Congenital Transmission by Protozoan

Guest Editors: Ricardo E. Fretes, Ulrike Kemmerling, and Demba Sarr
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Editorial

Congenital Transmission by Protozoan

Ricardo E. Fretes,1,2 Ulrike Kemmerling,3 and Demba Sarr4

1 Cell Biology, Histology, and Embryology Department, Medicine School, Universidad Nacional de Córdoba, 5000 Córdoba, Argentina
2 Universidad Nacional de La Rioja, 5300 La Rioja, Argentina
3 Program of Anatomy and Developmental Biology, Institute for Biomedical Sciences, Faculty of Medicine, University of Chile, 8380453 Santiago, Chile
4 Department of Infectious Diseases and Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA 30602, USA

Correspondence should be addressed to Ricardo E. Fretes, rfretes@yahoo.com

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Congenital transmission by protozoan parasites is a worldwide important public health problem. Congenital infections affect the mother and the fetus or newborn. It is still surprising that despite the abundant immunoepidemiological knowledge of congenital transmission of protozoan parasite, no definite etiology or predictive diagnostic tests have been identified. Understanding the mechanisms by which host/parasites infection and interaction occurs is one of the most important topics that will help find specific biomarkers of infection, prevent congenital transmission, maintain the health of the newborn, and develop safe and efficient treatments. In addition, understanding of the biological mechanisms of host/parasite interactions will facilitate protection of mothers and their families and reduce costs in health services. Moreover, this will lead to gain insight into epidemiological aspects and association with other pathologies and preserve the wellbeing of the newborn. In this special issue we have invited a few papers that address such issues.

Congenital malaria is underestimated and usually associated with low-density cord parasitemia. However, it is increasingly recognized as a potentially serious complication of maternal malaria during pregnancy in Sub-Saharan Africa. Earliest epidemiological studies have reported prevalence varying widely in malaria-endemic areas from 0% to 33%. At birth, infections are usually asymptomatic with low parasitemia and the diagnosis by microscopy is often missed. Infection may occur by transplacental passage of parasites during disruption of the placental barrier at the time of delivery, with subsequent clinical illness in the newborn baby.

A paper of this issue assessed the prevalence of congenital malaria during the dry season (period of low-mosquito density and low-malaria incidence) using peripheral and cord blood smears of new born babies in association with peripheral and placental blood smears of, respectively, near-term and term pregnant women. This study is interesting because the authors found that congenital malaria is not rare in their study area. Another paper is a literature review on the prevalence, burden, diagnosis, prevention, and control of congenital malaria in Sub-Saharian Africa. This review is of interest to individuals working in clinics and laboratory diagnostic of malaria but also to national governments and partners in Sub-Saharian Africa. The authors highlight the challenges in parasitological and clinical diagnosis, the challenges in prevention with the use of intermittent preventive treatment (IPT) and insecticide-treated nets (ITNs) and provides recommendation on how to strengthen the health system in Africa. Another paper reports the prevalence of transplacental malaria in Burkina Faso, a West African country, and determines the real burden of transplacental transmission. The authors show associations between levels of parasite in the maternal, placental, and umbilical cord blood. All papers about malaria published in this special issue show the interest of pursuing investigations for a better understanding of vertical transmission by malarial parasites.

During congenital Chagas transmission, the parasite reaches the fetus by crossing the placental barrier. In the past few years congenital transmission of T. cruzi has increasingly become more important, and partly responsible for the “globalization of Chagas’ disease,” constituting a public
health problem of increasing relevance. The fact that only
a percentage of the infected mothers transmit parasites
to their fetuses raises the question of the ability of the
placenta as well as the immunological status of mother
and fetus/newborn to impair the parasite transmission.
Therefore, it is thought that congenital Chagas disease is
the product of a complex interaction between the parasite,
the maternal, and fetus/newborn immune responses, and
placental factors. Additionally, a paper of this special issue
constitutes a morphological analysis by immunohistochem-
ical and histochemical methods of placentas from women
with chronic Chagas’ disease. *T. cruzi*-infected placentas
present destruction of the syncytiotrophoblast and villous
stroma, selective disorganization of the basal lamina, and
disorganization of collagen I in villous stroma. Changes in
the extracellular matrix of placental tissues, together with the
immunological status of mother and fetus, and parasite load
may determine the probability of congenital transmission
of *T. cruzi*. Another paper assayed the effect of *T. cruzi*
on glucose transporter protein-1 (GLUT1), which is the
main isoform involved in transplacental glucose transport.
High glucose media as well as *T. cruzi* infection reduce
GLUT1 expression. The effect of *T. cruzi* infection on GLUT1
expression may explain some of the clinical manifestation
of congenital Chagas disease. Finally, a paper analyzing the
congenital transmission of Chagas disease is a review about
the role of possible local placental factors that contribute
to the vertical transmission of the parasite. Additionally,
in that review different available methods for studying the
congenital transmission of *T. cruzi* are analyzed. In that
context, the *ex vivo* infection of human placental chorionic
villi by *T. cruzi* trypomastigotes constitutes an excellent tool
for studying parasite infection strategies as well as possible
local antiparasitic mechanisms.

*Ricardo E. Fretes*
*Ulrike Kemmerling*
*Demba Sarr*
Review Article

Mechanism of *Trypanosoma cruzi* Placenta Invasion and Infection: The Use of Human Chorionic Villi Explants

Ricardo E. Fretes\(^1,2\) and Ulrike Kemmerling\(^3\)

\(^1\) Department of Histology and Embryology, Faculty of Medicine, Universidad Nacional Córdoba, 5000 Córdoba, Argentina
\(^2\) IICSHUM and Cathedra of Histology, Embryology and Genetic, Health Department, Universidad Nacional La Rioja, 5300 La Rioja, Argentina
\(^3\) Program of Anatomy and Developmental Biology, Institute for Biomedical Sciences, Faculty of Medicine, University of Chile, 8380453 Santiago, Chile

Correspondence should be addressed to Ricardo E. Fretes, rfretes@cmefcm.uncor.edu and Ulrike Kemmerling, ukeimeterling@med.uchile.cl

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Congenital Chagas disease, a neglected tropical disease, endemic in Latin America, is associated with premature labor and miscarriage. During vertical transmission the parasite *Trypanosoma cruzi* (*T. cruzi*) crosses the placental barrier. However, the exact mechanism of the placental infection remains unclear. We review the congenital transmission of *T. cruzi*, particularly the role of possible local placental factors that contribute to the vertical transmission of the parasite. Additionally, we analyze the different methods available for studying the congenital transmission of the parasite. In that context, the *ex vivo* infection with *T. cruzi* trypomastigotes of human placental chorionic villi constitutes an excellent tool for studying parasite infection strategies as well as possible local antiparasitic mechanisms.

1. Introduction

Chagas disease was first described by the Brazilian physician, Carlos Chagas, in 1909. He identified the causal agent, the protozoan *Trypanosoma cruzi* (*T. cruzi*), the vectorial transmission and insect reservoirs as well as the clinical signs and symptoms. In other words, he described the complete cycle of the disease and suggested the possibility of congenital transmission [1].

In the human villous hemochorial placenta, fetal and maternal tissues are separated by a fetal epithelium (the trophoblast). Within the villous placenta, a single multinucleated cell layer (syncytiotrophoblast) contacts maternal blood within the intervillous space. Beneath the syncytiotrophoblast reside replicating progenitors (cytotrophoblast) that are separated by a basal lamina from the connective tissue of villous stroma containing vascular endothelium, fibroblasts, and macrophages. The syncytiotrophoblast forms a surface of about 12 m\(^2\) that contacts maternal blood. Therefore, in case of women with Chagas disease, the parasite has the opportunity to interact with a large cellular surface.

The mechanism of *T. cruzi* congenital transmission can be studied in different ways. One of the possible ways is through the dual placental perfusion system. The dual perfusion that simulates the maternal and fetal circulation could be an excellent method to study the mechanism of the transmission of the parasite [2]. However, this method requires complex equipment and very experienced users. Another possibility is the *ex vivo* infection of human chorionic villi explants, in which samples of placental tissue (chorionic villi) can be challenged *in vitro* with *T. cruzi* or other pathogens. This system is preferred to analyze the process of infection of the placental barrier, although the immune system does not participate. The analysis of infection in animal models is not treated in this paper because their placentas are not similar to human placenta. Also, pathological findings of placentas are not deeply described because they represent the final
rates of congenital infection vary from 1–21% [8–11]. In prevalence among pregnant women may reach 80%, and system, arrhythmias, and abnormal growth of the heart mega esophagus, degeneration of the autonomous nervous 30% of infected individuals, is associated with mega colon, can last for months or years. The chronic phase, present in 30% of infected individuals, is associated with mega colon, mega esophagus, degeneration of the autonomous nervous system, arrhythmias, and abnormal growth of the heart with progressive insufficiency, with evident negative impact on the patient’s health. In this phase the disease can be handicapping, and can either be the concurrent or the direct cause of death. The course of the disease depends on diverse factors: parasite load at the site of inoculation, both the parasite’s genetic group and strain, whether it is an infection de novo or reinfection, the host’s immunologic status, and the type of vector (triatomid) [3, 4].

2. Chagas Disease

Chagas disease develops in three phases. The acute phase is first developed, immediately after infection, with high levels of parasitemia and symptoms in only some patients (regional lymph node enlargement, bipalpebral unilateral edema, or Romaña’s sign, and characteristic electrocardiogram alterations). In most cases, acute infection is not accompanied by clinical findings, thus moving onto the latent phase that can last for months or years. The chronic phase, present in 30% of infected individuals, is associated with mega colon, mega esophagus, degeneration of the autonomous nervous system, arrhythmias, and abnormal growth of the heart with progressive insufficiency, with evident negative impact on the patient’s health. In this phase the disease can be handicapping, and can either be the concurrent or the direct cause of death. The course of the disease depends on diverse factors: parasite load at the site of inoculation, both the parasite’s genetic group and strain, whether it is an infection de novo or reinfection, the host’s immunologic status, and the type of vector (triatomid) [3, 4].

3. Congenital Chagas Disease

Congenital T. cruzi infection is associated with premature labor, low birth weight, and stillbirths [5–7]. Serologic prevalence among pregnant women may reach 80%, and rates of congenital infection vary from 1–21% [8–11]. In Argentina the transmission rate is estimated between 2–12% [12]. In Chile, in two of the endemic regions (IV and V regions), the congenital transmission rate of the parasite is 8.4% [13]. According to WHO/PAHO the number of infected women at fertile age is approximately 1.8 million and it is estimated that 14,400 neonates are being infected each year [14], this is another reason why this form of transmission becomes epidemiologically more important. Additionally, congenital transmission is partly responsible for the “globalization of Chagas’s disease” [10, 15].

Congenital Chagas transmission implies a T. cruzi seropositive mother and a postpartum detection of parasites in the newborn. Congenital Chagas disease is diagnosed by direct microscopic examination of blood samples, PCR, or standard serological assays. The latter can be carried out in infants when IgG antibodies transferred from the mother have been eliminated (8-9 months after birth).

During congenital transmission, the parasite reaches the fetus by crossing the placental barrier [16–22]. The fact that only a percentage of the infected mothers transmit parasites to their fetuses raises the question of the ability of the placenta as well as the immunological status of mother and fetus/newborn to impair the parasite transmission. Therefore, it is thought that congenital Chagas disease is the product of a complex interaction between the parasite, the maternal and fetus/newborn immune responses, and placental factors [9, 18].

4. The Parasite

T. cruzi is a haemoflagellated protozoan of the Kinetoplastida order and Trypanosomatidae family [22]. The parasite’s biological cycle includes three cellular forms characterized by the relative positions of the flagellum, kinetoplast, and nucleus [23]: (1) trypomastigotes: approximately 20 µm in length and sub terminal kinetoplast. They constitute the nonreplicative, mammalian infecting cellular form that is found in the blood and in the posterior intestine of triatomids. In mammals, this is the cellular form that disseminates infection through blood. (2) Epimastigotes: also 20 µm in length with a kinetoplast anterior to the nucleus. They represent the multiplying parasite form in the triatomid intestine. (3) Amastigotes: approximately 2 µm in diameter, rounded, with no emergent flagellum. It multiplies within the mammalian host cells, forming nests, until they rupture after several cell divisions. Before their release from the host cells, amastigotes differentiate into trypomastigotes which once released, invade the blood stream; they may then enter any other nucleated cell. Epimastigotes can be grown in axenic cultures while amastigotes are grown in cultured mammalian cells, releasing trypomastigotes that can be harvested to perform in vitro assays.

T. cruzi displays great biological, biochemical, and genetic diversity; therefore different strains of the parasite have been identified and classified into six discrete typing units (DTUs) [24, 25]. Strains of T. cruzi have been involved in different clinical forms of Chagas disease [26, 27], thus implicating a different genetic population in tissue tropism, replication, and virulence, and in consequence in disease outcome. T. cruzi strains corresponding to different DTUs might have relevant consequences on congenital transmission and fetal/neonatal pathology, even though Virreira et al. [28] and Burgos et al. [9] concluded that congenital transmission of T. cruzi is not associated with genetic polymorphism of T. cruzi. However, Solana et al. [29] and Triquell et al. [21] described biological differences among subpopulations of T. cruzi in experimental vertical transmission and placental infection.

5. Mother and Fetus/Newborn Immune Response

The immune system is fundamental to protect the mother against the environment, and to prevent damage to the fetus. During pregnancy the maternal immune system is characterized by a reinforced network of cellular and molecular recognition, communication, trafficking and repair; it raises the alarm to maintain the wellbeing of the mother and the fetus. On the other hand, the fetus provides a developing active immune system that will modify the way the mother responds to the environment, providing a uniqueness of the immune system responses during pregnancy [30].
A crucial factor to stop, limit, or permit the development of fetal/neonatal infection relates to the capacity of the mother and fetus/newborn to mount innate and/or specific immune response(s) against pathogens. Clinical studies have shown a strong association between intrauterine infections and pregnancy disorders such as abortion, preterm labor, intrauterine growth retardation, and preeclampsia [31]. As described above, congenital T. cruzi infection is associated with some of these pathologies [5–7, 15]. Production of proinflammatory cytokines can be observed in uninfected newborn from infected mothers. On the other hand, maternal T. cruzi–specific IgG antibodies play protective roles in mothers and in fetuses when antibodies are transferred through the placenta [33] and also may contribute to a reduction in parasitaemia [15].

6. Placenta

The placenta is the principal site for the exchange of nutrients and gases between the mother and fetus. This organ plays an important role in hormone, peptide, and steroid synthesis necessary for a successful pregnancy [34]. The human placenta is classified as a hemochorial villous placenta in which the free chorionic villi, formed by the trophoblast and the villous stroma, are the functional units. The trophoblast contacts maternal blood in the intervillous space, and it is separated by a basal lamina from the villous stroma, which is connective tissue containing the vascular endothelium, fibroblasts, and macrophages (Figure 1) [35]. Trophoblast, basal lamina, and villous stroma with the endothelium of fetal capillaries form the placental barrier that must be crossed by different pathogens, including T. cruzi, to infect the fetus during vertical transmission [16–22, 36–41].

Placentas from mother with acute Chagas disease (high parasitaemia) show severe histopathological changes, such as extensive necrosis, inflammatory infiltrate, and amastigote nests [5]. Contrarily, placentas from mother with chronic Chagas disease do not present necrotic foci and inflammatory infiltrate. Although parasite antigens can be visualized in the villous stroma, the typical amastigote nests are not present [22]. In accordance with these results, in ex vivo infected placental explants, though parasite antigens and DNA can be detected [17, 18, 42], amastigote nests are not observed. Only few individual parasites can be detected. This evidence suggests that antiparasite mechanisms may exist in the placental tissue of women suffering chronic Chagas disease.

7. Possible Antiparasitic Mechanisms of the Placenta

We updated the importance of the presence of the causal agent of Chagas disease in the intervillous space of human placentas, the viability of the parasite in this environment, and the process of infection of the placental tissue, mainly by ex vivo and in vitro studies.

1. Clearance of T. cruzi from the intervillous space is associated with the risk of congenital transmission. Thus, a high parasitaemia, as in acute infection, correlates with a higher transmission rate [15, 18, 43, 44]. Thus the amount of parasites could be an important risk factor for mother to fetus transmission of T. cruzi. There are only few publications analyzing the survival of T. cruzi in the placental environment. Triquell et al. [21], employing chorionic villi ex vivo and in vitro infection model cocultured with trypomastigotes from two different strains of T. cruzi, observed that one of the strains presents a better survival rate than the other in the placental environment. Furthermore, the two strains of T. cruzi respond differently when they were treated with placental media. Therefore, the great biological, biochemical, and genetic diversity of the parasite may determine, at least partially, the capacity of placental infection. These results open a new concept, that placenta might exert a clearance of the parasite from the intervillous space, and that different populations of T. cruzi have different survival capacities in that environment.

2. Contact time between T. cruzi and the trophoblast in the intervillous space: the time that the parasite remains in the intervillous space in contact with the syncytiotrophoblast is poorly known. Placental barrier is constituted by the trophoblast tissue, that comprises a continuous multinucleated, nonreplicating cell layer, the syncytiotrophoblast, a replicating
Cardiac output in woman 4250 mL/min

In pregnancy: 20% increase in circulating blood volume and 40% increase in cardiac output

Cardiac output in pregnant woman 5950 mL/min

10% of cardiac output reaches the uterus and 80% of this volume reaches the placenta

475 mL/min of blood reaches the placenta

Low parasitemia (0.1–1 parasite/mL): 68544–685440 parasites circulate through the placenta in 24 hours

High parasitemia (over 40 parasite/mL): more than 27 million parasites circulate through the placenta in 24 hours

Figure 2: Estimation of T. cruzi contact with the placenta in infected mothers.

layer of cytotrophoblasts that fuses with the STB, a basal lamina, and an underlying villous stroma or connective tissue, that includes vascular endothelium [35]. The placental barrier must be crossed by the parasites, therefore the time that T. cruzi trypomastigotes stay in the intervillous space and interact with the syncytiotrophoblast is of outmost importance. Shippey et al. [45] in a dual perfusion system of placental cotyledons observed T. cruzi DNA in the maternal effluents at 30 min, 60 min, and 90 min after injection an only one bolus of T. cruzi trypomastigotes through the maternal perfusate. There was no parasite DNA in the fetal effluent, indicating there was no passage to fetal circulation despite the great concentration of parasites injected. However, the perfusion time was only 120 min in these experiments. Contrarily, in the ex vivo infection of chorionic villi explants, a reproducible infection is obtained after 24 hours of coincubation with the parasite [17]. However, the perfusion experiments indicate that T. cruzi is present in the intervillous space at least for an hour and half. Despite this long time of interaction, T. cruzi was not able to invade or survive in the placental barrier, indicating a defense mechanism of the placental barrier against the causal parasite of Chagas.

In our laboratories, we have established the optimal conditions for the ex vivo infection of chorionic villi explants with T. cruzi [17, 18, 20, 21, 36–41, 47]. The coincubation of 10^5 or 10^6 trypomastigotes produces a reproducible infection of the chorionic villi [17]. This parasite's concentration may seem to be extremely high, but if we consider the amount of blood that circulates through the placenta every day, and then calculate the number of parasites that reaches the placenta, the parasite concentration recommended for ex vivo infection is not high. Therefore, if we consider that the cardiac output in women is 4250 mL/min, and that during pregnancy the circulating blood volume increases in 20% and the cardiac output in 40%. Then the cardiac output in pregnant women is 5950 mL/min. From this output, 10% reaches the pregnant uterus and 80% of this volume reaches the placenta. Taking into account all the data, a volume of 475 mL/minute of blood reaches the placenta [48]. Considering a parasitemia as low as 0,1 to 1 parasite/mL, a total of 68544 to 685440 parasites circulate through the placenta in 24 hours (Figure 2). On the other hand, in pregnant women with acute Chagas disease, Torrico et al. [11] have reported parasitemias over 40 parasites/mL; therefore, in this condition a total of 27 million parasites circulate in 24 hours in the placenta. If we consider all these data, our experimental conditions are not far from in vivo conditions.

The trophoblast, the first tissue that is in contact with the parasite in the intervillous space, constitutes a potential barrier to T. cruzi. We observed that the most notable tissue damage induced by T. cruzi in the chorionic villi explants is the trophoblast detachment and destruction. Additionally, the parasite induces selective disorganization of basal lamina, collagen I destruction [17], and apoptosis (especially in the trophoblast) in infected chorionic villi explants [49]. In accordance, we detected similar histopathological changes in
placentas from women with chronic Chagas disease ([22] manuscript in this number of J. of Tropical Medicine). Therefore, the similar histopathological changes observed in chagasic mothers and in ex vivo infected chorionic villi, validates the latter model. The extracellular matrix alteration produced by T. cruzi not only promotes its motility in tissues and its entrance into cells, but also alters the presence of cytokines and chemokines, which in turn permits T. cruzi to modulate and evade both the inflammatory and immune responses [16, 50, 51]. Alternatively, these changes in ECM function may be part of a local placental defense mechanism, which could explain why only very few parasites can be detected in the placenta. Similar effects can be observed in the chorionic villi explants during ex vivo infection, since placental explants do not allow a sustained infection by T. cruzi [19]. Thus, the placenta controls the productive infection of T. cruzi in chorionic villous and exerts a protective function to fetus.

In order to understand the mechanism by which T. cruzi fuses with trophoblast plasma membrane, Calderón and Fabro [52] studied the interaction between syncytial plasma membranes from the human placenta and from the parasites, founding modifications of membrane lipids and proteins of the syncytiotrophoblast. Additionally, modifications of enzyme activities in the chorionic villi have been described [37–39]. For instance, placental alkaline phosphatase (PLAP) is a glycosylphosphatidylinositol anchored plasma membrane protein present in the trophoblast that decreases its activity in chagasic women and is related to congenital transmission [53]. In ex vivo infected chorionic villi, pretreatment of the placental tissue with phospholipase C prevents the parasite-induced decrease of PLAP activity and significantly reduces the infectivity of T. cruzi. These results are consistent with a pathogenetic role for placental alkaline phosphatase in congenital Chagas disease [36, 54]. Additionally, T. cruzi induces in the ex vivo infection model an increase of lyosomal vesicles in the trophoblast, which are fundamental during cell invasion of the parasite [36–39].

Analyzing the process of trophoblast infection by T. cruzi in an in vitro system culturing monolayer trophoblasts cells in interaction with infective trypomastigotes, it was shown that two types of chorionic villi trophoblasts, syncytiotrophoblast, and cytotrophoblast have a differential susceptibility to infection by the causal agent of congenital Chagas disease [55]. The reduced infection in the syncytiotrophoblast was associated to fewer viable parasites in the culture medium and increased levels of nitric oxide. These results emphasize the importance of the integrity of the first placental barrier, the syncytiotrophoblast, in order to avoid a T. cruzi infection of the inner trophoblasts or stromal chorionic villi cells. As it was described above, structural trophoblast alternation is a common sign of miscarriages and premature births in placentas of chagasic women and strongly associated to the congenital transmission of T. cruzi. In these clinical situations, the detachment of the first placental barrier is a common sign which is also associated to parasitism of the placental tissue [9]. Thus, differential infection between the first placental barrier with respect to the inner trophoblast or stromal cells could represent a mechanism of invasion of the human placenta by T. cruzi.

8. Conclusion

Congenital Chagas transmission constitutes an increasing public health problem, and it is responsible for the urbanization and spreading of the disease to nonendemic areas of Latin America, United States of America, and Europe [18, 21]. The fact that the ex vivo infection of the chorionic villi explants with the parasite reproduces the in vivo infection in terms of cellular changes and infectivity makes it an excellent tool for studying parasite infection strategies as well as possible local antiparasitic mechanisms.

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Research Article

Transplacental Transmission of *Plasmodium falciparum* in a Highly Malaria Endemic Area of Burkina Faso

Alphonse Ouédraogo,¹ Alfred B. Tiono,¹ Amidou Diarra,¹ Edith C. Christiane Bougouma,¹ Issa Nébié,¹ Amadou T. Konaté,¹ and Sodiomon B. Sirima¹,²

¹Centre National de Recherche et de Formation sur le Paludisme, 1487 Avenue de l’Oubritenga, BP 2208, Ouagadougou 01, Burkina Faso
²Groupe de Recherche Action en Santé, BP 10248, Secteur 25, Songandé, Rue 25.26, Porte 79, Ouagadougou 06, Burkina Faso

Correspondence should be addressed to Sodiomon B. Sirima, s.sirima.cnlp@fasonet.bf

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Malaria congenital infection constitutes a major risk in malaria endemic areas. In this study, we report the prevalence of transplacental malaria in Burkina Faso. In labour and delivery units, thick and thin blood films were made from maternal, placental, and umbilical cord blood to determine malaria infection. A total of 1,309 mother/baby pairs were recruited. Eighteen cord blood samples (1.4%) contained malaria parasites (*Plasmodium falciparum*). Out of the 369 (28.2%) women with peripheral positive parasitemia, 211 (57.2%) had placental malaria and 14 (3.8%) had malaria parasites in their umbilical cord blood. The umbilical cord parasitemia levels were statistically associated with the presence of maternal peripheral parasitemia (OR = 9.24, *P* ≪ 0.001), placental parasitemia (OR = 10.74, *P* ≪ 0.001), high-density peripheral parasitemia (OR = 9.62, *P* ≪ 0.001), and high-density placental parasitemia (OR = 4.91, *P* = 0.03). In Burkina Faso, the mother-to-child transmission rate of malaria appears to be low.

1. Introduction

In malaria high-transmission areas, some population groups are at considerably higher risk of infection with *Plasmodium falciparum* and development of malaria morbidity or mortality than others. These include children less than five years of age and pregnant women. Malaria contributes significantly to perinatal disease burden in terms of pregnancy loss, prematurity due to preterm labor, and intrauterine growth retardation [1]. Malaria infection during pregnancy poses a substantial risk to the mother, her fetus, and the neonate. In areas of stable malaria transmission such as Burkina Faso, where adult women have considerable acquired immunity, *Plasmodium falciparum* infection during pregnancy does not cause symptomatic malaria, but may lead to maternal anemia as well as placental and cord blood malaria infection, especially among primigravidae and secundigravida [2–4].

Placental malaria is defined as the accumulation of *Plasmodium*-infected erythrocytes in the intervillous space in the placenta, causing histologic changes including leukocyte-induced damage to the trophoblastic basement membrane. The placental infection does not reflect the existence of peripheral infection over a short period preceding the delivery or whether it is related to infection during pregnancy. Susceptibility to this may be correlated to high exposure to malaria and repeated episodes of parasitemia during the pregnancy [5].

Vertical transmission of malaria from mother to foetus through the placenta and umbilical cord is defined as umbilical cord blood parasitemia. The transplacental transmission of *Plasmodium falciparum* from mother to fetus has long been well-described [6, 7].

The direct burden of neonatal malaria infection in terms of prevalence is not well-described in malaria endemic areas. In fact, the method used to identify congenital transmission is peripheral blood of newborns or umbilical cord blood. Malaria parasites have been detected only rarely in the peripheral blood of newborns, whether the blood specimen
is collected at the time of birth or hours later [8, 9]. In studies in which both umbilical cord blood and infant peripheral blood were obtained at the time of birth, the parasite load in the babies’ peripheral blood has always been lower than that in the umbilical cord blood [9–11]. Studies published so far have documented contradictory levels of this burden. In countries without endemic malaria, congenital malaria has occurred in children born to women who have immigrated from malarious areas. However, transplacental transmission of *Plasmodium falciparum* has been found to be rare in malaria-endemic areas, ranging from about 1 to 5% [12–15]. In contrast, data from recent studies on the burden of congenital and neonatal malaria, while scarce and contradictory, have indicated a high burden (more than 15%) in parts of sub-Saharan Africa [13, 15–18].

In Burkina Faso, the real prevalence of neonatal malaria is unknown but is estimated to be even higher. This assessment is based on presumptive malaria diagnosis. The present study was designed to determine the real burden of transplacental transmission, the risk factors associated with transplacental transmission, and the prevalence of cord blood and placental malaria parasitemia in malaria holoendemic area of Burkina Faso.

These results represent a pooled analysis of studies on malaria prevention in pregnant women [4, 19, 20] examining umbilical cord blood to determine the frequency of transplacental transmission of *Plasmodium falciparum*.

2. Materials and Methods

2.1. Study Site. The first study took place within six delivery units (DUs) of the Koupela health district, which is located around 120 km east of Ouagadougou. The second study was conducted in one DU of the Health District of Bousse. Both study sites have been extensively described elsewhere [4, 19, 20]. Malaria transmission is stable, with marked seasonality in both sites. Transmission is intense during the rainy season (June to October). The main malaria vectors are *Anopheles gambiae*, *Anopheles arabiensis*, and *Anopheles funestus*. The annual entomological inoculation rate ranges from 10 to 500 infective bites per individual. *Plasmodium falciparum* is responsible for more than 90% of malaria infections.

2.2. Study Population. The study participants were pregnant women who voluntarily consented to participate in trials of malaria prophylaxis during pregnancy. They were encouraged to deliver at the health facility where the study samples were collected for processing.

2.3. Clinical Procedures. Women delivering at the health facility, after giving informed consent, were asked a standard series of questions focused on sociodemographic characteristics, history of fever, antimalarial drug use, and the use of antimalarial chemoprophylaxis and bednets. Capillary blood was obtained by fingerstick for malaria blood film preparation. Placental blood films were prepared by identifying the maternal side of the placenta, wiping away excess blood, cutting into the surface, and placing pooled blood onto a slide. Umbilical cord blood samples were obtained by wiping away excess blood from a clamped cord, piercing it with a lancet, and placing a drop of expressed blood on a slide.

A detailed clinical examination was done on each newborn infant within 24 hours of delivery. Neonates were weighed with an electronic digital scale (+10 grams) (Tanita Corporation, Tokyo, Japan). The Dubowitz scoring system was used to estimate gestational age, using findings from physical and neurologic examinations. Scoring by APGAR index was performed at delivery but was not recorded in this study.

2.4. Lab Methods. All blood films (maternal, placental, and cord) were stained with Giemsa and examined for parasites at the “Centre National de Recherche et de Formation sur le Paludisme” immunoparasitology laboratory in Ouagadougou. For thick films, parasites and leukocytes were counted in the same fields until 500 leukocytes were counted. parasite densities were estimated using an assumed leukocyte count of 8,000 leukocytes/µL. Parasite densities were calculated according to the following formula: number of parasites counted × 8000/number of leukocytes counted. Thin films were then used to determine species when thick films were positive. All slides were double-read by two independent microscopists. If the ratio of densities from the first two readings was greater than 1.5 or less than 0.67, or if fewer than 30 parasites were counted with a difference of more than 10 parasites between the two readings, the slide was evaluated a third time. The geometric mean of the parasite density of the two most concordant results of the three readings was taken as the final result. When the discordance was only in terms of positivity, the slide was also evaluated a third time and the definitive result was based on the majority verdict for positivity.

2.5. Definitions. Blood film results were considered to be positive if any asexual-stage parasites were identified and negative if no parasites were seen in 100 high-power fields. Prematurity was defined as a gestational age <37 weeks as estimated by Dubowitz examination.

We defined low birth weight as a birth weight of <2,500 g with a gestational age ≥37 weeks.

2.6. Statistical Analysis. Data collected in the study questionnaire were verified, then double-entered, and validated with EpInfo, version 6.04 fr (Centers for Diseases Control and Prevention, Atlanta, USA). Data were analyzed using Epi-info and Stata 7.0. The analysis included data from births of all enrolled participants who delivered at the study centre clinic for whom data were available. Continuous, normally distributed data were described by mean and standard deviation and nonnormally distributed data by the median or geometrical mean and range. Proportions were compared using the Chi-square test and normally distributed continuous variables were compared using Student’s *t*-test. Statistical results were considered significant, when the two-sided *P* value was <0.05.
2.7. Ethical Considerations. The study was discussed with health authorities and local leaders to obtain their assent. The study was reviewed and approved by the Burkina Faso Ministry of Health ethical committee. Informed consent (by signature or thumbprint) was obtained after the consent document was read to the women in the local language. For illiterate mothers, the informed consent discussion process document was read to the women in the local language. For illiterate mothers, the informed consent discussion process was witnessed by an impartial individual.

3. Results

3.1. Characteristics of Women at the Delivery Unit. In total, 1,374 women delivered at the health unit; 1,309 women delivered singleton infants and provided data in the form of peripheral blood smear, placental blood, and umbilical cord blood. The profile of enrolled women is summarized in Table 1. Most of the women spoke Moore, a language indigenous to this region of Burkina Faso. The mean age (SD) of the women was 23.90 ± 5.89 years. The self-report indicated that ownership and use of insecticide-impregnated materials (bed nets and/or curtains) was reported by approximately one-third of the women. The rate of use of insecticide-impregnated materials (bed nets and/or curtains) was very low in the study population. All of the women used drug prophylaxis during their pregnancy (IPTp/SP or weekly prophylaxis with chloroquine).

Primigravidae comprised 35% of the total sample size, and primigravidae and secundigravida together made up 56% of the total sample size. The mean duration of gestation was 38.66 ± 3.89 weeks. The mode of delivery of babies was spontaneous for 97.5% of the mothers.

Two women died during delivery as a consequence of eclampsia. Other complications during the delivery included peripartum haemorrhage, placental retention, and retroplacental haemorrhage. Fifty stillbirths (3.6%) and 18 (1.3%) miscarriages occurred at the DUs.

The weight range of babies was 700 g to 4,700 g with a mean weight of 2,875 ± 450 g. Sixteen percent of the babies presented low birth weight at delivery (Table 1).

3.2. Maternal Peripheral Parasitemia: Placental and Umbilical Cord Blood Parasitemia. Over one-third of the women were parasitaemic. Indeed, of the 1,309 pregnant women, Plasmodium falciparum was detected in peripheral blood from 369 (28.2%) of the women at the time of delivery. Overall, the geometric mean of the maternal peripheral parasite density was 1,106.47 (95% CI 871.58–1404.67) parasites/µL. Table 2 shows the prevalence of peripheral, placental, and umbilical cord parasitemia. Plasmodium falciparum was detected in the placentas of 255 (19.5%) women; 28.5% of these belonged to primigravidae and 11.4% to multigravidae. The difference in infection between the primigravidae and the multigravidae was statistically significant (P < 0.0001). All of the women received malaria prophylaxis during pregnancy.

Umbilical cord blood parasitemia with Plasmodium falciparum was detected in 18 (1.4%) babies, and the geometric mean umbilical cord blood parasitemia was 315.69 (95% CI 79.38–1245.20) parasites/µL. Of these, 2.4% (11) belonged to primigravidae and 0.5% (3) to multigravidae. Of the 18 babies with Plasmodium-falciparum-positive umbilical cord blood parasitemia, 17 were positive for trophozoites and 1 was positive for schizonts only. Both schizonts and trophozoites were present in seven babies, and one newborn had a triple-positive association (trophozoite + schizont + gametocyte). Five newborns (27.8%) out of the 18 infected with Plasmodium falciparum had low birth weight.

Out of 369 (28.2%) women with peripheral positive parasitemia, 211 (57.2%) had positive placental malaria and 14 (3.8%) had malaria parasites in the umbilical cord blood. Eleven (5.2%) of the 211 (57.2%) women with positive placental smears for malaria had malaria parasites in the umbilical cord blood. Of the 18 babies infected with Plasmodium falciparum, 14 (77.8%) were born from mothers with

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
<th>2</th>
<th>&gt;2</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (years)</td>
<td>19.24 ± 2.25</td>
<td>21.68 ± 2.60</td>
<td>28.71 ± 5.38</td>
<td>23.90 ± 5.89</td>
</tr>
<tr>
<td>Mean gestational age (weeks)</td>
<td>38.16 ± 4.24</td>
<td>38.79 ± 3.79</td>
<td>39.02 ± 3.59</td>
<td>38.66 ± 3.89</td>
</tr>
<tr>
<td>Mossi ethnic group (%)</td>
<td>95.8</td>
<td>96.2</td>
<td>94.7</td>
<td>95.5</td>
</tr>
<tr>
<td>Sleeps under bednets (%)</td>
<td>22.8</td>
<td>28.1</td>
<td>19.4</td>
<td>22.4</td>
</tr>
<tr>
<td>Mother death (N)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Pregnancy outcome

<table>
<thead>
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<th>1</th>
<th>2</th>
<th>&gt;2</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stillbirth (N)</td>
<td>19</td>
<td>8</td>
<td>23</td>
<td>50</td>
</tr>
<tr>
<td>Miscarriage (N)</td>
<td>8</td>
<td>3</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>2,719 ± 430</td>
<td>2,893 ± 390</td>
<td>2,995 ± 440</td>
<td>2,875 ± 450</td>
</tr>
<tr>
<td>Number of live babies (N)</td>
<td>460</td>
<td>270</td>
<td>579</td>
<td>1309</td>
</tr>
<tr>
<td>LBW* (N/%)</td>
<td>111 (24.1%)</td>
<td>39 (14.4%)</td>
<td>57 (9.8%)</td>
<td>207 (15.8%)</td>
</tr>
</tbody>
</table>

LBW*: low birth weight.
Table 2: The proportion of newborns with *Plasmodium falciparum* parasitemia in umbilical cord blood, placental parasitemia, and maternal parasitemia, along with parasite density.

<table>
<thead>
<tr>
<th>Gravidity</th>
<th>1</th>
<th>2</th>
<th>&gt;2</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral parasitemia (%)</td>
<td>34.3</td>
<td>35.2</td>
<td>20.3</td>
<td>28.2</td>
</tr>
<tr>
<td>Geometric mean (parasites/µL) (CI 95%)</td>
<td>2,143.18 (1,552.86–2,957.92)</td>
<td>894.71 (548.60–1,459.19)</td>
<td>495.09 (318.44–769.74)</td>
<td>1,106.47 (871.58–1,404.67)</td>
</tr>
<tr>
<td>Negative peripheral parasitemia (%)</td>
<td>32.4</td>
<td>18.8</td>
<td>48.8</td>
<td>71.7</td>
</tr>
<tr>
<td>Peripheral parasitemia 1–999 (%)</td>
<td>30.9</td>
<td>29.5</td>
<td>39.6</td>
<td>11.4</td>
</tr>
<tr>
<td>Peripheral parasitemia 1,000–4,999 (%)</td>
<td>53.3</td>
<td>19.6</td>
<td>27.2</td>
<td>7.1</td>
</tr>
<tr>
<td>Peripheral parasitemia 5,000–9,999 (%)</td>
<td>52.5</td>
<td>22.5</td>
<td>25.0</td>
<td>3.1</td>
</tr>
<tr>
<td>Peripheral parasitemia ≥10,000 (%)</td>
<td>58.8</td>
<td>25.0</td>
<td>16.2</td>
<td>5.2</td>
</tr>
<tr>
<td>Placental parasitemia (%)</td>
<td>28.5</td>
<td>21.9</td>
<td>11.4</td>
<td>19.5</td>
</tr>
<tr>
<td>Geometric mean (parasites/µL) (CI 95%)</td>
<td>1384.55 (880.97–2,176.00)</td>
<td>396 (223.30–703.17)</td>
<td>591.71 (289.60–1,209.00)</td>
<td>830.52 (599.66–1,150.27)</td>
</tr>
<tr>
<td>Negative placental parasitemia (%)</td>
<td>30.9</td>
<td>20.0</td>
<td>49.1</td>
<td>19.9</td>
</tr>
<tr>
<td>Placental parasitemia 1–999 (%)</td>
<td>46.5</td>
<td>26.4</td>
<td>27.1</td>
<td>10.1</td>
</tr>
<tr>
<td>Placental parasitemia 1,000–4,999 (%)</td>
<td>51.2</td>
<td>26.8</td>
<td>22.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Placental parasitemia 5,000–9,999 (%)</td>
<td>50.0</td>
<td>29.2</td>
<td>20.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Placental parasitemia ≥10,000 (%)</td>
<td>69.7</td>
<td>6.1</td>
<td>24.2</td>
<td>2.6</td>
</tr>
<tr>
<td>Umbilical cord parasitemia (%)</td>
<td>2.4</td>
<td>1.5</td>
<td>0.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Geometric mean (parasites/µL) (CI 95%)</td>
<td>263.74 (40.85–1,702.50)</td>
<td>644.08 (5.85–70,803.72)</td>
<td>222.16 (0.76–64,635.57)</td>
<td>315.69 (87.02–1,145.20)</td>
</tr>
<tr>
<td>Negative umbilical cord parasitemia (%)</td>
<td>34.9</td>
<td>20.5</td>
<td>44.7</td>
<td>98.6</td>
</tr>
<tr>
<td>Umbilical cord parasitemia 1–999 (%)</td>
<td>61.5</td>
<td>15.4</td>
<td>23.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Umbilical cord parasitemia 1,000–4,999 (%)</td>
<td>0.0</td>
<td>100</td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Umbilical cord parasitemia 5,000–9,999 (%)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Umbilical cord parasitemia ≥10,000 (%)</td>
<td>66.7</td>
<td>33.3</td>
<td>0.0</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Positive peripheral parasitemia. For two newborns with *Plasmodium falciparum* infection, no peripheral or placental infection was found in the mother. None of the babies with cord blood positive for malaria had fever within the first 24 h of life.

The number of pregnant women with peripheral, placental, and umbilical infection and high peripheral parasitemia (>10,000 parasites/µL) decreased with increasing gravidity (Table 2).

The combination of being born to a mother with both peripheral and placental infection conferred a greater likelihood of infection of the baby (61%). Being born with only maternal peripheral infection reduced the likelihood of having umbilical cord parasitemia (17%). There was an association between umbilical cord blood infection and maternal peripheral infection (P < 0.001). Being born with only placental infection reduced the rate of umbilical cord parasitemia (11%). There was also an association between umbilical cord blood infection and placental infection (P < 0.001) (Table 3).

Being born to a mother with maternal peripheral parasite density ≥5,000 parasites/µL or placental parasite density ≥10,000 parasites/µL conferred a greater likelihood of umbilical cord parasitemia (Table 3).

### 3.3. Risk Factors.

We further examined the correlation between risk factors and the presence or absence of umbilical cord parasitemia to determine possible factors that might affect infection with malaria parasites in umbilical cord blood. Univariate logistic regression analysis indicated that being born with maternal parasitemia, being born with maternal parasite density ≥5,000 parasites/µL, being born with placental parasitemia, being born with placental parasite density between 1,000–4,999 parasites/µL, being born with placental parasite density ≥10,000 parasites/µL, and being born in a first pregnancy were all associated with the presence of umbilical cord blood parasitemia. Low birth weight was not associated with umbilical cord blood parasitemia (Table 3).

Low maternal peripheral and placental parasitemia, prematurity, use of impregnated bednets, low birth weight, and female sex were independent risk factors for infection in the babies.
Table 3: Univariate logistic regression of risk factors associated with *Plasmodium falciparum* parasitemia in umbilical cord blood.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Proportion of newborns with umbilical cord blood parasitemia and the risk factor specified</th>
<th>Odds ratio</th>
<th>95% confidence intervals</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral positive parasitemia</td>
<td>77.8%</td>
<td>9.24</td>
<td>3.02–28.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peripheral parasitemia 1–999</td>
<td>22.2%</td>
<td>2.23</td>
<td>0.75–7.20</td>
<td>0.13</td>
</tr>
<tr>
<td>Peripheral parasitemia 1,000–4,999</td>
<td>11.1%</td>
<td>1.64</td>
<td>0.37–7.28</td>
<td>0.50</td>
</tr>
<tr>
<td>Peripheral parasitemia 5,000–9,999</td>
<td>22.2%</td>
<td>9.62</td>
<td>3.01–30.69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peripheral parasitemia 10,000</td>
<td>22.2%</td>
<td>5.29</td>
<td>1.69–16.52</td>
<td>0.004</td>
</tr>
<tr>
<td>Placental positive parasitemia</td>
<td>72.2%</td>
<td>10.74</td>
<td>3.79–30.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Placental parasitemia 1–999</td>
<td>22.2%</td>
<td>10.74</td>
<td>3.79–30.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Placental parasitemia 1–999</td>
<td>22.2%</td>
<td>10.74</td>
<td>3.79–30.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Placental parasitemia 1–999</td>
<td>22.2%</td>
<td>10.74</td>
<td>3.79–30.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Placental parasitemia 1–999</td>
<td>22.2%</td>
<td>10.74</td>
<td>3.79–30.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PBW*</td>
<td>27.8%</td>
<td>2.10</td>
<td>0.74–5.97</td>
<td>0.16</td>
</tr>
<tr>
<td>Female sex of infant</td>
<td>61.1%</td>
<td>1.78</td>
<td>0.68–4.63</td>
<td>0.33</td>
</tr>
</tbody>
</table>

LBW*: low birth weight.

Table 4: Multivariate logistic regression of risk factors, associated with *Plasmodium falciparum* parasitemia in umbilical cord blood.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Odds ratio</th>
<th>95% confidence intervals</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral positive parasitemia</td>
<td>2.43</td>
<td>0.50–11.66</td>
<td>0.26</td>
</tr>
<tr>
<td>Peripheral parasitemia 5,000–9,999</td>
<td>3.71</td>
<td>0.92–14.98</td>
<td>0.06</td>
</tr>
<tr>
<td>Peripheral parasitemia ≥10,000</td>
<td>1.60</td>
<td>0.38–6.66</td>
<td>0.51</td>
</tr>
<tr>
<td>Placental positive parasitemia</td>
<td>3.30</td>
<td>0.73–14.87</td>
<td>0.11</td>
</tr>
<tr>
<td>Placental parasitemia 1,000–4,999</td>
<td>2.27</td>
<td>0.58–8.77</td>
<td>0.23</td>
</tr>
<tr>
<td>Placental parasitemia ≥10,000</td>
<td>0.95</td>
<td>0.15–5.69</td>
<td>0.95</td>
</tr>
<tr>
<td>Primigravid</td>
<td>1.91</td>
<td>0.70–5.21</td>
<td>0.20</td>
</tr>
</tbody>
</table>

On multivariate logistic regression, none of the seven factors that were significant on univariate logistic regression remained significant (Table 4).

4. Discussion

4.1. Low Levels of Umbilical Cord Parasitemia. This study shows the burden of malaria in pregnant women at the delivery unit in a malaria-endemic area of Burkina Faso. This investigation demonstrated the burden of malaria during pregnancy. The maternal peripheral parasitemia rate found in this study was 28.2%. This is similar to the rate reported in other countries in sub-Saharan Africa [2, 21].

The prevalence of placental parasites was 19.5%. Previously reported prevalence rates of placental parasitemia in Africa have been highly variable, ranging from 17.2% to 57% [11, 22–24]. The placenta is a site for *Plasmodium falciparum* sequestration. Many hypotheses, based on a systemic or local failure of the immunological response to malaria, have been proposed to explain the preference of the parasites for replication in the placenta [25].

The malaria rate in umbilical cord blood was low in the study population, occurring in 1.4% of all newborns, in 5.2% of babies born from mothers with peripheral and placental malaria infection, and in 5.1% of babies born from women with placental malaria infection only. Previously reported rates from different parts of Africa are highly variable, ranging from 0% to 54% [11, 22, 24, 26, 27]. The low rate in the present study, which is comparable to rates reported in some previous manuscripts, could be explained by effective prevention of malaria during pregnancy. In fact, it has now been proven that malaria parasites identified in cord blood are acquired antenatally by transplacental transmission of infected erythrocytes. The mechanisms underlying congenital transmission, according to an earlier report, include maternal transfusion into the fetal circulation either at the time of delivery or during pregnancy, through direct penetration through the chorionic villi, or through premature separation of the placenta [28, 29]. Pregnant women, who have received antimalarial treatment, should be able to clear parasitemia to avoid umbilical cord transmission. Immunity in the mother could also provide an explanation. Transplacental transmission of malaria appears to occur...
infrequently; it may be possible that after transplacental transmission, some elements of immunity, acquired from the mother, protect the infants from becoming infected [24]. This resistance may reflect, among other things, the physical barrier of the placenta to infected red cells, the passive transfer of maternal antibodies, and/or the poor environment afforded by fetal erythrocytes for plasmodial replication, due to their fetal hemoglobin composition and low free-oxygen tension [28].

Some studies have found high rates of transplacental malaria infection. The difference between those studies and our report could be explained by several factors. First, the high efficacy of malaria prophylaxis during this study may have allowed the women to clear their parasitaemia and therefore prevent placental and transplacental transmission of the infection to their babies. Second, our sole use of microscopy may have led to underdiagnosis, since this method has lower sensitivity than the newer molecular methods, such as real-time PCR, used in other studies [22, 29]. Finally, it might be that our numbers reflect the true prevalence of infection, as compared with data collected during routine services, in which lack of quality control of the reading of smears could lead to an overestimation of the infection rate.

4.2. Risk Factors. Univariate analysis of the association of risk factors with the presence or absence of umbilical cord parasitemia revealed seven risk factors that was associated with *Plasmodium falciparum* parasitemia in umbilical cord blood. The presence of maternal peripheral parasitemia, placental parasitemia, high-density peripheral parasitemia, high-density placental parasitemia, and primigravid status was associated with an increased risk of malaria parasitemia in cord blood. However, in multivariate analysis, these associations failed to reach statistical significance.

Maternal peripheral blood parasitemia was associated with umbilical cord blood parasitemia (OR = 9.24, *P* < 0.001). Four cases of umbilical cord blood parasitemia occurred in pregnancies not complicated by maternal peripheral malaria infection. There were also associations between placental and cord blood parasitemia (OR = 10.74, *P* < 0.001), and between cord blood/placental and maternal peripheral parasitemia. Five cases of umbilical cord blood parasitemia occurred in pregnancies not complicated by placental malaria infection. Only two newborns had mothers with neither peripheral malaria infection nor placental malaria infection. Earlier studies of transplacental malaria reported these associations [11, 18, 24, 30]. A strong association between placental malaria and umbilical cord blood parasitemia has been reported, and this was suggested to be responsible for congenital malaria. However, whether the presence of *Plasmodium falciparum* malaria parasites in umbilical cord blood denotes infection acquired antenatally or contamination with infected maternal blood at delivery is not clear [15]. These associations suggest that clearing maternal and placental parasitemia with effective antimalarial drugs before delivery could prevent transplacental transmission of parasitemia. The density of maternal peripheral parasitemia (>5,000 parasites/µL) was an important determinant of the likelihood of umbilical cord blood parasitemia (OR = 5.29, *P* = 0.004). The density of placental parasitemia (>10,000 parasites/µL) was also an important determinant of the likelihood of umbilical cord parasitemia (OR = 4.91, *P* = 0.03). High density parasitemia seems to facilitate umbilical cord infection. Conversely, previous reports, showing low rates of transplacental transmission of malaria, suggest that the placental barrier is very effective in the case of very low malaria parasite density [22].

Finally, univariate analysis showed that being born from a first pregnancy was also linked to umbilical cord blood parasitemia (OR = 2.96, *P* = 0.02).

5. Conclusions

Our data indicate that the rate of mother-to-child transmission of malaria, defined as positive umbilical cord blood parasitemia, appears to be low. Clinicians are therefore advised to investigate other aetiologies of fever in neonates. The low level of parasitaemia in cord blood suggests that contamination probably occurs during the labour period. Maternal, placental, and high density parasitemia were all associated with umbilical cord parasitemia. Prevention of malaria during pregnancy with effective antimalarial drugs should reduce the risk of infection for newborns.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

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References


Research Article

Reorganization of Extracellular Matrix in Placentas from Women with Asymptomatic Chagas Disease: Mechanism of Parasite Invasion or Local Placental Defense?

Juan Duaso,¹ Erika Yanez,¹ Christian Castillo,¹ Norbel Galanti,² Gonzalo Cabrera,² Gabriela Corral,³ Juan Diego Maya,⁴ Inés Zulantay,² Werner Apt,² and Ulrike Kemmerling¹, ⁵

¹ Programa de Anatomía y Biología del Desarrollo, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Independencia, Región Metropolitana, 1027 Santiago de Chile, Chile
² Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Independencia, Región Metropolitana, 1027 Santiago de Chile, Chile
³ Servicio de Obstetricia y Ginecología, Hospital de Illapel, Independencia, IV Región, 0512 Illapel, Chile
⁴ Programa de Farmacología Molecular y Clínica, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Independencia, Región Metropolitana, 1027 Santiago de Chile, Chile
⁵ Departamento de Estomatología, Facultad de Ciencias de la Salud, Universidad de Talca, Avenida Lircay s/n, VII Región, 3460000 Talca, Chile

Correspondence should be addressed to Ulrike Kemmerling, ukemmerling@med.uchile.cl

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Chagas disease, produced by the protozoan Trypanosoma cruzi (T. cruzi), is one of the most frequent endemic diseases in Latin America. In spite of the fact that in the past few years T. cruzi congenital transmission has become of epidemiological importance, studies about this mechanism of infection are scarce. In order to explore some morphological aspects of this infection in the placenta, we analyzed placentas from T. cruzi-infected mothers by immunohistochemical and histochemical methods. Infection in mothers, newborns, and placentas was confirmed by PCR and by immunofluorescence in the placenta. T. cruzi-infected placentas present destruction of the syncytiotrophoblast and villous stroma, selective disorganization of the basal lamina, and disorganization of collagen I in villous stroma. Our results suggest that the parasite induces reorganization of this tissue component and in this way may regulate both inflammatory and immune responses in the host. Changes in the ECM of placental tissues, together with the immunological status of mother and fetus, and parasite load may determine the probability of congenital transmission of T. cruzi.

1. Introduction

American Trypanosomiasis, or Chagas disease, is a zoonosis caused by Trypanosoma cruzi (T. cruzi). Currently, 10 million people in the Americas, from Mexico in the north to Argentina and Chile in the south, are estimated to be infected [1]. For thousands of years, Chagas disease was known only in the Region of the Americas, mainly in Latin America, where it has been endemic [2]. In past decades, it has been increasingly detected in other non-endemic countries in the American (Canada and the United States), the Western Pacific (mainly Australia and Japan) and the European continents. The presence of Chagas disease outside Latin America is the result of population mobility, notably migration, but cases have been reported among travelers returning from Latin America and even in adopted children [3]. Subsequent transmission occurs through transfusion, vertical, and transplantation routes [3].

The vertical transmission of T. cruzi cannot be prevented, but early detection and treatment of congenital infection achieves cure rates close to 100 per cent [4].

Fetal and maternal tissues are separated by a fetal epithelium (the trophoblast), the greatest area of which is in the villous placenta, the site of nutrient and gas exchange [5]. The human placenta is classified as a hemochorial villous placenta in which the free chorionic villi are the functional units.
These chorionic villi are formed by the trophoblast and the villous stroma. The trophoblast is formed by a single multinucleated cell layer (syncytiotrophoblast) which contacts maternal blood in the intervillous space, and by the cytotrophoblast which contains replicating progenitor cells. The trophoblast is separated by a basal lamina from the villous stroma, which is connective tissue containing vascular endothelium, fibroblasts, and macrophages [5]. Trophoblast, basal laminae, and villous stroma with endothelium of fetal capillaries form the placental barrier that must be crossed by different pathogens, including T. cruzi, in order to infect the fetus during vertical transmission [6, 7].

Damage to the villous placenta is almost always accompanied by inflammation, either in the intervillous space or within the fetal villi, and in severe cases is accompanied by loss of the protective trophoblast. Extensive placental damage may lead to fetal loss, intrauterine growth retardation [5], and clinical manifestations that can be observed in acute congenital Chagas disease [8, 9].

We have previously demonstrated that T. cruzi induces syncytiotrophoblast destruction and detachment in chorionic villi explants, and selective disorganization of basal lamina and disorganization of collagen I in the connective tissue of villous stroma [6]. However, no studies observing similar histopathological alterations in the different tissue compartments of chorionic villi from placentas of mothers with Chagas disease have been reported.

The aim of the present study was to determine whether the tissue disorganization and destruction of chorionic villi, described previously in ex vivo infected chorionic villi explants, can also be observed in placentas from Chagasian women. The diagnosis of Chagas disease in the women studied was performed by standard serological testing and PCR. Additionally, the parasite was detected in the placenta by immunofluorescence and PCR. Histopathological analysis, immunohistochemical studies of basal lamina, and histochemical studies of carbohydrate-rich molecules and collagen I in villous stroma of these placentas were performed.

2. Material and Methods

2.1. Patients and Diagnosis of T. cruzi Infection. Three pregnant women with asymptomatic Chagas disease were enrolled from the hospitals of Illapel, Servicio de Salud de Coquimbo, IV Region of Chile. Informed consent for the experimental use of the placentas was given by each patient as stipulated by the Code of Ethics of the Faculty of Medicine of the University of Chile. Maternal T. cruzi infection was assessed by standard serological techniques and PCR (Figure 1(a) and Table 1). T. cruzi infection in neonates was diagnosed by detection of parasites in umbilical cord or in peripheral blood by microscope examination using heparinized microhematocrite tubes and PCR (Figure 1(a) and Table 1). All of the neonates were negative for the presence of the parasite by direct parasitological examination. Two mothers and their respective newborns were positive for parasite DNA detection by PCR (Figure 1 lines 5 & 7 and 8 & 10), the third mother and her newborn were negative for parasite DNA detection by PCR (Figure 1 lines 11 & 13).

![Figure 1: Detection of T. cruzi in mother, newborn, and placenta by PCR. A 330-base pair fragment of the T. cruzi satellite DNA was amplified as described in Material and Methods. MW: molecular marker, lane 1: Negative control without DNA; lane 2: DNA from T. cruzi epimastigotes; lane 3: Noninfected human blood DNA; lane 4: Noninfected human placenta DNA; lanes 5–7: parasite DNA detected in peripheral blood (lane 5), in placenta (lane 6), and respective umbilical chord from neonate (lane 7) of mother 1; lanes 8–10: parasite DNA detected in peripheral blood (lane 8), in placenta (lane 9), and respective umbilical chord from neonate (lane 10) of mother 2; lanes 11–13: parasite DNA detected in peripheral blood (lane 11), in placenta (lane 12) and respective umbilical chord from neonate (lane 13) of mother 3. Note that in mother 3 parasite DNA can only be detected in the placenta. The PCR product was subjected to electrophoresis in 1.6% agarose gels and stained with ethidium bromide. PCR markers from Promega were employed as molecular weight standards.](image)

Nevertheless, this last mother was positive using standard serological techniques. Gestational age was determined by clinical and ultrasound analysis. Clinical data of the mothers and newborns are shown in Table 1.

For parasite DNA detection by PCR, a 330-base pair fragment of the T. cruzi satellite DNA was amplified as described previously [10]. The sequence of the oligonucleotides is the following: forward (5-AAAATGTACGGTACG(T/G)GAGATGCATGAA-3, specific primer 121) and backward (5-GGTTCGATTGGGGTTGGTGTAATATA-3, specific primer 122). The PCR product was subjected to electrophoresis in 1.6% agarose gels and stained with ethidium bromide. PCR markers from Promega were employed as molecular weight standards [10].

2.2. Placentas. We used three placentas from asymptomatic chagasic women. Placentas from control healthy women were obtained from vaginal or caesarean deliveries of uncomplicated pregnancies from the Hospital San José, Servicio de Salud Norte; Region Metropolitana, Santiago, Chile. Exclusion criteria for the control placentas were the following: major fetal abnormalities, placental tumor, intrauterine infection, obstetric pathology, and any other maternal disease. Villous tissue was obtained from the central part of cotyledons and immediately fixed in 10% formaldehyde in 0.1 M phosphate buffer (pH 7.3) for 24 hours.

2.3. Histological and Histochemical Techniques. The fixed tissues were dehydrated in alcohol, clarified in xylene, embedded in paraffin, and sectioned at 5 μm. Paraffin-embedded histological sections that were stained with hematoxylin-eosin for routine histological analysis, Picrosirius red-hematoxylin [6] and Arteta [11] for collagen histochemistry, and periodic acid-Schiff (PAS) for carbohydrate containing tissue elements (Sigma-Aldrich kit 395B) were applied.
were fixed, dehydrated, and embedded in paraaffin. The primary antibodies were applied individually to each section at 4°C overnight (anti-collagen IV Novocastra NCL-COLL-IV, dilution 1:100 v/v; anti-heparan sulphate Novocastra NCL-CD44-2, dilution 1:40 v/v; anti-fibronectin ABR MA1-83176, dilution 1:50 v/v). Immunostaining was performed using a horseradish peroxidase-labelled streptavidin-biotin kit (RTU-Vectastain kit) following the manufacturer’s directions using diaminobenzidine as the chromogen. Controls were performed by replacing the primary antibodies with phosphate buffered saline. All controls were negative. All sections were examined by light microscopy (Leitz Orthoplan), and images were captured with a Canon 1256 camera.

2.4. Immunohistochemistry. The placental chorionic villi were fixed, dehydrated, and embedded in paraffin as described above. Standard immunoperoxidase techniques were used to show collagen IV, heparan sulphate and fibronectin. The primary antibodies were applied individually to each section at 4°C overnight (anti-collagen IV Novocastra NCL-COLL-IV, dilution 1:100 v/v; anti-heparan sulphate Novocastra NCL-CD44-2, dilution 1:40 v/v; anti-fibronectin ABR MA1-83176, dilution 1:50 v/v). Immunostaining was performed using a horseradish peroxidase-labelled streptavidin-biotin kit (RTU-Vectastain kit) following the manufacturer’s directions using diaminobenzidine as the chromogen. Sections were counterstained with Mayer’s haematoxylin and her newborn were negative for PCR (Figure 1 lines 11 and 13) but the placenta was positive (Figure 1 line 12). The negative result for parasitic DNA detection by PCR in this mother and newborn may be due to a very low parasitemia, characteristic in the asymptomatic and chronic phases of Chagas disease. In these phases, the sensitivity of the techniques depends on the varying concentration of parasites in blood [1, 10]. For this reason, negative results do not necessarily indicate a lack of parasites in the blood or absence of infection. Serological diagnosis of T. cruzi infection is more sensitive than PCR [10, 12]; all three mothers analyzed by us had positive serology.

3. Results

3.1. T. cruzi Detection in Mothers, Newborns, and Placentas. The diagnosis of Chagas disease of the three mothers enrolled in our study was performed by standard serological techniques. After delivery, blood samples from the mothers and umbilical cords from respective neonates were processed for parasite DNA detection by PCR (Figure 1 lines 5 & 7 (mother & neonate 1), 8 & 10 (mother & neonate 2), 11 & 13 (mother & neonate 3)). DNA from the placentas was obtained from formalin-fixed placental tissue samples (Figure 1 lines 6, 9 and 12 show the results from placentas from mother 1, 2, and 3, resp.). Interestingly, one of the mothers (mother 3) and her newborn were negative for PCR (Figure 1 lines 11 and 13) but the placenta was positive (Figure 1 line 12). The negative result for parasitic DNA detection by PCR in this mother and newborn may be due to a very low parasitemia, characteristic in the asymptomatic and chronic phases of Chagas disease. In these phases, the sensitivity of the techniques depends on the varying concentration of parasites in blood [1, 10]. For this reason, negative results do not necessarily indicate a lack of parasites in the blood or absence of infection. Serological diagnosis of T. cruzi infection is more sensitive than PCR [10, 12]; all three mothers analyzed by us had positive serology.

Figure 2 shows the detection of the parasite by immunofluorescence. The placental chorionic villi were fixed, dehydrated, and embedded in paraffin as described above. A monoclonal antibody (mAb 25, dilution 1:100 v/v) specific for the T. cruzi flagellar calcium-binding protein (a gift from Dr. Schenkman, Universidade de Sao Paulo, Sao Paulo, Brasil) was applied to each section overnight at 4°C. The preparations were washed with PBS and incubated with anti-mouse IgG conjugated with fluorescein (ScyTek, ACA) in the presence of 1 μg/mL of 4′,6-diamidino-2-phenylindole (DAPI). Afterwards, sections were mounted in VectaShield (ScyTek, ACA) and observed in a Nikon Eclipse E400 microscope (Tokio, Japan).
Figure 2: Detection of T. cruzi in human placental chorionic villi from infected mothers. The presence of parasites in villous stroma of chorionic villi from T. cruzi infected placentas was shown by immunofluorescence, detected using mAb 25 antibodies. In (a–d), noninfected chorionic villi (controls) are shown. Panels (a–d) and (e–h) show images of the same fields. Panels (a) and (e): phase contrast; (b) and (f): nuclear staining with DAPI; (c) and (g): detection of the parasite by immunofluorescence; (d) and (h): merged images of (b-c) and (f-g), respectively. Inserts show an amplified region of the detection of the parasite by immunofluorescence (g-h). Bar scale (a–d): 50 μm; (e–h): 25 μm.

Figure 3: Histopathological analysis of placentas from mothers infected with T. cruzi. Chorionic villi from T. cruzi-infected placentas (b) show severe tissue damage compared to control villi from healthy noninfected woman (a). Detachment and disorganization of the syncytiotrophoblast (arrow) as well as fetal connective tissue destruction of the villous stroma (arrowhead) is observed. Chorionic villi were processed for routine histological techniques and stained with hematoxylin-eosin. Bar scale: 25 μm.

3.2. Histopathological Analysis of Placentas from Women Infected with T. cruzi. Figure 3(b) shows severe tissue disorganization and destruction of the chorionic villi from T. cruzi-infected mothers, detachment of the syncytiotrophoblast (arrows), and destruction of villous stroma (arrowhead). No inflammatory infiltrate is observed in placentas from infected women or control placentas. The control placentas from healthy women show an intact structure of the chorionic villi (Figure 3(a)).

3.3. Placental Chorionic Villi from Women Infected with T. cruzi Present Disorganization of the Extracellular Matrix. The extracellular matrix (ECM) is the noncellular component present in all tissues and organs and not only provides essential physical scaffolding for the cellular constituents, but also initiates crucial biochemical and biomechanical cues that are required for different tissue functions [16]. The ECM is composed of collagens, elastin, proteoglycans (including hyaluronan), and noncollagenous glycoproteins forming a complex, three-dimensional network among the cells of different tissues [17]. The non-collagenous proteins and proteoglycans are highly glycosylated and especially abundant in basal lamina [6, 18]. Several lines of evidence have shown that T. cruzi interacts with host ECM components producing breakdown products that play an important role...
Figure 4: Placentas from mothers infected with T. cruzi present severe disorganization of chorionic villi ECM. Placental tissue from healthy noninfected (a–c) and infected (d–f) women were processed for routine histochemical methods and stained with the PAS method for the detection of glycosylated components (a, d) with the Arteta trichromic (b, e), and picrosirius red-hematoxylin (c, f) methods for collagen histochemistry. Placental chorionic villi from infected mothers show reduced staining of glycosylated molecules (d), especially in basal lamina (arrows) compared to controls (a). Samples from infected mothers also show reduced histochemical reaction for collagen in basal lamina (e) and in villous stroma (f) compared to controls (b, c). Bar scale: 20 μm.

3.4. Placental Chorionic Villi from Women Infected with T. cruzi Present Selective Disorganization of Basal Lamina. The basal lamina is part of the placental barrier that parasites have to cross in order to invade the fetal connective tissue of the villous stroma containing fetal capillaries. PAS and Arteta histochemical stains showed alteration in this specialized structure (Figures 4(d) and 4(e)). Collagen IV, heparan sulphate and fibronectin are components of the basal lamina [18]. We have previously shown that the parasite affects the immunoreactivity of heparan sulphate and collagen IV in ex vivo infected chorionic villi explants in a parasite concentration-dependent manner, and that fibronectin is not affected [6]. Placentas from T. cruzi-infected women show similar results; the immunoreactivity for heparan sulphate is loose or markedly decreased (Figure 5(d)). The immunoreactivity of collagen IV is decreased (Figure 5(e)) and no change in this parameter can be observed for fibronectin (Figure 5(f)) with respect to placenta from healthy women.

4. Discussion

During congenital T. cruzi infection, the parasite reaches the fetus by crossing the placental barrier [6–8]. Congenital Chagas disease is a product of a complex interaction between the maternal immune response, placental factors, and the parasite [20].

Congenitally infected newborns develop a parasite-specific T-cell immune response comparable to that of adults [21], as well as phenotypic and functional modifications of their NK cells [22]. On the other hand, newborns of T. cruzi-infected mothers are prone to produce higher levels of proinflammatory cytokines in comparison to those born to non-infected mothers [23]. From these observations, some authors postulated that some newborns from T. cruzi-infected mothers might naturally autocure their congenital infection [24]. Interestingly, the placentas from women
Figure 5: Placentas from mothers infected with T. cruzi present selective basal lamina disorganization in chorionic villi. Placental tissue from control non-infected (a–c) and infected (d–f) women were immunostained for heparan sulphate (a, d), collagen IV (b, e), and fibronectin (c, f). Placental chorionic villi from infected mothers completely lost their immunoreactivity for heparan sulphate (d) and showed reduced immunoreactivity for collagen IV (e), compared to controls (a, b). No difference in immunoreactivity for fibronectin was observed between controls and infected chorionic villi (c, f). Chorionic villi were processed for routine immunohistological techniques and counterstained with Mayer’s hematoxylin. Bar scale: 20 μm.

with asymptomatic Chagas disease analyzed by us do not present inflammatory infiltrate. This aspect might represent an interesting aspect of the placental infection by T. cruzi, because of scarce invasion and/or destruction of the parasite within placenta. The lack of inflammation may be due to low parasite load in blood and tissues, characteristic of asymptomatic and chronic phases of the disease. Thus, other factors may also determine the capacity of the parasite to infect the placenta or, alternatively, impair the congenital transmission.

Parasitemia is one factor associated with the risk of congenital transmission of the parasite. A high parasitemia, as can be detected in acute infection, correlates with a higher transmission rate of around 50%. In chronic infected patients, with very low parasitemia, the transmission rate is between 1 and 21% [25].

Recently, increasing evidence of the presence of local placental antiparasite factors has been reported. Among them, nitric oxide synthesis in placental tissue [15] and “heatsensitive” agents [26] have been involved in local placental defense mechanisms.

The ECM is another factor that should be considered during tissue invasion and pathogen infection. The ECM is a dynamic structure that interacts with cells and generates signals through feedback loops to control the behavior of cells. Thus, ECM macromolecules are bioactive and modulate cellular events such as adhesion, migration, proliferation, differentiation, and survival [27]. Additionally, ECM molecules are strictly organized, and this organization determines the bioactivity of the ECM. Even minor alterations such as a single amino acid substitution in a single ECM component can lead not only to altered physicochemical properties of tissues but also to changes in cellular phenotype and cell-matrix interactions [17]. It has been proposed that these changes in ECM structure and bioactivity in tissue function ultimately lead to development of disease [17]. It has been proposed that ECM alterations produced by T. cruzi not only promote its motility in tissues and its entrance into cells, but also alter the presence of cytokines and chemokines, which in turn permit T. cruzi to modulate and escape both the inflammatory response and the immune response [6, 19]. Alternatively, these changes in ECM function may be part of local placental defense mechanisms, which could explain why only very few parasites can be detected in the placenta.

During tissue invasion, T. cruzi is able to interact with different elements of the ECM. Thus, the parasite presents surface molecules, such as gp85 [19] and gp83 [28], glycoproteins that bind to laminin and fibronectin [19, 28] as well as to sulphated glycosaminoglicans such as heparan sulphate [29]. The parasite can induce the expression of ECM
molecules or decrease their presence [19]. The more obvious explanation for the decrease of ECM is that the parasite destroys the ECM by secretion of proteases like cruzipain [6, 30], which can degrade collagen I and IV [30], or by induction of matrix metalloproteases [31]. However, another possibility is that T. cruzi decreases the expression of these molecules by modulation of signal transduction pathways or cytoskeleton rearrangement. Probably, during tissue invasion T. cruzi first binds to molecules of the basal lamina, facilitating its internalization to the different cells in the underlying connective tissue. T. cruzi infection decreases fibronectin expression in cardiomyocyte culture. On the contrary, laminin protein expression levels do not change, but the distribution of this ECM component changes dramatically in this type of cell culture [32]. We have shown previously that in ex vivo infected human chorionic villi, the immunoreactivity for heparansulphate decreases or disappears completely in a parasite-concentration-dependent manner. Fibronectin does not present changes in its immunoreactivity or in its distribution pattern [6]. Here we confirm these results in placentas from mothers with asymptomatic Chagas’ disease. In the ex vivo infection of chorionic villi, the immunoreactivity for collagen IV decreases in the basal lamina between the trophoblast and villous stroma, but not around fetal capillaries [6]. In the placentas from the Chagasic women, collagen IV immunoreactivity is decreased in both basal laminae. It is probable that chronic exposure to the parasite during the entire pregnancy causes a more severe change in ECM than a 24-hour challenge in ex vivo infected placental tissue.

5. Conclusion

The evidence that placentas from T. cruzi-infected women present severe ECM alterations indicates that the parasite induces reorganization of this tissue component in such a way that may regulate inflammatory and immune responses in the host. If this is the case, changes in placental tissue ECM, together with the immunological status of mother and fetus, and parasite load may determine the probability of congenital transmission of T. cruzi.

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References


Prevention of Congenital Transmission of Malaria in Sub-Saharan African Countries: Challenges and Implications for Health System Strengthening

Kayode O. Osungbade and Olubunmi O. Oladunjoye

1 Department of Health Policy and Management, Faculty of Public Health, College of Medicine and University College Hospital, University of Ibadan, PMB 5017 General Post Office, 200212 Ibadan, Nigeria
2 Department of Community Medicine, University College Hospital, PMB 5116, 200212 Ibadan, Nigeria

Correspondence should be addressed to Kayode O. Osungbade, koosungbade@yahoo.com

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1. Introduction

Malaria remains a significant burden in sub-Saharan Africa as it continues to be the leading cause of infant morbidity and mortality in Africa. It is believed that malaria contributes up to about 25% of infant mortality in Nigeria [1]. Furthermore, in areas of Africa with stable transmission, Plasmodium falciparum infection during pregnancy is estimated to cause as many as 10,000 maternal deaths each year, 8% to 14% of all low birth weight babies, and 3% to 8% of all infant deaths [2]. Thus, malaria burden, if not reduced, poses a threat to the attainment of the Millennium Development Goals 4 and 6.

Congenital malaria is defined as malaria in a newborn or infant, transmitted from the mother [3]. Congenital malaria is generally defined as malaria acquired by the fetus or newborn directly from the mother, either in utero or during delivery [4]. It is also defined as the presence of malarial parasites in the peripheral smear of the newborn from twenty four hours to seven days of life [5]. Malaria is considered to be congenital in the neonate when asexual parasites are detected in the peripheral blood within the first week of life [6, 7].

Congenital malaria was thought to be rare in developing countries [8, 9]. This might be due to a number of reasons. Firstly, the protective effect of the foetal haemoglobin (HbF) in a newborn is expected to exert its influence during the immediate neonatal period [10, 11]. Secondly, local health facilities in resource-limited settings often lack the capacity to diagnose malarial infection [12–14]. Thirdly, the clinical signs of neonatal malaria may be indistinguishable from those of neonatal sepsis [15]. Thus, congenital malaria might pass unnoticed, and this might be responsible for its under-reporting. Therefore, the aim of this paper was to highlight the prevalence and burden of congenital malaria in sub-Saharan Africa as well as the challenges of its diagnosis and
congenital malaria [16]. The situation is however di

3.1. Prevalence of Congenital Malaria in Sub-Saharan African Countries. Congenital malaria is rare in developed countries such as the United States of America, where only three infants out of 4.1 million live births were reported to have congenital malaria [16]. The situation is however different and widely varied in sub-Saharan African countries. To this end, recent reports have suggested that congenital malaria is not as rare in developing countries as previously thought [17]. For example, in a survey covering seven sites in sub-Saharan Africa, Fischer showed a mean prevalence rate of 7% for congenital malaria (range 0–23%) [9].

Furthermore, while a study in Kenya reported a prevalence of malarial parasitaemia in <0.5% of neonatal admissions [18], high prevalence of congenital and neonatal parasitaemia (>20%) was reported in Uganda and Zambia [19]. A study done in Ghana revealed an incidence of 13.6% congenital malaria infections [20]. In Nigeria, congenital malaria was documented in 13.6% of babies at delivery [21]. In another multicentre study in Nigeria, the overall incidence of congenital malaria was 5.1% (range 1.1%–11.5%) [6]. In addition, Runsewe-Abiodun et al. have reported a congenital malaria prevalence rate of 17.4% among sick neonates in a tertiary hospital in southwestern Nigeria [17].

3.2. Burden of Congenital Malaria in Sub-Saharan Africa. It is estimated that placental malaria is responsible for 35% of preventable low birth weight in developing countries [22]. It is also associated with intrauterine growth restriction, prematurity due to preterm labour and intrauterine foetal death [23]. Malaria in pregnancy is reported to cause between 75,000 and 200,000 infant deaths each year in sub-Saharan Africa [22].

3.3. Challenges in Detection of Congenital Malaria. The detection of malaria parasites in the infant’s blood is essential for diagnosis, although blood smears can be negative if there are low parasite counts (50 parasites/µL blood) [23]. Pengsaa suggested that three repeated blood smears over 48 hours should be reported negative before excluding the diagnosis [19]. The capacity to conduct blood test for the diagnosis of congenital malaria is however limited in sub-Saharan African countries. This is because studies have shown a persistent lack of capacity to conduct quality malaria diagnostic tests by local health facilities [12–14].

Cord malaria can also be detected by polymerase chain reaction (PCR). The use of PCR has suggested that congenital malaria may be more frequent, although it is unclear if a positive PCR represents an active infection [4]. Though, PCR is a more sensitive and accurate diagnostic technique than blood smear microscopy, its use is however very limited in developing countries as it is expensive and requires a specialized laboratory [4].

Apart from the laboratory constraints, low antenatal care attendance and skilled delivery rates in African countries will also affect detection and prevention of congenital malaria. Antenatal care utilization in the developing countries is about 65%; this is low compared to that of the developed countries, which is 97% [24]. The implication is that in about two thirds of pregnant women in sub-Saharan Africa, opportunities for early recognition and prompt treatment of malaria infection are missed.

3.4. Challenges in the Clinical Diagnosis. The clinical diagnosis of congenital malaria usually poses a challenge. This is because its clinical findings may be indistinguishable from those of neonatal sepsis [15]; hence, the condition is either detected late or not even suspected unless neonates are specifically screened for it. Furthermore, as with many congenital infections, the new born child can manifest with fever, irritability, feeding problems, hepatosplenomegaly, anemia, jaundice, and low birth weight [25].

Thus, the clinical distinction from other congenital infections rests primarily on maternal history of exposure to malaria and absence of a skin rash [23]. A study done in Calabar, Nigeria, reported that 35% of newborns with clinical signs of neonatal sepsis were found to have congenital malaria [26]. Fever was the most common feature reported by studies in Kenya and Nigeria among others, which include refusal to feed or poor feeding, irritability, anaemia, hepatosplenomegaly, and jaundice [6].

3.5. Challenges in Prevention of Congenital Malaria in Sub-Saharan African Countries. Prevention of any disease condition requires the availability of methods for prediction of those at high risk of the disorder. If there were tests which are adequately sensitive for detecting placenta malaria in the antenatal period, it would have helped in assessing the efficacy of antimalarial drug during pregnancy and identifying the infants at risk of congenital malaria [27]. However, there are no tests which are adequately sensitive for detecting placental malaria; hence, the difficulty in identifying those at high risk of this condition.

Therefore, malaria preventive measures in pregnancy still remain as priority interventions required to protect the foetus and the newborn against the adverse effects of congenital malaria. The World Health Organization (WHO) has recommended a three-pronged strategic framework in
areas of high or moderate (stable) malaria transmission of sub-Saharan Africa: intermittent preventive therapy (IPT), insecticide-treated nets (ITNs), and case management of malaria illness and anaemia [28].

3.5.1. Intermittent Preventive Therapy (IPT). Intermittent preventive therapy (IPT), also known as chemoprophylaxis for pregnant women, especially those in their first pregnancies, has been widely used in sub-Saharan Africa. In line with WHO recommendation, most national guidelines stipulate that all pregnant women should receive at least two doses of IPT given as sulphadoxine-pyrimethamine (SP) combination after quickening as part of preventive treatments at antenatal care [28]. Evidence abound that this measure has recorded successes. For example, researchers in Kenya and Malawi have shown that intermittent preventive therapy with sulphadoxine-pyrimethamine significantly reduces the prevalence of maternal anaemia and placental parasitaemia, and the incidence of low birth weight [29–32]. Furthermore, sulphadoxine-pyrimethamine has been found to be safe in pregnancy and efficacious in reproductive-age women; hence, its strong acceptance and coverage levels of greater than 80% for the first dose [29].

Despite the beneficial impact of sulphadoxine-pyrimethamine on maternal and infant health, its utilization is threatened by weak health systems and sociocultural issues in sub-Saharan Africa. Studies have shown that a substantial proportion (20% to 80%) of pregnant women in this setting make their first antenatal visit in their third trimester [33–35]. Furthermore, unbooked pregnancies are common [36, 37], and antenatal care utilization rate (i.e., antenatal care clinic attendance of at least once during most recent pregnancy) varies from 60% to 90% [1, 38, 39]. These findings could be partly attributed to low quality of service and sociocultural reasons. While evidence abound on the persistent declining quality of antenatal services provided to pregnant women [33, 35, 40], other researchers have found that perceived quality of service was the most important factor which influenced the choice of facility for obstetric care [41]; similarly, a perceived lack of quality in the antenatal care was associated with a late first antenatal visit in Kenya [35].

Within the sociocultural context, it is thought that non-initiation of care in the first trimester seems to be a widespread cultural practice in sub-Saharan Africa. In rural Gambia, women do not usually "announce" pregnancy but wait for other family members to discover it, thereby presenting for care well into the third trimester [42]. While further qualitative research is required to explore the extent to which late first antenatal visit is affected by cultural practices, the implication of the findings is that a substantial proportion of pregnant women always remains unprotected against malaria infection because of missed opportunity of treatment in the second trimester. This therefore exposes fetuses of such unprotected women to placental malaria and its adverse effects, thereby contributing to the challenges militating against prevention of congenital malaria. This situation is further worsened by the practice of partial preventive treatments, including sulphadoxine-pyrimethamine (SP) therapy provided by health workers at antenatal care service [33, 35, 43].

3.5.2. Insecticide-Treated Nets (ITNs). Randomized control trials in sub-Saharan Africa have consistently shown the effectiveness of insecticide-treated nets (ITNs) in the prevention of malaria in pregnancy. Studies in Ghana and Kenya have documented reduced placental malaria, low birth weight, and fetal loss resulting from use of ITNs [44]. Though the use of insecticide-treated nets (ITNs) is reportedly considered as the most cost-effective method of malaria prevention in highly endemic areas, there have always been some concerns about the preventive strategy in sub-Saharan Africa; these concerns include access, cost, retreatment, and gaps between ownership and use of ITN.

Access to ITNs by vulnerable populations including pregnant women continues to increase due to the efforts of national governments and supporting development agencies through multiple approaches such as stand-alone campaigns, health facilities, and antenatal clinic [1, 38]. Access is also enhanced by free distribution and sales of ITNs at subsidized cost or through discounted voucher systems [1, 38, 45], while the introduction of long-lasting insecticidal nets (LLINs) has reduced the burden of frequent retreatments.

Despite these efforts, recent findings revealed low utilization of ITNs among pregnant women. Though increasing trends in ownership and use of ITNs by households were reported in national demographic surveys, the reports indicated that 5% to 57% of pregnant women aged 15–49 years slept under an ITN the past night. Furthermore, gaps between ownership and use of an ITN continue to exist as about 50% or less of pregnant women aged 15–49 years in households with an ITN slept under the net the past night [1, 38, 46, 47].

3.5.3. Case Management of Malaria Illness. Supporting and promoting access to correct, affordable, and appropriate treatment within 24 hours of the onset of symptoms of malaria is the third essential component of malaria prevention and control during pregnancy in malarious endemic countries of sub-Saharan Africa [28]. The World Health Organization (WHO) had recommended chloroquine (CQ) in chloroquine-sensitive areas and sulphadoxine-pyrimethamine (SP) in areas with CQ resistance for treatment of uncomplicated malaria in pregnancy. Quinine is another alternative in areas where both CQ and SP are not effective [28].

However, prompt and effective case management of malaria illness is hinged on early and correct diagnosis of the condition. As noted above, this is seriously hindered by lack of capacity to conduct quality malaria diagnostic tests by local health facilities in sub-Saharan African countries [12–14]. While this situation might have justified the presumptive treatment of fever with antimalarials (i.e., treating all febrile episodes suspected of malaria with a full therapeutic dose of antimalarials), such practice might have led to a reduced
susceptibility of parasites to the commonly used antimalarial drugs in these settings.

3.6. Recommendations

3.6.1. Health System Strengthening. The World Health Organization (WHO) recently recommended prompt parasitologic confirmation by microscopy or alternatively by rapid diagnostic tests (RDTs) in all patients suspected of malaria before treatment is commenced unless parasitological diagnosis is not accessible [48]. Findings from the previous review revealed that many peripheral health facilities in resource-poor settings of sub-Saharan Africa lacked the capacity to conduct quality parasitological diagnosis of malaria by microscopy; this is because the procedure is labour-intensive requiring trained staff and quality equipment [49, 50].

On the other hand, malaria rapid diagnostic testing (RDT) has been found useful as an attractive alternative to routine microscopy with good sensitivity and specificity profiles [48, 51]. In addition, RDTs have the potential to be cost-effective, require no capital investment or electricity, are simple to perform, and are easy to interpret [51, 52]. If appropriately introduced and implemented, it also has been shown to have the potential of improving drug-prescribing behaviour of health workers [53], thereby reducing overtreatment of malaria and the problem of drug resistance.

From the foregoing, utilization of RDTs in resource-poor settings would seem to be an appropriate technology as it has the potential of improving the quality of malaria diagnosis and treatment services provided to all cases of malaria, including pregnant women and newborns. Therefore, it is suggested that national governments and development partners in sub-Saharan Africa should support widespread use of rapid diagnostic tests (RDTs) for malaria diagnosis. The capacity of local health facilities providing maternal and child health services should be strengthened with the provision of adequate supplies of equipment and consumables required to provide the diagnostic services. In addition, targeted trainings and supportive supervision of local health staff are highly desirable in order to meet up with the challenges of newly-assigned task.

In order to increase uptake of IPTs and facilitate prompt diagnosis and treatment of malaria in pregnancy, national governments and development partners in sub-Saharan African countries should also consider improving the poor maternal health service indicators—nonbooking, late antenatal visits and low antenatal service rate—as an essential task to be concertedly pursued. To achieve this task, strategies aimed at improving maternal health service should be implemented. For example, proven-specific interventions such as the World Health Organization (WHO) training on Life Saving Skills [54] are suggested for use of maternal health service managers in addressing the weak health systems. Periodic trainings conducted for local health staff can help address and strengthen health systems, particularly if targeted at previously reported areas of deficiency such as declining quality of maternal health service, poor record keeping practices of health workers, and poor utilization of intrapartum monitoring tools such as a partograph [33, 34, 55].

In addition, health staffs’ familiarization and adherence to WHO guidelines on provision of effective, efficient, safe, and culturally appropriate services to pregnant women and newborn under the Integrated Management of Pregnancy and Childbirth (IMPAC) would assist to ensure best practices [56]. It is expected that these efforts would improve utilization of maternal health services, increase IPT uptake, enhance prompt diagnosis and treatment of malaria in pregnancy, and consequently prevent congenital malaria.

On a long term basis, strategies which would bring about over 80% antenatal service utilization rate should be pursued vigorously. Therefore, it is suggested that redirecting and repackaging health education and service content of antenatal care service using a social marketing approach acceptable within the sociocultural context would be found useful. Therefore, placing the antenatal care service content and its benefits on public agenda through different media and matrices may be helpful in the study setting as opposed to the current practice of restricting information to women who make antenatal visits.

Despite male economic dominance and decision making power in developing countries, their involvement in reproductive health issues is reportedly low [57]. However, studies had shown that male involvement would significantly improve indicators of maternal health service utilization, [58] and, therefore, it could also improve uptake of IPTs and case management of malaria in pregnancy. Hence, it is recommended that father’s roles in maternal health be defined, packaged, and incorporated in the antenatal care service messages meant for dissemination to the public.

With regard to closing the gap between ownership and use of an ITN, a carefully designed qualitative research may be useful in eliciting the factors responsible or reasons for not sleeping under an ITN despite its availability in the household. While it is suggested that national governments and development partners should not relent on their efforts in making ITNs universally accessible to pregnant women either free of charge or at a subsidized price, experiences of other researchers on factors which promote or inhibit ITN usage may be found useful in packaging educational messages aimed at promoting its usage. ITN usage promoting factors such as high perception on the seriousness of malaria and its effect on pregnant women and children, high perceived benefit of ITNs in protecting children and pregnant women against malaria, and high awareness of the prevention of malaria as a better and cheaper option compared with treatment should be intensified. Whereas inhibitory factors such as fear of the chemical that is used to treat nets and unsupportive spouses should be demystified [59].

4. Conclusion

Evidence abound that congenital malaria constitutes a public health burden in sub-Saharan Africa. However, efforts of the national governments and development partners at
instituting the recommended cost-effective interventions are continuously thwarted by challenges brought about by weak health systems and sociocultural factors among others, thereby, mitigating against the progress towards attainment of Millennium Development Goal (MDG) 6 (Indicator 22, Target 8). Health system strengthening and appropriate public health promoting and educating messages delivered through a social marketing approach may be found useful in putting back on track the race towards 2015 with respect to attainment of MDG 6.

References


Research Article

In Vitro Infection of Trypanosoma cruzi Causes Decrease in Glucose Transporter Protein-1 (GLUT1) Expression in Explants of Human Placental Villi Cultured under Normal and High Glucose Concentrations

Luciana Mezzano, Gastón Repossi, Ricardo E. Fretes, Susana Lin, María José Sartori, and Sofía G. Parisi de Fabro

1 Cátedra de Biología Celular, Histología y Embriología, Instituto de Biología Celular, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Ciudad Universitaria, 5016 Córdoba, Argentina
2 Cátedra de Histología, Embriología y Genética, IICSHUM, Universidad Nacional de La Rioja, 5300 La Rioja, Argentina
3 Division of Environmental Health and Occupational Medicine, National Health Research Institutes, 35053 Zhunan Town, Miaoli County, Taiwan

Correspondence should be addressed to Luciana Mezzano, lucianamezzano@hotmail.com

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Trypanosoma cruzi, the etiologic Chagas’ disease agent, induces changes in protein pattern of the human placenta syncytiotrophoblast. The glucose transporter protein-1 (GLUT1) is the primary isomor involved in transplacental glucose transport. We carried out in vitro assays to determine if T. cruzi infection would induce changes in placental GLUT1 protein expression under normal and high concentration of glucose. Using Western blot and immunohistological techniques, GLUT1 expression was determined in normal placental villi cultured under normal or high concentrations of glucose, with or without in vitro T. cruzi infection, for 24 and 48 hours. High glucose media or T. cruzi infection alone reduced GLUT1 expression. A yet more accentuated reduction was observed when infection and high glucose condition took place together. We inform, for the first time, that T. cruzi infection may induce reduction of GLUT1 expression under normal and high glucose concentrations, and this effect is synergic to high glucose concentrations.

1. Introduction

Chagas’ disease, endemic in Latin America, is caused by Trypanosoma cruzi a flagellated protozoan with a life cycle involving an insect vector and a mammalian host. The congenital Chagas’ disease is associated with premature labor, miscarriage, and placentitis [1]. In endemic countries, maternal-fetal transmission of T. cruzi is between 1 and 17% of pregnancies in chronically infected mothers, depending on geographic area [2]. The infectious form of the parasite (trypomastigote) adheres to specific receptors on the outer membrane of host cells previous to intracellular invasion [3], and the process of invasion requires activation of signal transduction pathways in the parasite and the host cell [4–10].
higher in patients with the cardiac form of Chagas’ disease [16].

Hyperglycemia during pregnancy is a well-recognized pathogenic factor. Adequate glucose transfer from the maternal to the fetal compartment is crucial for the normal survival and development of the fetus during pregnancy [17]. GLUT1 is the primary isoform involved in the transplacental movement of glucose and distributed asymmetrically on the microvillus and basal membranes syncytiotrophoblast. The microvillous membrane contains more transporters than the basal. GLUT is inversely regulated by glucose concentration, and basal membrane GLUT1 is positively regulated by insulin-like growth factor I, placental growth hormone, and hypoxia [18]. In vivo, basal membrane GLUT1 is upregulated over gestation, increased in diabetic pregnancy, and decreased in chronic hypoxia, while microvillous membrane GLUT1 is unaffected [18, 19]. As the rate-limiting step in transplacental glucose transport, changes in the density of basal membrane GLUT1 will have a significant impact on transplacental glucose flux [18, 20].

Although increased expression of GLUT1 in placenta from diabetic pregnant women has been reported [18, 20], decrease in GLUT1 mRNA and protein levels in diabetic mice compared with the control and placental cell cultures under high glucose concentration conditions was also informed [20, 21]. Glucose would also alter GLUT1 partitioning between the plasma membrane and intracellular sites in favor of the latter [22].

In the invasion process, T. cruzi has been found to affect numerous surface molecules of the placental villi, probably causing placent dysfunction as consequence. We wondered if this parasite would somehow also affect GLUT1 expression pattern, especially under high glucose (HG) concentration, since T. cruzi has been reported to affect pancreatic function and alters the insulin-glycemia axis, and there is not yet a marker for placental or fetus infection. In this work, we compared the protein expression of GLUT1 of human placental explants infected in vitro with trypomastigotes, cultured under normal and high glucose (HG) concentration.

2. Materials and Methods

2.1. Placentas. Placentas (n = 17) from clinically and serologically healthy women at 38 to 40 weeks of gestation were obtained by caesarian delivery, in order to assure asepsis. Women signed an informed consent. Placentas were kept in glucose solution 0.29 mM at 4°C for transportation. Once in the laboratory, central villi of placental cotyledons were isolated, rinsed with PBS several times, and cut into pieces of 3 mm in diameter.

2.2. Parasites. Trypomastigotes of T. cruzi (Tulahuen strain) were isolated according to Andrews and Colli [23] and Fretes and Fabro [24], from infected Albino/Swiss mice bloodstream at the peak of parasitism. Briefly, mice blood was centrifuged for 10 minutes at 100 g and kept still for 1 h at 37°C. Plasma was then separated and centrifuged for 10 minutes at 590 g. The pellets containing the parasites were washed twice and suspended in MEM-199 (Gibco Lab., NY, USA).

2.3. Treatments of Placental Explants. Explants of normal human placental villi were cultured at 37°C, in normal atmosphere supplemented with 5% CO₂, in MEM-199 (pH 7) with 0.1% penicillin and 0.01% streptomycin and either with 5 mM D-glucose (normal D-glucose: NG) (J.T. Baker, NJ, USA) or 25 mM D-glucose (high glucose: HG) concentrations [22].

After 24 hours, 7 × 10⁵ trypomastigotes Tulahuen strain of T. cruzi were added with the refresh of culture media, prepared as mentioned above. Controls without parasites were carried out for both normal and HG concentrations. Cocultures with trypomastigotes and controls were incubated for another 24 h or 48 h.

After treatment, the placental explants were rinsed with PBS. Part of the explants were fixed with 10% formaldehyde and included in paraffin. Approximately 30 mg of placental explants from each treatment were homogenized in 0.25 mL PBS with an OMNI 1000 homogenizer for five cycles of high speed application, each lasting ten seconds.

2.4. Analysis of Placental Explants Infection. At the end of cultures, placental explants were collected, fixed, and stained with hematoxylin/eosin and PAS/hematoxylin and observed under low and high magnifications in a Zeiss Axioskop 20 microscope. Infection of placental explants was assessed observing amastigote groups of the T. cruzi in trophoblast or stromal cells of the chorionic villi.

2.5. Immunostaining of GLUT1 Protein Expression in Placental Explants. Deparaffinized histological sections were embedded in TBS (pH 7.2), pretreated with 0.05% saponin (15 min) for the unmasking of antigens, then with 3% H₂O₂ (15 min) to block internal peroxidases, and treated with 3% nonfat dry milk in TBS (15 min) to block nonspecific epitopes. GLUT1 protein was detected by incubating the treated sections with an anti-GLUT1 polyclonal antisemur (rabbit, CHEMICOM International Inc, Temecula, Calif, USA) diluted in TBS/Tween (1 : 500) for overnight, at 4°C, and revealed with a secondary antirabbit immunoglobulin conjugated with peroxidase (Sigma-Aldrich Co, Mo, USA). Peroxidase activity was developed using H₂O₂/4-Cl-1-naphthol. Background control without addition of anti-GLUT1 antiserum was carried out. We also used precultured placenta controls. Images stored as jpg format were analyzed with “Image Tool” UTHSCSA version 3.00 (downloadable from http://ddsdx.uthscsa.edu/dig/download.html) as described previously [25]. Five optical fields were measured per slide; each treatment for each placenta provided 3 slides. Immunostained area was expressed as ratio of total area (manually selected surface area).

2.6. Western Blot for GLUT1. Homogenized placental villi were mixed with lysis buffer containing protease inhibitors (1% w/v de SDS, 1 mM EDTA, 1 µg/mL of leupeptin,
Figure 1: Paraffin-embedded placental villi cultured for 48 hs, Pas/H stained. (a) Control placental villi without *T. cruzi* infection cultured with normal glucose concentration media; (b) normal placental villi *in vitro* infected with *T. cruzi*, cultured with normal glucose media; (c) normal placental villi cultured under high glucose concentration media; (d) normal placental villi cultured under high glucose concentration media, *in vitro* infected with *T. cruzi*. Amastigotes nests were observed inside the villous from infected placentas (arrows). Arrow points indicate increased glycogen deposits in placental villi explants cultured under HG concentration (c and d). Original magnification: 1000x. The scale bar represents 1 µm.

100 mM of Hepes pH 7.4) and centrifuged at 12000 g for 10 minutes. Protein content was measured with Lowry protein assay [26]. The samples were not heated prior electrophoresis; 40 µg of sample were loaded per lane in a SDS-PAGE gel that was carried out with 3% stacking gel and 10% resolving gel at 200 V for 1 hr [27] and blotted onto nitrocellulose at 300 mA for 1.5 hr using a Trans Blot Mini-Protean II apparatus (BioRad, Richmond, Calif, USA). GLUT1 on nitrocellulose was detected with rabbit anti-GLUT1 polyclonal antiserum (CHEMICOM International Inc, Temecula, Calif, USA), diluted 1:5000 in TBS-Tween and revealed with a secondary antirabbit immunoglobulin conjugated with peroxidase (Sigma-Aldrich Co, Mo, USA). Peroxidase activity was developed using H2O2/4-Cl-1-naphthol. Background control without addition of anti-GLUT1 antiserum and precultured placenta controls were carried out. Actin was detected as a positive control for protein content present in samples, due the stable expression of this protein.

GLUT1 expression was evaluated with the “Scion image for windows” program (version: Beta 4.0.2) to measure the area units marked by Western blot.

2.7. Statistical Analysis. Data were expressed as the mean ± SE and were analyzed statistically by ANOVA test followed by “post hoc” LSD Fisher test. Paired t-test was performed, to establish significance between groups (different placentas). A level of less than 5% (*P* < 0.05) was chosen to detect significant differences.

3. Results

Placental explants showed groups of the reproductive forms of the parasite (amastigotes) mainly in stromal cells of the chorionic villi under normal (NG) and high D-glucose (HG) concentrations, as they were seen in placental villi slides stained with PAS/H. Furthermore, the presence of the parasite was more evident when placental explants were cultured with high D-glucose concentration (Figure 1).

Placental villi explants cultured under HG concentration (HGC) underwent structural modifications mainly thickening of the syncytiotrophoblast and basal membranes, increased glycogen deposits, and a fetal capillary thickening, similar to those described in the bibliography [28–30]. These alterations were maintained and also were more notorious when these placental explants were infected with *T. cruzi*, compared to healthy controls incubated with normal glucose concentration (NGC), as shown in placental sections with PAS/H staining (Figure 1).

GLUT1 protein was intensively stained on the apical and basal membrane of the syncytiotrophoblast in controls of the chorionic villi under normal (NG) and high D-glucose (HG) concentrations, as they were seen in placental villi slides stained with PAS/H. Furthermore, the presence of the parasite was more evident when placental explants were cultured with high D-glucose concentration (Figure 1).

Image quantification of immunostained areas showed that placental explants cultured under HGC *per se* reduced the expression of GLUT1 to 34.7% at 24 h (Figure 2) and the
GLUT1 expression was intensively marked on the apical and basal syncytiotrophoblast cell membrane (arrows); (b) normal placental villi in vitro infected with *T. cruzi*; (c) normal placental villi cultured under high glucose concentration media; (d) normal placental villi cultured under high glucose concentration media, and in vitro infected with *T. cruzi*; (e) negative control placental villi (without GLUT1 antiserum incubation); (f) image quantification made by "Image Tool" UTHSCSA version 3.00, which indicates the percentage of the marked area; values shown are the mean ± SE of four samples from representative experiments (A: control placental villi, without infection; B: infected normal placental villi; C: normal placental villi cultured under high glucose media; D: infected normal placental villi cultured under high glucose media; E: negative control placental villi). Original magnification: 400x. The scale bar represents 1 µm. *Significantly different from the control placental villi (P < 0.05; n = 4; paired t-test).

Western-blotting image of one of the placental samples as representative of the experiment is shown in Figure 4. Placental explants cultured under HGC per se reduced the expression of GLUT1 to 40.5% at 24 h and the level of GLUT1 protein expression recovered to 56.5% at 48 h. In explants cultured with *T. cruzi*, the expression of GLUT1 protein was reduced under both NG and HG concentrations. At 24 h, the protein expression was reduced to 59.5% under NGC and to 20% under HGC. At 48 h, the GLUT1 protein expression was at 52.1% and 14.85% of the initial levels, in cultures under NG and HG concentrations, respectively (P < 0.05).
4. Discussion

Different authors have reported that diabetics have an increased susceptibility to a variety of infectious agents [31, 32]. Hyperglycemia has been previously observed to increase the morbidity and mortality of murine *T. cruzi* infection [15]. Diabetes and hyperglycemia were also reported to be more prevalent in chagasic human patients with the cardiac form of the disease, than in control ones [16]. But, according to the analysis of the bibliography, there is not any study analyzing an association between pregnant women affected with both Chagas’ disease and diabetes with congenital transmission or with the effect on the newborn. Furthermore, there is no marker for placental or fetus infection in Chagas’ disease. Due the effect of the Chagas parasite on some proteins located at the lipid raft of chorionic villi trophoblast [24, 33], as well as in trophoblast lipids [34], we aimed to analyze the possible modification of the main glucose transporter located at the syncytiotrophoblast, the GLUT1 protein, produced by placental *T. cruzi* infection.

*T. cruzi* crosses the placental barrier and infects the fetus, causing the congenital form of the disease [1]. In order to understand the mechanism used by the parasite to cross this barrier, the interaction between syncytiotrophoblast plasma

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**Figure 3**: GLUT1 immunodetection in paraffin-embedded placental villi sections cultured for 48 hours. (a) Uninfected control placental villi (GLUT1 expression was intensively marked on the apical and basal syncytiotrophoblast cell membrane); (b) normal placental villi *in vitro* infected with *T. cruzi*; (c) normal placental villi cultured under high glucose concentration media; (d) normal placental villi cultured under high glucose concentration media and *in vitro* infected with *T. cruzi*; (e) negative control placental villi (without GLUT1 antiserum incubation); (f) image quantification made by “Image Tool” UTHSCSA version 3.00, which indicates the percentage of the marked area; values shown are the mean ± SE of seven samples from representative experiments (A: control placental villi, without infection; B: infected normal placental villi; C: normal placental villi cultured under high glucose media; D: infected normal placental villi cultured under high glucose concentration media; E: negative control placental villi). Original magnification: 400x. The scale bar represents 1 µm. *Significantly different from the control placental villi (P < 0.05; n = 7; paired t-test).
membranes from the human placenta and the parasite was previously studied, with modifications in lipid and protein patterns from trophoblast membranes being found [35]. However, the mechanism by which the parasite infects the placenta as well as the effects upon the protein contents of the placental barrier is still not well understood.

Our experiments showed that GLUT1 protein expression was significantly diminished in normal placental villi cultured under HGC for either 24 or 48 hours. This corroborates previous work, a significant reduction in GLUT1 protein expression under high D-glucose concentration [22]. In the present study analyzing new born from pregnant women who have both conditions diabetes and Chagas.

Reduced GLUT1 expression observed in placental culture with parasites could imply a downregulated GLUT1 activity in pregnant women with Chagas’ disease. If this disease is causing damage to pancreas islets [11–13], or happens together with a previous diabetic condition, the adverse effects of reduced GLUT1 activity may be exacerbated.

The level of GLUT1 is regulated by glucose concentration, insulin-like growth factor I, placental growth hormone, and hypoxia [18]. It would be interesting to further study the mechanisms by which GLUT1 is affected in Chagas’ disease. It is likely that the parasite would perturb other components of the maternal and fetal glucose-insulin-GLUT1 axis. As glucose and GLUT are inversely regulated, changes in GLUT expression might alter the insulin level in plasma, pointing out to determine a marker for placental infection by T. cruzi in pregnant chagasic women. This matter could be of the utmost importance in congenital Chagas’ disease.

Histological studies have demonstrated that placentas from poorly controlled diabetic pregnancies show a thickening of the basal membranes and reduced vascularization of the villi [30]. Similar to observations reported by other authors [28, 29], in the present work we detected that placental villi cultured under high glucose conditions were

![Image](image-url)

**Figure 4:** Western blot to detect GLUT1 transporter protein expression in homogenates from normal placental villi, cultured for 24 hours (a) and 48 hours (b). Actin presence was also detected as a positive control for protein content in samples. GLUT1 expression was evaluated with the “Scion Image for Windows” program that measured the area units marked. WCN: without culture normal placental villi (positive control); N: normal placental villi; N + T: normal placental villi cocultured with T. cruzi; G: normal placental villi cultured under high glucose media; G + T: normal placental villi cocultured with T. cruzi under high glucose media. Values shown are the mean ± SE. *Significantly different from the control (P < 0.05; n = 8; ANOVA test).
morphologically altered, with thickening of the basement membranes. The presence of \textit{T. cruzi} maintains this alteration. The parasite causes disorganization of the basal membrane of the trophoblast and the stroma of the chorionic villi [44], as was observed in \textit{in vitro} experiments similar to those performed in this work. So, both conditions hyperglycemia and \textit{T. cruzi} can modify the stroma of the chorionic villi. According to our results, the alteration of the trophoblast basal membrane in the presence of \textit{T. cruzi} under high D-glucose concentration might be caused by two different mechanisms or not. This important matter should be elucidated.

The protein membrane pattern was also altered, as noted in others works on Placental Alkaline Phosphatase (PLAP) [7–9, 24, 25, 39–41]; and in the present study with GLUT1 protein. These characteristics could modify the human placenta efficacy as a barrier against infections. It has been described that chorionic villi \textit{in vitro} has low susceptibility to infection by \textit{T. cruzi} in normal D-glucose concentration [45, 46]. In order to clarify if high D-glucose concentration can increase the infection of chorionic villi by \textit{T. cruzi}, it is necessary to quantify the infection by \textit{T. cruzi} of the placental explants under high glucose concentration, in order to analyze the susceptibility of the invasion and the reproduction of the parasite in the placenta, aspect that we are planning to do in further experiments.

With the present work, we report for the first time the effect of \textit{T. cruzi} on GLUT1 protein expression, adding it to the increasing list of affected proteins altered by this parasite [7–9, 24, 25, 40, 41].

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Prevalence of Congenital Malaria in Minna, North Central Nigeria

Innocent Chukwuemeka James Omalu, Charles Mgbemena, Amaka Mgbemena, Victoria Ayanwale, Israel Kayode Olayemi, Adeniran Lateef, and Victoria I. Chukwuemeka

1 Department of Biological Sciences, Federal University of Technology, Minna, Nigeria
2 Dentistry Department, Niger State General Hospital, Minna 900002, Nigeria
3 Department of Biochemistry/Physiology, University of Abuja, FCT, Nigeria

Correspondence should be addressed to Innocent Chukwuemeka James Omalu, omalu_icj@hotmail.com

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The study was designed to determine the true prevalence of congenital, cord, and placental malaria in General Hospital Minna, North Central Nigeria. Peripheral blood smears of near-term pregnant women, as well as the placental, cord, and peripheral blood smears of their newborn babies, were examined for malaria parasites, using the Giemsa staining technique. Out of 152 pregnant women screened, 21 (13.82%) of them were infected with malaria parasites. Of the 152 newborn babies, 4 (2.63%) showed positive peripheral parasitaemia. Placental parasitaemia was 7/152 (4.61%), while cord blood parasitaemia was 9/152 (5.92%). There were strong associations between peripheral and cord malaria parasitaemia and congenital malaria \( P < 0.05 \). Plasmodium falciparum occurred in all, and none had mixed infection. The average birth weights of the babies delivered of nonmalarious pregnant women were higher than those delivered by malarious pregnant women, though not significant \( P > 0.05 \). Malaria parasitaemia occurred more frequently in primigravidae than multigravidae.

1. Introduction

Congenital malaria was first described in 1876 [1]. It can be acquired by transmission of parasites from mother to child during pregnancy or perinatally during labour [2].

Congenital malaria has been documented for many years, but it was previously thought to be uncommon especially in indigenous populations; more recent studies, however, suggest that incidence has increased, and values between 0.30 to 33.00% have been observed from both endemic and nonendemic areas [3].

Malaria and pregnancy are generally believed to be mutually aggravating conditions. The pathological changes due to malaria and the physiological changes associated with pregnancy have a synergistic effect on the course of each other [4].

Pregnancy exacerbates malaria through a nonspecific hormone-dependant depression of the immune system; protective antiplasmodial activity is suppressed at pregnancy [5].

The following hypotheses to explain the altered immunity associated with pregnancy were offered.

Reduced lymphoproliferative response sustained by elevated levels of serum cortisol, loss of cell-mediated immunity in the mother, the presence of placenta, a new organ in the primigravidae, allows the parasite to bypass the existing host immunity, or allows placenta-specific phenotypes of \( P. falciparum \) to multiply; pregnant women display a bias towards type-2 cytokines and are therefore susceptible to diseases requiring type-1 responses for protection like TB, malaria, and \( P. falciparum \) has the unique ability of cytoadhesion; chondroitin sulfate A and hyaluronic acid are the adhesion molecules for parasite attachment to placental cells [4].

In pregnancies complicated by malaria, both fetal growth retardation and preterm birth contribute to low birth weight [6]. Malaria control still remains the foremost public health challenge in Africa, and world malaria report, which indicated that Nigeria accounts for a quarter of all malaria cases in the 45 malaria endemic countries in Africa, clearly showed the enormity of the socioeconomic and health burdens of the disease in the country, even in the face of dearth of
information on the epidemiology of the disease. This, thus, informed this study on the prevalence of congenital malaria in Minna, an often neglected but germane aspect of the epidemiology of malaria in endemic communities [7].

2. Materials and Methods

2.1. Study Site and Population. The study was carried out at General Hospital Minna, North Central Nigeria. Minna, the capital of Niger State, Nigeria, is located within longitude 6°33′E and latitude 9°37′N, covering a land area of 88 km² with a population of 1.2 million. Minna has a tropical climate with mean annual temperature, relative humidity, and rainfall of 30.20°, 61.00%, and 1334.00 cm, respectively. The climate presents two distinct seasons: a rainy season (April–October) and dry season (November–March). Minna is an endemic area for malaria.

The study was conducted from October 2010 to February 2011. The subjects were near-term (close to delivery) pregnant women who were delivered of their babies at the hospital. Sample sizes were determined from the number of pregnant women that attended antenatal care during the period of study. The nonpregnant women served as control group and the pregnant women were divided into primigravidae and multigravidae. The prevalence of malaria infection in blood smears of near-term pregnant mothers, their newborns, cords, placentae, and peripheral blood of neonates was collected by heel pricking using a sterile blood lancet on clean glass slides. Labelled ethylene-diamine tetraacetic acid (EDTA) bottles were used in collecting blood samples for parasitological examinations. Blood collections were made possible with the assistance of midwives on duty during child delivery. Blood samples were also collected from 100 nonpregnant women.

The ages, types of birth, and weights of newborn babies were recorded.

2.4. Parasitological Examination. Thick and thin blood films were stained with 10% Giemsa and read for malaria parasites by two trained microscopists following standard quality control procedure. Parasitaemia was expressed as the number of asexual forms of *P. falciparum* per microlitre; a result was considered negative after a reading of 1000 leucocytes in the microscope (×1000). Parasitaemia was graded as low (1–999/μL), moderate (1000–9999/μL), and high (>10000/μL). Transplacental passage of *Plasmodium falciparum* was confirmed by detection of malaria parasite within 7 days of birth.

2.5. Statistical Analysis. All data were analysed using SPSS version 10.1 for windows. Descriptive statistics were computed for all relevant data. Chi square analysis was used to compare proportions within and among groups, for statistical significance.

3. Results

The prevalence of malaria infection in blood smears of near-term pregnant mothers, their newborns, cords, placentae, and nonpregnant women was shown in Table 1. Out of a total of 152 near-term pregnant women screened, 21 (13.82%) had parasite in their peripheral blood. Among 100 nonpregnant women screened, 13 (13.00%) had peripheral malaria parasite. Infection rate was higher in pregnant women but not significant (*P* > 0.05) using the chi-square. The inclusion of nonpregnant women helped to compare the prevalence of malaria. Also, there was no significant difference in infection rates between the primigravidae (15.79%) and the multigravidae (11.84%), (*P* > 0.05).

### Table 1: Prevalence of *Plasmodium falciparum* in blood samples of different groups of women and tissues at General Hospital Minna, North Central Nigeria.

<table>
<thead>
<tr>
<th>Source of blood</th>
<th>Overall No examd</th>
<th>Overall No positive</th>
<th>Overall Infect rate (%)</th>
<th>Primigravidae No examd</th>
<th>Primigravidae No positive</th>
<th>Primigravidae Infect rate (%)</th>
<th>Multigravidae No examd</th>
<th>Multigravidae No positive</th>
<th>Multigravidae Infect rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Near-term Pregnant women</td>
<td>152</td>
<td>21</td>
<td>13.82</td>
<td>76</td>
<td>12</td>
<td>15.79</td>
<td>9</td>
<td>11.84</td>
<td></td>
</tr>
<tr>
<td>Nonpregnant women</td>
<td>100</td>
<td>13</td>
<td>13.00</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Neonate</td>
<td>152</td>
<td>4</td>
<td>2.63</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Placenta</td>
<td>152</td>
<td>7</td>
<td>4.61</td>
<td>76</td>
<td>3</td>
<td>3.95</td>
<td>76</td>
<td>4</td>
<td>5.26</td>
</tr>
<tr>
<td>Cord</td>
<td>152</td>
<td>9</td>
<td>5.92</td>
<td>76</td>
<td>4</td>
<td>5.26</td>
<td>76</td>
<td>5</td>
<td>6.58</td>
</tr>
<tr>
<td>Total</td>
<td>708</td>
<td>54</td>
<td>7.63</td>
<td>226</td>
<td>21</td>
<td>9.29</td>
<td>228</td>
<td>20</td>
<td>8.77</td>
</tr>
</tbody>
</table>
Table 2: Average parasite densities (Parasites/uL) of blood of different groups of women and tissues at General Hospital Minna, North central Nigeria.

<table>
<thead>
<tr>
<th>Parasite densities</th>
<th>Pregnant women</th>
<th>Nonpregnant women</th>
<th>Newborn</th>
<th>Placenta</th>
<th>Cord</th>
<th>PG</th>
<th>MG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasites/uL</td>
<td>1415</td>
<td>411</td>
<td>128</td>
<td>689</td>
<td>196</td>
<td>1281</td>
<td>134</td>
</tr>
</tbody>
</table>

PG: Neonate with primigravid mothers,
MG: Neonate with multigravid mothers.

Table 3: Erythrocytic stages of *Plasmodium falciparum* in blood samples of different groups of women and tissues at General Hospital Minna, North central Nigeria.

<table>
<thead>
<tr>
<th>Source of blood</th>
<th>No positive</th>
<th>TR</th>
<th>GM</th>
<th>SCH</th>
<th>TR &amp; SCH</th>
<th>TR &amp; GM</th>
<th>TR &amp; GM &amp; SCH</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant women</td>
<td>21</td>
<td>60</td>
<td>64.5</td>
<td>25</td>
<td>26.8</td>
<td>—</td>
<td>—</td>
<td>93</td>
</tr>
<tr>
<td>Nonpreg women</td>
<td>13</td>
<td>15</td>
<td>46.8</td>
<td>12</td>
<td>37.5</td>
<td>—</td>
<td>—</td>
<td>32</td>
</tr>
<tr>
<td>Newborn</td>
<td>4</td>
<td>5</td>
<td>45.5</td>
<td>5</td>
<td>45.5</td>
<td>—</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Placenta</td>
<td>7</td>
<td>20</td>
<td>62.5</td>
<td>10</td>
<td>31.2</td>
<td>—</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>Cord</td>
<td>9</td>
<td>12</td>
<td>36.3</td>
<td>10</td>
<td>30.3</td>
<td>—</td>
<td>3</td>
<td>33</td>
</tr>
</tbody>
</table>


Of the 152 newborn babies screened, 4 (2.63%) had positive peripheral parasitaemia. Examination of the 152 placentae gave a prevalence of 4.63% (7) for malaria parasite, out of which 4 placentae were for the 4 babies with positive peripheral parasitaemia and known showed any clinical symptom. The only malaria parasite encountered in this study was the *Plasmodium falciparum*.

The prevalence of cord blood parasitaemia was 5.92% (9/152), 5 of which came from multigravidae (6.58%) and 4 from primigravidae (5.26%). The average parasite density was 1,415 p/uL for pregnant women which was significantly ($P < 0.05$) higher than the 411 p/uL recorded for nonpregnant women. Average parasite density for newborns was 128 p/uL, slightly lower than that for the cords which was 196 p/uL; however, both were significantly lower than parasite density recorded in the placentae (mean = 689 p/µL). Parasite density was significantly ($P < 0.05$) higher in neonates from primigravid (1281/uL) mothers than multigravid (134/uL) newborns. Parasite stages encountered were dominated by trophozoites (64.50%), distantly followed by gametocytes (26.80%) in pregnant women; the same trend was observed for nonpregnant women, placenta, cord, and neonates (Table 3).

The 4 parasitized newborns all had birth weights above 3 kg. On the average, however, the birth weights of babies from parasitized mothers were lower than those from non-parasitized mothers, albeit, the difference was not significant ($P > 0.05$). Also, newborns from primigravid mothers weighed averagely less than those from multigravid mothers; again the difference was not significant (Table 4).

### 4. Discussion

Many researchers have reported high prevalence of malaria in pregnancy in different parts of Nigeria, ranging from 19.70% to 72.00% [8–11]. The prevalence of malaria in pregnant women in this study was 14%; though consistent with the reported Nigerian situation, the relatively low percentage could be due to the fact that the study was carried out during the dry season, a period of low mosquito density and, perhaps, low level malaria transmission rates. This observation supports the position that in areas of malaria endemicity, pregnancy is associated with increased susceptibility to malaria, arising from pregnancy-induced altered immunity [6], Immunosuppression from raised serum cortisol, loss of cell-mediated immunity, effects of a new organ, the placenta, and loss of type-1 cytokine responses [4].

The prevalence of malaria in nonpregnant controls in this study was 13%, although this was lower than the pregnant subjects; the difference was not significant. The inclusion of nonpregnant women in the study allows comparison of malaria prevalence in pregnancy with nonpregnant female subjects. Parasite densities for pregnant women were comparatively higher than that of nonpregnant women. This is consistent with the findings of Agomo et al. [8], in a study done in Lagos, Nigeria. Although none of the pregnant women screened took malaria chemoprophylaxis during pregnancy, nor used any form of mosquito nets, the parasite density figures were low. However, one may conclude that this may have to do with the period of the study, October to February, that is, early to mid-dry season. A season that coincides with decreasing densities of female *Anopheles* species and therefore reduced inoculation with parasites.

Low parasites densities were also recorded for the placentae, cords, and peripheral blood of newborns.

Each placenta of the 4 parasitaemia neonates in this study also had positive parasitaemia. This agrees with the position of Chedraui et al. [6], that placental infection is a prerequisite for, but does not predict, congenital malaria. A strong correlation between placental and congenital parasitaemia has been shown [12].
However, an increasing trend in prevalence of congenital malaria has been reported recently. In a multicentre study done at Ibadan a prevalence of 5.10% was reported in University College Hospital [13]. A prevalence of 46.70% was reported in a study of 120 newborn babies at Ile-Ife, Southwestern Nigeria [14]. Also a prevalence of 13.00% was reported among 546 in-born neonates at Calabar Teaching Hospital [15]. These findings represent a new trend since parasitaemia in peripheral blood of newborns was considered rare in highly endemic areas.

Infection with *Plasmodium falciparum* was the only one encountered in this study. This agrees with a similar study done in Libreville, Gabon by Bouyou-Akotet et al. [16], wherein only *Plasmodium falciparum* was encountered. It also agrees with widely accepted view that *Plasmodium falciparum* is the predominant species in Nigeria [16]. The predominant forms of this parasite seen were the trophozoites (ring stages).

The only stillbirth encountered in this study resulted from foetal distress. The mother of the baby was negative for malaria parasites (nonmalarial).

On the average, the birth weights of babies from parasitaemia mothers were lower than those of babies from nonparasitaemia mothers, but not significantly so. This disagrees with the widely held view that babies with parasitized placentae often have low birth weight. It also contradicts the findings of Adebami et al. [17], who stated that babies of mothers with parasitized placentae have mean birth weight significantly lower than babies of mothers with nonparasitized placentae. The generally low parasite densities found in this study may explain the minimal effect on birth weight, as observed by Opara [2] who recorded parasitized neonate twins with normal birth weights. Primigravidae are more susceptible to malaria infection than multigravidae in endemic areas [18]. The findings of this study supports this position, albeit, the difference in prevalence was not significant.

Congenital malaria was previously thought to be rare, especially, in areas of malaria endemcity. Though this study appears not to support this view, an increasing prevalence of congenital malaria among newborns in other areas of malaria endemcity has been observed.

### Conflict of Interests

The authors declare that there is no conflict of interests.

### Acknowledgments

The authors gratefully acknowledge the assistance of the technologists and midwives and for the use of laboratory facilities of General Hospital Minna and the Department of Biological Sciences, Federal University of Technology, Minna.

### References


15. A. D. Ekanem, M. U. Anah, and J. J. Udo, "The prevalence of congenital malaria among neonates with suspected sepsis in

<table>
<thead>
<tr>
<th>Pregnant women</th>
<th>Primigravidae</th>
<th>Multigravidae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Positive pregnant women</td>
<td>3.03 ± 0.5</td>
<td>2.83 ± 0.44</td>
</tr>
<tr>
<td>Negative pregnant women</td>
<td>3.01 ± 0.52</td>
<td>2.89 ± 0.25</td>
</tr>
</tbody>
</table>
