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REVIEW

The pregnancy-associated secretome in malaria pathogenesis and immunity during gestation

[version 1; peer review: 1 approved, 1 approved with reservations, 2 not approved]

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v1 **First published:** 17 Oct 2024, **4**:224
https://doi.org/10.12688/openreseurope.18351.1
Latest published: 17 Oct 2024, **4**:224
https://doi.org/10.12688/openreseurope.18351.1

Abstract

In pregnancy, fetal health, growth and development are dependent on normal placental development. However, the mechanisms that support maternal immune tolerance for the fetus, fetal nourishment, and pregnancy maintenance to term are not fully understood. Placental secretions into the intervillous space play a key role in shaping early placental function. In their entirety, they represent the pregnancy-associated secretome (PAS). PAS alterations have been associated with various complications such as fetal growth restriction, maternal anemia, and preterm birth. Placental malaria (PM), caused by the sequestration of *Plasmodium falciparum*-infected erythrocytes in the intervillous space, affects PAS composition. In this review, we explore how *P. falciparum*-induced changes of PAS may contribute to pathogenesis and immune dysregulation during pregnancy, as well as the possibilities of leveraging PAS components as biomarkers for the prevention, diagnosis, and management of PM.

Keywords

Placental malaria, pregnancy-associated secretome, Plasmodium falciparum, maternal physiology, sequestration

Open Peer Review

Approval Status ✓ ✗ ✗ ?

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version 1 17 Oct 2024	✓ view	✗ view	✗ view	? view

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Competing interests: No competing interests were disclosed.

Grant information: This project has received funding from the European and Developing Countries Clinical Trials Partnership progamme under grant agreement number TMA2020CDF3203 EndPAMAL (to BNK). It was also supported by US National Institutes of Health grant number R01HD103727 (to YS), and the Royal Society, Future Leaders African Independent Researchers (FLAIR) Scheme number FLR\R1\201314 (to JG). The funding sources had no role in study design, collection, analysis, interpretation of data, and publication.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Mwai L, Adamba P, Mordmüller B *et al.* **The pregnancy-associated secretome in malaria pathogenesis and immunity during gestation [version 1; peer review: 1 approved, 1 approved with reservations, 2 not approved]** Open Research Europe 2024, **4**:224 https://doi.org/10.12688/openreseurope.18351.1

First published: 17 Oct 2024, **4**:224 https://doi.org/10.12688/openreseurope.18351.1

Introduction

Malaria is caused by *Plasmodium* parasites, which are transmitted through the bites of infected female anopheline mosquitos. According to the World Health Organization, malaria causes >10,000 maternal deaths and >200,000 neonatal deaths annually[1], with most occurring in sub-Saharan Africa. Malaria in pregnancy (MiP) poses a significant threat to the mother and fetus because *Plasmodium falciparum*-infected red blood cells (iRBCs) can sequester in the placenta, causing placental malaria (PM)[2], which is associated with serious adverse outcomes, including maternal anemia, fetal growth restriction, preterm birth, stillbirth, and increased neonatal mortality[3]. Malaria parasite sequestration in the placenta is facilitated by variant surface chondroitin surface antigen 2 (VAR2CSA), a member of the *P. falciparum* membrane protein 1 (PfEMP1) family of proteins, which is mutually exclusively expressed on the surface of iRBCs[4]. The surface of the syncytiotrophoblast, the specialized outermost placental epithelial cell layer that is in direct contact with maternal blood[5], contains VAR2CSA's receptor, chondroitin sulfate A (CSA). Despite CSA being expressed on many cell types in the human host, VAR2CSA preferentially binds to chondroitin sulfate carrying 4-O-sulfation of the N-acetyl-D-Galactosamine (GalNAc) residues found in the placenta[6]. The interaction between VAR2CSA on the surface of iRBCs and CSA, facilitates the sequestration of iRBCs in the intervillous space, causing PM[6,7]. Additionally, the upregulation of intercellular adhesion molecules (ICAMs), leads to a syncytium-like adhesion of iRBCs to endothelial walls, which impairs malaria parasite clearance as they evade splenic destruction contributing to the persistence of MiP[4].

Embryo implantation, early growth and development, and adaptive changes in maternal physiology depend on the pregnancy-associated secretome (PAS) in the intervillous space (Figure 1). PAS has a complex composition including proteins, hormones, growth factors, antibodies, and immunomodulatory molecules like cytokines and chemokines[8]. PAS associated components, such as endometrial and uterine-derived cells express heat shock proteins (HSPs), including HSP27, HSP60, HSP70, and HSP90, which are abundant in the first trimester of pregnancy[9] and are involved in decidualization, implantation, and placentation[9,10]. Dysregulated expression of these HSPs has been associated with placental necrosis, implantation failure, and loss of pregnancy[9]. MiRNA-containing placenta-derived extracellular vesicles, which are released by the trophoblast cells into the uterine cavity, are another PAS component thought to facilitate fetal growth[11–13]. In addition, secretions connected with Placenta-Associated Maternal Monocytes/Macrophages (PAMM1a) cells localize at sites of syncytium layer when damaged by infections to facilitate placental tissue repair[14]. Other PAS components are part of immune adaptations that are implicated in the prevention of fetal immunological rejection and the promotion of successful implantation[11,15], among them are immune mediators derived from T helper 1 and T helper 2 immune cells that promote fetal implantation and development, and regulatory T cells, which promote tolerance for paternal alloantigens[16–19].

The intricate interaction between PAS components and placenta must be maintained throughout pregnancy and disruptions of this interaction cause implantation failure, fetal growth restriction, and preterm birth[8,20]. Recent studies have implicated various *P. falciparum*-induced PAS-alterations in PM pathogenesis[19,21]. In this review, we discuss the potential role of PAS components and their alterations in the pathogenesis of PM and immunity against PM, as well as their implications for PM diagnosis, prevention, and therapy.

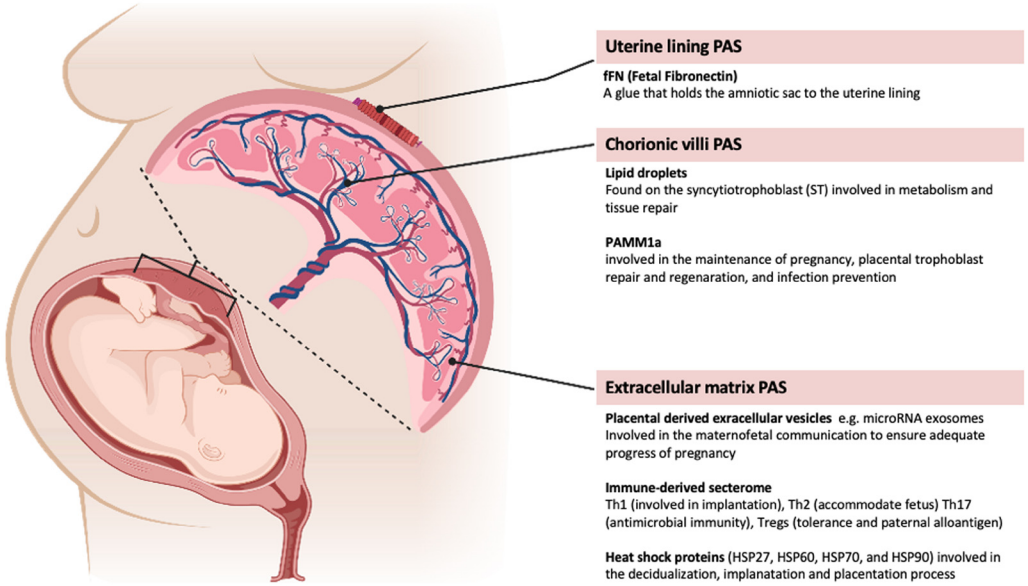


Figure 1. Pregnancy-associated secretome (PAS) function during fetal growth and development. (Image created with BioRender.com).

The PAS components implicated in the pathogenesis of PM

P. falciparum infection during pregnancy has been shown to affect various PAS components. Following iRBCs sequestration in the intervillous space, tumor necrosis factor-alpha (TNF-α) and reactive oxygen species (hydrogen peroxide, the hydroxyl radical, and the superoxide anion) elevate oxidative stress[19,21,22]. This causes monocyte and macrophage infiltration, which triggers placental apoptosis[18,19] and activates leukocytes[23], which drives further cytokine release. In addition, TNF-α-dependent monocyte recruitment can damage RBCs through oxidative stress or erythropoiesis inhibition[21]. Although TNF-α also attracts natural killer cells, which are beneficial in facilitating uterine vasculature adaptation to fetal demands for gas and nutrients at the maternal–fetal interface[24], high TNF-α levels can induce fetal necrosis[17].

P. falciparum causes placental inflammation through several pathways. The main immune signaling pathways include the activation of interferon (IFN)-β and IFN-α (type I IFNs) and IFN-γ (type II IFN)[25]. IFN-γ activates proinflammatory cytokines, chemokines, and growth factors, which are important for tissue regeneration and the clearance of intracellular pathogens[25]. Overactivation of the IFN-γ pathway may damage placental tissues through excessive inflammation[25]. Interleukin (IL)-10, an anti-inflammatory cytokine produced throughout pregnancy, along with the activation of IFN-β, counteracts this risk by attenuating IFN-γ overactivation[26]. IL-10 suppresses pro-inflammatory responses by elevating type 1 regulatory cells and restricting antibody response during blood-stage infection[25]. Further, IFN-α and TNF-α are produced by cytotoxic T lymphocytes, which are elevated in *P. falciparum*-infected placentas[19,27]. Indeed, overactivation of placenta and cord blood cytokines have been shown to affect fetal birth weight[28]. The overactivation of IFN-γ, TNF-α, IL-1β, IL-12p70, and IL-10 have been associated with reduced birth weight, whereas the overactivation of IL-28A (also known as IFN-L2/IFNλ2), IL-27, and IL-17E are associated with increased birth weight[27–29].

PM-induced chemokines, which accumulate in the intervillous space during iRBCs sequestration and mobilize monocytes, macrophages, cytotoxic T cells, granulocytes, and fetal and maternal B cells, are another important PAS component (Figure 2)[14,30]. Their effects may cause intervillositis (prolonged inflammation in the intervillous space), which is associated with increased fibrin deposition and syncytial knotting, which are implicated in fetal growth restriction, miscarriage, and stillbirth[14,31]. ICAM4 expression also increases the adhesion of PAMM1a to the syncytium via the expression of lymphocyte function-associated antigen-1, which drives further inflammation and may allow placental infection by opportunistic microbes[14].

PM activates PAS components belonging to the complement system, which also activate innate immunity[32,33]. This probably takes place via complement 3-independent pathways, specifically the cleavage of complements 3 and 5, mediated by thrombin

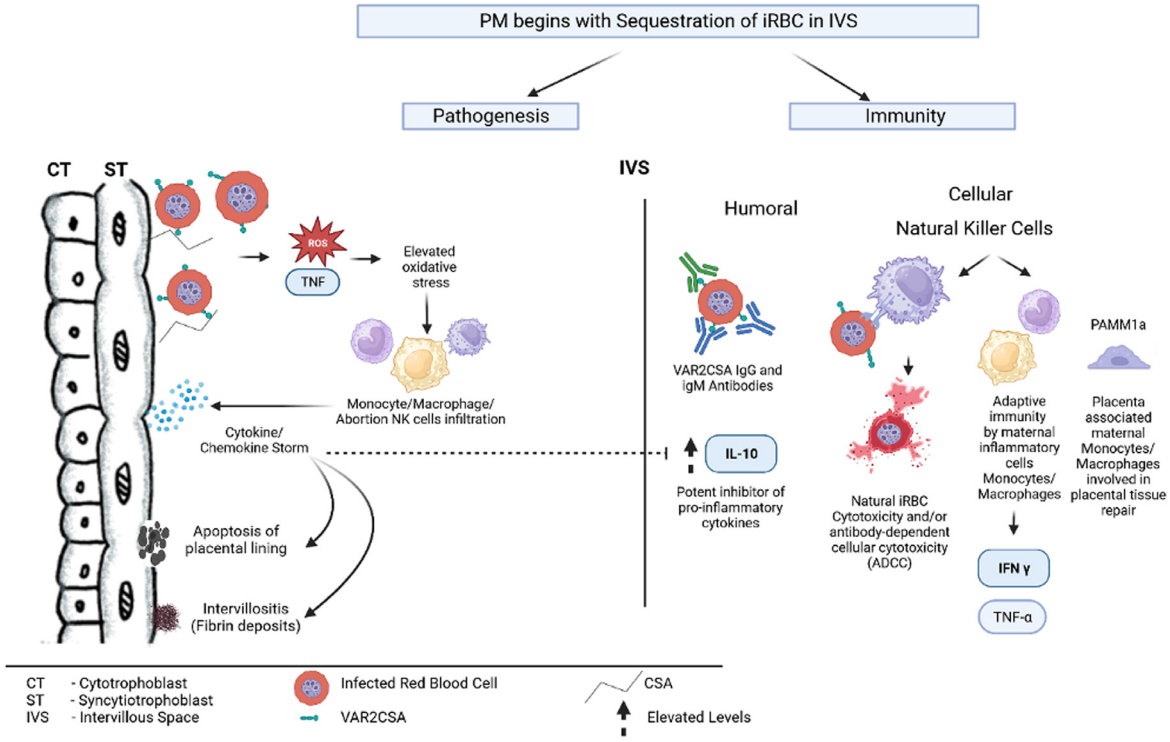


Figure 2. Activated immune components during PM. The image shows the involvement of the pregnancy-associated secretome (PAS) in PM pathogenesis and immunity. (Image created with BioRender.com).

and serine proteases from phagocytic leukocytes or possibly, by *P. falciparum* itself[34]. Although this complement activation plays a critical role in neutralizing invading *P. falciparum*, excessive activation is associated with malaria exacerbation through overwhelming complement regulatory proteins, leading to adverse maternal and fetal outcomes[33].

Malaria parasites-specific components, such as pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), are known to trigger immune responses through pathogen-recognition receptors (PRRs)[35], although they are not placenta-specific. For instance, the glycosylphosphatidylinositol (GPI) anchor, a molecule essential for parasite survival and immune activation[35,36] by facilitating key processes such as erythrocyte invasion, is a PAMP. GPI interacts with the host's toll-like receptors (TLRs), particularly TLR2-TLR1, triggering signaling pathways like Mitogen-activated protein kinase (MAPK) and nuclear factor-κB (NF-κB). These pathways result in the production of pro-inflammatory cytokines, such as TNF-α and Interleukin (IL)-1[36], contributing to the systemic inflammation observed in malaria, which could also affect the placenta.

Immune response is further amplified by other malaria parasite components such as hemozoin (DAMP), lipid peroxides (PAMP), and parasite nucleic acids (PAMP)[35]. Hemozoin, is a byproduct of hemoglobin digestion by the parasite that acts as a danger signal, activating the NOD-like receptor protein 3 (NLRP3) inflammasome and promoting the production of Interlukin (IL)-1β and other inflammatory mediators[37,38]. Lipid peroxides, released during oxidative stress in malarial infection, also play a crucial role in immune activation, causing cellular damage and modulating immune cell activity, thereby sustaining the inflammatory environment[21,35]. Furthermore, malaria parasite DNA and RNA are recognized by host pattern recognition receptors like cyclic GMP-AMP synthase (*cGAS*), leading to the activation of the stimulator of interferon genes (STING) pathway and the production of type I interferons and other cytokines, which amplify the immune response[35,39].

PAS-associated immunity during the peripartum and postpartum periods

Immunity to PM is mainly driven by the acquisition of anti-VAR2CSA antibodies, which can act through several mechanisms, including the blocking of iRBCs adherence to CSA by anti-VAR2CSA adhesion-blocking opsonic antibodies[40] and antibody-mediated effector functions through the activation of complement pathways against various stages of the parasite's life cycle, which may clear parasites and contribute to acquired anti-malaria immunity (Figure 2)[33]. Successive pregnancies are associated with the development of resistance to PM and unlike primigravida, women with multiple pregnancies have protective antibodies against *P. falciparum* antigens, such as anti-VAR2CSA antibodies, which might block iRBCs adherence to the placenta[41,42]. These anti-VAR2CSA antibodies are being targeted in the development of placental malaria vaccines (PMV)[43]. Thus far, there is no approved PMV for use in pregnant women, but two subunit vaccine candidates, namely the placental malaria vaccine (PAMVAC) based on interdomain region 1 through the duffy binding like domain 2 to interdomains 2 (ID1- DBL2X-ID2a) fragment, a 73-kDa derivative of the VAR2CSA from the *P. falciparum* FCR3 clone, and the Priming malaria vaccine (PRIMVAC) based on the DBL1X–DBL2X fragment, a 105-kDa derivative of the VAR2CSA from the 3D7 *P. falciparum* clone[43]. The anti-VAR2CSA antibodies induced by these vaccines block parasite binding to CSA[44,45].

In response to malaria infection, recruited NK cells promote the onset of adaptive immunity through the clearance of *P. falciparum* iRBCs by natural cytotoxicity and antibody-dependent cellular cytotoxicity processes enhanced by the production of regulatory cytokines such as IFN-γ and TNF-α[46–49].

PM has been associated with the mobilization of monocytes and macrophages to the placenta[50]. PAMM1a cells localize at sites of syncytium layer damage and contribute to placental tissue repair through the secretion of matrix metalloprotease-9 and fibronectin[14]. It is also hypothesized that PAMM1a contributes to placental protection against infection by potently responding to Toll-like receptor-mediated innate immune responses, which activate proinflammatory cytokines, chemokines and costimulatory molecules[14,50]. Although evidence linking PM and PAMM1a is currently lacking, macrophages/monocytes have been found to infiltrate the placenta in response to hemozoin, the malarial pigment[51]. In response to PM, these phagocytic macrophages/monocytes are recruited by β and α chemokines, monocyte chemoattractant protein 1, macrophage-inflammatory protein 1α, interferon-inducible protein 10, and IL-8[26,52].

Little is known about the immunity to malaria and how it affects malaria pathogenesis in the postpartum period. A study done by Ramharter *et al.* showed that women have a 10-fold increased risk for developing malaria in the postpartum period due to factors like changes in immune responses and perinatally acquired infections postpartum, compared to control non-pregnant women who had not delivered within the last 6 months[53,54]. Interestingly, there is evidence that *Pf* VAR2CSA specific IgG secretory B cell are maintained beyond birth[54]. Data suggest that more than 40% of women who had not been pregnant for 1–6 years were seropositive to *Pf* VAR2CSA, indicating prolonged antibody immunity[55].

Conclusion and future perspectives

The dysregulation of PAS components during PM can negatively impact pregnant women, who are at elevated risk to experience severe malaria and have serious consequences for the fetus, including fetal growth restriction, preterm birth, and stillbirth. Thus, strategies that modulate PAS components may offer critical avenues for mitigating such adverse outcomes and improving pregnancy outcomes in high-risk areas.

Currently, diagnosing placental malaria (PM) is challenging because the gold standard test requires histological examination of placental tissue after delivery[56]. Additionally, since PM

may be present even in a pregnant woman with almost no peripheral parasitemia, malaria parasite detection based on microscopy the gold standard test as well as rapid diagnostic tests, have decreased sensitivity as most parasites are sequestrated[57]. This argues for the development of tests that specifically detect PM and are less invasive than placental biopsy. Leveraging emerging strategies such as liquid biopsies (during pregnancy)[58] has a possibility of identifying early infections. Placenta liquid biopsies also represent a promising, non-invasive approach to analyze maternal blood for PAS components that may provide crucial insights into placental health in the presence of *Plasmodium* parasites.

Other promising approaches include the noninvasive prenatal testing (NIPT) and the analysis of extracellular vesicles (EVs), which carry placental molecular signatures[59,60]. Integrating these methods with a deeper understanding of the pregnancy-associated secretome will enhance our ability to combat the complex challenges posed by *P. falciparum* during gestation, ultimately improving maternal and fetal health. These approaches may as well provide valuable insights into potential therapeutic avenues. For instance, it has been shown that treating necrotizing enterocolitis, a neonatal disease with considerably high morbidity and mortality, with ST266, an amnion-derived multipotent PAS component thought to upregulate critical genes during gut remodeling, can prevent early pregnancy loss[11,61].

Encouragingly, the two promising placental malaria vaccines named previously; PAMVAC and PRIMVAC prevent *Plasmodium* malaria parasite sequestration[43,62]. These PM vaccine candidates underwent first-in-human, trials in Germany and Benin (PAMVAC)[45], France and Burkina Faso (PRIMVAC). Both candidates showed increased anti-VAR2CSA antibodies[41,63] that prevented *Plasmodium* parasite sequestration in an *in vitro* assay, offering a strategy for reducing PM prevalence and severity, although immunogenicity and breadth of protection was not optimal. Currently, a European Union funded project is aimed at improving the two vaccine candidates and performing Phase 1 and 2 studies[64]. At the moment, the main interventional tool to improve pregnancy outcomes is intermittent preventive treatment in pregnant women (IPTp). It has been implemented to prevent *P. falciparum* infection in most highly malaria-endemic regions[65,66]. When paired with education on mosquito bite prevention, it can enhance the efficacy of malaria infection prevention[1]. However, the effectiveness of chemoprophylaxis is at risk of increasing parasite resistance to sulfadoxine–pyrimethamine, which is recommended by the World Health Organization for malaria prophylaxis in pregnant women[66].

In summary, future research should focus on developing specific biomarkers for PM diagnosis, understanding the role of PAS components in mediating maternal-fetal immune responses, and exploring the therapeutic potential of PAS-based interventions to improve maternal and child health in malaria-endemic regions.

Data availability statement

No data are associated with this article.

Author contributions

All authors have made substantial, direct, and intellectual contributions to the work. All authors approved the manuscript for publication.

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Open Peer Review

Current Peer Review Status: ✔ ✘ ✘ ?

Version 1

Reviewer Report 13 December 2024

https://doi.org/10.21956/openreseurope.19830.r47330

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?

Olukemi K Amodu
University of Ibadan, Ibadan, Nigeria

The review discusses PAS components implicated in placental malaria pathogenesis. There is a good description of the PAS with focus on the inflammation pathways for immune signaling, relevant immune mediators, chemokines, and the complement system. It also explores the role of malaria parasite-specific components like PAMPs and DAMPs in triggering immune responses and examines immunity to placental malaria during and after delivery through antibody acquisition. In addition, the authors discuss the underlying mechanisms and rationale for malaria vaccine targets. The figures are clear and detailed.

However, the review is very short with sections lacking in extensive or distinct details or just given a cursory mention. For example, the mention of upregulation of ICAM and adhesion of iRBC is just a quick mention with background details significantly lacking.

Some sections end abruptly with lack flow into the next sections, particularly in the sections under the subheading – *PAS components implicated in the pathogenesis of PM*

Details should be expanded on in the different sections with subheadings providing clearer details and for smooth reading.

The message on the section on the PAS-associated immunity during the peripartum and postpartum periods should be well defined, describing the gaps and main points of similarities or differences.

Is the topic of the review discussed comprehensively in the context of the current literature?
Partly

Are all factual statements correct and adequately supported by citations?
Yes

Is the review written in accessible language?
Yes

Are the conclusions drawn appropriate in the context of the current research literature?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Genetics; Molecular Biology; Malaria

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 13 December 2024

https://doi.org/10.21956/openreseurope.19830.r47336

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Eliana María Arango Flórez
Facultad de Ciencias de la Salud, Fundacion Universitaria San Martin Facultad de Medicina (Ringgold ID: 469507), Bogotá, Colombia
Olga María Agudelo García
Departamento de Microbiología y Parasitología, Universidad de Antioquia, Medellín, Antioquia, Colombia

The review aimed to explore how Plasmodium falciparum-induced changes of pregnancy-associated secretome (PAS) may contribute to pathogenesis and immune dysregulation during pregnancy, as well as the possibilities of leveraging PAS components as biomarkers for the prevention, diagnosis, and management of PM.

In general, the article is well written but too summarized, these aspects must be reviewed and described deeply in order to achieve the proposed objective:

- Definition of PAS and its role in embryo implantation, early growth and development, and adaptive changes in maternal physiology. Mention some components and its localization as in Figure 1 is not enough.
- Molecular events of the interaction between VAR2CSA on the surface of *P. falciparum* infected red blood cells and CSA.
- Mention of the intercellular adhesion molecules (ICAMs) in the context of placental malaria is confuse.
- What are the implications of the PAS components in placental malaria diagnosis, prevention, and therapy?
- The presence of the parasite in placental tissue cannot be reduced only to mentioning

inflammation and the association with the production of proinflammatory cytokines, since these are primary and poorly regulated events of the immune response during placental malaria. It is necessary to consider the immune, modulatory, and endocrine role of the placenta.

Other minor edits suggested are:

- Update bibliography about association of malaria with maternal and neonatal deaths; authors used a paper of 2007.
- Replace MiRNA by microRNA.
- These acronyms are unnecessary because only appear once in the text: NIPT, EVs, IPTp.
- Erythropoiesis inhibition is not a mechanism of RBCs damage.
- Conclusions must be restricted to *P. falciparum*, the reference to "*Plasmodium* parasite" would not be correct.

Is the topic of the review discussed comprehensively in the context of the current literature?
Partly

Are all factual statements correct and adequately supported by citations?
Partly

Is the review written in accessible language?
Yes

Are the conclusions drawn appropriate in the context of the current research literature?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Report prepared by Olga María Agudelo García, PhD, Professor of Medicine Faculty, Universidad de Antioquia, and Eliana María Arango Flórez, PhD, Professor of Health Sciences Faculty, Fundación Universitaria San Martín. Both are expert in prengancy associated malaria

We confirm that we have read this submission and believe that we have an appropriate level of expertise to state that we do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 25 November 2024

https://doi.org/10.21956/openreseurope.19830.r46618

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Andrew Teo
University of Melbourne, Melbourne, Australia

Authors attempted for a short review on placental malaria. The flow could be improved, sections are not well described. Please consider enhancing the descriptions in each section, more subheadings may aid in readability

"Malaria parasite sequestration in the placenta is facilitated by variant surface chondroitin surface antigen 2 (VAR2CSA), a member of the P. falciparum membrane protein 1 (PfEMP1) family of proteins, which is mutually exclusively expressed on the
surface of iRBCs4. The surface of the syncytiotrophoblast, the specialized outermost placental epithelial cell layer that is in direct contact with maternal blood5, contains VAR2CSA's receptor, chondroitin sulfate A (CSA)." - this section is condensed, from VAR2CSA to CSA, might be worth explaining what is unique about P.falciparum, PfEMP1, then to the receptors IEs bind to.

The sentence on ICAM1 seems out of place.

A heading is needed for - Embryo implantation, early growth.... - This is not introduction.

"P. falciparum infection during pregnancy has been shown to affect various PAS components. Following iRBCs sequestration in the intervillous space, tumor necrosis factor-alpha (TNF-α) and reactive oxygen species (hydrogen peroxide, the hydroxyl radical, and the superoxide anion) elevate oxidative stress"- I am not sure what the sentences mean. Can the authors please explain and elaborate?
Overall, the authors' focus on only TNF is not entirely clear. Next section they suddenly discussed about PM inflammation. The flow could be improved here. Clarifying the connection between these topics would help strengthen the overall coherence of the discussion.

"PM-induced chemokines, which accumulate in the intervillous space during iRBCs sequestration and mobilize monocytes, macrophages, cytotoxic T cells, granulocytes, and fetal and maternal B cells, are another important PAS component" - This section is not accurate, its not only chemokines that are associated with intervilositis - its the overall inflammation.

"PM activates PAS components belonging to the complement system, which also activate innate immunity." - suddenly on immunity, no flow in here

"Malaria parasites-specific components, such as pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), are known to trigger immune responses through pathogen-recognition receptors (PRRs)35, although
they are not placenta-specific" - how is this section on PM? it seems to be just a random section on inflammation.

"PAS-associated immunity during the peripartum
and postpartum periods" - this section here is about acquired immunity, how is this about PAS? It's unclear

The conclusion could benefit from a more clearly defined take-home message that succinctly summarizes the key insights or implications of the study, many different topics were discussed in

the conclusion from PAS, to diagnostics to vaccines.

Is the topic of the review discussed comprehensively in the context of the current literature?
Partly

Are all factual statements correct and adequately supported by citations?
Partly

Is the review written in accessible language?
Partly

Are the conclusions drawn appropriate in the context of the current research literature?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: placental malaria

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 09 November 2024

https://doi.org/10.21956/openreseurope.19830.r45299

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Jeremiah Laktabai
Moi University School of Medicine, Eldoret, Kenya

The authors are reviewing the important subject of malaria pathogenesis and immunity during pregnancy, delving into the pregnancy associated secretome (PAS), and how the changes induced by *P. falciparum* infection contribute to immune dysregulation.

The introduction section covers malaria epidemiology, pathogenetic mechanisms implicated in malaria in pregnancy, and a description of PAS and its components.
The PAS components implicated in the pathogenesis of placental malaria are discussed. These include inflammation pathways for immune signaling and the relevant immune medicators, the chemokines and the complement system.
The potential role of malaria parasite specific components such as PAMPs and DAMPs in triggering various immune responses is explored.

Immunity to placental malaria during and after delivery through antibody acquisition, and the underlying mechanisms, and the rationale for malaria vaccine targets are also discussed.

The article is well written, in appropriate detail with relevant literature cited. The two figures are clear, and reinforce the message in the text.

The conclusions are appropriate.

Is the topic of the review discussed comprehensively in the context of the current literature?
Yes

Are all factual statements correct and adequately supported by citations?
Yes

Is the review written in accessible language?
Yes

Are the conclusions drawn appropriate in the context of the current research literature?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Malaria implementation research, malaria in pregnancy

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.