The Severity of Malaria and Toxoplasmosis Co-Infections among Pregnant Women in Yaounde, Cameroon

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ABSTRACT

Malaria and toxoplasmosis are two diseases caused by parasites of the same phylum (Apicomplexa). They have severe consequences on the health of pregnant women and their unborn babies. As such, they are of importance for the public health, especially in Sub-Saharan Africa and Cameroon. The aim of this study was to evaluate the severity of toxoplasmosis among malaria-positive pregnant women attending the Biyam-Assi District Hospital. It was a cross-sectional study that took place from May to November 2019 involving 232 pregnant women who voluntarily accepted to take part in the study. Demographic data was collected using structured questionnaires and blood was collected by finger prick. Thick blood films were prepared for the detection of malaria and the Giemsa-stained slides were read microscopically. A drop of blood was used for the detection of toxoplasmosis using the chromatographic cassette (Rapid Diagnostic Test) and the results were read after 15 minutes. The data obtained was analysed using SPSS version 24. The results showed that the prevalence of toxoplasmosis was 22.84 %. This
prevalence didn’t depend on the trimester of pregnancy ($p = 0.08$). The prevalence of malaria was 38.9%. No significant difference was observed for either the prevalence or severity of malaria over the three trimesters of pregnancy ($p = 0.60$ and $p = 0.9$ respectively). The prevalence of coinfection with malaria and toxoplasmosis was 9.05%. Women without toxoplasmosis were more prone to severe malaria (20.29%) than their counterparts who had toxoplasmosis (4.78%) and the difference between these two groups was statistically significant ($p = 0.02$). The severity of toxoplasmosis was not influenced by the presence of *Plasmodium* ($p = 0.20$). It was concluded that the occurrence and severity of toxoplasmosis in pregnant women does not depend on their malaria serological status. However, the presence of severe malaria in pregnant women depends on their toxoplasmosis status. Indeed, women already infected with *T. gondii* are less susceptible to malaria than their counterparts who are negative for toxoplasmosis. These findings suggest that *T. gondii* in a person confers some form of resistance to infections with *Plasmodium*. It was recommended that the Public Health Ministry in Cameroon could include screening for toxoplasmosis among the routine test for pregnant women in order to improve on the health of mother and baby.

Keywords: Malaria; toxoplasmosis; plasmodium; *T. gondii*; severity.

1. INTRODUCTION

Parasitism is one of the most widespread biological phenomena. To date, many phyla of parasites have been identified among which is the phylum of Apicomplexa. It contains many parasites that cause a multitude of diseases in humans and animals [1]. In particular, *Toxoplasma gondii*, the causal agent of toxoplasmosis, and *Plasmodium*, the causal agent of malaria, are the two parasites of interest in our study.

Toxoplasmosis is a cosmopolitan antropozoonosis caused by a coccidia, *Toxoplasma gondii* affecting more than 30% of the world population [2]. Although generally asymptomatic, it can be very dangerous in immunocompromised individuals [3] and in pregnant women who can transmit it to their unborn children, in which case it is called congenital toxoplasmosis [4]. Congenital toxoplasmosis is the infection of the fetus by the parasite *Toxoplasma gondii* transmitted by the mother; this implies that the mother has been in contact with the parasite during pregnancy without being previously immunized [5]. This is the most severe form and 85% of children born to infected mothers appear normal at birth but develop the disease later [4]. Indeed, toxoplasmosis has been reported as an abortifacient disease of sheep and women whose definitive host is the cat [5]. In Cameroon, a study revealed that the prevalence of toxoplasmosis in pregnant women was 70% for IgG and 2.73% for IgM [6] and the ever increasing number of unexplained abortions is becoming a major concern for obstetric gynecologists.

Malaria is a parasitosis caused by haematzoa of the genus *Plasmodium* and transmitted by mosquitoes of the genus *Anopheles* [7]. This disease is beneficially important to populations in intertropical zones as well as travelers. Malaria is indeed a serious public health problem in sub-Saharan Africa and Cameroon resulting in 80% of hospital consultation and 89% of deaths [8]. Pregnant women and children under five are the most vulnerable and there are approximately 3,000 child deaths each day from malaria [9]. In Cameroon, malaria is present in all regions and is the leading national endemic with a national morbidity of 25.9% in 2018 [10].

Malaria therefore captures the attention of public authorities because, its consequences are visible and direct compared to those of parasitic zoonoses such as toxoplasmosis which has silent, indirect consequences running at a low level in the human and animal populations. Toxoplasmosis is therefore neglected. Thus, a number of strategies have been put in place to fight malaria in endemic areas, particularly in our country, but this is not the case for toxoplasmosis, which remains somewhat unknown to the general public. Moreover, poverty, environment, and lifestyle are factors that can favor co-infection by these two diseases [11]. Thus, the co-infection of pregnant women with these two diseases can be a real economic, social and scientific problem for our country in view of the singular negative impacts that they cause. However, no information is available on the prevalence of co-infection by these two diseases and even less on the severity of toxoplasmosis in pregnant women suffering from malaria in Cameroon. The general objective of
our study was therefore to determine the severity of toxoplasmosis in malaria-positive pregnant women attending antenatal clinics at the District Hospital of Biyem-assi Yaounde. The specific objectives were to evaluate the prevalence of toxoplasmosis in pregnant women in antenatal consultations at Biyem-Assi District Hospital, assess the prevalence of malaria among pregnant women attending antenatal clinics at the Biyem-Assi District Hospital, assess the prevalence of malaria and toxoplasmosis (coinfection) among pregnant women attending antenatal clinics at Biyem-Assi District Hospital, evaluate the severity of malaria in pregnant women with toxoplasmosis and evaluate the severity of toxoplasmosis in pregnant women with malaria at the HDB.

2. MATERIALS AND METHODS

2.1 Study Design and Study Site

It was a cross-sectional study from March 2019 to December 2019 involving a total of 232 pregnant women. The blood samples were collected at the Biyem-assi District Hospital, a public health facility that is very popular with women from various parts of Yaoundé. The samples were handled in the hospital's parasitology laboratory for the diagnosis of toxoplasmosis and in the Zoology laboratory of the Ecole Normale Supérieure de Yaoundé for the diagnosis of malaria. The choice of the sample collection site was made on the basis of the climatic and environmental conditions prevailing in the area, which are favorable for the development of female anopheles and the stability of malaria transmission. Indeed, Yaoundé is a city in the Central Region of Cameroon, located in the Division of Mfoundi. This region has a particular equatorial climate characterized by the presence of two rainy seasons (a long one from March to June and a short one from September to November) and two dry seasons (a long one, December-February and a short one, July-August).

2.1.1 Study population and inclusion criteria

The study population consisted of pregnant women presenting in the district hospital for antenatal consultation. The inclusion criteria were:

- To have voluntarily given written consent;
- To be HIV and hepatitis negative;

2.1.2 Administrative

An authorization was obtained from the Director of the Higher Teacher Training college, University of Yaounde 1, through the Head of Department for Biological Science. Another authorization for sample collection was obtained from the Director of the District Hospital Biyem Assi/The Health District through the Rector of the University of Yaounde 1 and the the Ministry of Public Health Cameroon. Data were processed with strict respect for anonymity. As an incentive, the Rapid Diagnostic Test (RDT) for toxoplasmosis and a thick blood sample were performed free of charge for all patients. All patients who tested positive were sent to the appropriate medical doctors for treatment and follow up within the hospital facility.

2.1.3 Subject recruitment procedure and sample collection

Women attending ANC who met the inclusion criteria were registered and an oral interview was conducted to explain the importance of the study and the experimental protocol.

The sample size was calculated according to the Lorentz formula [11], $N = \frac{Z^2 \times P \times Q}{d^2}$

Where $Z$ is the statistical power (1.96), $P$ is the estimated prevalence of disease (23% from Tonga et al [12], $Q=1-P$, $d$ is the significance level (0.05).

$N = \frac{(1.96 \times 1.96 \times 0.23 \times 0.77)}{(0.05)^2}$

$N = 272$.

A total of 300 questionnaires were administered and in the end, 232 pregnant women who consented to the study were sampled.

2.1.4 Sample collection and handling

The blood samples were collected following a gentle prick with a sterile lancet on the second or third finger previously cleaned with alcohol-soaked cotton. Using a pipette, 1 drop of this blood was collected and deposited directly on a Gold colloidal chromatographic cassette (TOX IgG/IgM rapid test by Labpro Pharma, LLC) in order to look for anti-Toxoplasma gondii antibodies. In addition, a portion of the blood collected was deposited on one end of a previously labeled slide. With the end of another slide, circular movements were performed to defibrinate this blood and spread it on a surface of about 1 cm diameter in order to obtain the
thick drop for the microscopic diagnosis of malaria. The slides obtained were air-dried and transported to the zoology laboratory of the ‘Ecole Normale Supérieure de Yaounde’ for analysis.

2.2 Laboratory Examinations

The examinations carried out were the RDT of toxoplasmosis and the microscopic examination of malaria (thick drop).

2.2.1 Performing the toxoplasmosis RDT

2.2.1.1 Components and principle

The Toxo IgG/IgM rapid on-site test is a lateral flow chromatography immunoassay. The test strip consists of:

- a burgundy colored conjugate pad containing recombinant *T. gondii* antigen conjugated to colloidal gold and rabbit IgG-gold conjugates.
- a nitrocellulose membrane strip containing two test strips (strip T1 and T2) and a control strip (strip C). The T1 strip is precoated with monoclonal anti-human IgM for the detection of anti- *T. gondii* IgM, the T2 strip is precoated with reagents for the detection of anti- *T. gondii* IgG, and the C strip is precoated with goat anti-rabbit IgG.

When an adequate volume of test specimen is applied to the sample pad of the strip, it migrates through the strip by capillary action. IgM anti-*T. gondii*, if present in the specimen, will bind to the *T. gondii* conjugates. The immunocomplex will then be captured on the membrane by the pre-coated anti-human IgM antibody, forming a burgundy T1 band, indicating a positive *T. gondii* IgM test result. Anti-*T. gondii* IgG, if present in the sample, will bind to *T. gondii* conjugates. The immunocomplex will then be captured by the pre-coated reagents on the membrane, forming a burgundy-colored T2 band, indicating a positive test result for anti- *T. gondii* IgG.

The absence of T bands (T1 and T2) suggests a negative result. The test contains an internal control (C-band) which must show a burgundy band of the goat anti-rabbit IgG regardless of color development on any of the T bands. Otherwise, the test result is invalid and the specimen should be retested.

2.3 Procedure

During handling, the test reagent, buffer and samples were left at room temperature (15-30°C) as the sensitivity of the analysis could be reduced at low temperatures. The test device was then placed on a clean, flat surface. The dropper was held vertically and a drop of sample of about 10 microliters was put in the sample well of the test device. Two drops of buffer were added and timing was done. After 10 to 15 minutes, the results were read. The result thus obtained was marked on each participant’s card.

2.3.1 Interpretation of the results

The results were considered as follows:

- Positive IgG indicated immunity to toxoplasmosis and therefore the infection could not be transmitted to the fetus;
- Positive IgM indicated recent infection;
- Positive IgG and IgM indicated either re-infection or chronic infection;
- Negative IgG and IgM indicated an absence of *Toxoplasma gondii*.

2.3.2 Performance of microscopic examination of malaria

2.3.2.1 Principle

The thick blood film is a technique for concentrating red blood cells in order to detect and count *Plasmodium* parasites in the blood.

2.3.3 Procedure

2.3.3.1 Realization of the thick blood film

A portion of the blood collected from each participant was used to perform the thick blood film. To do this, the drop of blood was collected and placed on a slide labelled with the same name and code as the strip used for the toxoplasmosis RDT. To do this, the drop of blood was collected and placed on a slide labelled with the same name and code as the strip used for the toxoplasmosis RDT. Then, using the edge of a second slide, the drop was spread over a diameter of about 1 cm by turning it for a few seconds and the resulting spread was carefully dried, without being fixed. Drying time was around 15 minutes at 37°C.

2.4 Staining of the Slides

In order to observe the *Plasmodia*, staining was necessary and it was done with a Giemsa solution diluted to 10%. For this purpose, a 500 mL stock solution of Giemsa rapide, at 100% concentration was available. To obtain the 10% diluted solution, 1 volume of Giemsa was taken for nine volumes of water. Staining was done for 10 minutes. After staining, the slides were rinsed with clear water without running the jet directly on the stained blood, to avoid detaching it from the slide. The slides were drained in an upright position on a slide rack.
2.4.1 Reading of the slides and interpretation of the results

The slides were read using a microscope with a 100x objective.

The parasite density was determined on 200 leukocytes and then related to 8000 leukocytes to obtain the parasitemia per microliter of blood. The interpretations of the observations were made as follows:

- Low parasitemia: (<500 parasites per microliter of blood)
- Moderate parasitaemia: (501- 5000 parasites per microliter of blood)
- High parasitemia: (> 5000 parasites per microliter of blood) [13].

2.5 Data Analysis

Data were collected from the report forms and entered into a computer database using Microsoft Excel 2019. The database created was cleaned by R software version 3.4.1. To format the tables and graphs, Excel 2019 software was again used.

3. RESULTS

3.1 Socio-Demographic Characteristics of the Study Population

Regarding the socio-demographic characteristics of the study population, the results showed that the majority of pregnant women (27.16%) were between 21 and 25 years of age; the most represented educational levels were secondary and higher education, 45.25% for each. There were more married women (57.33%) than single women (42.67%). Christian women were the most numerous (94.83%) and the most represented trimester of pregnancy in the study population was the first trimester (0-3 months) (38.79%) as shown in Table 1.

3.1.1 Prevalence of toxoplasmosis among pregnant women in Biyem-assi, Yaounde

The overall prevalence of toxoplasmosis in the study population was 22.84%, n = 53 (Table 2). This prevalence varied from one trimester to another with the highest number of positive cases recorded in the second trimester (32.81%, n = 21). In the third trimester, the prevalence was (17.95%, n = 14). Nevertheless, the difference observed was not significant as P = 0.07 (Table 2).

3.1.2 Severity of toxoplasmosis in pregnant women in biyem-assi yaounde

The presence of IgM (marking cases of recent contamination and therefore dangerous for the pregnant woman and her baby) was recorded during the three trimesters of pregnancy without much variation (P = 0.56). However, a higher prevalence was recorded in the third trimester (6.67%). Cases of old toxoplasmosis and therefore immunity (IgG) were more frequent (Table 3). There were 21 samples that did not
read neither positive or negative (no control band and no test band). The test was repeated and the same results were obtained, so they were considered non-applicable.

3.1.3 Prevalence of malaria in pregnant women in biyem-assi yaounde

For malaria prevalence, 90 women tested positive for malaria giving a prevalence of 38.79% (Table 4). The prevalence of malaria varied according to the trimester of pregnancy. It was highest in the first trimester of pregnancy at 42.22%, n = 38. It was observed to be lower in the second trimester and third trimesters of pregnancy (Table 4). Nevertheless, the difference observed was not significant because P was 0.6 (> 0.05).

3.1.4 Severity of malaria by trimester of pregnancy in biyem-assi yaounde

Over the three trimesters of pregnancy, the severity of malaria varied, but in general, simple malaria cases were the most frequent (60%). The greatest number of severe cases was observed in the second trimester (20%). However, these observed differences were not significant (P = 0.97) (Table 5).

Table 1. Socio-demographic characteristics of the study population

| Characteristic          | Categories | No. sampled | Percentage |
|-------------------------|------------|-------------|------------|
| Age                     | < 21 years | 35          | 15.09      |
|                         | 21-25 years| 63          | 27.16      |
|                         | 26-30 years| 58          | 25.00      |
|                         | 31-35 years| 43          | 18.53      |
|                         | 36-40 years| 17          | 7.33       |
|                         | >40 years  | 16          | 6.90       |
| Total                   |            | 232         | 100.00     |
| Primary                 |            | 22          | 9.48       |
| Level of education      | Secondary  | 105         | 45.26      |
|                         | University | 105         | 45.26      |
|                         | Total      | 232         | 100.00     |
| Marital status          | Single     | 99          | 42.67      |
|                         | Married    | 133         | 57.33      |
|                         | Total      | 232         | 100.00     |
| Religion                | Christians | 220         | 94.83      |
|                         | Muslims    | 7           | 3.02       |
|                         | Pagans     | 2           | 0.86       |
|                         | Others     | 3           | 1.29       |
|                         | Total      | 232         | 100.00     |
| Trimester of Pregnancy  | 0-3 months | 90          | 38.79      |
|                         | 4-6 months | 64          | 27.59      |
|                         | 7-9 months | 78          | 33.62      |
|                         | Total      | 232         | 100.00     |

Table 1. Prevalence of toxoplasmosis in relation to age of pregnancy

| Trimester of pregnancy | No. Effective | Prevalence (%) | P-value |
|------------------------|---------------|----------------|---------|
| 0-3 mois               | 90            | 18(20)         | 0.07    |
| 4-6 mois               | 64            | 21(32,81)      |         |
| 7-9 mois               | 78            | 14(17,95)      |         |

Table 3. Severity of toxoplasmosis in relation to the age of pregnancy

| Toxoplasmosis | Trimester of pregnancy | P-value |
|---------------|------------------------|---------|
|               | 0-3 mois               | 4-6 mois | 7-9 mois |
| Negative      | 72(85,71%)             | 43(82,69%) | 64(85,33%) |         |
| IgG positive  | 7(8,33%)               | 6(11,54%) | 6(8,00%)   | 0.56    |
| IgM positive  | 5(5,95%)               | 3(5,77%) | 5(6,67%)   |         |
### Table 4. Malaria prevalence in relation to age of pregnancy

| Trimestre de grossesse | No Sampled | Prevalence (%) | P-value |
|------------------------|------------|----------------|---------|
| 0-3 months             | 90         | 38 (42.22)     | 0.60    |
| 4-6 months             | 64         | 25 (39.06)     |         |
| 7-9 months             | 78         | 27 (34.62)     |         |
| Total                  | 232        | 90 (38.79)     |         |

### Table 5. Malaria severity with respect to age of pregnancy

| Severity of Malaria | Trimestre de grossesse | P-value |
|---------------------|------------------------|---------|
|                     | 0-3 months             | 4-6 months | 7-9 months |
| Simple              | 23 (60.53%)            | 15 (60.00%) | 16 (59.26%) | 0.97     |
| Moderate            | 9 (23.68%)             | 5 (20.00%)  | 7 (25.93%)  |         |
| Severe              | 6 (15.79%)             | 5 (20.00%)  | 4 (14.81%)  |         |
| Total (positive)    | 38                     | 25         | 27         |         |

#### 3.1.5 Malaria and toxoplasmosis co-infection in biyem-assi yaounde

A minority of the women (110, 47.41%) had neither malaria nor toxoplasmosis, 69 (29.74%) women had only malaria, 32 (13.79%) women had only toxoplasmosis, and 21 women tested positive for both malaria and toxoplasmosis (Fig. 2).

#### 3.1.6 Severity of malaria in pregnant women according to their toxoplasmosis status in Biyem-Assi Yaounde

Women without toxoplasmosis were significantly more prone to severe malaria (with 20.29% of severe cases) than women with toxoplasmosis (with only 4.76% of severe cases). The observed difference was statistically significant with \( p = 0.02917 \) (Table 6).

#### 3.1.7 Severity of toxoplasmosis in pregnant women according to their malaria status in biyem-assi yaounde

The majority of women with toxoplasmosis (IgM or IgG) had moderate malaria (\( n = 9 \) for IgG and \( n = 5 \) for IgM). The difference was significant between toxoplasmosis positive and toxoplasmosis negative participants for both IgG and IgM antibodies as \( p = 0.01455 \) and \( p = 0.06291 \) respectively (Table 6).

The detection of IgM during pregnancy indicates recent cases and therefore of serious consequences. Table 7 shows that IgM positive cases are more present in malaria positive women (47.62%) than in malaria negative women (28.13%). However, this difference is not statistically significant (\( p = 0.24 \)).

![Fig. 2. Prevalence of coinfection malaria/toxoplasmosis among pregnant women](image-url)
Table 6. Severity of malaria with respect to toxoplasmosis (IgM&IgG)

| Toxoplasmosis | Severity of malaria | P-value |
|---------------|---------------------|---------|
|               | Simple              | Moderate | Severe    |         |
| Negative      | 43(62,32%)          | 12(17,39%) | 14(20,29%) | 0,02    |
| Positive      | 11(52,38%)          | 9(42,86%) | 1(4,76%)  |         |
| Total (cas positif) | 54                  | 21        | 15        |         |
| Positif       | 8(44,44%)           | 9(50,00%) | 1(5,56%)  | 0,01    |
| Negative      | 46(63,89%)          | 12(16,67%) | 14(19,44%) |         |
| Total         | 54                  | 21        | 15        |         |
| IgG           | Positive            | 5(50,00%) | 5(50,00%) | 0(0,00%) | 0,06    |
|                | Negative            | 49(61,25%) | 16(20,00%) |         |
|                | Total               | 54        | 21        | 15        |         |
| IgM           | Positive            | 5(50,00%) | 5(50,00%) | 0(0,00%) |         |
|                | Negative            | 49(61,25%) | 16(20,00%) |         |
|                | Total               | 54        | 21        | 15        |         |

Table 7. Severity of toxoplasmosis in pregnant women according to their malaria status

| Toxoplasmosis | Positive | IgM+ | IgG+ | P-value |
|---------------|----------|------|------|---------|
| Malaria       | Negative | 32   | 23(71,88%) | 0,24    |
|               | Positive | 21   | 10(47,62%) |         |
|               |          |     | 11(52,38%) |         |

4. DISCUSSION

In this study, the prevalence of toxoplasmosis in pregnant women at Biyem-assi, Yaounde was 22.84%. This prevalence was much lower than those observed in studies conducted by Nguefack et al. (78.6%) in 2016 [14] and Njunda et al. (70%) in 2011 in Douala [6], Cameroon. Nevertheless, this rate is close to that observed in the latest studies on toxoplasmosis in pregnant women in Cameroon published by Ndamukong-Nyanga et al. (32.5%) [15]. Indeed, the latter already noted a sharp decline in the prevalence of toxoplasmosis among pregnant women in the study region. The present result therefore supports this conclusion. This regression could be explained by the fact that women are much more aware today of the dangers of toxoplasmosis and therefore take preventive measures seriously. Already, the age group most represented was very young, 21-25 years old, and the vast majority of them were educated, which could explain this regression in prevalence observed.

In addition, the prevalence of toxoplasmosis was higher in the second trimester of pregnancy (32.81%). However, our study showed no statistically significant association between Toxoplasma gondii infection and the trimester of pregnancy. Despite the statistical insignificance, the rate of IgG-positive women across all three trimesters of pregnancy was significantly higher than that of IgM-positive women. This is in agreement with the results of other researchers such as Ndassi and Kamga in 2014 [16] in Cameroon. Nevertheless, in their studies, the prevalence of women with both IgM and IgG antibodies was even lower which is not the case for the present study. Also, in more detail, the highest number of women with IgG antibodies without IgM detection was found in the second trimester of pregnancy with a sharp regression in the third trimester. This could be because IgG antibodies are markers of old infections. On the other hand, the greatest number of IgM positive women was found in the second trimester, which would indicate a recent infection with T. gondii because IgM antibodies are the first to be secreted in case of a new infection and therefore have a high chance of transmitting the disease to the fetus. In addition, a very low rate of IgM-positive women was recorded in the third trimester. This could be related to diagnosis and treatment following ill-health in the first two trimesters of pregnancy.

The prevalence of malaria among pregnant women was 42.2% in the first trimester of pregnancy, which then gradually declined in subsequent trimesters. However, the difference was not statistically significant and therefore the prevalence of malaria did not depend on the trimester of pregnancy considered. This result does not corroborate the results obtained by Cumber et al. [17] in Nkolbisson, Yaounde. Indeed, their result showed a prevalence of 9% in the first trimester of pregnancy, then an increase in the second trimester and finally a decrease in the third trimester. The peak prevalence was therefore observed in the second trimester, i.e. 20%, which is still lower than the
prevalence observed in the current study. The high prevalence observed in this study in the first trimester of pregnancy is of concern given that there is no chemoprophylaxis during this period of pregnancy [18].

The present study indicates that the severity of malaria defined on the basis of the number of trophozoites taken on 200 leukocytes [13] did not depend on the trimester of pregnancy. Indeed, the most severe malaria cases were recorded in the second trimester of pregnancy, i.e. 20% (n = 25), but no statistically significant difference was observed over the three trimesters. Severe malaria represents a major public health problem in endemic areas given the serious consequences on the mother, the fetus and the newborn.

Malaria and toxoplasmosis are two parasitoses known to be real dangers and obstacles to a favorable pregnancy outcome. The present study indicates that the prevalence of co-infection with these two diseases was 9.05%. Some (29.74%) of the women tested positive for malaria only and 13.79% of them had toxoplasmosis only. Thus, women with both diseases were less present in the study population. The prevalence of malaria was the highest, as in the present study. This low prevalence of toxoplasmosis-malaria co-infection suggests that survival of both parasites in the same pregnant woman is rare. This result is quite surprising and difficult to explain because in a pregnant woman, the immune defenses are already diminished which exposes her to a good number of diseases sometimes at the same time [19,20,21]. These two parasites of the same family were expected to therefore have a greater facility of attack in these women. Notwithstanding, the present results suggest that the occurrence of one of the two diseases would inhibit the other.

The present study showed that the most severe cases of malaria were found in women without toxoplasmosis (20.29%) compared to only 4.76% of severe malaria cases in women with toxoplasmosis. In fact, the difference in malaria severity between women without toxoplasmosis and those with toxoplasmosis was statistically significant. Previous results already showed that women with toxoplasmosis and malaria were less represented in the population, suggesting an inhibition of one parasite by the other. In addition, the results indicate that the presence of toxoplasma would reduce cases of malaria and even more severe malaria cases that are a real danger for the pregnancy; severe malaria in a pregnant woman causes abortions, anemia and exposes the fetus to a low birth weight [22]. Pregnant women who tested positive for toxoplasmosis would therefore be less prone to severe malaria cases. The same observation was made for simple malaria, but the opposite was observed for moderate malaria cases. This result could reflect a kind of immunization, of protection against malaria due to the presence of anti T. gondii antibodies. Also, taking into account the presence of anti-T. gondii antibody, severe malaria cases were more numerous in women without IgG than in those with IgG. This was a statistically significant difference suggesting that anti-T. gondii IgG is a factor in the variation of malaria severity in pregnant women. This could be explained by the fact that anti T. gondii IgG immunizing the woman against toxoplasmosis would play the same role with respect to Plasmodium falciparum infections. In addition, the most severe cases of malaria were observed in women who did not have anti T. gondii IgM, without however having a statistically significant difference with women who had anti T. gondii IgM.

The detection of anti T. gondii IgM suggests cases of recent infection and therefore severe toxoplasmosis in a current pregnancy, and the present study indicates that women with malaria are at greater risk of toxoplasma contamination. However, there was no statistically significant relationship between the presence of malaria and toxoplasma contamination marking the cases of severe toxoplasmosis in ongoing pregnancies. P. falciparum infection would therefore not be a factor in the occurrence of toxoplasmosis in pregnant women.

5. CONCLUSION

From this study, it was concluded that:

- The occurrence of toxoplasmosis as well as its severity does not depend on the trimester of pregnancy considered; identical finding for malaria;
- Co-infection by malaria and toxoplasmosis is rare in pregnant women;
- The severity of malaria in pregnant women depends on the toxoplasmosis status;
- The severity of toxoplasmosis in pregnant women does not depend on malaria status.
- These results suggest that although the two parasites incriminated here are of the same family, with many points in common taken
singly, they do not react in the same way to each other. *T. gondii* in a person confers some form of resistance to infections with *Plasmodium* but *Plasmodium* does not confer resistance to toxoplasmosis.

6. RECOMMENDATIONS

It was recommended that the Public Health Ministry in Cameroon could include screening for toxoplasmosis among the routine test for pregnant women in order to improve on the health of mother and baby.

CONSENT AND ETHICAL APPROVAL

This study was conducted in strict compliance with the ethical principles of the Ministry of Public Health of Cameroon regarding good practices in human clinical research. Voluntary participants who met the inclusion criteria for the study signed an informed consent form before registration.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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