ORIGINAL ARTICLE

SUBMICROSCOPIC PLACENTAL MALARIA: HISTOPATHOLOGY AND EXPRESSION OF PHYSIOLOGICAL PROCESS MEDIATORS

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ABSTRACT

Objetives: To relate histopathological events of placental malaria (PM), immune cell behavior and gene expression associated with cytokines, hypoxia, inflammation and angiogenesis in placentas with or without plasmodial infection. **Materials and methods:** Transversal design, with three independent groups. Women were recruited, and their placentas were collected in 2009-2016, in the hospitals of Puerto Libertador and Tierralta, northwestern Colombia. The sample size was defined by convenience. The malaria diagnosis was based on real-time quantitative PCR. **Results:** We studied 20 cases of PM by *P. vivax* (PM-V), 20 cases of PM by *P. falciparum* (PM-F) and 19 without PM; 95% of the cases of PM are submicroscopic placental plasmodial infection (SPPI). The three groups differ in frequency and number of histopathological events. Physiological process mediators showed significant difference between groups, except IL-2, VEGF, VEGFR-1 and C5a. **Conclusions:** Infected placentas are clearly different from uninfected ones. P. vivax behaves as pathogenic as *P. falciparum*. The approximation to the integral approach of the problem of PM is underlined. Submicroscopic placental plasmodial infection causes tissue and physiological mediator alterations as does microscopic infection, although probably to a lesser degree.

Keywords: Malaria; Plasmodium; Placenta; Histopathology; Pathology; Mediator; Colombia (source: MeSH NLM).

INTRODUCTION

Submicroscopic placental plasmodial infection (SPPI) by *Plasmodium falciparum* is frequent in endemic countries and contributes to the development of maternal anemia and low birth weight [1]. SPPI by *P. vivax* (SPPI-*vivax*) is almost unknown in the world.

The standard technique for malaria diagnosis in endemic areas is the thick blood smear, which, by definition, does not detect submicroscopic infections, which are usually asymptomatic. These two conditions, submicroscopic and asymptomatic, had kept SPPI in absolute oblivion until the beginning of the 21st century. But there are already strong arguments suggesting that asymptomatic infections have important consequences for health and society, and should be renamed as chronic malaria infections [2].

The immunopathogenesis of placental malaria (PM) should consider pregnancy to be a unique physiological state in which the maternal immune system must protect the mother against infection and other noxae, while modulating her immune response to prevent rejection by the semi-allogeneic fetus [3]. The presence of *Plasmodium* and its byproducts (such as hemozoin) in the placental tissue essentially alters the immune environment that regulates the placenta [4].

In the case of gestational and placental malaria, increased cytokines TNF-α, IFN-γ and IL-10 are associated with trophoblastic damage, low birth weight and prematurity [5]. The expression of placental proinflammatory cytokines is high in women with PM and the expression of anti-inflammatory cytokines is low [5-9].

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PM alters angiogenic remodeling [10,11]. Mononuclear-cell infiltration and its inflammatory products are associated with low birth weight; and a shorter and less impactful inflammatory process in multigravid women, when comparing with primigravid women. These differences may explain the different outcomes that these women and their offspring undergo [12-14].

In normal pregnancy, the evident physiological processes of apoptosis, inflammation, hypoxia, vasculogenesis and angiogenesis, among others, are strictly controlled. In these processes the role of cellular communication (CC) markers/mediators is decisive [15-19]. The CC system is a complex network between cells, that sends signals to other cells, the latter with receptor functions, producing a physiological response and a change in cellular activity. In CC, there are many markers of physiological or physiopathological processes and they have different origins (hormones, growth factors, cytokines, prostaglandins, leukotrienes, etc.) [20].

The objective of the study was to determine the association of submicroscopic infection by *P. vivax* or *P. falciparum* with histopathological events, the behavior of immune cells, cytokine-associated gene expression, hypoxia, angiogenesis and inflammation in placentas.

MENSAJES CLAVE

Motivation for the study: There is limited evidence on the histopathological and inflammatory effects of submicroscopic placental infection by *P. falciparum* and *P. vivax*.

Main findings: Placentas of women who live in the largest endemic area of Colombia, in the northwest of the country, were affected by submicroscopic plasmodial infection (SPPI), not detected with thick drop, but with quantitative polymerase chain reaction (qPCR). The SPPI causes tissue damage in the placenta and affects mediators of processes, such as inflammation, hypoxia, angiogenesis, among others, compared to non-infected placentas. Both *P. vivax* and *P. falciparum* act as pathogens.

Implications: Diagnostic and treatment actions for gestational plasmodial infection in prenatal consultation need to be greatly improved and should necessarily include the thick drop test in every control.

MATERIALS AND METHODS

Design and study population

A cross-sectional study was conducted by comparing three independent groups. The sample size was defined for convenience, according to the existing placental samples in the tissue bank of our research group. The selection included twenty samples with PM by *vivax* (PM-V), 20 with PM by *falciparum* (PM-F) and 19 samples without PM (PM-no) or control. The diagnostic test for plasmodial infection was the real-time quantitative polymerase chain reaction (qPCR) in placental blood.

The women were enrolled and their placentas collected in 2009 and 2016, in the hospitals of Puerto Libertador and Tierralta, municipalities in the south of Córdoba, in northwestern Colombia, a region where malaria is highly endemic [21]. South Córdoba, Urabá Antioqueño and Bajo Cauca Antioqueño make up the eco-epidemiological region that generates the most cases of malaria annually in Colombia [21].

The inclusion criteria were the following: permanent residence in this region for at least the last year; no history of pre-eclampsia/eclampsia, hypertensive pregnancy disease, diabetes, HIV, and toxoplasmosis, rubella, cytomegalovirus, herpes simplex (TORSCH); and giving birth in one of the hospitals having a 36-to-41-week gestation.

Gestational age was taken from the medical record. Women in the control group had to be afebrile and those in the plasmodial infection group may or may not have had a fever (only 1% had a fever); they should also voluntarily agree to participate in the study. The exclusion criteria were withdrawal of consent or the occurrence of any complication or disease.

Malaria diagnosis

The maternal peripheral blood was obtained at the time of delivery. The placenta was cleansed with saline solution (0.9%) and blood and tissue samples were taken from the maternal side of the placenta for diagnosis (thick drop, qPCR, histopathology) as indicated in other reports [22 25]. The researchers read the thick drop samples and considered them negative when 200 fields, with 100x magnification, were free of parasites.

The DNA was extracted using the Chelex-saponin method and qPCR [22,23]. The qPCR was run on the ABI 7500 FAST platform. Samples with a cycle threshold (Ct) <45 were analyzed in duplex species-specific reactions for *P. falciparum* and *P. vivax* [23]. Amplification of the 18S rRNA genes of the DNA was used for quantification. The DNA copy number was quantified from the genus-specific reaction against a standard curve using a plasmid containing a fragment of the 18S gene for *P. falciparum*.

Histopathological study

Placental tissue was processed according to standardized procedures [24,25]. Two fragments were taken from each placenta and from each one a plate was made for histological

study by light microscopy with classic procedures. One fragment came from a point near the umbilical cord insertion and the other from the middle zone (equidistant between the cord and the placental border). A total of 40 fields were read (20 per fragment). The reading was compared against the results of thick drop and qPCR. Total magnification of 400X was used for general histological reading. Total magnification of 1000X was used to determine the presence of infected erythrocytes (iE) or hemozoin.

Quantification of the expression of genes associated with markers/mediators

A tissue fragment preserved with RNA Later® (Qiagen) at 4 °C was used to quantify the expression of genes associated with markers/mediators and cytokines in placental tissue. The mediators were grouped as follows: proinflammatory (IL-2, TNF, IFNɣ, COX-1, COX-2, C5a), anti-inflammatory (IL-10, IL-4), angiogenic (VEGF, VEGFR-1) and hypoxic (HIF).

Different markers, except C5a, were measured by relative quantification of mRNA by qPCR. Relative quantification was done to determine the expression levels of the study mediators in relation to the expression levels of the constituent gene and to obtain the relationship between the gene of interest and the constituent gene. The Pfaffl procedure was applied to determine delta-delta TC as follows [26]:

$$\text{Gene expression} = \frac{\triangle (\text{Efficiency of the gene of interest})^{\text{CT of the gene of interest (control CT - sample CT)}}}{\triangle (\text{Efficiency of the constituent gene})^{\text{CT of the constituent gene (control CT - sample CT)}}}$$

By following the manufacturer's recommendations, the commercial kit Human C5a Elisa Kit was used to quantify the complement C5a fraction in placental serum samples.

Secondary information sources and study groups

After inclusion, a clinical-epidemiological questionnaire was applied. The medical record was used as a data source. Parturient patients and their placentas were evaluated, distributed in three groups: 19 in the PM-no group or control group, 20 in the PM-F group and 20 in the PM V group.

Statistical analysis

SPSS 18.0 and GraphPad Prism 5 were used. Significance decisions were made with a probability of less than 0.05. The Kolmogorov-Smirnov test was used to assess the normal distribution of quantitative variables. Levene's test was used to assess the homoscedasticity of variances. The Mann-Whitney test was used to determine if there was a difference between two independent groups. The non-parametric Kruskal-Wallis test was applied to determine if there was a difference between three independent groups, then the Dunn test was used to identify the pairs of groups that differed.

Ethical considerations

The project was endorsed by the Bioethics Committee of the SIU University Research Headquarters, University of Antioquia (Medellín, Colombia) (Act of Approval No. 07-32-126, Colciencias Project Code No. 111540820495, Contract No. 238-2007).

RESULTS

From the total, 95% (38/40) of PM cases are submicroscopic infections. From the pregnant women total, 25% (15/59) had history of malaria during the current pregnancy.

The age range was from 14 to 41 years and the average in the three groups was similar (PM no: 22; PM-F: 25; PM-V: 23). The average gestational age was 38.6 weeks (range from 36 to 41). Previous pregnancies averaged 2.8. From the total, 36% were in their first gestation, 20% in their second and 44% were multi-gestational (3 to 9). Of the 59 deliveries, 5 were by caesarean section.

At the time of delivery, the average hemoglobin value was 11.1 g/dL. Women with PM had lower hemoglobin levels than uninfected women (10.86 g/dL and 11.69 g/dL, respectively). Hemoglobin was lower in the PM-V group (10.75 g/dL) than in the PM-F group (10.97 g/dL), but without significant difference (p=0.506). The mean neonatal weight in the PM-no group was 2,974 g; in the PM-F group, 2,852 g; and in the PM-V group, 2,737 g. The difference in mean neonatal weight was 237 g between children in the PM-V and PM-no groups, and 115 g between children in the PM-V and PM-F groups.

The control group (PM-no) had absence of necrosis and higher frequency of atherosis, abruptio and thrombus than the other two. The PM-V group presented similar results to PM-F in atherosis, necrosis, infarction, fibrin deposits and thrombi, but less hemozoin and infected erythrocytes. Fibrin deposits were observed in all placentas. In the intervillous space, 95-100% of placentas showed hemorrhage and 6 out of 10 placentas showed thrombi and calcifications. There was significant difference ($p < 0.05$) between the three groups regarding abruption, syncytial nodes and hemozoin. The PM-F group generated this difference. In the species comparison, there was no significant difference ($p > 0.05$) (Table 1).

In the PM-no group, atherosis and abruption are rare, averaging 1.3 and 2.2 events, respectively. In the same order; infarction and villous edema had 9.1 and 8.7 events on average. In the same PM-no group, the amount of fibrin and

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syncytial node deposits is 72.5 and 109 on average. Placentas without infection have 366 villi, 1463 capillaries and 3.97 capillaries per villi. The average number of hemorrhages was 18, while the average number of thrombi and calcifications were 2 (they were in 60-70% of them) (Table 2).

In PM-F and PM-V groups, the appearance of placental infection reduces the amount of atherosis, villous edema, capillary hemorrhage and thrombi, but increases villous infarction, calcifications and immune cells. Only a few events show significant difference (Table 2).

Immune cells were found in all placentas, in all three compartments, and regardless of infection. The quantities were different, in the PM-no group, 9 immune cells in decidua, 22 in villous and 50 in intervillous space were observed; in the PM-V group, 58, 34 and 130 were observed respectively; in the PM-F group the highest amount of immune cells were found: 68, 45 and 157, respectively (Table 2).

Table 3 shows the expression of inflammatory, angiogenic and hypoxic mediators by group. Inflammation mediators (COX-1, COX-2, IL-10, IFNɣ, TNF, C5a) were significantly higher in PM. IL-2 and IL-4 varied little among the three groups. Although C5a did not show statistically significant difference between the groups, it had higher values in PM-V compared to the two other groups. Regarding angiogenesis, VEGF and VEGFR-1 showed no significant difference between groups, but their expression increased in groups with PM. Regarding hypoxia, HIF-1α was significantly different and with higher values in groups with PM. In general, the mediators that showed no significant difference between the three groups were IL-2, VEGF, VEGFR-1 and C5a. For VEGFR-1 and C5a, the high variability within each group is probably a factor affecting statistical significance when comparing the three groups.

Figures 1, 2 and 3 show the bivariate linear correlations between histological events and process mediators. In the group without infection, the predominance of negative and weak ($p < 0.10$) significant correlations (SC) is clear. Fibrin deposits, syncytial nodes and thrombi have the highest amount of SC. The 6 SC that fibrinoid deposits have are made with 7 process mediators, which are inflammation promoters (COX-2 [but not COX-1], C5a, IFNɣ [but not TNF], VEGF) and hypoxia (HIF) and angiogenesis mediators (VEGF, but not its receptor). The 5 SC of the syncytial nodes are made with 3 of the mediators that are also present in the relations with the fibrinoid deposits (COX-2, HIF and VEGF) and with cytokines 2 (pro-inflammatory) and 10 (anti-inflammatory). The 4 SC of thrombi occur with the inflammation mediators, TNF, IL-2, VEGF and the hypoxia mediator HIF.

On the other hand, it is necessary to highlight the SC that several mediators establish with some histological events. This is the case with HIF, which, in addition to being associated with syncytial nodes, fibrinoid deposits and thrombi, it is also associated with infarction. TNF is associated with abruptio, thrombi and villous capillaries.

In the PM-F group, SC reduce and the polarity changes; in this case, the poles are not the syncytial nodes, fibrinoid deposits and thrombi, but the only pole that stands out with

Table 1. Frequency of placental histological findings by study group

Zone and event	Placenta without malaria (PM-no)		Placental malaria by *falciparum* (PM-F)		Placental malaria by *vivax* (PM-V)		PM-No vs. PM-F vs PM-V	PM-V vs. PM-F
	n = 19	%	n = 20	%	n = 20	%	P value	P value
Decidua								
Atherosis	10	53	6	30	7	35	0.317	0.736
Abruption	12	63	8	40	3	15	0.009	0.077
Necrosis	0	0	1	5	2	10	0.350	1.000
Villosity								
Infarction	16	84	18	90	19	95	0.537	0.696
Edema	18	95	18	90	15	75	0.309	0.405
Syncytial nodes	19	100	20	100	15	75	0.005	0.056
Fibrinoid deposits	19	100	20	100	20	100	1.000	1.000
Intervillous space								
Thrombi	13	68	9	45	10	50	0.305	0.751
Calcifications	12	63	13	65	12	60	0.947	0.744
Hemorrhage	19	100	19	95	19	95	1.000	1.000
Hemozoin	0	0	10	50	6	30	0.002	0.197
Parasites	0	0	3	15	1	5	0.164	0.598

Table 2. Magnitude of placental histological events, according to the presence of plasmodial infection

Zone and event	Placenta without malaria (PM-no) n = 19 Mean ± SD	Placental malaria by *falciparum* (PM-F) n = 20 Mean ± SD	Placental malaria by *vivax* (PM-V) n = 20 Mean ± SD	MP-No vs. MP-F vs. MP-V p value	PM-No vs. PM-V; PM-F vs. PM-V [a] Significant differences
Decidua					
Atherosis	1.3 ± 1.5	0.5 ± 1	0.7 ± 1.2	0.198	
Abruption	2.2 ± 2.5	2.7 ± 4.0	0.8 ± 2.4	0.031	Yes; No
Necrosis	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.3	0.371	
DIC[b]	9 ± 6	68 ± 32	58 ± 30	0.000	Yes; No
Villosity					
Infarction	9.1± 8	11.1 ± 9	15.8 ± 11	0.068	
Edema	8.7 ± 6	6.9 ± 5	4.9 ± 5	0.095	
Fibrinoid deposits	72.5 ± 22	82.3 ± 34	65.5 ± 20	0.158	
Syncytial nodes	109 ± 67	150 ± 60	110 ± 42	0.037	No; No
Villosities	366 ± 44	364 ± 48	326 ± 61	0.056	
Capillaries	1463 ± 434	1,446 ± 634	1,043 ± 460	0.018	Yes; No
Capillaries / Villosity	3.97 ± 1.07	3.87 ± 1.35	3.15 ± 1.06	0.050	Yes; No
VIC[b]	22 ± 11.3	45 ± 19.8	34 ± 15.8	0.000	Yes; No
Intervillous space					
Hemorrhages	18 ± 8	13 ± 8	12 ± 9	0.090	
Thrombi	2.0 ± 3	1.1 ± 2	1.4 ± 2	0.345	
Calcifications	1.9 ± 1.8	11.1 ± 16.6	16.6 ± 22.6	0.492	
ISIC[b]	50 ± 24	157 ± 56	130 ± 54	0.000	Yes; No

[a] Indicates whether the Dunn test showed a difference between each pair of comparisons (PM-no vs. PM-V; PM-V vs. PM-F) with $p < 0.05$.
[b] DIC: Deciduous immune cells. VIC: villous immune cells. ISIC: immune cells in intervillous space. SD: standard deviation.

the villous capillaries (three SC: with TNF, HIF and IL-10). TNF had SC with edema, infarction, the number of villi and the number of capillaries per villi; IL-2 had SC with abruption, atherosis, thrombi and fibrinoid deposits.

For the PM-V group, SC abound as in the absence of infection, but with different focuses: abruption, thrombus, calcifications. The SC of abruption are formed with COX-1, C5a and HIF 1α; those of thrombi and calcifications with TNF and IL-2. Thrombi are also associated with VEGF, whereas calcifications are related to VEGFR-1.

DISCUSSION

This study found important differences in the placental effects of the two plasmodial species evaluated. The amount of abruption, syncytial nodes, villi, capillaries and capillaries

Table 3. Expression of inflammation, angiogenesis and hypoxia mediators according to study group

Mediator	PM-no n = 19 Mean ± SD	PM-F n = 20 Mean ± SD	PM-V n = 20 Mean ± SD	PM-No vs. PM-F vs. PM-V p value	PM-No vs. PM-V; PM-V vs. PM-F[a] Significant differences
COX-1	0.9 ± 0.3	13,2 ± 1.8	13.6 ± 1.1	0.000	Yes; No
COX-2	0.9 ± 0.4	5.9 ± 0.5	7.6 ± 0.7	0.000	Yes; No
IL-2	1.3 ± 0.5	1.0 ± 0.2	1.1 ± 0.2	0.413	
IL-4	1.3 ± 0.4	1.6 ± 0.4	1.6 ± 0.3	0.010	No; No
IL-10	1.2 ± 0.3	3.8 ± 0.6	3.8 ± 0.4	0.000	Yes; No
IFNɣ	1.4 ± 0.3	10.6 ± 1.6	8.9 ± 0.6	0.000	Yes; No
TNF	2.7 ± 0.5	10.2 ± 0.7	11.8 ± 0.5	0.000	Yes; No
HIF-1a	0.6 ± 0,5	1.2 ± 0.4	1.1 ± 0.3	0.000	Yes; No
VEGF	0.8 ± 0.4	1.0 ± 0.1	1.0 ± 0.1	0.179	
VEGFR-1	1.1 ± 2.6	2.4 ± 4.9	2.3 ± 5.1	0.891	
C5a [b]	77.7 ± 13.9	77.7 ± 37.6	94.7 ± 26.8	0.274	

[a] Indicates whether Dunn's test showed a difference between each pair of comparisons (PM-no vs. PM-V; PM-V vs. PM-F), with $p < 0.05$.
[b] For C5a, concentration in blood was measured and not the expression of the associated gene.

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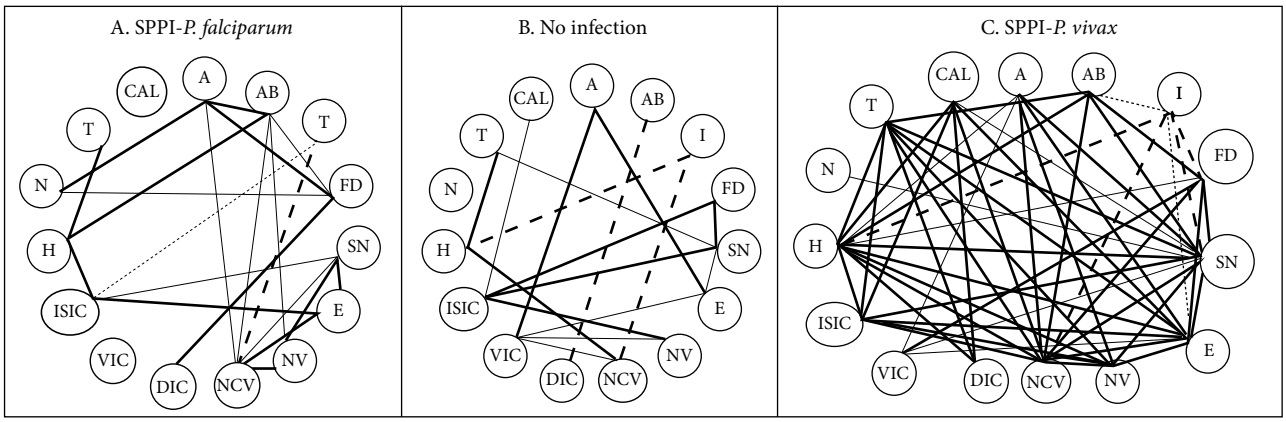
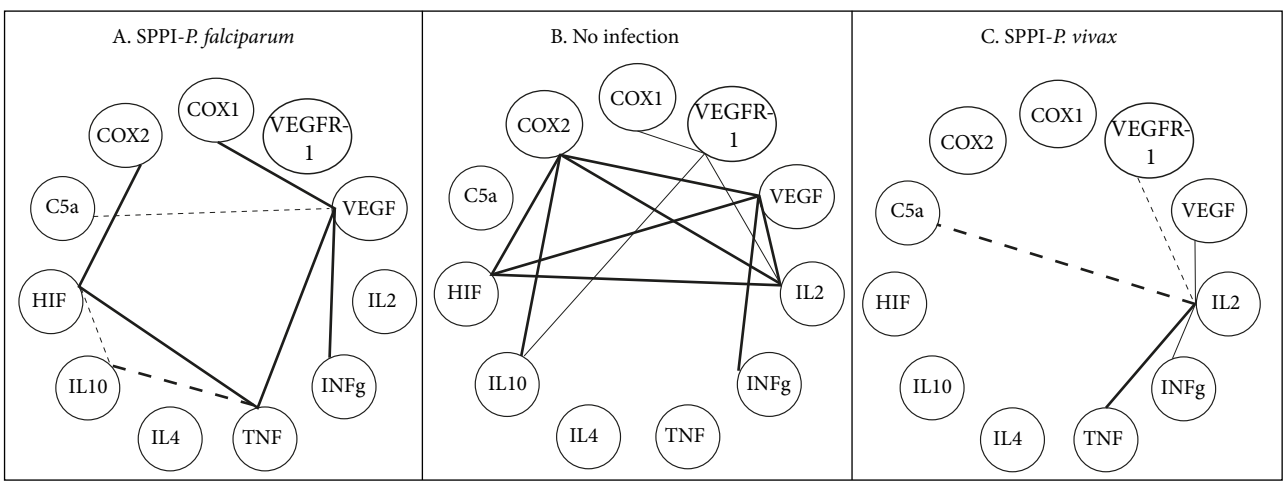


Each event always occupies the same position in the plane.
The circles represent the histological findings:
A: atherosis, AB: abruption, I: infarction, FD: fibrin deposits, SN: syncytial nodes, E: edema, NV: number of villi, NCV: number of capillaries per villus, DIC: decidual immune cells, VIC: villous immune cells, ISIC: intervillous space immune cells, H: hemorrhage, N: necrosis, T: thrombus, CAL: calcifications
Solid/continuous and dotted lines are positive and negative correlations, respectively.
The thickness or intensity of the line represents the degree of significance.
A. Group of placentas infected with *P. falciparum* B. Group of placentas without infection. C. Group of placentas infected with *P. vivax.*
In group B (without infection) all histological findings have a significant correlation (SC) except necrosis (N) which was the only finding not found in these placentas.
The amount of SC in the SPPI-*P. vivax* group is notorious in comparison to the other groups. In addition, most of the SC in PM-V are positive and only 4 of the total SC in this group are negative and refer to infarction with AB, H, NCV, SN, DF and E.

Figure 1. Significant bivariate linear correlations (SC) between placental histological even**ts**

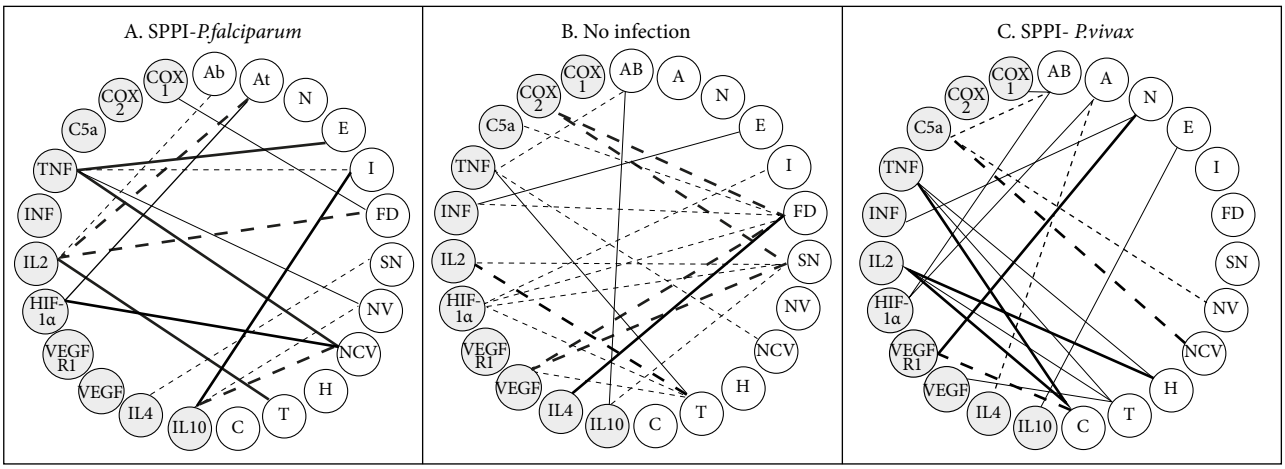
by villi is significantly lower when *P. vivax* is present, compared to *P. falciparum*. On the other hand, placentas infected with *P. vivax*, when compared to placentas without infection, generated more infarction and less abruption, edema, villi, capillaries and capillaries by villi. There is evidence of the pathogenic nature of PM-SPPI due to *P. falciparum* in pregnant women and their children [1]. Even in studies in the same geographical area where this study took place [27 29], submicroscopic placental infection by *P. vivax* causes harmful effects.

Comparisons of the *P. vivax* group against the *P. falciparum* group or against the group without infection strongly



The circles represent the mediators. The continuous and dotted lines are positive and negative SC, respectively. The thickness or intensity of the line represents the degree of significance.
In the group without infection all existing SC are positive, it is shown that the IL-2, COX-2, VEGF and HIF mediators have the highest amount of SC; followed by VEGFR-1, IL-10 and IFN**ɣ**. The SC absence from IL-4, TNF and C5a is notable. In the group with infection it is evident that SC change, and negative SC appear, which were not in the group without infection. In the SPPI-*P. vivax* group, less SC appear and IL-2 is the only center of existing SC. In the SPPI-P. *falciparum group* the SC are more decentralized. In both groups with infection, TNF and C5a present SC, which did not occur in uninfected placentas.

Figure 2. Significant bivariate linear correlations (CS) between mediators of placental processes of angiogenesis, inflammation and hypoxia



The circles represent the mediators. The solid and dotted lines are positive and negative significant correlations (SC), respectively. The thickness or intensity of the line represents the degree of significance.

In the group without infection all existing SC are positive, it is shown that mediators IL-2, COX-2, VEGF and HIF have the highest amount of CS; followed by VEGFR-1, IL-10 and IFNɣ. The absence of CS from IL-4, TNF and C5a is notorious. In the groups with infection it is evident that CS change and negative CS appear that were not in the group without infection. In IPP-*P. vivax*, fewer CS appear and IL-2 is the only centre of existing CS. In the IPP-*P. falciparum* group the CS are more decentralized. In both groups with infection, TNF and C5a present CS, which did not occur in placentas without infection.

Figure 3. Linear correlations between histological events and process mediators in the placenta.

support the placental pathogenic capacity of *P. vivax*. These findings provide a basis for explaining its effects by a mechanism that does not necessarily involve the cytoadhesion of infected erythrocytes to the placental tissue, as occurs in *P. falciparum* infections.

There is scarce information on the correlations between histological events and process mediators. When there is no infection, SC between histological events and process mediators are predominantly less than 10%. This can be interpreted as a homeostatic state, in which no specific response predominates, but all of them are active, in basal and balanced states. Otherwise gestation would be at risk.

When there is a plasmodial infection, the panorama for SC changes radically, both in the case of placental plasmodial infection by *P. falciparum* (SPPI-F), but more so in the case of placental plasmodial infection by *P. vivax* (SPPI-V). SC in the non-infection state disappear and new ones arise in plasmodial infections. The centers or poles of SC that were the syncytial nodes and fibrinoid deposits disappear and are replaced by poles that now correspond to the process mediators, mainly inflammation. IL-2, TNF and IL-10 are poles in SPPI-F, while IL-2, TNF and C5a are poles in SPPI-V. In the absence of infection, the villi and capillaries are virtually CS-free; when infection occurs, they emerge as poles. All this would express the exacerbated inflammatory state of the placenta as a consequence of the infection.

In this study, no hormones were measured at any point in gestation. Progesterone, estrogens, androgens and glucocorticoids are involved in gestation from implantation to delivery; their biosynthesis and metabolism are the result of complex pathways involving the fetus, the placenta and the mother [30]. Many sexual hormones clearly interact with the immune system and many mediators of physiological processes also interact with the immune system. There is, therefore, a complex physiological network between hormones, mediators of physiological processes and the immune system.

One of the strengths of this study is the comprehensive approach to PM, addressing the relationship between histopathological events and mediators of inflammation, angiogenesis and hypoxia. To our knowledge these relationships have not been studied before. We had a well-defined control group, which was negative for *Plasmodium* according to highly sensitive and specific technique (qPCR), without the presence of TORCH syndrome, HIV, eclampsia/preeclampsia, and diabetes. However, the presence of intestinal parasites and malnutrition could not be ruled out.

We consider the small sample size and the selection by convenience as limitations due to time and money available. The mediators evaluated basically represent the inflammatory process of the placenta at full-term, other process mediators were not sufficiently measured. The analysis of the correlations between histological findings and process mediators is superficial because there is little or no information available.

In conclusion, when SPPI occurs, there are placental tissue changes as well as changes in the expression of inflammatory process mediators, whether the causal agent is *P. falciparum* or *P. vivax*. If the parasite is pathogenic to the placenta, it is to be expected that when parasitemia increa-

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ses, effects will be stronger. Placental infection with *P. vivax* contributes to the increase in placental histological findings associated with tissue damage and deterioration. The alteration of the placental structure was mainly associated with the decrease of villi and the number of capillaries per villi, as well as with the increase of ischemic degenerative lesions associated with calcifications and infarction. These results could be correlated with low birth weight and low hemoglobin levels.

Authors' contributions: CLG and JCF conceived and executed all phases of the research, analyzed and interpreted the data, and produced the article.

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