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# Co-Infection of *Toxoplasma gondii* with HBV in HIV-Infected and Uninfected Pregnant Women in Burkina Faso

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**Abstract:** *Toxoplasma gondii* infections can induce serious complications in HIV-infected pregnant women, leading to miscarriage; favour the mother-to-child transmission of HBV and HIV and birth defects. The purposes of this study were: (1) to quantify IgM and IgG antibodies to *Toxoplasma gondii* in HIV-seropositive and seronegative pregnant women, (2) to identify hepatitis B antigens (HBsAg) in pregnant women and (3) to determine *T. gondii* and HBV co-infections among these patients. The study was conducted at Centre Medical Saint Camille, in Burkina Faso from January to June 2009. A total of 276 HIV-infected and uninfected pregnant women were included. All women had less than 32 weeks of amenorrhoea and were aged from 19 to 42 years. *Toxoplasma gondii* antibodies and HBsAg were detected using ELISA method. In addition, women freely agreed to answer a questionnaire. The results of our investigations revealed that, among these pregnant women, 38.8% were illiterates, 50.4% were housewives and only 5.4% were civil servants. Positive *T. gondii*-specific IgM (4.7%) and IgG (27.2%) were detected. In this study, we found that HIV-seropositive status seem to be associated with great prevalence rates of both *T. gondii* (31.9 vs. 22.5%) and HBV (13.0 vs. 5.8%). The elevated co-infection rate in HIV-positive women suggested that they are exposed to *T. gondii* and HBV infections prevalently because of their immune depression. Therefore, to reduce the prevalence of *T. gondii* and HBV among HIV-seropositive pregnant women, lamivudine could be included in their HEART and women should follow healthy lifestyle formation.

**Key words:** *Toxoplasma gondii*, HIV, HBV, co-infection, pregnant women, ARV, Burkina Faso

## INTRODUCTION

*Toxoplasma gondii* an obligate intracellular parasite found in many species throughout the world, causes a variety of clinical syndromes in both human and animals (Ashburn, 1992; Simpore *et al*., 2006; Nissapatorn *et al*., 2004). Thereby untreated acute toxoplasmosis among pregnant women can lead to infection of the foetus via transplacental transmission. Certainly, congenital toxoplasmosis may affect any organ, produce severe complications such as hydrocephalus and lead to ocular lesions which can appear late after birth (Berger *et al*., 2009). In this situation, foetal outcome can be fatal. *Toxoplasma gondii* seroprevalence estimated for human population varies greatly among different countries, among different geographical areas within the same country and among different ethnic groups living in the same area (Simpore *et al*., 2006). In sub-Saharan Africa the prevalence of *T. gondii* and HBV increased at the same time as HIV. Toxoplasmosis prevalence was found to be high in several African countries: 60% from AIDS patients in Côte d'Ivoire (Adou-Bryn *et al*., 2004); 75.4% in Nigeria (Onadeko *et al*., 1996); 58.4% in Tunisia (Bouratbine *et al*., 2001); 34.1% from pregnant women in Sudan (Elnahas *et al*., 2003); 40.2% in Senegal, at Dakar (Faye *et al*.,1998); 53.6% in Benin (Rodier *et al*., 1995) and 25.3% in Burkina Faso (Simpore *et al*., 2006). The prevalence of HBV is also high in the same countries: 85% in adult population in Senegal (Sall-Diallo *et al*., 2004); 9.4% in Cote d'Ivoire (Rouet *et al*., 2004); 14.3% HBV prevalence in Nigeria (Uneke *et al*., 2005); 12.4 and 9.8% in Burkina Faso, according respectively to Ilboudo *et al*.

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(2002) and Simpore *et al*. (2006). In African countries, high prevalence of *T. gondii* and HBV among HIV seropositive persons is expected; nevertheless, HIV-infected persons who are *Toxoplasma gondii* seronegative may be advised about preventive behavioural practices such as eating only well-cooked meats, washing their hands after outdoor activities involving soil contact and after contact with cats (Berger *et al*., 2009). *Toxoplasma gondii*, HIV and HBV co-infected pregnant women may influence each other, promoting or enhancing their vertical co-transmission through the placenta. This is why some scientific reports have suggested that HIV pregnant women or HBV-seropositive diagnosed with acute toxoplasmosis should be treated as soon as possible to reduce the risk and severity of congenital infection (Foulon *et al*., 1999; Gilbert *et al*., 2001; Gras *et al*., 2005). Others have argued that there is still no treatment capable of reducing vertical transmission (Foulon *et al*., 2000; Peyron *et al*., 2000; Gilbert *et al*., 2001). Nevertheless, a recent meta-analysis reported that a maternal treatment started within three weeks of seroconversion had a small effect in the reduction of *T. gondii* vertical transmission when compared to treatments that were started eight or more weeks after seroconversion (Thiebaut *et al*., 2007). The goal of this study was: (1) to detect IgM and IgG antibodies to *Toxoplasma gondii* in HIV-seropositive and seronegative pregnant women; (2) to identify hepatitis B antigens (HBsAg) in these women and (3) to determine *Toxoplasma gondii* and HBV co-infection among these patients.

## MATERIALS AND METHODS

**Samples:** Blood samples were collected at the Saint Camille Medical Centre (SCMC) in Ouagadougou, in Burkina Faso, from January to June 2009. Two hundred seventy-six pregnant women, aged from 19 to 42 years, with an average of 27.65±5.35 years, were enrolled at the Voluntary Counseling and Testing Programme (VCTP). One hundred thirty-eight women were VIH-seropositive and 138 were HIV-seronegative (control group). All women had less than 32 weeks of amenorrhea at sampling time.

**Laboratory studies:** After informed consent, 10 mL of venous blood were collected from each women in 2 tubes coated with EDTA. The first tube was centrifuged at 3000 rpm for 10 min to remove plasma that was frozen at -80°C, until use. IgM and IgG antibodies to *T. gondii* from plasma samples were quantified by an Enzyme Immunoassay technique (RADIM House, Italy), using a microplate spectrophotometer reader (Seac, Italy). Plasma was also tested for hepatitis B surface antigen (HBsAg). This test was carried out with rapid test (Hepatitis B virus combo Device test, House Laboratories Barge, Inc, USA). The second tube containing whole blood was used only for T CD4+ cells count performed on a FACS Calibur (Becton Dickinson, San Jose, CA, USA). In addition, these women voluntarily agreed to answer a questionnaire, referring to their school level, their function in the civil service, the number of living children, the number of deceased children and the number of previous miscarriages.

**Ethical committee:** Each person provided informed consent before blood taking and the study accomplishment was approved by the Ethics Committee of the Saint Camille Medical Centre and CERBA (Centre de Recherche Biomoléculaire Pietro Annigoni of Ouagadougou, in Burkina Faso).

**Statistical analysis:** Demographic and clinical profiles were recorded on computer files and analyzed by standard software SPSS-10 and EpiInfo 6.6.04 Statistical significance was set at $p<0.05$.

## RESULTS

The answers to the questionnaire and the ELISA test allowed us to obtain these different results:

Table 1 shows the information on the level of school training, occupation and maternity of the 276 pregnant women distributed according to the age. We note that 107 (38.8%; 95% CI 33.04-44.82) were illiterate and 15 (5.4%; 95% CI 3.18-8.99) were integrated in the public service; 139 (50.4%; 95% CI 44.32-56.39) carried out especially a

Table 1: Information on school training, occupation and maternity of pregnant women

| | School training of the pregnant women | | | Occupation of the HIV pregnant women | | | No. of children alive, died and abortions | | |
|---|---|---|---|---|---|---|---|---|---|
| Age group (years) | No. | Illiterates | Literates | Housewives | Commercial | Civil servants | No. of children alive | No. of dead children | Abortion No. |
| X<20 years | 23 | 9/23 (39.1) | 14/23 (60.9) | 18/23 (78.3) | 4/23 (17.4) | 1/23 (4.3) | 1.61 (0-3) | 0.35 (0-2) | 0.27 (0-1) |
| 20- 25 | 83 | 30/83 (36.1) | 53/83 (63.9) | 51/83 (61.5) | 25/83 (30.1) | 7/83 (8.4) | 2.35 (1-4) | 0.58 (0-2) | 0.56 (0-2) |
| 26-31 | 98 | 39/98 (39.8) | 59/83 (60.2) | 39/98 (39.8) | 57/98 (58.2) | 2/98 (2.0) | 2.48 (1-4) | 0.63 (0-3) | 0.79 (0-2) |
| 32-37 | 64 | 26/64 (40.6) | 38/64 (59.4) | 28/64 (43.8) | 31/64 (48.4) | 5/64 (7.8) | 2.59 (1-5) | 0.69 (0-3) | 0.74 (0-3) |
| X>37 | 8 | 3/8 (37.5) | 5/8 (62.5) | 3/8 (37.5) | 5/8 (62.5) | 0/8 (0.0) | 2.78 (1-5) | 0.71 (0-3) | 0.81 (0-3) |
| Total | 276 | 107/276 (38.8) | 169/276 (61.2) | 139/276 (50.4) | 122/276 (44.2) | 15/276 (5.4) | 2.46 (0-5) | 0.71 (0-3) | 0.64 (0-3) |

Values in brackets are percentage

Table 2: *Toxoplasma gondii* and HBV co-infection among pregnant women

| | | *Toxoplasma gondii* | | | | HBV | |
|---|---|---|---|---|---|---|---|
| Infection | No. | IgM- | IgM+ | IgG- | IgG+ | HBV- | HBV+ |
| HIV-* | 138 | 130 | 8/138 (5.8) | 107/138 (77.5) | 31/138 (22.5) | 130/138 | 8/138 (5.8) |
| HIV+° | 138 | 133 | 5/138 (3.6) | 94/138 (68.1) | 44/138 (31.9) | 120/138 | 18/138 (13.0) |
| Total | 276 | 263 | 13/276 (4.7) | 201/276 (72.8) | 75/276 (27.2) | 250/276 | 26/276 (9.40 |
| p-value | | 0.394 | | 0.079 | | 0.039 | |

Values in brackets are percentage

Table 3: Prevalence of *T. gondii* (IgM and IgG) and HBV for age groups

| | | | *Toxoplasma gondii* | | | | HBV | |
|---|---|---|---|---|---|---|---|---|
| Class | Years | Number | IgM- | IgM+ | IgG- | IgG+ | HBV- | HBV+ |
| 1 | X<20^ | 23-Jan | 23/23 | 0/23 (0.0) | 13/23 (56.5) | 10/23 (43.5) | 23/23 | 0/23 (0.0) |
| 2 | 20- 25 | 8300.00% | 80/83 | 3/83 (3.6) | 62/83 (74.7) | 21/83 (25.3) | 77/83 | 6/83 (7.2) |
| 3 | 26-31[&] | 9800.00% | 94/98 | 4/98 (4.1) | 78/98 (79.6) | 20/98 (20.4) | 90/98 | 8/98 (8.2) |
| 4 | 32-37 | 6400.00% | 58/64 | 6/64 (9.4) | 45/64 (70.3) | 19/64 (29.7) | 54/64 | 10/64 (15.6) |
| 5 | X>37° | 800.00% | 8-Aug | 0/8 (0.0) | 3/8 (37.5) | 5/8 (62.5) | 8-Jun | 2/8 (25.0) |
| Total | Total | 27600.00% | 263/276 | 13/276 (4.7) | 201/276 (72.8) | 75/276 (27.2) | 250/276 | 26/276 (9.4) |

IgG +: ^¬[&] : p = 0.021 and ¬° p = 0.024. Values in brackets are percentage

Table 4: Mean age and rates of T CD4 correlates with anti HBV antigens, antibodies to toxoplasmosis and HIV

| Antibodies | Age | p-value | CD4 | p-value |
|---|---|---|---|---|
| IgM+ | 30.62±4.86 | 4.10% | 299.2±39.54 | 0.325NS |
| N | 13 | | 5 | |
| IgM- | 27.51±5.34 | 358.5±133.7 | | |
| N | 263 | | 133 | |
| IgG+ | 28.00±6.07 | 0.508 (NS) | 363.9±119.7 | 0.723NS |
| N | 75 | | 44 | |
| IgG- | 27.52±5.06 | 352.8±190.9 | | |
| N | 201 | | 94 | |
| HIV- | 26.78±5.70 | 0.007 | - | - |
| N | 138 | - | | |
| HIV+ | 28.52±4.83 | 356.3±171.0 | | |
| N | 138 | | 138 | |
| HBV- | 27.35±5.24 | 0.40% | 360±180.4 | 0.506NS |
| N | 250 | | 120 | |
| HBV+ | 30.54±5.57 | 331.2±85.4 | | |
| N | 26 | | 18 | |

N = Number, NS = Not significant

life of housewives and 122 (44.2%; 95% CI 38.29-50.28) the commercial ones. Among them, several had dead children 0.71 (0-3) and many had several miscarriages during their preceding pregnancies 0.64 (0-3).

In the Table 2, are shown the results of *Toxoplasma gondii* and HBV coinfections among the pregnant women. For the test of toxoplasmosis, we obtained the following results: IgM+ 13 (4.7%; 95 CI 2.64-8.11); IgG+ 75 (27.2%; 95 CI 22.10-32.90) and for the HBsAg+ 26 (9.4%; 95 CI 6.36-13.65). For the frequency of HBV positive, we found a statistical difference between HIV seropositive and negative pregnant women (p = 0.039).

As shown in Table 3, the prevalence rate of toxoplasmosis (IgG) increases significantly with ages from class 1 to class 3 (p = 0.021) and from class 3 to class 5 (p = 0.024). The prevalence rate of hepatitis B (HBsAg) increases too significantly with ages: class 1 (0.0%); class 2 (7.2%); class 3 (8.2%); class 4 (15.6%) and class 5 (25.0%).

Table 4 shows the mean ages and rates of T CD4 cells on the basis of co-infections with HIV, HBV and *Toxoplasma gondii*. In terms of ages, the t-test reveals a statistically significant differences between those with IgM antibodies + (30.62±4.86 years) and IgM- (27.51±5.34 years) (p = 0.041); between HIV- (26.78±5.70 years) and HIV + (28.52±4.83 years) (p = 0.007) and between HBV- (27.35±5.24 years) and HBV + (30.54±5.57 years) (p = 0.004). Table 4 shows also no significant difference in T CD4 count for HIV-seropositives pregnant women co-infected with toxoplasmosis or hepatitis B (p>0.500). The rate of co-infections of HIV-seropositives / *T. gondii*-seropositives (44/138: 31.9%; 95 CI 24.36-40.43) (Table 5) was higher than that of HIV-seronegatives/*T. gondii*-seropositive (31/138: 22.5%; 95% CI 15.99-30.51) among these pregnant women (p<0.001) (Table 2). The rate of co-infections of HIV-seropositives/HBV-seropositives (18/138: 13.0%; 95 CI 8.12-20.09) (Table 5) was higher than

Table 5: Co-infections rates in HIV-positive pregnant women

| HIV : 138 | | *T. gondii* : 75 | | HBV : 26 | |
|---|---|---|---|---|---|
| *T. gondii* | HBV | HIV | HBV | HIV | *T. gondii* |
| 44/138 (31.9) | 18/138 (13.0) | 44/75 (58.7) | 13/75 (17.3) | 18/26 (69.2) | 2/26 (7.7) |

HIV/*T. gondii* ¬*T. gondii*/HIV: p<0.001, HIV/HBV¬HBV/HIV: p<0.001, HBV/*T. gondii*¬*T. gondii*/HBV: p = 0.384 (NS). Values in brackets are percentage

that of HIV-seronegatives/HBV-seropositives (8/138: 5.8%; 95% CI 2.72-11.48) among these pregnant women (p<0.001) (Table 2).

## DISCUSSION

The present study aimed to look for the presence of IgM and IgG antibodies to *Toxoplasma gondii*, to identify hepatitis B antigens in HIV-seropositive and seronegative pregnant women and to determine *Toxoplasma gondii* and HBV co-infection among these patients. Thus 276 women freely adhere to the study. The results revealed that, 138 of 276 pregnant-women were found HIV-seropositive. All these women come to the Voluntary Counselling and Testing Programme (VCTP) of SCMC for a specific consultation on their serological status and to adopt a preventive strategy for HIV vertical transmission. Among them 27.2% (75/276) were found to be positive for *T. gondii* IgG antibodies. This value is similar to seroprevalence data from some other Africans countries: 27.0% in Uganda (Zumla *et al*., 1991); 25.3% at Ouagadougou, in Burkina Faso (Simpore *et al*., 2006); 24.5% at Bobo-Dioulasso, in Burkina Faso (Millogo *et al*., 2000) and 21.0% from blood donors in Mali (Maiga *et al*., 2001).

On the other hand, we found difference of *T. gondii* prevalence between HIV positive and HIV-negative pregnant women: 31.9 versus 22.5%. An analogous difference of the *T. gondii* infection among HIV-positive and HIV negative patients has been widely reported in Uganda (34% versus 27%) (Zumla *et al*., 1991) and in Bamako (60% versus 21.0%) (Maiga *et al*., 2001). Considering also the rates of co-infection by the three pathogens assayed (Table 5), the risk for HIV-negative pregnant women to be infected by one of these 3 pathogenic agents (*T. gondii*, HIV and HBV) is high. Moreover, in our study, the risk for HIV-positive pregnant women to be co-infected by *T. gondii* and HBV is 2.6%.

This observation is not surprising since the HIV infection results in immunodeficiency which favors the *T. gondii* and HBV infections without a preferential way of transmission (Gutierrez-Zufiaurre *et al*., 2004). However, for adults in Burkina Faso, the sexual contact is a preferred source of transmission for both HBV than HIV. The influence of *T. gondii* on the evolution of HIV and vice versa is not well known, but the high rate of co-infection of *T. gondii* with HBV among the HIV infected women in Burkina Faso (4.1%) demonstrated the existence of a correlation between these two pathogenic agents which could influences the mother-to-child transmission of HIV (Simpore *et al*., 2006).

Thirteen women (4.7%) were found to be positive for *T. gondii* IgM antibodies. The prevalence rate of *T. gondii* IgM antibodies increases with age from 0.0% in the group aged less than 20 years to 9.4% in the 32-37 years old subjects; but we have no statistically significant difference. According to our results, most of these women are illiterate, unemployed and poor; many have had spontaneous abortions, but today, we do not know the causes. Almost 72% of pregnant women of our study are seronegative for IgG antibodies to *T. gondii* and in absence of prevention strategies, there is a serious risk of acquiring primary infection during pregnancy. They can bear malformed babies. Some countries allow therapeutic abortion in these circumstances (Wallon and Ilboudo, 2002); but, in Burkina Faso, abortion is permitted in only three cases: rape, incest or danger to life of the mother. Apart from these situations, the law severely punished offenders (SAWADOGO, 1999). Some traditional African societies practice eugenics systematically eliminating malformed children from birth. Others, however, believe that the child whether normal or malformed is always a gift of God and therefore should be home as such (Simporé and Ilboudo, 2002). According to Wallon *et al*. (2002), the event of a maternal infection during the first 8 weeks of pregnancy the risk of foetal infection is low and results mainly in a spontaneous termination of pregnancy. Future parents should be assured that conversely to a common opinion, the prognosis of congenital toxoplasmosis in live-born children is good. For these early maternal infections as for those acquired later, Wallon *et al*. (2002) recommend immediate treatment with spiramycin, monthly ultrasound surveillance, amniocentesis and treatment with pyrimethamine and sulphamides if the PCR is positive. For them, abortion should be restricted to cases with ultrasound lesions.

The lamivudine (3TC), the oldest nucleoside inhibitor of HBV polymerase is also effective on the reverse transcriptase of HIV, when used in dose of 100 mg daily for the treatment of hepatitis B (in opposition to 300 mg daily in the treatment of HIV). Under 3TC (lamivudineor Zeffix®, Epivir®), the HBV DNA quickly drops (Pessoa *et al*., 2008); thus, lamivudine is so active against HBV and must be taken into account as an important

factor for the treatment of people co-infected by HIV. So it is recommended to include in the HEART the molecule of lamivudine in order to simultaneously reduce the rates of vertical transmission of HIV and HBV and also, diminish *T. gondii* mother-to-child transmission. We should be advised not only the screening of HIV and *T. gondii*, but HBV in pregnancy to reduce the rate of transmission of these pathogens. An additional measure for all women HBV-positives, would also be vaccinating their children at birth, which is not yet systematically in Burkina Faso.

The present study demonstrates a high vulnerability of the pregnant women and their fetuses to *T. gondii*, the hepatitis B and HIV co-infection and suggests that the public health policy should take care of primary and secondary prevention for all pregnant women and their fetuses. Intrauterine infection by *T. gondii* is an important cause of some birth defects worldwide (Peng *et al*., 2004) and many cases of congenital toxoplasmosis can be prevented. Specific measures must be taken by the women and their health-care providers to decrease the risk for infection during pregnancy and prevent severe illness in newborns.

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