Majority of the women (62.50%) were between 30 and 39 years old. Of these, housewives (33.93%) and shopkeepers (48.21%) were the most represented. 71.43% had formal education while 48.21% had no formal education and thus were primarily traders (**Table 1**).

Table 1. Socio-demographic characteristics of the 56 pregnant HIV-infected women tested for *T. gondii* infection.

	Number	Percentage
Age Range (years)		
[20 - 29]	16	28.57
[30 - 39]	35	62.50
[40 - 43]	5	8.93
Total	56	100
Education		
Formal	40	71.43
Non-formal	16	28.57
Total	56	100
Occupations		
Households	19	33.93
Traders	27	48.21
Dressmakers	8	14.29
Planters	2	3.57
Total	56	100

3.3. Prevalence of Toxoplasmosis among HIV-Infected Women

In order to investigate the prevalence of Toxoplasmosis among HIV-infected pregnant women, anti-toxoplasma IgG and IgM antibodies were tested on HIV-infected women (n = 56). We found that 33.93% (19/56) of HIV-infected individuals were positive for *T. gondii*. The prevalence of IgM anti-toxoplasma antibody was 5.36% (3/56), 25% (14/56) for IgG anti-toxoplasma while 3.57% (2/56) were positive for both IgM and IgG (Figure 1).

3.4. *Toxoplasma gondii* Infection Did Not Influenced CD4+ T Lymphocytes in HIV Infected Women

To investigate the effect of *Toxoplasma gondii* infection on CD4+ T lymphocytes count in HIV-infected women (n = 56), CD4+ T lymphocytes counts were performed. Table 2 shows that there is no significant difference between HIV (+)/*T. gondii* IgM (-)/IgG (-), HIV (+)/*T. gondii* IgM (+)/IgG (-), HIV (+)/*T. gondii* IgM (-)/IgG (+) and HIV (+)/*T. gondii* IgM (+)/IgG (+) ($p = 0.39$).

3.5. *Toxoplasma gondii* Co-Infection Is Not Associated with Stillbirth

There were no cases of stillbirth in *T. gondii*/HIV co-infected women. In contrast, 7.14% (4/56) new born from *T. gondii* (-)/HIV (+) died (Table 3).

4. Discussion

The study population consisted of 4599 pregnant women under 24 weeks of

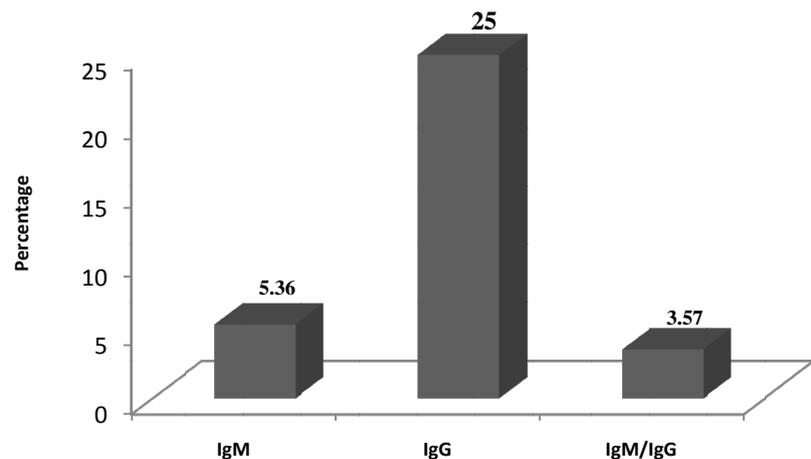
Table 2. Rate of CD4+ T lymphocytes in women infected with *T. gondii*.

	Number	CD4+ count (mean \pm SD) ^a	<i>p</i> -value ^b
HIV (+)/ <i>T. gondii</i> IgM (-)/IgG (-)	37	378.8 \pm 222.8	0.39
HIV (+)/ <i>T. gondii</i> IgM (+)/IgG (-)	3	457.3 \pm 183.3	
HIV (+)/ <i>T. gondii</i> IgM (-)/IgG (+)	14	419.4 \pm 287.3	
HIV (+)/ <i>T. gondii</i> IgM (+)/IgG (+)	2	480.5 \pm 252.4	
Total	56	382.2 \pm 218.2	

a. Minimum = 25; maximum = 1160; SD: standard deviation. Women were included regardless of their CD4+ T cell count. This is why a large difference is observed between the minimum and the maximum, explaining the high value of the standard deviation. b. Comparison of the four means of CD4+ count in the third column using 1 way ANOVA (Kruskal-Wallis test).

Table 3. Stillbirth in *T. gondii*/HIV co-infection.

	Stillbirths
HIV(+)/ <i>T. gondii</i> IgM(-)/IgG(-)	4
HIV(+)/ <i>T. gondii</i> IgM(+)/IgG(-)	0
HIV(+)/ <i>T. gondii</i> IgM(-)/IgG(+)	0
HIV(+)/ <i>T. gondii</i> IgM(+)/IgG(+)	0

**Figure 1.** Prevalence of anti-toxoplasma antibodies in HIV positive women.

amenorrhoea, who had consented to participate in the PMTCT program in the Lacs Health District. The prevalence of HIV in our population was 2.41%. However, our results (2.41% HIV prevalence) were close to that of the national prevalence of people living with HIV (2.4%) in Togo in 2015 [2]. The prevalence of 2.41% in our study is justified by the fact that HIV prevalence in the Maritime region (3%) is higher than the national prevalence (2.4%) as previously reported [2].

The prevalence of HIV types among the pregnant women in our study was 98.20% for HIV-1 and 1.98% for HIV-2. These are however relatively high compared with reports from Burkina Faso [14], a neighboring country in the

sub-Saharan Region. In Burkina Faso, the gender and age-specific HIV prevalence are reported as 7.3% among HIV-positive women, with 97.4% of them living with HIV-1 while 1.8% of had HIV-2.

Of the 111 HIV-positive women in the present study, 56 women were tested for *T. gondii* infection. Their age ranged from 20 to 43 years, with an average age of 32.29 ± 5.27 years. However, the 30 - 39 age group were the most represented (62.5%) followed by the 20 - 29 year-olds (28.57%). Therefore, the sexually active population was included in these age groups, which explains this strong representation.

Sixteen (28.86%) pregnant women ($n = 56$) in our studied population had no formal education and 48.21% (27/56) were traders. This finding is in agreement with studies which show that less educated women and women living in poverty were more likely to be infected with HIV [15]. The high rate of traders is explained by the fact that the Lacs District shares borders with Benin and that of Abidjan-Lagos, therefore serves as strategic commercial city.

Positive toxoplasmic serology with only the presence of IgM antibodies indicates a recent infection with toxoplasma [16]. This means that 5.36% of the women in our study who were positive for IgM suffer from a recent infection. Positive toxoplasmic serology with only the presence of IgG antibodies usually reflects an old infection by toxoplasma. This means that 25% of pregnant women that presented with positive IgG in our study suffer from an old infection, so probably contracted *T. gondii* infection before pregnancy. Toxoplasmic serology positive to both IgM and IgG is indicative of ongoing sero-conversion [17]. Thus, 3.57% of women in this study were in the sero-conversion phase. Our results are similar to sero-prevalence data of some African countries: For instance 27.00% to IgG in Uganda [18], 25% to IgG in Dakar (Senegal) according to Faye [12], 21% to IgG in blood donors in Mali [19]; 4.70% to IgM and 27.20% to IgG in Burkina Faso [14]. In Togo, few studies were carried out on cases of co-infections of toxoplasmosis and HIV, but Apetse found a prevalence of 54% in patients living with HIV (PLHIV) hospitalized at the neurological unit of Sylvanus Olympio Teaching Hospital of Lome, Togo [20].

The similarity of the results in other countries may be accounted for by the fact that the tropical region is favorable to the survival of oocysts in the soil but also by the consumption of contaminated food and to a lesser extent by the coat of animals (cats) [21]. Almost 75% of pregnant women in our study were negative for anti-IgG serology, therefore in the absence of adequate prevention strategies, these women would be at risk of contracting toxoplasma during pregnancy.

Concerning CD4+ T lymphocytes, the mean rate of 382.2 ± 218.2 cells/ μ l observed in our study is higher than 143 cells/ μ l, found by Dokekias in Brazzaville (Congo) [22]. It is known that HIV causes a slow and progressive deterioration of the host's immune system, which makes an individual prone to several opportunistic infections [23]. In our population, 9 (16.07%) of the 56 women had a CD4+ T lymphocytes cell count less than 200 cells/ μ l. Unlike our results, Djibril

et al. found 83.6% cases of CD4+ T lymphocytes cell count less than 200 cells/ μ l in patients living with HIV (PLHIV) who were hospitalized at the Sylvanus Olympio Teaching Hospital of Lome, Togo [24]. This difference is due to that it is a severe immunodepression that usually motivates hospitalization in these patients.

The prevalence of anti-*Toxoplasma gondii* antibodies in HIV infected women was 5.36% for IgM (3/56), 25% for IgG (14/56) and 3.57% for both IgM and IgG (2/56). Patients who had recent infection with *T. gondii* (IgM+) had a higher CD4+ T cell count but the difference was not statistically significant ($p = 0.39$). The co-infection of HIV/*T. gondii* did not influence the CD4+ T lymphocytes count in our study. This could be due to the PMTCT program. This program would also explain the fact that no stillbirths have been reported in women co-infected with HIV and *T. gondii*. The follow-up of HIV-infected women during the PMTCT program is an asset that allows at the same time the detection and treatment of active toxoplasmosis. This therefore limits the risk of transmission of the disease to the fetus.

5. Conclusion

Toxoplasmosis is a threat to pregnant women and the immunocompromised because of the risk of complications. This study reveals a high prevalence of HIV infection in pregnant women (2.41%) in the Health District of the Prefecture of Lacs. IgM seroprevalence estimated at 5.36% and IgG seroprevalence at 25% show that a large number of pregnant women are exposed to toxoplasma contamination. It is essential that health policies decide on the systematic monitoring of women during pregnancy in order to prevent fetal contamination for better expectations in the fight against vertical transmission of HIV and *T. gondii*. The study shows also that the PMTCT program could help in the maintaining of a good immunity for the purpose of fighting opportunistic infections.

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