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Seroprevalence of Latent *Toxoplasma gondii* Infection among HIV-infected Pregnant Women in Bobo-Dioulasso, Burkina Faso

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Abstract: The deficit of cellular immunity, as found in HIV infected individuals, may lead to the reactivation of latent *Toxoplasma gondii* cysts, with as consequence, the occurrence of toxoplasmosis and an eventual vertical transmission of the disease during pregnancy. The present study was designed for determining the occurrence of latent *Toxoplasma gondii* among HIV-infected pregnant women during the first trimester in Bobo-Dioulasso. Thus, 348 pregnant women aged from 17 to 47 years (average age of 6.64±4.75 years) were enrolled. The specific anti-*Toxoplasma gondii* IgG and IgM antibodies were quantified from whole blood specimens using the high-sensitivity direct agglutination and the enzyme linked fluorescent assays, respectively, the IgG avidity test being used for the dating of the primary infection. The results revealed that the seroprevalence of *Toxoplasma gondii* latent infection was 34.7%. It was significantly higher in HIV-infected women compared with uninfected ones (68,7%; CI 95%: 43,6%-88,9%) versus (33,1%; CI 95%: 28, 2%-38,3%). In addition, all the occurrences of the high IgG avidity were closely linked with the presence of IgM. These results underlined the need for the clinical follow-up of the maternal HIV diseases including the toxoplasmosis during the pregnancy since; the newborns are still exposed to vertical transmission of *Toxoplasma* infection in endemic areas like Burkina Faso.

Key words: Seroprevalence, *Toxoplasma gondii*, antibodies, IgG, IgM

INTRODUCTION

Toxoplasma gondii is a ubiquitous intracellular protozoan widely distributed around the world. The infection by *Toxoplasma gondii* is mainly acquired by ingestion of undercooked or raw meat containing viable tissue cysts, or by ingestion of food and water contaminated with oocysts shed by felids (Dubey and Jones, 2008).

The infection is responsible for a significant morbidity and mortality in certain groups (Weiss and Dubey, 2009). Toxoplasmosis can cause severe damages in fetuses of acutely infected pregnant women (Dunn *et al.*, 1999). Additionally, the reactivation of latent infection occurs in immune compromised patients, causing life-threatening disease, especially the encephalitis (Passos *et al.*, 2000). The encephalitis due to

reactivated toxoplasmosis is one of the most common opportunistic neurological infections in AIDS patients, typically observed in the latest stages of the human immunodeficiency virus infection (Maschke *et al.*, 2000). Once infected, the host normally acquires lifelong immunity induced by the persistence of the parasite in an encysted form indeed, maternal immunity appears to protect against fetal infections (Miller *et al.*, 1969). The congenital toxoplasmosis is usually the result of maternal acquisition of *Toxoplasma gondii* for the first time during gestation (Bachmeyer *et al.*, 2006). However, the vertical transmission of toxoplasmosis as a result of the reactivation has been described in immune compromised women (Mitchell *et al.*, 1990; Marty *et al.*, 2002) and more rarely in immune competent women (Kodjikian *et al.*, 2004).

The Clinical manifestations of congenital toxoplasmosis are numerous, including intracranial calcifications, convulsion, psychomotor retardation, strabismus, chorioretinitis, microcephaly and hydrocephaly observed in infancy or later. However, most neonates with congenital toxoplasmosis are asymptomatic or with subclinical presentation of the disease at birth (Kieffer and Wallon, 2013; Safadi *et al.*, 2003).

In Burkina Faso, reported medical literature about congenital toxoplasmosis is unknown. In addition, the occurrence of toxoplasmosis, which is currently estimated by anti-*Toxoplasma gondii* antibodies in pregnant women infected with HIV is limited. Some authors have indeed noted a prevalence of infection with *Toxoplasma gondii* in HIV-infected and uninfected pregnant women to 20.2% versus 28.5% (Simpore *et al.*, 2006) and 22.5% versus 31.9% (Lima and Viana, 2009).

We believe that defining the occurrence of toxoplasmosis, using anti-*Toxoplasma gondii* antibodies during antenatal care in pregnant women infected with HIV/AIDS in Bobo-Dioulasso, would reduce the risk of treatable damage of congenital toxoplasmosis and decrease its morbidity. Therefore, the objective of this cross-sectional study conducted from January to June 2012 was to determine the occurrence of latent *Toxoplasma gondii* among HIV-infected pregnant women during the first trimester of their pregnancy in Bobo-Dioulasso.

MATERIALS AND METHODS

Study area: Burkina Faso, a western African country, is bordered to the North and the West by the republic Mali, to the East by Niger and to the South by Ivory Coast, Ghana, Togo and Benin. It is one of the Sub-Saharan African countries most affected by the HIV/AIDS infection (Simpore *et al.*, 2006). The present cross-sectional study was conducted from January to June 2012 in Bobo-Dioulasso. It is the second city of Burkina Faso, located in the western part of the country, with 43.59% live births and 5,000 pregnant women per year in prenatal consultations.

The sampling and the socio demographic data gathering were performed in Burkina Faso in public centers for mothers and child health (MCH) and in Parasitology-Mycology Laboratory of Bobo-Dioulasso. The laboratory assays were performed in France in the Laboratory of Parasitology-Mycology of Reims.

Ethical committee: The study was approved by the National Research Ethical Committee of Burkina Faso,

under number 0123/10. Each pregnant woman was asked to sign informed consent form before blood taking. Those who could not write gave a verbal consent.

Studied population: We included 348 pregnant women aged from 17 to 47 years (average age of 6.64 ± 4.75), looking for the first trimester prenatal care in public centers for mothers and child health (MCH) of Bobo Dioulasso and carrying an evolving intra uterine pregnancy. The socio demographic data including the age, occupation, education level, marital status and the number of gravidity were gathered by direct interviews and completed from registers.

Samples collection: The 10 mL of venous blood were collected from each pregnant woman in 2 tubes, one being coated with EDTA, the second being anticoagulant free. The first tube was centrifuged at 3000 rpm for 10 min to remove plasma that was frozen at -20°C , until use for analyze IgG and IgM anti-*Toxoplasma gondii*. The second tube was directly used for CD4+T- lymphocyte counts with a FACS calibur (Becton Dikson, USA).

Serological test

On-site rapid HIV testing: On-site rapid HIV testing was performed by using two rapid assays according to a sequential testing algorithm recommended by the WHO for developing countries. We used a sensitive test followed by specific test, allowing the confirmation of initial positive results and the discrimination between HIV type 1, 2 and 1/2 infections as previously described (Koblavi-Deme *et al.*, 2001). Indeed, all serum samples were first tested by the immune chromatographic *Determine*® HIV1/2 test (Abbott Laboratories, Wiesbaden, Germany) and the positive samples were then tested by the immune filtration Genie II HIV-1/HIV-2 assay (Bio-Rad Laboratories, Marnes-la-Coquette, France).

High-sensitivity direct agglutination (HSDA): This was used for the quantitative determination of specific anti-*Toxoplasma gondii* antibodies. Samples were considered IgG reactive when the antibody titer was greater than or equal to 6 U ADHS mL^{-1} (Desmonts and Remington, 1980).

(Enzyme linked fluorescent assay (ELFA) using the VIDAS system: (BioMerieux-Lyon, France) was laboratory technique performed for the quantitative determination of specific IgM antibodies with the following reference value for positive results: $\text{IgM} > 0.65$.

Table 1: Seroprevalence of antibodies IgG anti-*Toxoplasma gondii* according to socio demographic characteristics, obstetrical data and HIV status among 348 pregnant women

Characteristics	Total (%)	No. IgG positive (%)	CI 95%
Age (years)			
<20	59	25 (42.3%)	30.2-55.2
20-25	141	49 (34%)	26.9-43.2
>25	148	47 (31%)	24.3-39.9
Instruction level			
Illiterates	135	53 (39.2%)	31.3-47.6
Primary level	117	32 (27.3%)	19.8-35.9
Secondary and plus	96	36 (37.5%)	28.5-47.4
Occupation*			
Housewives	197	89 (45.1%)	38.3-52.1
Civil servants	46	11 (23.9%)	13.2-37.7
Liberal profession	88	17 (19.3%)	12.1-28.5
Others	17	4 (23.5%)	7.9-47.5
Marital status			
Unmarried	285	107 (37.5%)	32.1-43.3
Married	63	14 (22.2%)	13.2-33.7
Gravidity			
Primigravida	75	33 (44%)	31.1-55.3
Multigravida	273	88 (32%)	26.6-37.6
HIV status			
Negative	332	110 (33%)	28.2-38.3
Positive	16	11 (68.7%)	43.6-88.9

VIDAS system: (BioMérieux-Lyon, France) was employed in order to confirm acute infection of samples those were reactive to both anti-*Toxoplasma gondii* IgG and IgM antibodies and results were interpreted according to the manufacturer's instructions with the reference value for high avidity: IgG>0.3 (avidity>0.3 exclude acute infection <4 months) (Pelloux *et al.*, 1998).

Statistical analysis: Demographic and serological profiles were recorded on computer files and analyzed by Epi Info version 6.0 software (Centers for Disease Control and Prevention, Atlanta, GA). Statistical significance was set at $p<0.05$.

RESULTS

A total of 348 blood samples from HIV-infected pregnant women aged were analyzed. Any cases of HIV-1 co-infection with HIV-2 were noted. The serological tests revealed that 16 samples (4.6, 95% CI: 2.7-7.2%) were infected with HIV and 121 samples contained anti-*Toxoplasma gondii* IgG. Overall, the seroprevalence of latent infection of *Toxoplasma gondii* was 34.7% (121/348, 95% CI: 29.9-39.6%). In addition, 65.3% samples did not contain neither anti-*Toxoplasma gondii* IgG nor IgM. Of the 121 IgG positive samples, 26 showed high avidity of IgG and stable titer associated with the presence of IgM in two iterative sera three weeks later. No statistically significant association was observed between overall rate of latent infection of *Toxoplasma gondii* and age, educational level, occupation, marital status and gestity of pregnant women Table 1. However, according

to HIV serology, the seroprevalence of latent infection of *Toxoplasma gondii* was significantly increased of 68.7% (95% CI: 28.2-38.2%) among HIV-infected pregnant women versus uninfected (33.1%, 95% CI: 43.6-88.9%) (Table 1). Also, no case of IgM antibodies was associated with IgG anti-*Toxoplasma gondii*. Further among the 16 HIV-infected pregnant women with a chronic *Toxoplasma gondii* infection, all of them had their CD4+T-lymphocyte counts. Within this group, the median CD4+T-lymphocyte count was 340 cells mm^{-3} .

DISCUSSION

The objective of this cross-sectional study was to determine the occurrence of latent *Toxoplasma gondii* among HIV-infected pregnant women during the first trimester of their pregnancy in Bobo-Dioulasso. Overall, the seroprevalence of toxoplasmosis among pregnancy in this study was 34.7%. This data suggested that 65.3% of pregnant women are exposed to a risk of seroconversion for toxoplasmosis because of the deficiency of the immunity due to HIV infection. Consequently, all babies from these pregnancies must also be submitted to screening at birth and completely investigated. This strategy would contribute towards immediate use of specific therapies, with better prognosis and lower frequencies of sequel of toxoplasmosis in the fetus.

Toxoplasma gondii antibodies profile was significantly influenced by HIV infection in pregnant women (68.7 vs. 33.1%). Our rates are in agreement with other studies carried out in Burkina Faso which reporting 20.2% vs. 28.5% by Simpore *et al.* (2006) and 22.5% vs.

31.9% by Lima and Viana, (2009) respectively among HIV-infected pregnant women and uninfected. A recent study performed in Mozambique found rates similar to those reported in the current study (31.3% vs. 10.9%) respectively among HIV-infected pregnant women and uninfected by Siteo *et al.* (2010). On the other hand, no statistically significant difference in prevalence was observed in Brazil (59.8% vs. 58.1%) respectively among HIV-infected pregnant women and uninfected (Fernandes *et al.*, 2012). Our results underlined the importance of follow-up of maternal HIV disease and toxoplasmosis during pregnancy because; children born from HIV-infected women may be at higher risk of vertical transmission of *Toxoplasma* infection in HIV endemic areas.

In addition, our results indicated that 68.7% of HIV-infected pregnant women had anti IgG *Toxoplasma gondii* (Table 1) and therefore are immunized. However, this immunity passes through a primary infection acquired by the encystment of *Toxoplasma gondii* in the muscles and central nervous system. The deficit of cellular immunity (AIDS), exacerbated by a pregnancy exposed to the reactivation of the latent cysts that exposed to the risk of transplacental contamination of the fetus may lead to congenital toxoplasmosis (Bachmeyer *et al.*, 2006). This rate suggests the implementation of a rigorous monitoring for toxoplasmosis among pregnant women in general and in particular, HIV-infected pregnant women. In fact, HIV is one factor contributing to the reactivation of cysts of *Toxoplasma gondii* exacerbated by pregnancy.

Indeed, immunodepression, even when discrete, may allow transmission of latent maternal infections to the newborn (Bachmeyer *et al.*, 2006) which could be prevented by adequate follow-up of maternal HIV and toxoplasmosis disease during pregnancy. However, all the 26 samples that had IgM were tested for avidity. All of the values obtained had high-avidity of IgG antibodies (>0.3), which suggests that the infection probably was acquired before gestation (Pelloux *et al.*, 1998; Liesenfeld *et al.*, 2001). Outside of subacute immunodepression (HIV), these women are immunized against toxoplasmosis and consequently it is almost no possibility of congenital toxoplasmosis in their fetus who receive maternal protection.

Moreover, in the present study, any IgM was found among HIV-infected pregnant women. The absence of IgM, a marker of evolutive infection reflects a latent toxoplasmosis in our setting. Our results illustrate the possibility of congenital toxoplasmosis by reactivation in a pregnant woman infected with both HIV and *Toxoplasma gondii*. Under these conditions, prophylaxis

or therapy is required for both infections. Clearly, adhesion to therapy should have been more efficiently in this case.

Despite the absence of evolutive markers for toxoplasmosis (IgM) among HIV-infected pregnant women, there is urgent need to implement a monitoring program for toxoplasmosis pergravida our setting. On the basis of our results, it would be advisable to implement a monitoring program as a first measure for prevention of congenital toxoplasmosis, associated with the education of non-immune pregnant women for toxoplasmosis. We could envisage a trimester serological surveillance of *Toxoplasma gondii*-seronegative women to detect a toxoplasmosis seroconversion occurred during pregnancy and in this case provide a neonatal screening for congenital toxoplasmosis for all the pregnant women in this setting.

Further, the mean CD4+T-lymphocyte count was 340 cells mm^{-3} among HIV-infected pregnant women. However, the authors reported rates of 369.9 (Simpore *et al.*, 2006) and 330 CD4 mm^{-3} (Lima and Viana, 2009) in Burkina Faso among women infected with HIV. The lower CD4 counts found among pregnant women infected with HIV who also presented with anti-*Toxoplasma gondii* antibodies, suggests intense HIV disease progression in this group. Progression could be caused by HIV infection and progressive disease in *Toxoplasma gondii* chronically infected patients.

Therefore, previous studies noted that *Toxoplasma* infection can promote an increase in CD4 cells by a strong specific immune response (Purner *et al.*, 1998).

CONCLUSION

Toxoplasma gondii antibodies profile was significantly influenced by HIV infection in pregnant women (68.7% vs. 33.1%). This rate should not be neglected in this context. Also, there appears high level of exposure (65.3% of absence of immunity) to *Toxoplasma* primary infection that can be fatal to the new born if the disease was contracted by women before the third trimester of pregnancy. Therefore, it would be advisable to implement a monitoring program as a first step to prevention of congenital toxoplasmosis associated with the education of pregnant women immunized for toxoplasmosis and HIV-infected pregnant women.

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