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Plasmodium falciparum genotypes in matched peripheral, placental and umbilical cord blood in an area characterised by unstable malaria transmission in eastern Sudan

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Abstract

Background: There are few published studies on *Plasmodium falciparum* genotypes in peripheral, placental and umbilical cord blood in areas characterised by unstable malaria transmission.

Method: A cross-sectional study was conducted to investigate *P. falciparum* genotypes in matched peripheral, placental and umbilical cord blood in eastern Sudan. Thick blood smears and *P. falciparum* merozoite surface protein 1 (MSP1) and 2 (MSP2) genes as polymorphic markers in polymerase chain reactions were investigated in 3 kinds of samples of 153 pregnant women at delivery.

Results: There was no significant difference in the prevalence of blood film-detected *P. falciparum* in which 5 (3.3%), 7 (4.6%) and 3 (2.0%) (P = 0.437) of the 153 samples

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were determined to be *P. falciparum*-positive by microscopy for maternal peripheral, placental and cord blood samples, respectively.

Out of these 145 samples, 24 (16.6%), 39 (26.9%) and 24 (16.6%) (P = 0.039) of the peripheral, placental and cord samples, respectively, had submicroscopic parasitaemia (blood films were negative). There was no association between submicroscopic parasitaemia and age or parity.

RO33 and K1 (MSP1 alleles) were detected in 21/29 (72.4%), 42/46 (85.7%), 26/27 (92.2%) and 6/29 (20.6), 16/46 (32.6) and 0(0) (P < 0.001) of the maternal, placental and cord samples, respectively. MAD20 was not detected in any of the samples.

While the 3D7/IC1 allele was detected in 12 (41.3%), 30 (65.2%) and 4 (14.8%) (P < 0.001) of the peripheral, placental and cord samples, respectively, the FC (MSP2) allele was detected in only the 6 (20.6) placental samples. Multi-clonal infection was detected in 10 (34.4), 27 (58.6) and 3 (11.1) (P < 0.001) of the maternal placental and cord samples, respectively.

Conclusion: Compared with the peripheral and cord samples, placental samples had a higher prevalence of submicroscopic parasitaemia. MSP1 alleles were predominant in the cord, while MSP2 alleles were predominant in the placental samples, which had a significant higher multiplicity of the infection.

Keywords: Medicine, Infectious disease

1. Introduction

Malaria, especially *plasmodium falciparum* infection during pregnancy, is a major public health problem in sub-Saharan Africa, where it is the major cause of perinatal and infant deaths (Desai et al., 2007; Bader et al., 2010; Adam et al., 2011). The sequestration of parasite-infected red blood cells in the placenta is the pathognomonic of malaria during pregnancy. Malarial parasites express a pregnancy-specific variant surface antigen (VAR2CSA) on the red blood cell surface, which is capable of binding to the chondroitin sulphate A in the placenta (Rogerson et al., 2007; Doritchamou et al., 2012).

Previous studies on *P. falciparum* genotypes in the matched peripheral, placental and umbilical cord blood in different settings showed varied results. While some of these studies have shown a large overlap between parasites found in each of these compartments, others have suggested that only a subset of peripheral infections sequester in the placenta (Kassberger et al., 2002; Arango et al., 2012; Mayengue et al., 2004; Schleiermacher et al., 2002; Guitard et al., 2010; Cohee et al., 2016).

Investigating the dynamics and genotypes of parasites in different settings is of importance for vaccine design. Malaria during pregnancy is one of the main causes of maternal perinatal morbidity and mortality in Sudan (Adam et al., 2005b; Adam et al., 2011; Mohammed et al., 2013). There is no published data on *P. falciparum* genotypes in matched peripheral, placental and umbilical cord blood in Sudan.

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The current study was conducted to investigate *P. falciparum* genotypes in matched peripheral, placental and umbilical cord blood in eastern Sudan, which is characterised by unstable malaria transmission (Himeidan et al., 2005).

2. Methods

A cross-sectional study was conducted at New Halfa Hospital in eastern Sudan during the post-rainy season (October) of 2014. The area is characterised by unstable malaria transmission, and *P. falciparum* is the main parasite species (Himeidan et al., 2005).

After signing an informed consent, consecutive parturient women were approached to participate in the study. Women with hypertension, diabetes mellitus or any chronic illness were excluded from the study. A questionnaire in the local language was administered by a trained medical officer to gather medical/obstetric histories.

Five millilitres of blood were withdrawn from the placenta and umbilical cord and used for the measurement of haemoglobin and blood film for malaria; and three to five drops were spread on a filter paper for DNA extraction and polymerase chain reaction (PCR).

2.1. Microscopy

Giemsa-stained thick and thin blood films were examined with a 100 × objective to identify the presence/absence of malaria parasites. The blood films were considered negative if no parasites were detected in 100 oil immersion fields of a thick blood film. In the malaria-positive slides, parasite density was measured by counting the number of asexual parasites per 200 leukocytes, based on a mean count of 8,000 leukocytes per microlitre of blood.

2.2. Parasite DNA extraction and PCR

P. falciparum DNA extraction and PCR assays were performed as previously described (Adam et al., 2005a; Elbashir et al., 2011; Mohammed et al., 2013). In summary, following delivery, three drops of blood were collected onto a filter paper (Whatman Grade 3) from maternal peripheral blood, the maternal side of the placenta and the umbilical cord. These drop samples were then air-dried and stored in individual sterile plastic bags until the analyses, which were conducted in our lab in Khartoum, Sudan. The blood spots (approximately 25 μl/one-third of a spot) were punched out. The piece of filter paper was washed with distilled water and placed directly in a PCR reaction tube containing 25 μl of all the PCR reaction components. Quality control was assured by using a negative control sample with no template DNA and an internal positive control was used for the same purpose. Genomic DNA was assayed by nested PCR for DNA from *P. falciparum*

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(Snounou et al., 1993a; Snounou et al., 1993b). PCR assays were performed by two team members (OEF and MIE), who were both blinded to the clinical data of the study.

2.3. Ethics

The study received ethical clearance from the Ethical Committee at the Medical College of Bahri University in Bahri, Sudan.

2.4. Statistics

Data analysis was performed using SPSS. Data were presented as mean (SD), and the proportions were compared between the groups using X^2. Logistic regression was performed where submicroscopic parasitaemia was the dependent variable and age, parity and the other variables were the independent variables. $P < 0.05$ was considered significant.

3. Results

3.1. General characteristics

The mean (SD) age and parity of the 153 enrolled women was 25.7 (6.5) years and 2.7 (2.5), respectively. Seventy (45.8%) of the 153 enrolled women were primiparous. The majority of the women were rural residents (75.2%), had < secondary educational levels (74.5%) and had no antenatal care (77.8%). Approximately one-third (36.6%) of these women had used bed nets in the index pregnancy. Over half (58.2%) of the women had a history of malaria (unconfirmed) in the index pregnancy.

The mean (SD) of the maternal body mass index (BMI), haemoglobin and birth weight was 23.0 (3.9) kg/m^2, 11.4 (1.8) g/dl and 3122.4 (538) g, respectively.

There was no significant difference in the prevalence of blood film-detected *P. falciparum* in the 153 collected blood samples, although 5 (3.3%), 7 (4.6%) and 3 (2.0%) ($P = 0.437$) were determined to be *P. falciparum*-positive by microscopy for maternal peripheral, placental and cord blood samples, respectively.

One hundred forty-five cases had blood films that were negative in all 3 compartments and were considered for submicroscopic parasitaemia. Out of these 145 cases, the prevalence of submicroscopic parasitaemia (blood films were negative) was significantly higher in the placenta, in which the prevalence was 24 (16.6%), 39 (26.9%) and 24 (16.6) ($P = 0.039$) in the peripheral, placental and cord samples, respectively.

Forty-five cases of submicroscopic parasitaemia were detected (in any of the three compartments). Only 16 (11.0%) cases of submicroscopic parasitaemia were

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detected in the peripheral, placenta, and cord samples. Submicroscopic parasitaemia was detected in 24 (16.6%) samples in both peripheral and placental samples.

Binary regression analysis showed no association between submicroscopic parasitaemia and age, parity, residence, antenatal care and use of bed nets (Table 1).

3.2. Genotyping of the parasitaemia

Both MSP1 (MAD20, K1 and RO33) and MSP2 (3D7/IC1 and FC27) alleles were genotyped in 29, 46 and 27 of the peripheral, placental and cord samples, respectively (in samples that contained either microscopic or submicroscopic parasites, Table 2).

3.3. MSP1 genotyping

MSP1 alleles were detected in 21/29 (72.4%), 42/46 (85.7%) and 26/27(92.2%) ($P = 0.014$) of the peripheral, placental and cord samples, respectively.

RO33 and K1 alleles were detected in 21/29 (72.4%), 42/46 (85.7%), 26/27 (92.2%) and 6/29 (20.6%), 16/46 (32.6%) and 0 (0) ($P < 0.001$) of the peripheral, placental and cord samples, respectively. No MAD20 allele was detected in any of the compartments (Table 2).

3.4. MSP2 genotyping

Eighteen (62.0%) of the peripheral, 30 (65.2%) of the placental and 4 (14.8%) ($P < 0.001$) of the cord samples had MSP2 genotypes.

Table 1. Factors associated with submicroscopic parasitaemia among parturient women in eastern Sudan in binary logistic regression.

Variables	Odds Ratio	95% Confidence Interval	P
Age, years	1.03	0.90–1.18	0.597
Parity	0.88	0.62–1.24	0.472
Body mass index, kg/m^2	1.01	0.91–1.13	0.772
Rural residency	0.47	0.15–1.44	0.190
Education < secondary level	0.74	0.41–1.34	0.328
Lack of antenatal care	0.52	0.14–1.83	0.310
Not using bed nets	1.00	0.39–2.56	0.995
Blood groups	0.87	0.314–2.45	0.805

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Table 2. MSP1 and MSP2 *P. falciparum* genotyping of the peripheral, placental and cord samples in eastern Sudan.

Alleles	Peripheral (n= 29)	Placental (n= 46)	Cord (n= 27)	P
Total MSP1	21 (72.4)	42 (85.7)	26 (92.2)	0.014
RO33	21 (72.4)	42 (85.7)	26 (92.2)	0.012
K1	6 (20.6)	16 (32.6)	0 (0)	
MAD20	0 (0)	0 (0)	0 (0)	
Total MSP2	18 (62.0)	30 (65.2)	4 (14.8)	< 0.001
3D7/IC1	12 (41.3)	30 (65.2)	4 (14.8)	< 0.001
FC	6 (20.6)	0 (0)	0 (0)	
Multiplicity	10 (34.4)	27 (58.6)	3 (11.1)	< 0.001

MSP = merozoite surface protein.

While the 3D7/IC1 allele was detected in 12 (41.3%), 30 (65.2%) and 4 (14.8%), $P < 0.001$ of the peripheral, placental and cord samples, respectively, the FC allele was only detected in the placental samples (6, 20.6%).

Thus, MSP1 alleles are predominant in the cord, while MSP2 alleles are predominant in the placental samples.

Multiplicity (more than one allele) of the infection was detected in 10 (34.4%), 27 (58.6%) and 3 (11.1%) ($P < 0.001$) of the peripheral, placental and cord samples, respectively. The multiplicity of the infection varied from 2 to 4, with a median of 1.

4. Discussion

The main findings of the current study were that in comparison with the peripheral (16.6%) and cord (16.6%) samples, placental samples had a significantly higher prevalence (26.9%) of submicroscopic *P. falciparum* infections and a significantly higher prevalence of multiplicity of *P. falciparum* infection. There was no association between submicroscopic parasitaemia and age or parity. We previously reported that (14.0%) and (27.6%) of the placentae investigated in eastern and central Sudan, respectively, had submicroscopic *P. falciparum* malaria infections (Kashif et al., 2013; Mohammed et al., 2013). Supporting the results of the current study, both the latter studies as well as studies from other African settings (Mayengue et al., 2004) reported no association between age, parity and submicroscopic *P. falciparum* malaria infections. This is an area of unstable transmission (in the current study), and the observation of no differences in submicroscopic infection by age or gravidity suggest that women of all gravidities

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are at risk of infection during pregnancy. This is very likely due to the lack of exposure during pregnancy and the absent of gravidity dependent immunity.

The current study showed that RO33, K1 (MSP1) and 3D7/IC1 (MSP2) alleles were predominant, and the MAD20 (MSP1) allele was not detected in any of the samples. Previous reports have shown that 32 (80%) of the pregnant women with submicroscopic infections studied in eastern Sudan had the FC 27 allele (MSP2), 6 (15%) showed only the ICI allele and 2 (5%) showed both of these alleles (Adam et al., 2005a). In Khartoum, the capital of Sudan, the distribution of MSP1 allelic families among pregnant women showed that the K1 allele represented 52.9% of cases, the MAD20 allele represented 44.7% and the RO33 represented 35.2% (Omer et al., 2011).

The FC27/MSP 2 was the predominant allelic family reported from other African settings and might reflect the geographical variation of the parasite genotypes (Kassberger et al., 2002; Mayengue et al., 2004).

In this study, multiplicity of the infection was detected in 10 (34.4%), 27 (58.6%) and 3(11.1%) ($P < 0.001$) of the peripheral, placental and cord samples, respectively. This finding is characteristic of areas with unstable malaria transmission and has also been reported among pregnant women in Senegal (Schleiermacher et al., 2001).

5. Conclusion

Compared with the peripheral and cord samples, placental samples had a higher prevalence of submicroscopic parasitaemia. The MSP1 allele was predominant in the cord samples, while MSP2 was predominant in the placental samples, which had a significant higher multiplicity of the infection. The use of insecticide-treated bed nets might help to reduce infection, importantly diagnostic assessment including microscopy and rapid diagnostic tests should be practiced during antenatal care visit to allow prompt treatment in infected pregnant women. A combination of preventive and treatment measures might help to reduce submicroscopic infection in areas of unstable transmission.

Declarations

Author contribution statement

Omar E. Fadlelseed: Conceived and designed the experiments; Wrote the paper.

Maha E. Osman: Performed the experiments.

Nahla M. Shamseldin: Analyzed and interpreted the data.

Amar B. Elhussein: Contributed reagents, materials, analysis tools or data.

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Ishag Adam: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Adam, I., A-Elbasit, I.E., Salih, I., Elbashir, M.I., 2005a. Submicroscopic Plasmodium falciparum infections during pregnancy, in an area of Sudan with a low intensity of malaria transmission. Ann. Trop. Med. Parasitol. 99, 339–344.

Adam, I., Khamis, A.H., Elbashir, M.I., 2005b. Prevalence and risk factors for anaemia in pregnant women of eastern Sudan. Trans. R Soc. Trop. Med. Hyg. 99, 739–743.

Adam, I., Elhassan, E.M., Haggaz, A.E.D., et al., 2011. A perspective of the epidemiology of malaria and anaemia and their impact on maternal and perinatal outcomes in Sudan. J. Infect. Dev. Ctries. 5, 83–87.

Arango, E.M., Samuel, R., Agudelo, O.M., et al., 2012. Genotype comparison of Plasmodium vivax and Plasmodium falciparum clones from pregnant and non-pregnant populations in North-west Colombia. Malar. J. 11, 392.

Bader, E., Alhaj, A.M., Hussan, A.A., Adam, I., 2010. Malaria and stillbirth in Omdurman Maternity Hospital, Sudan. Int. J. Gynecol. Obstet. 109, 144–146.

Cohee, L.M., Kalilani-Phiri, L., Mawindo, P., et al., 2016. Parasite dynamics in the peripheral blood and the placenta during pregnancy-associated malaria infection. Malar. J. 15, 483.

Desai, M., ter Kuile, F.O., Nosten, F., et al., 2007. Epidemiology and burden of malaria in pregnancy. Lancet Infect. Dis. 7, 93–104.

Doritchamou, J., Bertin, G., Moussiliou, A., et al., 2012. First-Trimester Plasmodium falciparum Infections Display a Typical "Placental" Phenotype. J. Infect. Dis. 206, 1911–1919.

http://dx.doi.org/10.1016/j.heliyon.2017.e00326

Elbashir, H.M., Salih, M.M., Elhassan, E.M., et al., 2011. Polymerase chain reaction and histology in diagnosis of placental malaria in an area of unstable malaria transmission in Central Sudan. Diagn. Pathol. 6, 128.

Guitard, J., Andersen, P., Ermont, C., et al., 2010. Plasmodium falciparum population dynamics in a cohort of pregnant women in Senegal. Malar. J. 9, 165.

Himeidan, Y.E.-S., Malik, E.M., Adam, I., 2005. Epidemiology and Seasonal Pattern of Malaria in an Irrigated Area of Eastern Sudan. Am. J. Infect. Dis. 1, 75–78.

Kashif, A.H., Adam, G.K., Mohmmed, A.A., et al., 2013. Reliability of rapid diagnostic test for diagnosing peripheral and placental malaria in an area of unstable malaria transmission in Eastern Sudan. Diagn. Pathol. 8, 59.

Kassberger, F., Birkenmaier, A., Khattab, A., et al., 2002. PCR typing of Plasmodium falciparum in matched peripheral, placental and umbilical cord blood. Parasitol. Res. 88, 1073–1079.

Mayengue, P.I., Rieth, H., Khattab, A., et al., 2004. Submicroscopic Plasmodium falciparum infections and multiplicity of infection in matched peripheral, placental and umbilical cord blood samples from Gabonese women. Trop. Med. Int. Heal. 9, 949–958.

Mohammed, A.H., Salih, M.M., Elhassan, E.M., et al., 2013. Submicroscopic Plasmodium falciparum malaria and low birth weight in an area of unstable malaria transmission in Central Sudan. Malar. J. 12, 172.

Omer, S., Khalil, E., Ali, H., Sharief, A., 2011. Submicroscopic and multiple plasmodium falciparum infections in pregnant Sudanese women. N. Am. J. Med. Sci. 3, 137–141.

Rogerson, S.J., Hviid, L., Duffy, P.E., et al., 2007. Malaria in pregnancy: pathogenesis and immunity. Lancet Infect. Dis. 7, 105–117.

Schleiermacher, D., Le Hesran, J.Y., Ndiaye, J.L., et al., 2002. Hidden Plasmodium falciparum parasites in human infections: Different genotype distribution in the peripheral circulation and in the placenta. Infect. Genet. Evol. 2, 97–105.

Schleiermacher, D., Rogier, C., Spiegel, A., et al., 2001. Increased multiplicity of Plasmodium falciparum infections and skewed distribution of individual msp1 and msp2 alleles during pregnancy in Ndiop, a Senegalese village with seasonal, mesoendemic malaria. Am. J. Trop. Med. Hyg. 64, 303–309.

Snounou, G., Viriyakosol, S., Jarra, W., et al., 1993a. Identification of the four human malaria parasite species in field samples by the polymerase chain reaction

http://dx.doi.org/10.1016/j.heliyon.2017.e00326

and detection of a high prevalence of mixed infections. Mol. Biochem. Parasitol. 58, 283–292.

Snounou, G., Viriyakosol, S., Zhu, X.P., et al., 1993b. High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Mol. Biochem. Parasitol. 61, 315–320.

 http://dx.doi.org/10.1016/j.heliyon.2017.e00326