**Plasmodium falciparum-isolates from Cameroonian pregnant women do not rosette**

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**Summary**: The placenta of pregnant women is frequently parasitized by erythrocytes infected by mature stages of *Plasmodium falciparum* (IE), a phenomenon associated with low birth weight of the offspring. The cytoadherence phenotype of the parasites from pregnant women suggests that placental sequestration may result from cytoadherence to the syncytiotrophoblast. However, as anatomopathological studies report that cytoadherence in the placenta is a rare event, we investigated whether placental parasites may sequester by forming rosettes with uninfected erythrocytes, another possible sequestration mechanism. Parasites from placental blood as well as parasites from the peripheral blood of pregnant and non pregnant subjects were assessed for their ability to rosette. In non pregnant subjects, the rosetting capacity of parasites was as reported in literature while, except in one case, parasites from pregnant women did not rosette. We conclude that the lack of rosetting is a new feature of IE's from pregnant women and that rosetting cannot be involved in the placental sequestration of IE's.

**KEY WORDS**: malaria, *Plasmodium falciparum*, human, placenta, pregnancy, rosette.

**ABBREVIATION**: IE, *Plasmodium falciparum*-infected erythrocyte.

In areas in which *Plasmodium falciparum* malaria is endemic, placental malaria is frequent and associated with low birth weight of the baby, a major cause of neonatal morbidity, thus posing a public health problem (for review, see Brabin, 1991 and Menendez, 1995). In the placenta, most of infected erythrocytes (IE) contain mature stages of the parasite, while IE's from the peripheral blood of the same woman contains only young stages (Garnham, 1938). The involvement of cytoadherence as a mechanism for the sequestration of IE's in the placenta has been reported (Fried & Duffy, 1996; Maubert et al, 1997), but other potential mechanisms have not been assessed. Rosettes (binding of at least two uninfected erythrocytes to an infected erythrocyte) are large cellular structures that facilitate the sequestration of IE's in microvasculature *ex vivo* (Kaul et al., 1991). We investigated whether malaria parasites from pregnant women could form rosettes, that could be trapped in the placental intervillous spaces. For this purpose, we compared the rosetting ability of parasites from pregnant and non pregnant subjects.

**METHODS AND RESULTS**: Parasites from non pregnant subjects were collected from nine women and five men attending at the Messa dispensary, Yaounde,
 Cameroon. They presented with acute but uncomplicated \textit{P. falciparum} malaria, a peripheral parasitaemia higher than 0.2 \%, and were 22.2 ± 6.6 years old (m ± SD). Blood samples were drawn and parasites were cryoconserved in liquid nitrogen. Before the assay, samples were thawed and cultured for less than one cycle to pigmented stages (late trophozoites to young schizonts) according to the standard method (Trager & Jensen, 1976). Among those 14 isolates, parasites from 12 showed mature stages within the first life cycle, and rosetting assay was performed using the standard procedure (Carlson & Wahlgren, 1992). Briefly, 50 µl of the parasite culture was mixed with 50 µl of a 0.01 \% acridine orange solution. 10 µl of the suspension were distributed under a 22 × 22 mm coverslip and at least 200 IEs were examined under fluorescence microscopy using × 500 magnification. The number of rosettes (i. e. IEs that bound at least two uninfected erythrocytes) was determined. Eight out of the 12 samples studied formed rosettes, the percent of IEs forming rosettes being: 1, 1, 1, 5.5, 6.5, 7.5, 14.5, and 17.5. These figures are in line with other reports (Hasler \textit{et al.}, 1990; Wahlgren \textit{et al.}, 1990), and the rosetting ability was not related to sex.

We assessed rosette formation in placental blood from parasitized placentas collected after delivery in two maternity hospitals (Nkolndongo and Etoudi) of Yaounde. Their age was similar to the one of non pregnant subjects (21.5 ± 5.6 years). An incision was made on the maternal face of the placenta and a drop of placental blood was diluted to a 5 \% hematocrit in \textit{P. falciparum} culture medium containing acridine orange. Rosetting rate was assessed as above. None of the 23 infected placental blood studied presented with rosettes.

As the absence of rosettes in placental blood may be related to alteration of the IEs during labor, we studied parasites from the peripheral blood of pregnant women. Twenty five women (age: 22.8 ± 4.6) presenting with a blood parasites were bled within one hour after delivery and parasites were cryoconserved. Rosetting assay was performed as mentioned for non pregnant subjects. After \textit{in vitro} culture, 16 samples showed mature parasites and at least 0.2 \% parasite density. Seven were the peripheral counterpart of studied placental parasites and nine were from other women. Only one formed rosettes and the percentage of rosette-forming IEs was low (2 \%).

**DISCUSSION**

The frequency of rosetting was higher in parasites from non-pregnant (8/12) than in peripheral blood from pregnant subjects (1/16) (Fisher's exact test $p = 0.001$), and than in placental blood (0/23) (Fisher's exact test $p < 0.0001$). As rosetting assay with parasites from peripheral blood was performed in a medium free of immune serum, the difference in rosetting ability cannot result from a difference in antibodies against rosettes.

In non-pregnant host, rosetting facilitates the sequestration of parasites \textit{ex vivo} (Kaul \textit{et al.}, 1991). However the implication of rosetting in human pathology remains uncertain as studies conducted in Gambia (Carlson, 1993) and in Kenya (Rowe \textit{et al.}, 1995) found an association between rosetting and the development of severe or cerebral malaria, while no such relationship was found in a larger study carried out in Papua New Guinea (Al-Yaman \textit{et al.}, 1995). Although pregnant women are at increased risk for malaria (Diagne \textit{et al.}, 1995), our data demonstrate that rosetting is not involved in pregnancy-related malaria morbidity which has to be explained by other mechanisms. Parasites from pregnant and non pregnant women were recently been shown to differ in their cytoadherence phenotype (Fried & Duffy, 1996). Our data show that the lack of rosetting ability is another feature of parasites from pregnant women and that rosetting cannot contribute to parasite concentration in the placenta.

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**REFERENCES**


P. FALCIPARUM-ROSETTES DURING PREGNANCY


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