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Interaction of histological events and physiological mediators in healthy placentas from malaria-endemic area in Colombia: An approach with a factorial model

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Abstract

The biological study of the placenta is fragmented and focused on morbid events. The interaction of histological events and mediators of physiological processes in healthy placentas in malaria-endemic areas is unknown. This study aimed to build a factorial model for the convergence of events and mediators in healthy placentas of women living in northwestern Colombia through a study of 44 placentas. Linear correlations and exploratory factor analysis were carried out with histological events and expression of genes related to mediators. The factor analysis allowed us the identification of three components. The first compound by the following histological variables: number of capillaries and villus, immune cells in villus, atherosis, and CD8+ lymphocytes. The second with articulation of histological variables (syncytyal nodes, fibrinoid deposits, thrombi and immune cells) and physiological mediators of apoptosis and regulation. The thirth included physiological mediators of hypoxia, angiogenesis, pro-inflammation and anti-inflammation. All components presented excellent predictive and construct validity, and excellent goodness of fit parameters. In healthy placentas, the factorial structure of histological events and physiological mediators in three underlying components that support their interactions was demonstrated. These findings are significant because they help improve the study of healthy placental biology in malaria endemic areas and evaluate mechanisms that alter its morphology and function, with their subsequent risk for pregnancy and maternal-fetal health. *(Afr J Reprod Health 2022; 26[11]: 92-105).*

Keywords: Placenta, histology, cytokines, factor analysis

Résumé

L'étude biologique du placenta est fragmentée et centrée sur les événements morbides. L'interaction des événements histologiques et des médiateurs des processus physiologiques dans les placentas sains dans les zones d'endémie palustre est inconnue. Cette étude visait à construire un modèle factoriel pour la convergence des événements et des médiateurs dans les placentas sains des femmes vivant dans le nord-ouest de la Colombie à travers une étude de 44 placentas. Des corrélations linéaires et une analyse factorielle exploratoire ont été réalisées avec des événements histologiques et l'expression de gènes liés à des médiateurs. L'analyse factorielle nous a permis d'identifier trois composantes. Le premier composé par les variables histologiques suivantes : nombre de capillaires et de villosités, cellules immunitaires dans les villosités, athérose et lymphocytes CD8+. La seconde avec articulation de variables histologiques (ganglions syncytyaux, dépôts fibrinoïdes, thrombi et cellules immunitaires) et médiateurs physiologiques de l'apoptose et de la régulation. Le troisième comprenait des médiateurs physiologiques de l'hypoxie, de l'angiogenèse, de la pro-inflammation et de l'anti-inflammation. Tous les composants présentaient une excellente validité prédictive et de construction, ainsi qu'une excellente qualité des paramètres d'ajustement. Dans les placentas sains, la structure factorielle des événements histologiques et des médiateurs physiologiques dans trois composants sous-jacents qui soutiennent leurs interactions a été démontrée. Ces résultats sont importants car ils aident à améliorer l'étude de la biologie placentaire saine dans les zones d'endémie palustre et à évaluer les mécanismes qui modifient sa morphologie et sa fonction, avec leur risque ultérieur de grossesse et de santé materno-fœtale. *(Afr J Reprod Health 2022; 26[11]: 92-105).*

Mots-clés: Placenta, histologie, cytokines, analyse factorielle

Introduction

The placenta is a very complex organ, of mixed maternal and fetal origin, with multiple functions from the beginning to the end of the pregnancy. The placenta has essential endocrine and immunological functions that modulate maternal-fetal physiology and metabolism. During the usual time of pregnancy, it presents morphological and functional transformations. At present, multiple

interactions between the placenta and maternal tissues are recognized like endocrine signals for the development of the placenta, migration of extravillous trophoblast cells towards the uterine wall where they interact with the cells of the mother's innate immune system resulting in multiple physiological effects[1-3].

Concerning the synthesis of scientific evidence in this field, it is worth highlighting some recent reviews[2-6]. Maltepe and Fisher described the emerging research in placental biology and its importance for fetal and adult health, highlighting the complexity of its conformation and functioning process[4]. Turco and Moffett reviewed the morphological, molecular, and functional aspects of human placental formation, emphasizing the trophoblast[2]. Costa's group analyzed the main infection routes and the associated adverse maternal and fetal outcomes, taking into account TORCH pathogens (Toxoplasmosis, Other agents, Rubella, Cytomegalovirus, Herpes simplex), including Zika virus, *Listeria monocytogenes*, *Treponema pallidum*, and Parvovirus B19 among the "other agents"[3]. Iacovelli's group conducted a systematic review to explore the strength of the association between different maternal and pregnancy characteristics with the appearance of the abnormally invasive placenta[5]. Reijnders *et al*. conducted a systematic review of the placenta's development and function in women with a history of complications related to this organ[6]. To these revisions are added other manuscripts that describes the complexity of the macroscopic and microscopic anatomy of the placenta and its histology[7].

However, there is insufficient evidence articulating various placental components in healthy women. A review in PubMed and Science-Direct (December 20, 2020) with the expressions (*normal placenta [Title]) AND (histology [Title / Abstract])), (healthy placenta [Title]) AND (histology [Title / Abstract])), (placenta [Title]) AND (physiology [Title / Abstract]*) and (*placenta [Title]) AND (immunology [Title / Abstract]*) generated few results and the majority of them around morbid events. The literature registers limited information on the relationship between the histological characteristics and the molecules involved in the physiological processes that occur in normal placentas of apparently healthy pregnancies from malaria endemic areas. Normal physiological processes involve many molecules or substances, such as cytokines, chemokines, receptor molecules, ligands and others mediators of placental processes as angiogenesis, hypoxia, inflammation, immunity or apoptosis, etc. (hereinafter mediators); as well as placental histological features such as atherosis, necrosis, abruption, villous edema, etc. (hereinafter events). The evaluation of the interrelationships of histological events with mediators in the placenta is necessary to advance knowledge of the functioning of that organ.

There are multiple situations where the problem under study cannot be adequately summarized in a few variables. To find a solution, methods for the analysis of multiple variables, such as factor analysis (FA), are used. The FA applies to numerical variables or *items* that can be grouped into a factor, component, dimension, or construct, terms that we use here as synonyms and that express an underlying characteristic common to the set of variables, which would be the focus of the inquiry in a study. The FA is an advanced statistical method to simplify the relationships between a group of indicators, identifying an underlying factor from those variables with the most significant explanatory power in a correlation matrix. The FA generates more easily interpretable information, indicating why some variables are more related to each other and less to others. This explanation refers to a *factor* that reveals a group of correlated items' underlying structure, indicating how they tend to cluster. By analyzing the conceptual content of the *items* that belong to the same *factor*, the underlying phenomenon that explains their interactions could be understood[8,9].

In this sense, research on the placenta that analyzes the relationship between mediators and events with FA is almost absent. It is essential to highlight the research made by Dobaño's group. They carried out a multifactorial study of the primary immune functions in a complete set of TH1, TH2, TH17 and regulatory cytokines, pro-inflammatory and anti-inflammatory cytokines and chemokines, and growth factors, in a cohort of 540 pregnant women living in five tropical malaria-endemic countries[10]. It is also worth mentioning the study by López-Guzmán and Carmona-Fonseca that relates events with mediators in placentas with and without infection by *Plasmodium*[11]. However, these two antecedents present the following

limitations: the first focuses on immune markers and does not perform measurements of the histological component, while the second performs univariate analysis and explores some correlations, without developing a multivariate analysis to identify clusters of physiological measurements. and histological.

Therefore this study was conducted to describe the interaction of histological features and physiological mediators in healthy placentas from malaria endemic areas, by constructing a factorial model.

Methods

Study design

A cross-sectional study, since all variables were measured at the same time.

Study setting

The study region is located Northwestern Colombia and it includes endemic areas of two departments Antioquia (Urabá and the Bajo Cauca) and Córdoba (upper basins of the Sinú and San Jorge rivers). This region is the one reporting the highest number of malaria cases in the country[12].

Subjects

During eight years (2009-2016), 334 placentas were obtained in this region and conserved in formaldehyde for histological slides, and RNA Later® (Qiagen) at 4°C for gene expressions associated with the mediators. From a total of 334 samples, 44 were selected, corresponding to the population of healthy placentas (Figure 1).

Eligibility criteria

For the selection of the placentas, the pregnant women met the following inclusion criteria: Being a permanent resident of the region (at least a year before the beginning of the study); not have diseases or infections that are screened in antenatal care (preeclampsia/eclampsia, high blood pressure, diabetes, HIV or other sexually transmitted infections, anemia, malaria, and TORCH - Toxoplasmosis, Other agents, Rubella, Cytomegalovirus, Herpes simplex-); being healthy according to a general clinical examination; without history of abortion to repeat or stillbirth (maximum one of each, in reproductive life); delivery at term (37.0 to 42.0 weeks), without fetal distress, and voluntarily participate in the study.

Histological features

Samples of placental tissue were obtained from the maternal face and were processed using standardized procedures; fragments were taken close to the insertion point of the umbilical cord and in the middle area (equidistant between the cord and the placental edge) for making histological slides of the fragments for reading with light microscopy in 40 fields with 400X magnification[11,13]; according to the guidelines reported by previous placental histology studies[14-19].

Many microscopic histological features appear in normal placentas, so their finding does not indicate placental pathology. Histological features were evaluated quantitatively in two ways: a) for atherosis (A), necrosis (N), abruption (Ab), villus edema (E), villus infarction (I), hemorrhage (H) in intervillus space (IVS), and thrombus (T) in IVS, the fields with the presence of the feature were added, and this sum was considered as the amount of each event; b) for fibrin deposits (FD), syncytial nodes (SN), chorionic villus (V), fetal capillaries (C), capillaries per villi (C/V), calcifications (Ca) in IVS, and immune cells (cellular infiltrates into the decidua, villus and IVS), and the number of cells in the process of apoptosis (apoptotic and pre-apoptotic); each feature was enumerated in each field, the total sum of features in the 40 fields was made, and the sum was divided by 40 to obtain the average. The variables evaluated were defined considering criteria on general placental histopathology and histopathology of placental malaria[20-39].

Immunohistochemical study

In formalin-fixed, paraffin embedded tissues, the EnVision system (Dako) was used for the quantification of lymphocyte clusters with anti-Human CD4+ (Clone 4B12, Dako), anti-Human CD8+ (Clone C8/144B, Dako), anti-Human CD14+ (Clone TÜK4, Dako), anti-Human CD56+ (Clone 123C3), and anti-Human CD68+ (Clone PG-M1, Dako). Briefly, the paraffin sections were deparaffinized and rehydrated in xylene and graded

alcohols. After blocking with peroxidase in ChemMate peroxidase-blocking solution (Dako), the slides were incubated with the primary antibodies. After the application of the peroxidase-labelled polymer, the slides were incubated with the diaminobenzidine substrate chromogen solution, counterstained with hematoxylin, washed again, dehydrated, and mounted. The immunohistochemical slides were observed using a Zeiss Axio Imager M2 light microscope equipped with a Zeiss Axio Cam HRc Camera to capture placenta images. Ten photos were collected per slide, with 40X objective lens. Subsequently, each photograph was analyzed and the number of cells was counted for each photo. The number of positive cells was calculated and analyzed using the Image J software[40].

Measurements of genes expressions associated with the mediators

The following measurements were made: i) apoptosis by Fas and FasL; ii) hypoxia (HIF1-α); iii) angiogenesis (VEGF and VEGF-R); iv) pro-inflammation (Cox1, Cox2, IFN-γ, and TNF-β); v) anti-inflammation (IL2, IL4, IL10, and TGF-β); v) immune regulation (FOXP3 and CTLA4; IL10 also has a regulatory role). These measurements were complemented with the measurement of gene expression of mediators in maternal peripheral blood: IFN-γ, TNF-β, IL2, IL4, IL10, and TGF-β.

The measurement of the genes expressions associated with these mediators of physiological processes was based on a tissue fragment preserved with RNA Later® (Qiagen) at 4°C, with relative quantification of mRNA by qPCR (expression levels of the mediators versus the expression levels of the housekeeping gene), following the manufacturer's recommendations and the model validated by Pfaffl 2001[11,41].

Bias control

Selection bias was controlled by applying the eligibility criteria by research personnel. Information bias was mitigated by training and standardization of fieldwork, internal quality control in the laboratory, implementation of the manufacturer's instructions for the tests, histological criteria validated in previous studies, and double data entry.

Analysis plan

Descriptive analysis

The variables were described with mean, standard deviation and range. The assumption of normality was evaluated using the Shapiro-Wilk test. Linear correlations of events and mediators were measured with the Pearson coefficient when the two variables presented normal distribution and with the Spearman coefficient when one or both variables did not fulfill the normality assumption. Inter-element relationships were determined with Cronbach's alpha, which is satisfactory for reliability with values ≥0.70.

Exploratory multivariate analysis

Exploratory FA was performed using the principals' components analysis extraction method, estimation of the proportion of the variance explained by the variables included in each factor, Varimax Rotation with Kaiser Normalization, goodness-of-fit with KMO (Kaiser-Meyer-Olkin), and Bartlett's Test of Sphericity. Factorial loads or coefficients λ ≥0.30, KMO ≥0.80, and statistically significant Bartlett interpreted as satisfactory[8,9]. KMO and Bartlett test defined that it was possible to factor the original variables efficiently[42,43].

Confirmatory multivariate analysis

To facilitate the interpretation of the constructs, once the components were identified by exploratory FA, the variables with λ coefficients ≥0.50 (which have a high probability of forming a construct in a confirmatory FA) were taken to determine the reliability (Cronbach's alpha), content validity (λ coefficients ≥0.30), predictive validity (percentage of explained variance) and goodness of fit of the domain that they make up [43]. In the confirmatory FA of the variables with λ coefficients ≥0.50, we used structural equations with the method of estimation of maximum likelihood; the goodness of fit was determined with CFI (comparative fit index), TLI (Tucker–Lewis index), NFI (normalized fit index) and IFI (incremental fit index), which are considered satisfactory for values ≥0.70. It was complemented with RMSEA (root mean square error of approximation), which is satisfactory with values

≤0.10; ECVI (expected cross-validation index) that indicated the potential for replication of the model (It does not have a reference value for interpretation), and Hoelter that calculates the sample size that would be sufficient for representing a model adequately[44].

The analyzes were done with SPPS (Statistical Package for Social Sciences) 27.0. A p-value lower than 0.05 was taken as significant.

Results

The placentas belong to women with a mean age of 22.6 ± 5.9 years (range of 14 to 42 years); 39% first pregnant, 28% second pregnancy, 17% third and 16% with 4-10 pregnancies; the average parity was 2.8 ± 2.3 (range between 1-10); total pregnancy (including current) 2.3 ± 2.0, term deliveries 1.2 ± 1.9, history of abortion 0.12 ± 0.47 (7,3% of women), history of stillbirth (2.8%). The newborns were all born alive, with an Apgar index 1 minute 7.8 ± 0.9, with height 49.3 ± 1.8 cm, with a 33.8 ± 0.7 cm head circumference and a weight of 3171 grams.

In the decidua, the highest average of events corresponded to the abruptio, in the villus were the capillaries and the number of villus, and in the IVS, the presence of hemorrhage predominated. The most significant cellular infiltrates occurred in the IVS; the predominant lymphocyte populations were CD56+ and CD68+. A high number of apoptotic cells were found in the placentas. Most of these variables presented a non-normal distribution, except for syncytial nodes, capillaries, capillaries per villus, hemorrhage, and CD14+ and CD56+ lymphocytes (Table 1).

Necrosis registered zero (0.0) in 42 placentas, one had a value of 2.0 and another 11.0 (it was not considered for the following analyzes). In the mediators, the highest average values (or median and interquartile range if the distribution is not normal) were FasL, Fas, and TNF-β, and those that fitted the normal distribution were Fas, FasL, VEGF, Cox2, IL2, and CTLA4. Finally, in the maternal peripheral blood measurements, the median was higher for the pro-inflammatory markers (Table 2).

Table 3 shows the factorial loads of the variables that made up the two components extracted in the FA: *Component 1*: composed of 20 events, of which 6 did not have loads of significant magnitude (λ <0.30 in abruptio, CD56+, cells in apoptosis, FOXP3-p, IFN-γ[Placenta] and TNF-β [placenta]), of the 14 items with significant λ only two (14%) were mediators in maternal peripheral blood (IFN-γ and TNF-β) and 12 histological events, some of the villus (edema, infarction, capillaries, villus, capillaries per villus, and immune cells in villus), the IVS (hemorrhage, calcifications), the decidua (atherosis) and lymphocyte cluster (CD8+, CD14+, and CD68+). *Component 2*: comprised of 22 events, of which 9 had a positive λ coefficient, and 13 presented negative loads, indicating that this component derives to two sub-domains, one in which histological events predominate, and another that predominantly grouped mediators.

By taking the variables with a high coefficient λ (≥0.50), it was possible to simplify the complexity of the histological events in six variables that make up domain 1 and explain 54.0% of the variance; this construct presents excellent goodness of fit parameters (it is confirmed that the factorial structure is statistically adequate for the data analyzed); even with 26 placentas valid and reproducible results could be obtained for this factor. The second domain is composed of four histological events, two apoptosis mediators and one regulation molecule; as in domain 1, high predictive validity was found according to the percentage of explained variance and excellent goodness of fit parameters in the confirmatory model of this factorial structure. Similar findings were observed in domain 3, consisting of 8 mediators of hypoxia, angiogenesis, pro-inflammation and anti-inflammation (Table 4).

In synthesis, domain 1 represents the histological, domain 3 the physiological mediators and domain 2 a transition between both types of biological measurements. Together, the three domains show that it is possible to reduce the complexity of placental events in malaria endemic areas, initially represented in 43 different variables, to simpler factorial structures with a maximum of 8 variables. This too is equivalent to saying that this research identifies the variables with the greatest explanatory power of the central physiological and histological processes to understand healthy placental biology in malaria endemic areas, for its later use as a comparator of the effects of placental malaria and other infectious diseases. The three domains are necessary to understand the histology-

Table 1: Description and normality test of histological events

Event	Mean±St dev	Median (IQR)	Range	p(Shapiro- Wilk)
Deciduous				
Atherosis	1,5±2,0	1,0 (0,0-2,0)	0,0-11,0	0,000
Necrosis	0,3±1,7	0,0 (0,0-0,0)	0,0-11,0	0,000
Abruptio	2,0±3,0	0,5 (0,0-2,5)	0,0-12,0	0,000
Villus				
Edema	9,5±8,4	8,0 (1,0-17,0)	0,0-26,0	0,001
Infarction	7,0±7,9	5,0 (1,0-10,5)	0,0-27,0	0,000
Fibrinoid deposits	73,5±31,0	70,0 (58,5-83,0)	0,0-218,0	0,000
Syncytyal nodes	107,3±52,2	90,5 (69,5-143,0)	0,0-233,0	0,093
Villus	384,8±101,7	358,0 (313,0-414,0)	0,0-643,0	0,000
Capillaries (thousands)	2,9±1,0	2,8 (2,-3,5)	1,1-6,0	0,404
Capillaries/Villus	7,8±2,3	8,2 (5,8-9,3)	3,9-13,9	0,078
Intervillous space (IVS)				
Hemorrhage	14,9±9,1	16,0 (9,0-20,5)	0,0-31,0	0,109
Thrombi	1,3±2,3	0,0 (0,0-2,0)	0,0-9,0	0,000
Calcifications	2,2±2,9	2,0 (0,0-3,0)	0,0-16,0	0,000
Cellular immunes infiltrates				
Immune cells in decidua	6,6±3,0	6,0 (4,0-9,0)	2,0-14,0	0,032
Immune cells in villus	29,8±28,0	25,5 (14,5-34,0)	0,0-143,0	0,000
Immune cells in IVS	87,7±84,9	65,0 (43,0-91,0)	17,0-376,0	0,000
Cluster of lymphocytes				
CD4+	10,9±0,9	11,0 (10,0-12,0)	10,0-13,0	0,003
CD8+	9,8±1,1	10,0 (9,0-11,0)	8,0-11,0	0,006
CD14+	30,6±3,7	32,0 (28,0-34,0)	23,0-37,0	0,534
CD56+	76,7±4,1	78,0 (74,0-79,0)	70,0-87,0	0,484
CD68+	45,0±3,0	46,0 (43,0-48,0)	40,0-49,0	0,011
Apoptosis				
Celapop (Cells in apoptosis)	42,6±2,8	42,0 (41,0-45,0)	39,0-49,0	0,007

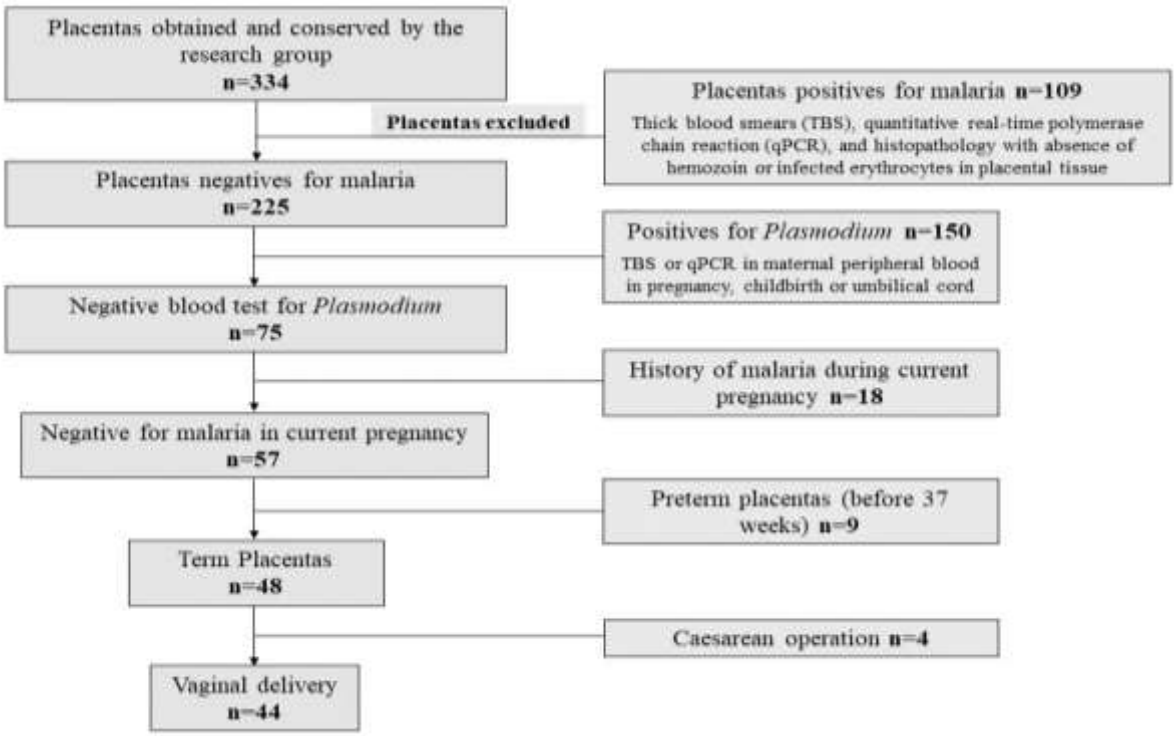

Figure 1: Selection flowchart of the study population of 44 placentas

Table 2: Description and normality test of the mediators of placental physiological processes and maternal peripheral blood

Mediator	Mean±St dev	Median (IQR)	Range	p(Shapiro- Wilk)
Placenta				
Apoptosis				
Fas	3,1±0,7	3,0(2,6-3,8)	2,0-4,6	0,297
FasL (Fas ligand)	6,8±0,9	6,5(6,2-7,5)	5,2-9,0	0,119
Hypoxia				
HIF1-α	0,6±0,4	0,7(0,2-0,9)	0,0-2,0	0,008
Angiogenesis				
VEGF	0,8±0,4	0,9(0,6-1,0)	0,1-1,4	0,054
VEGF-R	1,1±3,0	0,2(0,1-0,7)	0,0-11,7	0,000
Pro-inflammation				
Cox1	0,9±0,3	1,0(0,8-1,0)	0,1-1,5	0,003
Cox2	0,8±0,4	1,0(0,4-1,0)	0,1-1,7	0,072
IFN-γ	1,3±0,3	1,3 (1,2-1,4)	0,1-1,9	0,000
TNF-β	2,6±0,7	3,0(2,3-3,1)	0,1-3,4	0,000
Anti-inflammation				
IL2	1,3±0,5	1,2(0,9-2,0)	0,4-2,3	0,067
IL4	1,3±0,4	1,2(1,1-1,4)	0,0-2,0	0,002
IL10[a]	1,2±0,4	1,2(1,0-1,3)	0,1-2,0	0,007
TGF-β	1,3±0,3	1,3 (1,0-1,4)	1,0-2,0	0,001
Regulation				
FOXP3	0,3±0,1	0,3(0,2-0,3)	0,1-0,7	0,001
CTLA4	1,4±1,2	1,3(0,4-2,2)	0,0-4,0	0,087
Peripheral maternal blood				
Anti-inflammation				
IL2	1,8±0,4	1,9(1,4-2,1)	0,7-2,4	0,022
IL4	1,4±0,3	1,4(1,3-1,7)	1,1-1,9	0,054
IL10 [a]	0,8±0,5	1,0(0,2-1,0)	0,1-1,9	0,017
TGF-β	0,6±0,3	0,7(0,3-0,8)	0,0-1,0	0,013
Pro-inflammation				
IFN-γ	2,7±0,6	2,9(2,4-3,0)	1,1-3,2	0,000
TNF-β	2,8±0,7	3,0(2,6-3,0)	0,2-3,8	0,000

[a] It also has a regulatory role.

physiology relationship, but it is also shown that each one makes sense in itself, given its content validity, predictive validity, good goodness of fit of the confirmatory model, and adequate sample size.

Discussion

This study on the interaction of histological processes and physiological mediators in healthy placentas from study region shows multiple linear correlations between apoptosis, hypoxia, angiogenesis, inflammation, regulation, and others, which were grouped into three principal domains. This revealed several issues: (1) The complexity of the placenta and its anatomical, histological, and physiological processes (each with multiple variables or events to measure); (2) despite the morphological and functional complexity of this organ, the FA allowed obtaining three components in which the correlations between various variables can be grouped into underlying dimensions (new constructs to research). In exploratory FA, the FOXP3 receptor, CD56+ lymphocytes, IFN-γ and TNF-β, cells in apoptosis, and abruptio, all in the placenta, do not contribute significantly to the components generated. This situation is remarkable because CD4+ (with a high factorial load with component two) and Foxp3 are a unique subset of the immune response's regulatory elements that determine peripheral tolerance and are critical to the resolution of tissue inflammation. For this reason, subsequent studies will have to determine if FOXP3 in the placenta lacks the importance described in other organs or if the particularities of

Table 3: λ coefficients/factorial loads of histological events and physiological mediators in the placenta and maternal peripheral blood (with the extraction of two components) (ordered from highest to lowest value in each component)

Component 1		**Component 2**	
Variable	**Coefficient λ**	**Variable**	**Coefficient λ**
Capillaries	0,820	Syncytial nodes	0,752
Villus	0,756	Thrombi	0,739
CD+8	0,748	FasL	0,730
Capillaries/Villi	0,706	IL4 (mother)	0,704
ICV	0,656	Immune cells in villus	0,698
IFN-γ (mother)	0,610	Fas	0,691
Atherosis	0,534	CTLA4 (placenta)	0,523
TNF-β (mother)	0,510	Fibrinoid deposits	0,498
Hemorrhage	0,465	Immune cells in decidua	0,488
Calcifications	0,387	HIF1-α	-0,833
CD68+	0,337	VEGF	-0,827
Edema	0,315	IL2 (placenta)	-0,734
Infarction	-0,475	Cox2	-0,712
CD14+	-0,371	Cox1	-0,675
FOXP3 (placenta)	0,190	IL10 (mother)	-0,568
Abruptio	0,102	TGF-β (placenta)	-0,566
CD56+	0,094	IL2 (mother)	-0,512
Apoptotic cells	-0,068	CD4+	-0,485
IFNγ (placenta)	0,070	TGF-β (mother)	-0,424
TNF-β (placenta	-0,008	VEGF-R	-0,317
		IL4 (placenta)	-0,316
		IL10 (placenta)	-0,300

Table 4: Reliability, content and predictive validity, and goodness-of-fit parameters of three possible domains (by confirmatory factor analysis)

	Domain 1[a]	Domain 2[b]	Domain 3
Content validity			
Variables and Coefficient λ	C = 0,91 V = 0,70 C/V = 0,77 ICV = 0,78 A = 0,50 CD8 = 0,69	SN = 0,89 FD = 0,78 T = 0,51 ICIV = 0,75 FasL = 0,50 Fas = 0,53 CTLA4p = 0,83	HIF1-α = 0,77 VEGF = 0,76 Cox1 = 0,64 Cox2 = 0,76 IL10g = 0,55 IL2g = 0,55 IL2p = 0,75 TGFβ_p = 0,58
Reliability			
Cronbach's α	0,82	0,81	0,83
Predictive validity			
% Explained variance	54,0	49,0	45,8
Goodness-of-fit parameters **(default model)**			
NFI (normalized fit index)	0,840	0,72	0,65
IFI (incremental fit index)	0,88	0,94	0,84
TLI (Tucker–Lewis index)	0,71	0,83	0,62
CFI (comparative fit index)	0,89	0,92	0,79
RMSEA (root mean square error of approximation)	0,22	0,01	0,11
ECVI (expected cross-validation index)	1,49	1,36	1,84
HOELTER .05 (default/ independence)	26	31	44

The following variables were not included due to their Coefficient λ (<0.50):
[a] IFN-γ (mother)=0,40 and TNF-β (mother)=0,41. [b] IL4g = 0,40.

the group evaluated would support this result[45]. Concerning CD56+, it should be noted that it has been proven that fetal cytotrophoblasts can direct the migration of these maternal immune cells (in addition to natural killer (NK) cells, macrophages, and other T cells); their presence being more

critical during the development of pregnancy and probably less relevant at the end[46]. Something similar could happen with apoptosis, meaning its role is less relevant at the end of pregnancy. This process has traditionally been studied for pathological situations, and it would not be as decisive in the biology of healthy placentas[47,48]. Similarly, IFN-γ and TNF-β, which regulate the metabolism of placental calcitriol, the first favoring its production and the second through its catabolism[49], did not show relevance in the FA of this study, suggesting they are less relevant at the end of pregnancy. Despite the above, it must be kept in mind that these components have generally been studied with preclinical models (in vitro or in vivo) and in diseases or pregnancy complications, which makes their comparison with the findings of healthy placentas difficult.

In confirmatory FA domain 1 summarizes the main histological events to explain the structure of the healthy placenta in malaria endemic areas. In this study, it was observed that the number of chorionic villi and the vessels in them, the number of immune cells in these villi, and the number of CD8+ cytotoxic lymphocytes, in conjunction with deciduous atherosis, are the key variables to consider in the placenta normal. Now it is clear that villi and vessels work closely related because a healthy villu requires a certain number of vessels in it, as well as immune cells.

The second domain comprises four histological events (from villus and intervillous space), two apoptosis mediators and one regulation molecule; this constitutes important evidence of the convergence of histological events and their correlation with physiological mediators in what could be called healthy placental homeostasis. For this factor, it is essential to remember that the control of inflammation depends on the decrease in proliferation and maturation of immune cells, induction of apoptosis of active leukocytes, and inhibition of inflammatory mediators' secretion[50]. Furthermore, in relation to the mediators included in this domain, the Fas receptor and the Fas ligand (FasL) are key elements of the apoptosis process, which they activate. Apoptosis depends on *de novo* protein synthesis and the activation of biochemical factors resulting from a balance modification in favor of the expression of proapoptotic genes over antiapoptotic genes. A close relationship exists between the participation of FasL and cytotoxicity mediated by CD8+ T lymphocytes, an effector mechanism of great importance in the antitumor immune response[51,52]. In domain 3, made up of 8 mediators of hypoxia, angiogenesis, pro-inflammation and anti-inflammation; this factor shows a Th1 (pro-inflammatory) immune response, which makes it possible to appreciate that in the maternal-fetal interface, the Th2 immune response (anti-inflammatory/regulatory) does not always have to predominate. It also reflects the importance of pro-inflammatory cytokines' controlled response and its correlation with the histological events described in domain 1, for labor and a subsequent defense against infections[53,54]. The above is consistent with a previous review on the immunology of pregnancy that alludes to the Th1-Th2 cooperation theory, according to which Th1 cytokines participate in the induction of the Th2 response, which is based on studies that showed connective tissue decidual, Hofbauer cells, and other histological events as a source of cytokines that induce maternal-fetal tolerance, the local immune response against different microorganisms, and placental endocrine production. In the same direction, other authors have shown that pro-inflammatory cytokines' production increases during delivery, which increases the activation of macrophages, neutrophils, NK cells, prostaglandins, and metalloproteases that improve natural immunity processes, uterine contractions, tear and cervical dilation[55].

The placental mediators of hypoxia (HIF1-α), angiogenesis (VEGF), pro-inflammation (Cox1 and Cox2) and anti-inflammation (IL2, TGF-β), as well as maternal peripheral blood anti-inflammatory mediators (IL2, IL10), too could support the Th1-Th2 cooperation theory and its interaction with other placental processes that must coexist (without the predominance of any of them) to guarantee a physiological balance in which its basal levels ensure a pregnancy free of diseases or complications. Proof of the above can be found in studies that have documented the coexistence of Th1 responses with activation of cellular immunity (mainly macrophages) and a Th2 that modulates the antibody-mediated response and inhibits macrophage functions; for example, IL-4 (with feedback of the IL-10) which promotes a transition from Th0 to Th2 lymphocytes with subsequent inhibition of the cytotoxic response. For this

reason, pregnancy is currently explained by interrelated Th1/Th2/Th3/Tr1 systems, whose lack of regulation has been associated with abortions[55]. It has also been shown that VEGF and its receptors are determining factors for vasculogenesis and angiogenesis, specifically the vascular development of embryogenesis, where oxygen and oxidative stress modify their concentrations; this points to the need for a balance between hypoxia and angiogenesis[56]. There are no studies that evaluate this convergence. Therefore, this hypothesis on hypoxic, angiogenic, and inflammatory homeostasis could not be contrasted with other sources and requires additional studies. Furthermore, the convergence of apparently mutually exclusive biological events (for example, Th1/Th2 response, hypoxia, angiogenesis, apoptosis, etc.) is supported by the diversity and complexity of processes surrounding the end of pregnancy and delivery. In addition, VEGF-A is a mediator of chorionic vessel formation, HIF1-α expresses the normal hypoxia that occurs in relation to the proliferation of vessel-forming cells; Cox1 and 2 enzyme cyclooxygenases are key in the synthesis of prostaglandins, and these perform homeostasis functions in various organs, such as the formation of villi and vessels; IL2 is a T-cell growth factor and induces lymphocyte subpopulations and activates B-cell proliferation[51,52].

It should also be kept in mind that the immunological and histological activation described in this manuscript must be analyzed in the context of the origin of the healthy placentas, that is, endemic areas of malaria, which may show differences compared to healthy placentas from other areas. As can be seen in the Dobaño group's study, which reported that although samples from tropical and non-tropical areas show similar patterns of uncomplicated pregnancies, the first group presents higher concentrations of various immunological markers, accounting for an over-activation of some histological or physiological events in places like the one analyzed in this research. Dobaño's group also indicates that the factorial analysis of the control group (uncomplicated pregnancies from non-tropical areas) presented a more homogeneous grouping of variables than the samples from women from tropical countries, without being clear about the explanatory mechanism of such a finding[10]. These findings mean that the investigation of healthy placental biology should start from studies similar to the one presented in this manuscript in order to have a baseline for comparison of possible subsequent morbid events. Simultaneously, it would demonstrate the need for local observational studies given the possible morphological and functional variations between different areas following their nutritional, epidemiological (mainly morbidity profiles), socioeconomic, or other specificities.

The above-mentioned takes on greater relevance when bearing in mind that placental function is crucial for maternal-fetal health or the uncomplicated development of a pregnancy, and it is responsible for the replacement of respiratory gases, nutrients and waste materials, creates the immunological interface between mother and fetus, determines maternal tolerance towards the fetus carrying paternal antigens, supports mutual molecular interactions between mother and fetus, among other vital functions[57].

Finally, it should be noted that most studies in this field present isolated components or a limited (scarce) subgroup of biomarkers, with contradictory findings, which prevents a more detailed discussion of the results of this research. This very fact accounts for its novelty. In this regard, other authors have reported changes in the immune system in different trimesters of pregnancy and postpartum[58-60], and others have compared these events in peripheral blood, placenta, and cord at the time of delivery[61,62]. Only the study by Dobaño's group makes an effort to simultaneously study a series of broader responses to unravel the complex relationships between cytokine networks, but without accounting for the simultaneity of the immunological component with the histological one. Moreover, as they state in their publication, without applying exhaustive eligibility criteria to rule out infectious processes in the women included, which reduces the possibility of extrapolation to the context of healthy placental biology[10].

Along the same lines, a 2020 review provides an overview of the major immune cells present at the maternal-fetal interface and the mechanisms used by the placenta to promote maternal immune regulation, tolerance, and adaptation. It discusses how the deregulation of these pathways could lead to obstetric

complications such as miscarriage and pre-eclampsia[63].

Strength

This type of statistical analysis allow understand the biology of the healthy placenta in malaria endemic areas as an axis for the subsequent study of pathological mechanisms, especially placental malaria.

Limitations

A small number of placentas and the absence of clinical or epidemiological aspects that improve the extrapolation of the results. It must be kept in mind that there is no definitive rule on the number of subjects required in FA. In general terms, smaller samples may be adequate when either the factorial structure is apparent (that is, with at least four variables defining each factor and no variable in more than one factor) or it has variables that present a high number of intercorrelations[8]. Currently, there is not so much rigidity in estimating the adequate sample size for the FA[8]. In this research, despite the difficulties of finding a high number of healthy placentas, it was possible to sketch a clear factorial structure, with high λ coefficients, adequate goodness-of-fit statistics (even the Hoelter statistic allowed us to demonstrate that the structure of the three domains is achieved with sample sizes smaller than those of the current study), and agreement between the rotated and unrotated factorial structure, which would demonstrate that even with the low number of placentas, the internal validity required by this type of study was guaranteed.

Ethical considerations

The research was performed in accordance with relevant guidelines/regulations of the Bioethics Committee "Sede de Investigación Universitaria SIU", Minutes # 21-101-961, Universidad de Antioquia-Colombia (based on the principles of the Declaration of Helsinki and Resolution 8430 of the Ministry of Health of Colombia). In the period 2009-2016, our research group carried out several research projects on placental malaria, which ones samples were taken from healthy placentas as a control group. In these investigations, all the pregnant women signed the informed consent in which the use of the placentas for other studies was endorsed, with the condition that the new projects had the endorsement of a bioethics committee.

Conclusion

The statistical correlation of the histological and physiological events of healthy placentas and their factorial structure in three underlying components that support their interactions was demonstrated. These findings are essential for improving the study of healthy placental biology in malaria endemic areas and evaluating mechanisms that alter its morphology and function, with the subsequent risk for pregnancy and maternal-fetal health.

Declarations

Ethical approval and consent to participate: Written Informed Consent was always obtained to participate and for the samples to be used in research; all research was performed in accordance with relevant guidelines/regulations of the Bioethics Committee of "Sede de Investigación Universitaria SIU", Minutes # 21-101-961, Universidad de Antioquia-Colombia (based on the principles of the Declaration of Helsinki and Resolution 8430 of the Ministry of Health of Colombia).

Consent for publication

Not applicable.

Availability of data and material

The data used and/or analyzed during the current study are available under reasonable request to the author.

Competing interest

The authors declare that they have no competing interests.

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Authors' contributions

All authors contributed to the elaboration and approval of the final manuscript.

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