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Association between Placental Malaria and Severe Pre-Eclampsia in Two University Hospitals of Yaounde City

Ayissi Gregory[1*], Astrid Ruth Ndolo Kondo[2], Ndoumba Afouba Alice[3], Noa Ndoua Claude Cyrille[1], Essiben Félix[1], Meka Esther[1], Belinga Etienne[1], Metogo Junie[1], Engo Engo Samuel Désiré[4], Bodo Edmond Lemaire[1], Tchente Nguefack Charlotte[2], Foumane Pascal[1], Mboudou Emile Télésphore[1]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine and Biomedicals Sciences, University of Yaoundé I, Yaounde, Cameroon
[2]Department of Obstetrics and Gynecology, Faculty of Medicine and Pharmaceuticals Sciences, University of Douala, Douala, Cameroon
[3]Department of Morphological and Anatomo-Pathological Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Dschang, Dschang, Cameroon.
[4]Department of Morphological and Anatomo-Pathological Sciences, Faculty of Medicine and Biomedicals Sciences, University of Yaoundé I, Yaounde, Cameroon
Email: *ayissigregory@yahoo.fr

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Abstract

Context: Pre-eclampsia and placental malaria, are two diseases that share pathophysiological similarities, such as placental ischemia, endothelial dysfunction and production of pro-inflammatory cytokines. **Objective**: The objective of our study was to investigate the association between placental malaria lesions and severe pre-eclampsia. **Methodology**: We conducted a prospective analytical cross-sectional study in two University Hospitals in the city of Yaounde (Yaounde Central Hospital and the Gynaecological Obstetrics and Paediatrics Hospital), and in the laboratory of the Centre Pasteur in Yaounde over an eight-month period (1st January 2021 – 1st September 2021). All patients with pre-eclampsia diagnosed according to the criteria of the International Society for the Study of Hypertension (ISSHP) and free of chronic metabolic or infectious pathology were included in this study. The patients were divided into two groups: group 1 (mild pre-eclampsia) and group 2: severe pre-eclampsia. Socio-demographic, clinical and histopathological characteristics specific to pre-eclampsia and placental malaria were investigated. Statistical analysis was performed with SPSS 23.0 software, Chi 2 was used to compare categorical variables, Student t-test was used to compare means, and logistic

regression was used to assess the association between placental malaria lesions and PES. **Results**: The mean age of our study population was 29.93 ± 7.36 years versus 28.28 ± 7.18 years in patients with mild and severe pre-eclampsia respectively. Pre-eclampsia placental lesions (accelerated villous maturation, infarction) were significantly greater in patients with severe pre-eclampsia ($p < 0.001$), as were placental malaria lesions (syncytial knots, acute inflammation, chronic inflammation; $p < 0.001$). The mean placental weight was significantly lower in patients with severe pre-eclampsia (511.67 ± 125.01 g versus 454.37 ± 121.96 g; $P = 0.024$). On univariate analysis, the absence of antenatal care (OR: 2.33; 95% CI: 1.02 - 5.32) and the absence of intermittent preventative treatment (OR: 3.06; 95% CI: 1.22 - 7.77) were significantly associated with severe pre-eclampsia. After multivariate analysis and regardless of wide confidence intervals, there was a strong association between placental malaria lesions and severe pre-eclampsia (aOR: 31.92; 95% CI: 8.67 - 117.47; $p < 0.001$); and a strong association between placental malaria lesions and pre-eclampsia placental lesions in patients with severe PE (aOR: 10.45; 95% CI: 1.28 - 85.09; $p = 0.020$). **Conclusion**: Placental malaria lesions were significantly associated with severe pre-eclampsia and increased the risk of developing severe pre-eclampsia placental lesions by a factor of 10.

Keywords

Mild Pre-Eclampsia, Severe Pre-Eclampsia, Malaria, Placental Lesions, Association

1. Introduction

Pre-eclampsia (PE) refers to the onset of hypertension and proteinuria, or the development of hypertension and significant noble organ dysfunction with or without proteinuria after 20 weeks gestation, or postpartum in a previously normotensive woman [1]. Worldwide, the global prevalence of pre-eclampsia is 4.6%, in Africa 44.0‰ and in Cameroon 4.97% according to Mboudou *et al.* [2]-[4]. It is a nosological entity of hypertension in pregnancy that constitutes the second leading cause of mortality after postpartum hemorrhage worldwide [5].

On the other hand, malaria is a parasitic disease caused by the multiplication and development in the liver and then in the red blood cells of a plasmodium hematozoan. In sub-Saharan Africa (a high-transmission region), the median prevalence of maternal malaria (defined as peripheral or placental infection identified by microscopy) is 25% [6]; and in a study carried out in Cameroon between 1996 and 2001, it was 19.9% of pregnant women [7]. Nearly 200,000 women and 10,000 newborns die from the consequences of malaria during pregnancy every year; and the fetal mortality rate is twice as high in endemic countries compared with non-endemic countries [8].

The critical role of the placenta in the pathophysiology of pre-eclampsia, particularly in early pre-eclampsia, is supported by epidemiological and experimental

data showing that placental tissue is necessary for the development of the disease, but not the fetus, and that preeclampsia is always cured within days or weeks of placental delivery [9] [10]. In fact, in pre-eclampsia, cytotrophoblastic cells infiltrate the decidual portion of the spiral arteries but fail to penetrate their myometrial segment, resulting in their failure to develop into wide, tortuous vascular channels created by the replacement of the muscular-elastic wall by fibrinoid material [11]. On the contrary, the vessels remain narrow, resulting in hypoperfusion, hypoxia and placental ischemia responsible for the release of anti-angiogenic factors into the maternal circulation, giving rise to a generalized endothelial disease at the origin of the various symptoms [12] [13].

Inflammation, through infection or the release of circulating DNA after apoptosis or post-hypoxic trophoblastic necrosis, plays a key role in maintaining or exacerbating endothelial damage, as demonstrated by a meta-analysis by Conde-Agudelo *et al.* [14] [15]. As a result, malaria in pregnancy via this pathway could be responsible for severe forms of PE. Indeed, the median prevalence of malaria in pregnancy in endemic zones is 25%, where it overlaps geographically with that of pre-eclampsia [2]. Its particular tropism for the placenta results in intervillous erythrocyte sequestration, syncytial degradation, multinucleated aggregates of syncytial nuclei (syncytial knots), and the subsequent inflammatory reaction, most often leading to localized destruction of placental villi, thus doubly mortgaging the maternal-fetal outcome in association with pre-eclampsia [16]-[18]. The latter is histologically responsible for acute placental artherosis and other secondary lesions [19]. Moreover, Moellers *et al.*, in 2019, in a study of 138 placentas from women exposed to malaria during pregnancy, concluded that malaria in early pregnancy impaired the vascular development of the placenta [20].

Given these arguments, one might wonder about the role of placental malaria in the genesis of severe forms of pre-eclampsia, being in a country with a high malaria endemic. We will attempt to find answers to this question through a comparative analytical study, looking for placental histopathological lesions of malaria and PE, in parturients with severe and non-severe pre-eclampsia, with the aim of establishing the association between placental malaria lesions and PE, in the maternity wards of two University Hospitals in the city of Yaounde.

2. Methodology

2.1. Type of Study

We conducted an analytical cross-sectional study with prospective data collection.

2.2. Study Site

The study took place in two maternity wards of university hospitals in Yaounde: the Yaounde Central Hospital (YCH), the Yaounde Obstetrics Gynecologic and Pediatrics Hospital (YOGPH). The analytical phase was carried out at the anatomopathology laboratory of the Pasteur Centre of Yaounde.

2.3. Period/Duration of the Study

The study covered a period of 08 months from 1st January 2021 to 1st September 2021. The duration of the study was 08 months from 1st January 2021 to 1st September 2021.

2.4. Study Population

***a. Target population*:**

Placentas of patients with pre-eclampsia diagnosed according to International Society for the Study of Hypertension in Pregnancy (ISSHP) criteria, with or without biological evidence of plasmodium falciparum malaria (thick drop and/or rapid diagnostic test – RDT).

***b. Source population*:**

All parturients were admitted to maternity wards at our various study sites for pre-eclampsia.

***c. Sampling*:**

In a recently published study which aimed to assess whether Plasmodium falciparum placental lesions increased the risk of pre-eclampsia, Obiri *et al.* found proportions of 61% and 36.2% active placental malaria in patients with and without pre-eclampsia respectively [21]. This corresponds to a difference of 24.8% in favour of the group of patients with pre-eclampsia. What sample size would be required to detect this difference with 95% power and a significance level of 0.05?

Comparing the proportions p1 and p2 in groups of equal size requires the following equation:

$$n = \frac{\left[P1(1-P1)+P2(1-P2)\right]}{(p1-p2)^2} \times cp,\ power$$

n is the number of subjects required in each group and cp, power a defined constant [22].

Numerical application:

$$n = \frac{\left[P1(1-P1)+P2(1-P2)\right]}{(p1-p2)^2} \times cp,\ power$$

P1 = 0.61; P2 = 0.36; $cp,\ power = 13$,

$$n = \frac{\left[(0.61\times 0.39)+(0.36\times 0.64)\right]}{(0.61-0.36)^2} \times 13$$

$$n = 97.76$$

Thus 98 parturients were required in each study group, which is in line with the estimate provided by Altman's Normogram.

***d. Non-inclusion criteria*:**

Patients meeting the inclusion criteria who do not wish to participate in the

study, patients with chronic or gestational hypertension, additional pre-eclampsia and patients with other chronic metabolic or infectious pathologies were not included in the study.

3. Procedure

3.1. Data Collection

a. Approach to patients

We approached the parturients included in our study with a polite greeting, then put them at ease before explaining the purpose of our study. We then obtained their signed or verbal informed consent, before assigning them a code number to ensure anonymity and sample matching.

b. Clinical examination

Anamnesis. We looked for socio-demographic data (age, gender parity, socio-economic level, religion, region of origin) and past medical history contributing to malaria in pregnancy and pre-eclampsia (antenatal care ≤ 4, Intermittent Preventive Treatment for malaria ≤ 4, primipaternity, primiparity, use of long-lasting impregnated mosquito nets LLINs, use of Aspirin, post IVF pregnancy).

Physical examination. We looked for functional signs (headache, blurred vision, scotomas, photophobia, epigastric pain, decreased active fetal movements, oliguria, polyarthralgia, chills, pelvialgia, per vaginal bleeding), then we assessed the general condition, then we took parameters (BP, pulse, temperature, respiratory rate), finally we assessed the Bishop score.

Diagnosis. The diagnosis of mild pre-eclampsia was defined as hypertension (systolic blood pressure ≥ 140 mm Hg and < 160 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg and < 110 mm Hg at least 4 h apart under ideal blood pressure measurement conditions) and proteinuria (≥ 30 mg on at least two urine dipstick readings) after 20 weeks gestation or postpartum in a previously normotensive woman; in accordance with the criteria of the International Society for the Study of Hypertension in Pregnancy (ISSHP) for the Diagnosis of Clinical Pre-eclampsia [23] [24].

The diagnosis of severe pre-eclampsia was made according to the following ISSHP criteria [23] [24]:

- Severe arterial hypertension: systolic blood pressure ≥ 160 mm Hg or diastolic blood pressure ≥ 110 mm Hg on two occasions at least 4 hours apart while the patient is in bed.
- Clinical signs of severity: severe headache unresponsive to usual analgesics, visual blur, scotoma or photophobia, tinnitus, epigastric bar pain, oliguria defined as diuresis of less than 0.3 cc/hour.
- Biological signs of severity: HELLP syndrome (Hemolysis, Elevated Liver Enzymes, Low Platelet), renal failure (serum creatinine >1.1 mg/dL [97.2 micromol/L] or a doubling of the serum creatinine concentration in the absence of other renal diseases).
- HELLP syndrome has been defined according to the Tennessee classification,

[25] and given by the following criteria:

- Haemolysis is established by at least two of the following:
- Peripheral smear with schisocytes.
- Serum bilirubin ≥1.2 mg/dL (20.52 micromol/L).
- Low serum haptoglobin (≤25 mg/dL) or lactate dehydrogenase (LDH) ≥ 2 times normal (based on laboratory-specific reference ranges).
- Severe anaemia, unrelated to haemorrhage.
- Elevated liver enzymes: Aspartate Aminotransferase (ASAT) or Alanine Aminotransferase (ALAT) ≥ 2 times normal (based on laboratory-specific reference ranges).
- Low platelets: < 100,000 cel/microL.

The diagnosis of clinical malaria in labour was made on the basis of functional signs (fever, chills, polyarthralgia, muscle aches) and physical signs (tachycardia, tachypnea, signs of maternal anaemia, and signs of fetal distress). A thick drop was taken to assess the parasite load.

Management. Data on management (mode of delivery, type of anesthesia, type of induction, magnesium sulphate, antihypertensive drugs, rehydration and antimalarials), on intrapartum fetal complications (acute foetal distress, IUGR, IUFD) and post-partum complications (early neonatal death, low birth weight, anoxic-ischaemic encephalopathy), and on maternal outcomes (death, stroke, retinal detachment, liver haematoma, haemorrhage, kidney damage, eclampsia) were collected.

NB. Missing data from the interview and physical examination were taken from the patients' medical records.

c. Placental tissue sampling

To study the association between severe PE and placental malaria, placental tissue was collected from women suffering from severe PE and mild PE after delivery. Two squares of placental tissue of 2 or 3 cm^3 were taken, one from the center of the placenta and the other from the periphery. These were then washed in an isotonic saline solution to remove excess blood and immediately placed in a sterile, well-labeled vial which was filled with 100 ml of 10% neutral formalin for tissue fixation. The biopsies were immediately transported to the pathology laboratory, where the tissue was processed.

d. Macroscopy

Macroscopy was performed when fixation was deemed adequate. The fragments were then placed in cassettes legibly identified with the registration number. The number of cassettes was noted on the worksheet, together with details of whether or not the sample had been placed in its entirety.

e. Dehydration, thinning and impregnation

The aim of this phase was to replace the water in the tissues with a neutral substance that hardens the samples in order to obtain thin sections, in this case paraffin. It was carried out in an apparatus called a histokinette or automatic dehydration machine (Leica model TP 1020). It consists of 12 trays. The cassettes are

placed in a basket, which is in turn immersed in each of the trays for a specific length of time, thanks to an automatic rotation system. The trays are divided as follows:

- 1st tray: buffered formalin, to perfect fixation (1 h 30).
- 2nd to 8th trays: alcohols with degrees increasing from 50 degrees to 100 degrees, for dehydration (7 h).
- 9th and 10th trays: Xylene for lightening, *i.e.* eliminating the alcohol to allow the paraffin to penetrate (2 h).
- 11th and 12th trays: liquid paraffin to impregnate the tissues (3 hours).

f. Coating

It was carried out using a coating station (Leica Histocore Arcadia) which includes a paraffin dispenser and a cooling plate. A small quantity of paraffin was poured into a mould, and then the sample was placed in it and then fixed to the bottom by placing the mould on the cooling plate. A variable quantity of paraffin was added, and the mould was placed on the plate for 15 minutes to obtain a block that could be cut.

g. Microtome sectioning

This was done using a Leica RM 2245 microtome. The block was inserted firmly into the microtome holder. First of all, roughing was carried out with cuts of 15 to 20 microns, and then the actual cuts were made with strips of 3 to 5 microns thick.

h. Slides making

The selected cut was detached from the ribbon with a scalpel and immersed in a water bath for 30 seconds. It was then spread out on a slide bearing an identification number. It was then drained and dried in an oven.

i. Staining

We began by dewaxing the slide by passing it through 3 trays of xylene, then passing it through 3 alcohol baths of increasing degrees, followed by rehydration in 2 tanks of water. Then:

- Haematoxylin (5 to 7 minutes).
- Rinsing under running water for 1 to 3 minutes.
- Brief immersion in 1% acetified water.
- Rinsing with running water for 1 to 3 minutes.
- Passage through ammonia water to turn nuclei blue for 5 seconds.
- Rinsing under running water for 1 to 3 minutes.
- Eosin for 2 to 4 minutes.
- Rinsing under running water for 1 to 3 minutes.
- Passage through an absolute alcohol bath.
- Passage through a xylene bath.

j. Mounting

After applying a few drops of synthetic resin (Eukitt), a coverslip was placed on the slide. The slides were then labelled and placed in trays before being given to the pathologists for reading.

k. Slide reading, interpretation and iconography

The slides were read on a microscope (LEICA DM 1000 LED) by the laboratory's pathologist. Images were obtained using standard cellSens software and a d27 camera (**Figure 1**).

l. Lesions

- The histological lesions of pre-eclampsia [26]-[32] sought in these patients were as follows:
- Acute arthrosis (*i.e.* fibrinoid necrosis of the vessel wall with an accumulation of lipid-laden "foamy" macrophages and a perivascular infiltrate of monocytes).
- Infarction.
- Signs of accelerated villous maturation.
- calcifications.
- Placental malaria lesions [16] [33]:
- Deposits of haemozoin (malaria pigment) in phagocytes and on fibrin in the intervillous space.
- Syncytial degradation, syncytial knots and localised destruction of villi.
- Signs of acute and chronic inflammation.
- Calcifications.

3.2. Statistical Analysis of the Data

The data collected using the data collection form were entered and analysed using statistical software:

- Excel 2013 for forming the input mask.
- SPSS 23.0: The means of the two independent groups were compared using the Student test; the categorical variables were compared using the Chi-square. The association between placental malaria and PE was evaluated by logistic regression.
- OFFICE 13 for data entry, table formatting, creation of graphs and statistical diagrams.

3.3. Ethical and Administrative Considerations

All methods were applied in accordance with the guidelines and regulations in force in Cameroon. Ethical approval to conduct this study was obtained from the ethical and institutional committee of the University of Yaoundé I (UYI). Demographic data and biological samples were obtained after informed consent from the selected participants. Participation was strictly voluntary, with no restrictions if the participant decided to withdraw from the study.

4. Results

4.1. Socio-Demographic Data

4.1.1. Age, Socio-Economic Level, Level of Education

Patients with mild PE were slightly younger than those with severe PE (mean

Table 1. Distribution of the study population according to age, socio-economic level and level of education.

Variable	Total N = 101	Pre-eclampsia		p
		Mild n = 40	Severe n = 61	
Age (mean ± standard deviation)	28.93 ± 7.18	29.93 ± 7.36	28.28 ± 7.18	0.267
Age range				0.455
[15 – 24]	28 (27.7)	9 (22.5)	19 (31.1)	
[24 - 33]	36 (35.6)	17 (42.5)	19 (31.1)	
[33 - 42]	37 (36.6)	14 (35.0)	23 (37.7)	
Socio-economic level				
Low	59 (58.4)	21 (52.5)	38 (62.3)	0.317
Medium	36 (35.6)	15 (37.5)	21(34.4)	
High	6 (5.9)	4 (10.0)	2 (3.3)	
Level of education				
None	2 (2.0)	1 (2.5)	1 (1.6)	0.440
Primary	29 (28.7)	15 (37.5)	14 (23.0)	
Secondary	47 (46.5)	16 (40.0)	31 (50.8)	
University	23 (22.8)	8 (20.0)	15 (24.6)	

*P = p-value, * = p-value < 0.05.*

age: 29.93 ± 7.36 years versus 28.28 ± 7.18 years; p = 0.267), with no statistically significant difference between the two groups. Low socio-economic status was more common in both groups, although there was no significant difference between the two groups (Table 1).

4.1.2. Gestity, Parity and Gestational Age

Median gestity was 3 and median parity 1 in patients with mild and severe PE, with no significant difference between the two study groups. Severe pre-eclamplsia patients had relatively younger pregnancies than patients with mild pre-eclampsia (Table 2).

Table 2. Distribution of the study population according gestity, gestational age and parity.

Variable	Total N = 101	Pre-eclampsia		p
		Mild n = 40	Severe n = 61	
Gestity (Mean/QI)	3.00 (1.00 - 5.00)	3.00 (1.00 - 4.75)	3.00 (1.00 - 5.00)	0.653
Gestity				0.220
G1	35 (34.7)	11 (27.5)	24 (39.3)	
G2 - 3	22 (21.8)	12 (30.0)	10 (16.4)	
G4 - 5	29 (28.7)	13 (32.5)	16 (26.2)	
G6+	15 (14.9)	4 (10.0)	11 (18.0)	

Continued

Parity (Mean /QI)	1.00 (1.00 - 3.00)	1.00 (1.00 - 3.00)	1.00 (1.00 - 3.00)	0.909
Parity				0.723
P0	21 (20.80)	7 (17.50)	14 (23.00)	
P1	34 (33.70)	16 (40.00)	18 (29.5)	
P2 – 3	26 (25.70)	10 (25.00)	16 (26.20)	
P4+	20 (19.80)	7 (17.50)	13 (21.30)	
GA (mean ± SD)	36.89 ± 3.48	37.55 ± 3.08	36.46 ± 3.68	0.126
Range				
<28	1 (1.0)	0 (0.0)	1 (1.6)	
[28 – 34]	18 (17.8)	5 (12.5)	13 (21.3)	
[34 – 37]	22 (21.8)	8 (20.0)	14 (23.0)	
[37 - 41]	57 (56.4)	25 (62.5)	32 (52.5)	
> 41	3 (3.0)	2 (5.0)	1 (1.6)	

P = p-value, * = *p-value* < 0.05, *QI = quartile interval*, *SA = weeks of amenorrhoea*, *G*0 =*nulligravida*, *G*1 = *primigravida*, *G*2 - 3 = *paucigravida*, *G*-5 = *multigesta*, *G*6+ = *large multigesta*, *P*0 = *nulliparous*, *P*1 = *primiparous*, *P*2 - 3 = *pauciparous*, *P*4+ = *multiparous*, *GA*: *gestational age*, *SD*: *standard deviation*.

4.2. Clinical, Diagnostic and Biological Characteristics of the Study Population

4.2.1. Past Medical History of the Study Population

According to medical history, patients with severe PE had poor antenatal follow-up and took less IPT than patients with mild PE (p < 0.05) (Table 3).

Table 3. Distribution of the study population according to past medical history.

Variable	Total N = 101	Pre-eclampsie		p
		Mild n = 40	Severe n = 61	
Antenatal follow-up				0.041*
Yes	53 (52.5)	26 (65.0)	27 (44.3)	
No	48 (47.5)	14 (35.0)	34 (55.7)	
History of malaria				0.604
Yes	25 (24.8)	11 (27.5)	14 (23.0)	
No	76 (75.2)	29 (72.5)	47 (77.0)	
New partner				0.809
Yes	6 (5.9)	3 (7.5)	3 (4.9)	
No	95 (94.1)	37 (92.5)	58 (95.1)	
Aspirin intake				0.591
Yes	52 (51.5)	20 (50.0)	32 (52.5)	

Continued

No	49 (48.5)	20 (50.0)	29 (47.5)	
LLIN				0.687
Yes	48 (47.5)	20 (50.0)	28 (45.9)	
No	53 (52.5)	20 (50.0)	33 (54.1)	
IPT				0.016*
Yes	25 (24.8)	15 (37.5)	10 (16.4)	
No	76 (75.2)	25 (62.5)	51 (83.6)	

*P = p-value, * = p-value* < 0.05, *LLIN*: *long-lasting insecticidal net*, *IPT*: *intermittent preventive treatment of malaria.*

4.2.2. Vital Parameters, Symptoms and Signs by Group

Table 4. Distribution of the study population according to general conditions and vital parameters.

Variable	Total N = 101	Pre-eclampsia Mild n = 40	Pre-eclampsia Severe n = 61	p
General condition				0.000
Good	71 (70.3)	36 (90.0)	35 (57.4)	
Impaired	30 (29.7)	4 (10.0)	26 (42.6)	
Fever				0.156
Yes	7 (6.9)	1 (2.5)	6 (9.8)	
No	94 (93.1)	39 (97.5)	55 (90.2)	
Systolic BP (mean ± SD)	165.7 ± 22.19	149.70 ± 11.23	176.21 ± 21.34	0.000
Diastolic BP (mean ± SD)	109.51± 15.71	97.70 ± 6.60	117.26 ± 15.12	0.000
HR (mean ± SD)	98.21 ± 16.44	90.80 ± 16.01	103.07 ± 14.95	0.000

*P = p-value, * = p-value* < 0.05, *BP = blood pressure*, *HR*: *heart rate*, *SD*: *standard deviation.*

General condition was poorer in patients with severe PE than in the control group. Systolic blood pressure, diastolic blood pressure and pulse rate were significantly higher in the severe PE group. There was no significant difference in fever between the two groups (Table 4).

Table 5. Distribution of clinical signs of severity in patients with severe PE.

Variables	Pre-eclampsia severe N (61)	
	Frequence (n)	Pourcentage (%)
Headache	35	57.4
Visual blur	15	24.6
Epigastralgia	11	18.0
Scotoma	2	3.3
Tinnitus	2	3.3

Headache, visual blur and epigastralgia were the most frequent signs of severity in patients with severe PE (Table 5).

Table 6. Other functional and physical signs by group.

Variable	Total N = 101	Pre-eclampsia Mild n = 40	Pre-eclampsia Severe n = 61	p
Chills				0.882
Yes	3 (3.0)	1 (2.5)	2 (3.3)	
No	98 (97.0)	39 (97.5)	59 (96.7)	
Polyarthralgia				0.604
Yes	1 (1.0)	0 (0.0)	1 (9.8)	
No	100 (99.0)	40 (100.0)	60 (1.6)	
Pelvic pain				0.604
Yes	10 (9.9)	4(10.0)	6 (9.8)	
No	91 (91)	36 (90.0)	55 (90.2)	
vaginal bleeding				0.416
Yes	1 (1.0)	0 (0.0)	1 (1.6)	
No	100 (99.0)	40 (100.0)	60 (98.4)	
Lower limbs oedema				0.559
Yes	44 (43.6)	16 (40.0)	28 (45.9)	
No	57 (56.4)	24 (60.0)	33 (54.1)	
Decrease in featal kicks				0.542
Yes	4 (4.0)	2.5 (1)	3 (4.9)	
No	97 (56.4)	97.5 (39)	58 (95.1)	

P = p-value, * = *p-value* < 0.05.

For the other clinical signs, there was no significant difference between the two groups. For example, for oedema of the lower limbs, there was no difference between the two groups p = 0.559 (Table 6).

4.2.3. Diagnosis of Clinical Malaria, Parasitaemia and Average Placental Weight

Table 7. Distribution of the study population according to clinical diagnosis of associated malaria.

Variable	Total N = 101	Pre-eclampsia Mild n = 40	Pre-eclampsia Severe n = 61	p
Clinical malaria				0.576
Yes	7 (6.9)	3 (7.5)	4 (6.6)	
No	94 (93.1)	37 (92.5)	57 (93.4)	

P = p-value, * = *p-value* < 0.05.

As regards the diagnosis of clinical malaria, a small proportion of the total sample was concerned (7.9%) and there was no significant difference between the two groups (Table 7).

Table 8. Distribution of the population according to a positive thick smear test.

Variable	Total N = 101	Pre-eclampsie Mild n = 40	Pre-eclampsie Severe n = 61	p
Positive thick smear test				0.576
Yes	28 (27.7)	9 (22.5)	19 (31.1)	
No	73 (72.3)	31 (77.5)	42 (68.9)	

P = p-value, * = *p-value* < 0.05.

There was no significant difference between the two groups in terms of the positive thick smear test (Table 8).

Table 9. Distribution of the population according to average parasitemia.

Variable	Total N = 101	Pre-eclampsia Mild n = 40	Pre-eclampsia Severe n = 61	p
Parasitemia (average ± SD)		576.88 ± 1073.92	2132.95 ± 2682.40	0.107

P = p-value, * = *p-value* < 0.05, *SD*: *standard deviation.*

There was no significant difference in mean parasitemia between the two groups (Table 9).

Table 10. Distribution of the study population according to placenta weight.

Variable	Total N = 101	Pre-eclampsia Mild n = 40	Pre-eclampsia Severe n = 61	p
Placenta weight (g)	483.02 ± 123.48	511.67 ± 125.01	454.37 ± 121.96	0.024*

P = p-value, * = *p-value* < 0.05, *g = grams.*

The placentas of patients with severe pre-eclampsia were significantly smaller than those of patients with mild pre-eclampsia (Table 10).

Table 11. Distribution of the study population according to HELLP syndrome and acute kidney injury in patients with Severe PE.

Variables	Severe pre-eclampsia N (61)	
	Frequence (n)	Pourcentage (%)
HELLP	17	27.9
Acute kidney injury	9	14.8

Around 15% of patients with severe PE developed acute kidney injury and more than a quarter of them had HELLP syndrome (Table 11).

4.2.4. Placental Lesions in PE and Malaria

Table 12. Distribution of patients according to PE placental lesions.

Variable	Total N = 101	Pre-eclampsie Mild n = 40	Pre-eclampsie Severe n = 61	p
Villi maturation				0.000*
Absent	11 (10.9)	10 (25.0)	1 (1.6)	
Low	34 (33.7)	17 (42.5)	17 (27.9)	
Moderate	44 (43.6)	13 (32.5)	31 (50.8)	
Intense	12 (11.9)	0 (0.0)	12 (19.7)	
Infarction				0.024*
Yes	15 (14.9)	2 (5.0)	13 (21.3)	
No	86 (85.1)	40 (95.0)	48 (78.7)	
Pre-eclampsia lesions				0.000*
Yes	90 (89.1)	30 (75.0)	60 (98.4)	
No	11 (10.9)	10 (25.0)	1 (1.6)	

P = p-value, * = *p-value* < 0.05.

Placental lesions due to pre-eclampsia were significantly increased in patients with severe PE (p<0.001) (Table 12).

Table 13. Distribution of the study population according to placental malaria lesions.

Variable	Total N = 101	pre-eclmpsia Mild n = 40	pre-eclmpsia Severe n = 61	p
Syncytial knots				0.000*
Yes	46 (45.5)	3 (7.5)	43 (70.5)	
No	55 (54.5)	37 (92.5)	18 (29.5)	
Acute inflammation				0.017*
Yes	8 (7.9)	0 (0.0)	8 (13.1)	
No	93 (92.1)	40 (100.0)	53 (86.9)	
Chronic inflammation				0.011*
Yes	9 (8.9)	0 (0.0)	9 (14.8)	
No	92 (91.1)	40 (100.0)	52 (85.2)	
Malaria lesions				0.000*
Yes	47 (46.5)	3 (7.5)	44 (72.1)	
No	54 (53.5)	37 (92.5)	17 (27.9)	

P = p-value, * = *p-value* < 0.05.

Placental malaria lesions were significantly increased in patients with severe PE

compared with those with mild PE (p < 0.001) (Table 13).

Table 14. Distribution of the study population according to placental calcifications.

Variable	Total N = 101	Pre-eclampsia		p
		Mild n = 40	Severe n = 61	
Calcifications				0.000
Yes	39 (38.6)	7 (17.5)	32 (52.5)	
No	62 (61.4)	33 (82.5)	29 (47.5)	

P = p-value, * = *p-value* < 0.05.

Calcifications were significantly higher in patients with severe PE than in those with mild PE (p < 0.05) (Table 14).

4.2.5. Associations between Pre-Eclampsia and Placental Malaria

Table 15. Factors associated with severe pre-eclampsia after univariate and multivariate analysis.

	Severe pre-eclampsia		OR (CI 95%)	*P*	Adjusted OR (CI 95%)	*P*
	Yes	No				
Age in years (mean ± standard deviation)	28.3 ± 7.1	29.9 ± 7.4	1.03 (0.97, 1.09)	0.265	1.00 (0.92, 1.08)	0.93
Number of antanata visits ≥ 4						
Yes (n = 53)	27 (50.9%)	26 (49.1%)	Reference	/	Reference	/
No (n = 48)	34 (70.8%)	14 (29.2%)	2.33 (1.02, 5.32)	0.043*	1.66 (0.46, 5.94)	0.431
Intermittent preventive treatment						
Yes (n = 25)	10 (40.0%)	15 (60.0%)	Reference	/	Reference	/
No (n = 76)	51 (67.1%)	25 (32.9%)	3.06 (1.20, 7.77)	0.001*	2.56 (0.59, 11.16)	0.208
Presence of calcifications						
Yes (n = 39)	32 (82.1%)	7 (17.9%)	5.20 (1.99, 13.55)	0.001*	6.36 (1.83, 22.07)	0.004*
No (n = 62)	29 (46.8%)	33 (53.2%)	Reference	/	Reference	/
Presence of syncytial knots						
Yes (n = 46)	43 (93.5%)	3 (6.5%)	29.46 (8.03, 107.98)	<0.001*	32.78 (8.0, 134.31)	<0.001*
No (n = 55)	18 (32.7%)	37 (67.3%)	Reference	/	Reference	/

OR = Odds Ratio, CI = Confidence Interval, P = p-value, * = *p-value* < 0.05.

On univariate analysis, absence of antenatal visits, absence of IPT, presence of calcifications and syncytial nodes were significantly associated with severe PE.

After multivariate analysis, the presence of calcifications increased the risk of severe PE by a factor of 6, while the presence of syncytial knots increased this risk by a factor of 32 (Table 15).

Table 16. Association between pre-eclampsia lesions and malaria lesions.

	Pre-eclampsia lesions		OR (CI 95%)	*P*
	Yes	No		
Malaria lesions				
Yes (n = 47)	46 (97.9%)	1 (2.1%)	10.45 (1.28, 85.09)	0.02*
No (n = 54)	44 (81.5%)	10 (18.5%)	Reference	/

*OR = Odds Ratio, CI = Confidence Interval, P = p-value, * = p-value* < 0.05.

After logistic regression, malaria lesions were significantly associated with pre-eclampsia. (OR: 10.45; 95% CI: 1.28 - 85.09; P = 0.020) (Table 16).

Table 17. Association between severe clinical pre-eclampsia and malaria lesions.

	Severe clinical PE		OR (IC 95%)	*P*
	Yes	No		
Malaria lesions				
Yes (n = 47)	44 (93.6%)	3 (6.4%)	31.92 (8.67, 117.47)	<0.001*
No (n = 54)	17 (31.5%)	37 (68.5%)	Reference	/

*OR = Odds Ratio, CI = Confidence Interval, P = p-value, * = p-value* < 0.05.

The presence of placental malaria lesions increased the risk of developing pre-eclampsia by a factor of almost 30 (Table 17).

Table 18. Association entre prééclampsie sévère clinique et paludisme clinique.

	Severe clinical PE		OR (IC 95%)	*P*
	Yes	No		
Clinical malaria				
Yes (n = 7)	4 (57.1%)	3 (42.9%)	0.86 (0.18 – 4.09)	0.855
No (n = 94)	57 (60.6%)	37 (39.4%)	Reference	/

*OR = Odds Ratio, CI = Confidence Interval, P = p-value, * = p-value* < 0.05.

Clinical malaria was not associated with sever PE (Table 18).

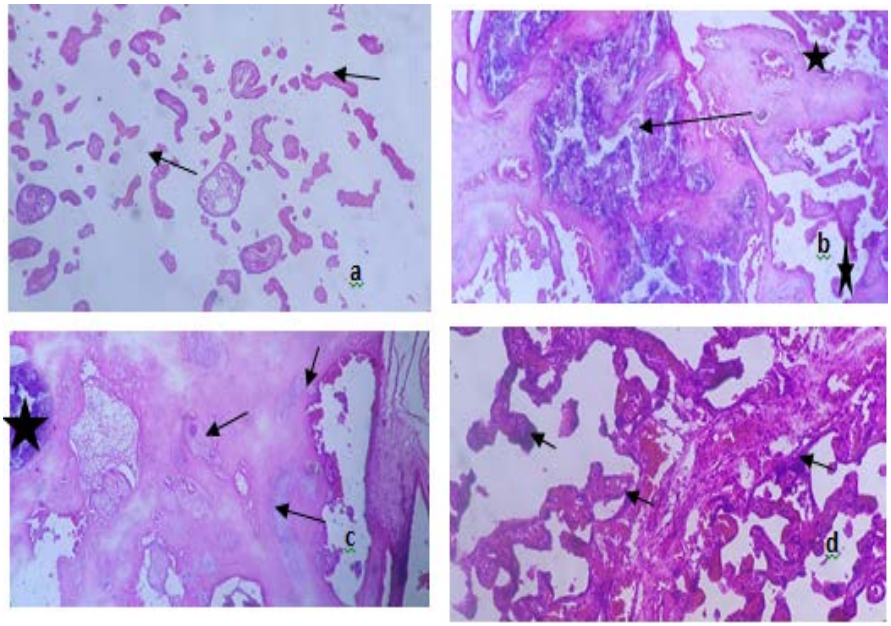

Figure 1. Some placental lesions found in our study. **a**: accelerated villous maturation, with atrophic and thinned villi (arrows) and an increase in the inter-villous space. **b**: foci of calcification (arrow) with accelerated villous maturation (stars). **c**: placental infarction with ghostly villi (arrows) associated with a focus of calcification (star). **d**: multiple syncytial nodes (arrows).

5. Discussion

In our study, patients with mild and severe pre-eclampsia had a mean age close to 30 years (29.93 ± 7.36 vs 28.28 ± 7.18) and a low socio-economic level, with no statistically significant difference between the two groups. Several authors in the literature have similar results [21]; indeed in their studies, Silva *et al.* and Lamminpää *et al.* respectively found that a low socio-economic level increased the risk of developing PE by a factor of 5 (OR: 4.91; 95% CI: 1.93, 12.52), while an advanced maternal reproductive age increased the risk of PE by a factor of 1.5 in a population of almost 18,000 women [34] [35]. This can be explained by the decidualisation anomalies associated with advanced maternal age, which are implicated in the occurrence of pre-eclampsia; and the low socio-economic level, which is associated with poor antenatal care, as well as other risk factors for PE [36] [37].

Most patients in our study had a parity of less than 1 (n = .55; 54.5%) reflecting the study median parity of 1.00 (QI: 1.00-3.00) with no significant difference between the two groups ($p > 0.05$). This is in line with the literature on pre-eclampsia, which states that nulliparity and primiparity are established risk factors for preeclampsia. The reasons for this are thought to be related to a lack of immune adaptation to pregnancy, higher insulin resistance in primiparous women, and angiogenic and genetic factors [38]. On the other hand, primiparity and nulliparity are also factors associated with malaria in pregnancy, as primiparous patients have not yet developed antibodies specific to malaria in pregnancy (VSA-PAM) [39].

Lack of pregnancy follow-up and failure to take IPT were factors significantly associated with severe pre-eclampsia in the univariate analysis in our study, but after adjustment they were found to be non-associated, certainly because of the low power of the study. In fact, regular antenatal monitoring would prevent or prevent pregnant women from developing severe forms of pre-eclampsia, although Manandhar *et al.* have found the opposite in a study [40]; while IPT chemoprophylaxis would prevent them from developing placental malaria by ensuring clearance of blood trophozoites and residual hepatic forms [41].

It was expected that there would be significant differences between the altered general condition, blood pressure and pulse rate in favour of the severe PE group, which was confirmed after analysis of the results. There was no significant difference between the signs of clinical malaria in the two groups (fever, chills, pelvic pain, etc.). The most frequent functional signs of severe pre-eclampsia were headache (57.4%), visual blur (24.6%), and epigastric pain (18%), which is in close line with the data in the literature and shows the extent of the reversible posterior encephalopathy in severe PE (PRES syndrome) responsible for the first two symptoms [42] [43]. With regard to biology in our study, the rate of HELLP syndrome was 27.9% and that of acute kidney injury 14.8% in patients with severe PE. This is in line with the data in the literature and highlights the severity of the biological risks to which these patients are exposed [44] [45].

Patients with severe PE had a significantly lower mean placental weight than patients with mild PE (454.37 ± 121.96 vs 511.67 ± 125.01; = 0.024). Similar results were found by Rehman *et al.*, Kishawa *et al.* and Dahlstrϕm *et al.*, although the latter compared patients with PE versus patients without PE [46] [47]. This highlights the effect or consequence of the severity of EP on placental weight without obscuring the harmful effect that associated placental malaria could have, as demonstrated by Moellers *et al.* [20]. In fact, in pre-eclampsia, hypoperfusion secondary to the lack of invasion of the spiral arteries is responsible not only for the clinical and biological spectrum of the disease, but also for the placental development defect responsible for low placental weight. Furthermore, it was demonstrated in 2019 that malaria in early pregnancy impairs the vascular development of the placenta and consequently its growth and weight [20].

Placental lesions of malaria (prominent syncytial Knots, acute inflammation, chronic inflammation) and pre-eclampsia (accelerated villous maturation, infarction) were significantly present in patients with severe PE compared with the mild PE group ($p < 0.001$). In addition, confounding lesions such as calcifications were significantly higher in patients with severe PE. Several authors have reported similar results, whereas Ndao *et al.* reported opposite results [6] [48]-[51], although we are the first study to compare severe versus mild pre-eclampsia patients. They explained their contradictory results by the seasonal nature of malaria in their area [52] [53].

Our aim was to highlight the association between placental malaria lesions and placental pre-eclampsia lesions in patients with PES. It was interesting to note:

- the lack of association between clinical malaria and clinical PE (OR: 0.86; 95% CI: 0.18 - 4.09; p = 0.855), which is supported by the fact that a non-pregnant patient with clinical malaria does not have pre-eclampsia;
- the strong association between the presence of placental malaria lesions and clinical severe PE (aOR: 31.92; 95% CI: 8.67 - 117.47; p < 0.001), this demonstrates that placental malaria is an independent risk factor for severe PE;
- and finally, a strong association between placental malaria lesions and PE placental lesions in patients with severe PE (aOR: 10.45; 95% CI: 1.28 - 85.09; p = 0.020), which implies that there is a 10-fold increase in the risk of having PE placental lesions when placental malaria is present.

Several authors had results similar to ours [6] [48]-[51], and this could be explained by the fact that there are pathophysiological similarities between PE and placental malaria, such as placental ischemia, endothelial dysfunction and the production of pro-inflammatory cytokines. As a result, the additive effect between PE placental lesions and malaria placental lesions would be responsible for severe forms of PE. Indeed, in the course of our research we observed the geographical overlapping of pre-eclampsia and malaria on the world map [4] [54], which corroborates the observations of Wickramasuriya GAW, who since 1936 has described "an epidemic of pregnancy toxemia following that of malaria" [55].

6. Conclusions

At the end of our study, we can say that:

- The patients in our study were mostly elderly primipara women with a low socio-economic level.

- Most of them had poor pregnancy follow-up and were not taking IPT correctly.

- Clinical malaria was poorly expressed in the study population and there was no significant difference in parasitaemia between the two groups.

- There was no association between clinical malaria and severe pre-eclampsia.

- Placental lesions of malaria and pre-eclampsia were significantly greater in patients with severe pre-eclampsia.

- Finally, the association between placental malaria lesions and severe pre-eclampsia placental lesions was very strong, suggesting that placental malaria could be responsible for severe forms of pre-eclampsia.

Limitation

The limitations of our study were the low power of the study, the limited financial resources and the difficulty in evaluating certain specific histopathological lesions such as haemozoin due to the lack of birefringence and the risk of confounding bias because the two placental pathologies share common lesions such as calcifications.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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