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RESEARCH ARTICLE

Leptin, insulin like growth factor-I levels and histology-diagnosed placental malaria in an area characterized by unstable malaria transmission in central Sudan [version 1; peer review: 2 approved with reservations]

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Abstract

Background: There are few published data on the association between leptin, insulin like growth factor-1 (IGF-1) and malaria during pregnancy. This study aimed to investigate maternal and umbilical cord leptin and IGF-1 levels and malaria during pregnancy, and their association - if any - with birth weight.

Methods: A cross-sectional study was conducted at Medani, Sudan. Medical and obstetrics history was gathered from each parturient woman (n=175) and malaria was investigated by blood film and placental histology. Maternal and umbilical cord leptin and IGF-1 levels were measured using ELISA.

Results: Upon histological examination, 48 women were infected with placental malaria, and 127 were found free from the disease. Out of the 48, 2 of the patients showed signs of active infection, 3 of chronic infection and 43 of previous infection. Placental malaria and preterm delivery were associated with low birth weight (< 2500 g). Younger mothers and primigravidae had a higher risk for placental malaria infection. There was no significant difference in maternal and umbilical cord leptin and IGF-1 levels between women infected with placental malaria and those free from the disease.

Conclusions: The current study showed that low birth weight was significantly associated with placental malaria. Young mothers and primigravidae had a higher risk to develop the infection. There was no significant difference in the levels of maternal and umbilical cord leptin and IGF-1 levels between women infected with placental

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malaria and those free from the disease. Both the levels of maternal and cord leptin and IGF-1 were found not to be associated with birth weight.

Abbreviations: IGF-1: Insulin like growth factor-1; LBW: Low birth weight; ELISA: Enzyme-linked immunosorbent assay; PM: Placental malaria.

Keywords

placental malaria, birth weight, leptin, Insulin-like growth factor 1, IGF-1

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Introduction

Malaria during pregnancy is a major public health concern, especially in sub-Saharan Africa where there are approximately 125 million pregnant African women living in malaria-endemic regions. Almost one fifth of these pregnant women are at risk of malaria (Dellicour *et al.*, 2010; Desai *et al.*, 2007). Malaria during pregnancy is the main cause of maternal, perinatal and neonatal adverse effects, especially anemia and low birth weight (LBW) (Ahmed *et al.*, 2014; Menendez *et al.*, 2000; Rogerson *et al.*, 2003).

The pathogenesis of placental malaria and LBW is not fully understood. Leptin is a hormone secreted mainly by adipocytes (Zhang *et al.*, 1994) that can potentiate inflammation by enhancing macrophage phagocytosis (Loffreda *et al.*, 1998; Pacifico *et al.*, 2006). Previous reports have shown that leptin levels were decreased during malarial attack in pregnant women (Conroy *et al.*, 2011), and that these decreased leptin levels were associated with placental malaria infection, as well as low birth weight (Kabyemela *et al.*, 2008a; Kabyemela *et al.*, 2008b).

Insulin-like growth factor-I (IGF-1), also called somatomedin C, is a polypeptide with a sequence similar to that of insulin (Rinderknecht & Humbel, 1978). Recently, maternal and umbilical cord blood levels of IGF-1 were investigated in malaria during pregnancy as possible determinants of birth weight (Ayoola *et al.*, 2012; Umers *et al.*, 2011).

Research on malaria during pregnancy and its associated adverse effects e.g. LBW is highly valuable for researchers and clinicians as it can yield basic data needed for the future vaccine.

Pregnant Sudanese women are susceptible to malaria regardless of their age and parity, and malaria is associated with increased maternal mortality, anemia, LBW, and stillbirths (Adam *et al.*, 2005a; Ali *et al.*, 2011; Bader *et al.*, 2010; Mohammed *et al.*, 2013).

Central Sudan is characterized by unstable malaria transmission, and *P. falciparum* is the main malaria parasite species reported in the area (Malik *et al.*, 2004). To add to the research on placental malaria during pregnancy that has been carried out already (Alim *et al.*, 2015; Mostafa *et al.*, 2015; Salih *et al.*, 2011), the current study was conducted in central Sudan to investigate the maternal and umbilical cord levels of leptin and IGF-1 in placental malaria infection.

Methods

A cross-sectional study was conducted from August to October 2014 in the labor ward of the Medani Maternity Hospital. After signing an informed consent form, women with singleton pregnancies were approached to participate in the study. Women with twins, hypertension, diabetes mellitus and antepartum hemorrhage were excluded from the study. Socio-demographic data (age, parity, residence and gestational age) and data on obstetric history, medical history, and bed net use were gathered using a structured questionnaire that was completed by a trained medical officer in the local language (Arabic). Maternal weight and height were measured and body mass index (BMI) was calculated and expressed as weight(kg)/height(m)². Maternal hemoglobin concentrations were estimated (HemoCue AB, Angelholm, Sweden). Newborns were

weighed immediately following birth using the Salter scale and the sex of each newborn was recorded. The total sample size was calculated assuming that at least 23% of parturient women would have placental malaria infection. To have over 80% power to detect a difference of 5% at $\alpha=0.05$, we recruited 175 women. We assumed that 10% of women might not respond or have incomplete data.

Giemsa-stained blood smears for light microscopy

Maternal, placental, and umbilical cord blood films were prepared for testing. Slides were stained by 10% Giemsa. In the slides positive for malaria the number of asexual parasites was counted per 200 leukocytes, assuming a leukocyte count of 8000 leukocytes per μ l (for thick films) or per 1000 red blood cells (for thin films). Blood films were considered negative if no parasites were detected in 100 oil immersion fields of a thick blood film.

The maternal and umbilical cord blood was then allowed to clot, centrifuged for 10 minutes at 3000 rpm and the serum separated and stored at - 20°C until further analysis.

Placental histology

The details on placental histology have been mentioned previously (Alim *et al.*, 2015; Mostafa *et al.*, 2015; Salih *et al.*, 2011). In summary, a 3cm³ full thickness sample was obtained from the maternal surface approximately half the distance between the umbilical cord and the edge of the placenta. The placental biopsy samples were immediately placed in 10% neutral buffered formalin. Buffer was used to prevent formation of formalin pigment, which might be difficult to differentiate from malaria pigment (Bulmer *et al.*, 1993a). The placental biopsy samples were then embedded in paraffin wax/sections. In every case, the thick paraffin sections were stained with hematoxylin-eosin and Giemsa stains. Slides were read by a pathologist who remained blind to the clinical characteristics of each of these samples. Placental malaria infection was characterized using parameters previously described by Bulmer *et al.*: uninfected (no parasites or pigment), acute (parasites in intervillous spaces), chronic (parasites in maternal erythrocytes and pigment in fibrin, or cells within fibrin and/or chorionic villous syncytiotrophoblast or stroma), and previous (no parasites, and pigment confined to fibrin or cells within fibrin) (Bulmer *et al.*, 1993b).

ELISA for measuring leptin and IGF-1 levels

Maternal and umbilical cord serum levels of leptin and IGF-1 were measured using ELISA Kits and the manufacturers' instructions were strictly followed (DRG Diagnostics, Marburg, Germany).

Statistical analysis

The data analyses were performed using SPSS statistical software for windows (version 18.0). Statistical significance was set at P value < 0.05. To compare means and proportions between groups, student's t-test and Chi-square test were used, respectively. For non-parametric data significant differences in means between two groups were calculated using the Mann-Whitney test. Univariate and multivariate analyses were performed with a logistic regression model where placental malaria infection was the dependent variable and expected risk factors (mother's age, parity, mother's weight, mother's haemoglobin, educational level, residence, use of bed net, antenatal care attendance, use of folic acids supplements and mother's serum leptin and IGF-1) were the independent variables.

Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Linear regression models were set to investigate the factors associated with the level of mother's haemoglobin and birth weight. Predictor variables for mother's haemoglobin model were; antenatal care attendance, parity, BMI, maternal serum leptin and use of folic acid supplements. Predictor variables for birth weight were; mother's age, antenatal care attendance, mother's haemoglobin, placental malaria, mother's height, delivery gestational age.

Ethics

The study received ethical clearance from the Research Board at the Faculty of Medicine, University of Khartoum, Sudan. (Approval number: 2-2011).

Selection of participants

Pregnant women who delivered at Medani Maternity hospital from August through to October 2014 were recruited for this study, following written informed consent. All participants finally included in the study had to satisfy the selection criteria and have none of the exclusion criteria.

Results

Out of the 175 women enrolled in the study, 77 (44%) were primiparae. The majority of them (105; 60.0%) had rural residency and used bed nets (157; 89.7%) during the index pregnancy (Dataset 1 (Elsheikh *et al.*, 2017)).

In total, 36 (20.6%) had blood group A, 21 (12%) had blood group B, four (2.3%) had blood group AB, and 113 (64.6%) had blood group O. The mean (SD) hemoglobin level was 10.2 (1.1) g/dL, and 129 (73.7%) of the women were anemic (hemoglobin <11 g/dL). Eighteen (10.5%) women delivered low-birth weight neonates (<2500 g)(Dataset 1 (Elsheikh *et al.*, 2017)).

Forty-eight women were infected with placental malaria (PM⁺), and 127 were free from the disease (PM⁻). Out of the 48, 2(4%) of PM⁺ patients had active infection, 3(6%) had chronic and 43 (90%) past infection (Dataset 1 (Elsheikh *et al.*, 2017)).

The mean age ($\pm$ SD) of PM⁺ patients was 26 ± 4.8 years and ranged from 17 to 38 years. The mean age ($\pm$ SD) of PM⁻ patients was 28 ± 6 years with a range of 18 to 41 years (Table 1). Younger women (25 – 30 years) were significantly more often infected with PM (P = 0.02)(Dataset 1 (Elsheikh *et al.*, 2017)).

Moreover, babies born to women with PM tended to be in the LBW (< 2500 g) category more often than those born to non-infected women, but the p-value failed to reach the significance level (p = 0.054). Maternal weight, BMI, gravidity, gestational age at delivery and hemoglobin levels were unchanged significantly between groups (Dataset 1 (Elsheikh *et al.*, 2017)).

Placental malaria associated low birth weight

Low birth weight (< 2500g) was significantly associated with placental malaria (N = 172, p = 0.006)(Dataset 1 (Elsheikh *et al.*, 2017)).

Risk factors for placental malaria

Univariate and multivariate analysis demonstrated that only the mother's age and parity were significant risk factors (p-values were 0.008 for mother's age and 0.009 for parity).

Younger mothers and primigravidae had a higher risk for PM. The risk of infection was lower for older mothers, with an odds ratio (OR) of 0.881 (p = 0.008, 95%CI: 0.802 – 0.968). For each additional year in age, the odds of getting placental malaria lowered by a factor of 0.881. The OR for parity was 4.3 (p = 0.009, 95%CI: 1.45 – 12.998) (Table 2, Dataset 1 (Elsheikh *et al.*, 2017)).

Serum levels of leptin and IGF-1

The levels of leptin were higher in LBW infants and their mothers, whilst IGF-1 levels were higher in normal weight infants and their mothers. However, these differences failed to reach statistical significance (Table 3). Non-infected mothers and their infants showed higher levels of leptin and IGF-1 than infected ones (Figure 1 and Figure 2), but these differences also failed to reach statistical significance (Table 4, Dataset 1 (Elsheikh *et al.*, 2017)).

Table 1. Maternal characteristics according to placental malaria histopathology (Dataset 1). Data are means (SD). Women with placental malaria (PM) infection were on average younger (p= 0.02) and delivered lighter neonates than uninfected women (p= 0.054, not significant).

Malaria histopathology	All women (n= 175) Mean n= 171	Uninfected (n= 127) Mean n= 124	Infected (n= 48) Mean n= 47	P (t-test)
Maternal age, years	27.3 (5.8)	27.9 (6.1)	25.7 (4.8)	0.02*
Maternal weight, Kg	63.0 (5.6)	63.4 (5.8)	62 (5.1)	0.1
BMI	23.5 (4.2)	23.7 (3.7)	23.0 (5.3)	0.33
Gravidity number of pregnancy	2.2 (1.5)	2.3 (1.6)	2.1 (1.2)	0.3
Delivery gestational age, weeks	38.9 (1.6)	38.9 (1.6)	38.7 (1.7)	0.4
Maternal hemoglobin level, g/dL	10.3 (1.1)	10.3 (1.1)	10.3 (1.3)	0.9
Birth weight, Kg	3.1 (0.5)	3.1 (0.5)	3.0 (0.6)	0.054

Table 2. Factors associated with placental malaria infections using univariate and multivariate analyses (Dataset 1).

	Univariate analysis			Multivariate analysis		
	OR	95.0% CI	P-value	OR	95.0% CI	P-value
Maternal age	0.934	0.879 – 0.993	0.028*	0.881	0.802 – 0.968	0.008* ¹
Parity (primigravidae or multigravidae)	1.23	0.621 – 2.44	0.553	4.345	1.452 – 12.998	0.009* ²
Maternal weight	0.953	0.894 – 1.016	0.137	0.933	0.858 – 1.014	0.104
Maternal haemoglobin	1.011	0.753 – 1.357	0.944	1.257	0.827 – 1.911	0.284
Education level	0.000	0.00	0.999	0.000	0.000	0.999
Residence	1.26	0.63 – 2.51	0.517	1.342	0.536 – 3.359	0.529
Use of bed net	0.905	0.301 – 2.7	0.859	0.795	0.139 – 4.560	0.797
Antenatal care attendance	0.859	0.433 – 1.702	0.662	1.581	0.551 – 4.539	0.394
Use of folic acid supplements	0.620	0.142 – 2.700	0.524	0.389	0.045 – 3.343	0.390
Maternal serum leptin	0.987	0.958 – 1.02	0.388	0.980	0.938 – 1.023	0.355
Maternal serum IGF-1	0.999	0.996 – 1.00	0.430	0.999	0.995 – 1.002	0.498

*: Maternal age showed statistically significant association with placental malaria in univariate and multivariate analysis.

*¹: Adding one year in age decreases the risk of getting placental malaria by about 11.9%.

*²: The risk for primigravidae to get placental malaria is 4.3 higher than for multigravidae.

Table 3. Leptin and IGF-1 levels in women who delivered low birth weight (LBW) babies and those who delivered normal birth weight babies (Dataset 1). The data is shown as median (interquartile range).

Birth weight	Mother delivered normal birth weight (n=148)	Mother delivered LBW (n= 18)	P
Maternal serum Leptin, ng/ml	5.1 (2.4 – 13.9)	7.1 (3.4 – 13.0)	0.292
Cord serum Leptin, ng/ml	5.3 (2.8 – 8.7)	6.2 (1.1 – 10.6)	0.864
Maternal serum IGF-1, ng/ml	122.3 (29.5 – 246.1)	67.0 (32.3 – 166.0)	0.123
Cord serum IGF-1, ng/ml	38.0 (15.7 – 71.7)	27.2 (15.0 – 64.8)	0.395

Linear regression analysis showed that gestational age had the strongest positive effect on birth weight ($\beta = 0.191$, $p = 0.01$), followed by antenatal care attendance ($p = 0.043$) and mother's age ($p = 0.85$, not significant) (Table 5, Dataset 1 (Elsheikh *et al.*, 2017)).

Dataset 1. The file contains data on socio-demographics (age, parity, residence and gestational age), obstetric and medical history, bed net use, maternal weight, height and BMI, maternal hemoglobin, infant birth weights and maternal and umbilical cord leptin and IGF-1 levels for each participant

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HME has confirmed that all raw data provided with this manuscript has been de-identified.

Discussion

The main findings of the current study are that placental malaria is significantly associated with LBW. Younger mothers and primigravidae had a higher risk for PM infection. There was no significant difference in leptin and IGF-1 levels between PM+ and PM– women and their infants, as well as between LBW infants and their mothers and normal weight infants and their mothers. Maternal and umbilical cord leptin and IGF-1 levels were not associated with birth weight.

Our results coincide with what has been reported previously about LBW being significantly associated with placental malaria (Albitiet *et al.*, 2010; Aribodor *et al.*, 2009; Menendez *et al.*, 2000). Although some studies conducted in different areas in Sudan did not report

Figure 1. (A) Boxplot of maternal serum leptin concentrations in women with and without placental malaria. (B) Boxplot of umbilical cord serum leptin concentrations in women with and without placental malaria. Maternal and cord leptin levels were measured in serum samples of non- (PM⁻, n= 122, 5 missed samples) and women with placental malaria (PM⁺, n= 47, 1 missed sample). The Mann-Whitney test was used to compare the levels of maternal and umbilical cord leptin between the two groups. PM⁺ women showed lower levels of maternal and cord leptin but these differences were not statistically significant (Dataset 1).

Figure 2. (A) Boxplot of maternal insulin-like growth factor-I (IGF-I) concentrations in women with and without placental malaria. (B) Boxplot of umbilical cord IGF-I concentrations in women with and without placental malaria. Maternal and cord IGF-1 levels were measured in serum samples of non-infected women (PM⁻, n= 122, 5 missed samples) and women with placental malaria (PM⁺, n= 47, 1 missed sample). The Mann-Whitney test was used to compare the levels of maternal and umbilical cord IGF-1 between the two groups. PM⁺ women showed lower levels of maternal and cord IGF-I but these differences were not statistically significant (Dataset 1).

Table 4. Leptin and IGF-1 levels in women with placental malaria and those who had no malaria (Dataset 1). The data is shown as median (interquartile range).

Malaria Histopathology	Uninfected (n= 122)	Infected (n= 47)	P
Maternal serum Leptin, ng/ml	5.5 (2.8 – 14.1)	4.9 (2.3 – 10.8)	0.268
Cord serum Leptin, ng/ml	5.5 (2.8 – 8.9)	4.5 (1.8 – 8.8)	0.599
Maternal serum IGF-1, ng/ml	114.3 (25 – 245.8)	95.8 (46.9 – 197.0)	0.724
Cord serum IGF-1, ng/ml	38.2 (16.9 – 64.7)	29.1 (14.4 – 70.8)	0.623

Table 5. Factors affecting birth weight and maternal haemoglobin (Dataset 1).

	Birth Weight			Maternal Haemoglobin		
	B Coefficients	Std. Error	P	B Coefficients	Std. Error	P
Maternal age	0.139	0.007	0.085			
Antenatal care attendance	-0.176	0.092	0.043*	-0.365	0.162	0.000
Parity				0.172	0.153	0.015*
BMI				0.090	0.036	0.193
Maternal haemoglobin	0.098	0.039	0.255			
Placental malaria	-0.099	0.089	0.196			
Maternal serum leptin				-0.221	0.069	0.002*
Use of folic acid supplements				-0.123	0.703	0.080
Maternal height	0.078	0.006	0.331			
Delivery gestational age	0.191	0.026	0.018*			

*: Birth weight was significantly affected by gestational age and antenatal care attendance.

this association. A study conducted in Gadarif hospital in an area characterized by unstable malaria transmission in eastern Sudan found that placental malaria affects pregnant women regardless of their parity and had no effects on birth weight (Salih *et al.*, 2011). Another study showed that, while placental malaria infections that were positive by histology were not associated with LBW, submicroscopic malaria infections (diagnosed by PCR) were (Mohammed *et al.*, 2013). Moreover, a study conducted by Batran *et al.* (Batran *et al.*, 2013) found that placental infections had no effect on LBW or anemia.

Many other studies also observed that the mother's age and parity are risk factors for placental malaria (Falade *et al.*, 2010; Ndeserua *et al.*, 2015; Ojurongbe *et al.*, 2010; Tako *et al.*, 2005; Walker *et al.*, 2013), which is in contrast with our previously published results (Adam *et al.*, 2005a; Adam *et al.*, 2005b; Adam *et al.*, 2007; Adam *et al.*, 2009; Adam *et al.*, 2011; Albiti *et al.*, 2010).

The study showed that leptin levels were higher in non-infected mothers and their infants than in infected ones, however these differences failed to reach statistical significance. This concurs with a study conducted in Malawi which found a significant reduction of leptin levels in mothers infected with PM, and according to this

the authors suggested leptin to be an informative biomarker for diagnosis of PM (Conroy *et al.*, 2011). Another study in Tanzania also reported the same finding (Kabyemela *et al.*, 2008a). Kabyemela and his colleagues (Kabyemela *et al.*, 2008b) also investigated the effect of PM in the relationship between cord leptin levels and birth weight. They found that cord leptin had a strong positive relationship with birth weight in offspring of PM⁻ women ($P = 0.02$ to $P < 0.0001$) but not in offspring of PM⁺ women, however the differences did not reach significance level.

Although the current study failed to detect a significant differences in IGF-1 levels between PM⁺ and PM⁻ women, one study have shown that placental malaria-associated inflammation disturbs maternal and fetal levels of IGFs, which regulate fetal growth (Umbers *et al.*, 2011). This may be one mechanism by which placental malaria leads to fetal growth restriction, but this study did not report the effect size of PM on IGF-1 levels.

It is worth mentioning that the differences between the current study and later ones that reported low leptin (Conroy *et al.*, 2011; Kabyemela *et al.*, 2008a; Kabyemela *et al.*, 2008b) and IGF-1 levels in maternal and umbilical cord serum (Umbers *et al.*, 2011) might be due to the duration of malaria infection itself. While the

majority of malaria infections in the current study were past placental infection (4% of PM⁺ patients had active infection, 6% had chronic and 90% past infection), the later studies reported results from active placental infections. Furthermore, submicroscopic placental malaria infection using PCR (Polymerase Chain Reaction) was not investigated in the current study.

We have recently reported that women with submicroscopic malaria were at higher risk to have LBW (Mohammed *et al.*, 2013). Likewise Adegnika *et al.* have reported that microscopic and submicroscopic *P. falciparum* infection, but not inflammation (C-reactive protein) caused by infection, is associated with low birth weight (Adegnika *et al.*, 2006).

The main limitation of this study was that we relied on a single measure of leptin and IGF-1 levels at delivery; however it was not feasible to obtain the levels of these infants before birth. Most studies relating umbilical cord blood IGF-1 levels and birth weight reported single measurements at birth (Ong *et al.*, 2000; Yang & Yu, 2000), and findings are similar. Another limitation was that we did not measure the concentrations of any other components of the IGF axis, including growth hormone, insulin, IGF binding proteins (IGFBP1-5), and receptors (IGF-1R and 2R) to further establish the potential implication of the IGF system in fetal growth. More limitation is that although we are interested only in the biologically active IGF-1 (free form), the ELISA technique used in this study measures the total amount of IGF-1 (free and protein-bound) in serum.

Conclusions

The current study shows that there is no statistically significant difference in the levels of maternal and cord leptin and in the levels of IGF-1 between PM⁺ women and PM⁻ women, and between women who delivered LBW infants and those who delivered normal weight ones. The main finding is that placental malaria is significantly associated with LBW. Neither maternal and umbilical cord leptin levels nor IGF-1 levels were associated with birth weight.

Data availability

Dataset 1. The file contains data on socio-demographics (age, parity, residence and gestational age), obstetric and medical history, bed net use, maternal weight, height and BMI, maternal hemoglobin, infant birth weights and maternal and umbilical cord leptin and IGF-1 levels for each participant. HME has confirmed that all raw data provided with this manuscript has been de-identified. DOI, [10.5256/f1000research.10641.d158697](https://doi.org/10.5256/f1000research.10641.d158697) (Elsheikh *et al.*, 2017)

Author contributions

HME and IA designed the experiments. IA conceived the study and participated in study coordination. EME conducted the clinical work. AAM performed the pathological analysis. HME carried out the laboratory work, statistical analysis and study coordination. HME, IA and MIE prepared the first draft of the manuscript. MIE contributed to the experimental design. AHK contributed in statistical analysis. All authors were involved in the revision of the draft manuscript and have agreed to the final content.

Competing interests

No competing interests were disclosed.

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References

- Adam I, Adamt GK, Mohammed AA, *et al.*: **Placental malaria and lack of prenatal care in an area of unstable malaria transmission in eastern Sudan.** *J Parasitol.* 2009; **95**(3): 751–752.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Adam I, A-Elbasit IE, Salih I, *et al.*: **Submicroscopic *Plasmodium falciparum* infections during pregnancy, in an area of Sudan with a low intensity of malaria transmission.** *Ann Trop Med Parasitol.* 2005b; **99**(4): 339–344.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Adam I, Babiker S, Mohammed AA, *et al.*: **ABO blood group system and placental malaria in an area of unstable malaria transmission in eastern Sudan.** *Malar J.* 2007; **6**: 110.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Adam I, Elhassan EM, Mohammed AA, *et al.*: **Malaria and pre-eclampsia in an area with unstable malaria transmission in Central Sudan.** *Malaria J.* 2011; **10**: 258.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Adam I, Khamis AH, Elbasher MI: **Prevalence and risk factors for *Plasmodium falciparum* malaria in pregnant women of eastern Sudan.** *Malaria J.* 2005a; **4**: 18.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Adegnika AA, Verweij JJ, Agnandji ST, *et al.*: **Microscopic and sub-microscopic *plasmodium falciparum* infection, but not inflammation caused by infection, is associated with low birth weight.** *Am J Trop Med Hyg.* 2006; **75**(5): 798–803.
[PubMed Abstract](#)
- Ahmed R, Singh N, ter Kuile FO, *et al.*: **Placental infections with histologically confirmed *Plasmodium falciparum* are associated with adverse birth outcomes in India: a cross-sectional study.** *Malar J.* 2014; **13**: 232.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Albiti AH, Adam I, Ghouth AS: **Placental malaria, anaemia and low birthweight in Yemen.** *Trans R Soc Trop Med Hyg.* 2010; **104**(3): 191–4.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ali AA, Elhassan EM, Magzoub MM, *et al.*: **Hypoglycaemia and severe *Plasmodium falciparum* malaria among pregnant Sudanese women in an area characterized by unstable malaria transmission.** *Parasit Vectors.* 2011; **4**: 88.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Alim A, E Bilal N, Abass AE, *et al.*: **Complement activation, placental malaria infection, and birth weight in areas characterized by unstable malaria transmission in central Sudan.** *Diagn Pathol.* 2015; **10**: 49.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

- Aribodor DN, Nwaorgu OC, Eneanya CI, *et al.*: **Association of low birth weight and placental malarial infection in Nigeria.** *J Infect Dev Ctries.* 2009; 3(8): 620–623.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ayoola OO, Whatmore A, Balogun WO, *et al.*: **Maternal malaria status and metabolic profiles in pregnancy and in cord blood: relationships with birth size in Nigerian infants.** *Malar J.* 2012; 11: 75.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bader E, Alhaj AM, Hussan AA, *et al.*: **Malaria and stillbirth in Omdurman Maternity Hospital, Sudan.** *Int J Gynaecol Obstet.* 2010; 109(2): 144–6.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Batran SE, Salih MM, Elhassan EM, *et al.*: **CD20, CD3, placental malaria infections and low birth weight in an area of unstable malaria transmission in Central Sudan.** *Diagn Pathol.* 2013; 8: 189.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bulmer JN, Rasheed FN, Francis N, *et al.*: **Placental malaria. I. Pathological classification.** *Histopathology.* 1993a; 22(3): 211–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Bulmer JN, Rasheed FN, Morrison L, *et al.*: **Placental malaria. II. A semi-quantitative investigation of the pathological features.** *Histopathology.* 1993b; 22(3): 219–25.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Conroy AL, Liles WC, Molyneux ME, *et al.*: **Performance characteristics of combinations of host biomarkers to identify women with occult placental malaria: a case-control study from Malawi.** *PLoS One.* 2011; 6(12): e28540.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Dellicour S, Tatem AJ, Guerra CA, *et al.*: **Quantifying the number of pregnancies at risk of malaria in 2007: a demographic study.** *PLoS Med.* 2010; 7(1): e1000221.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Desai M, ter Kuile FO, Nosten F, *et al.*: **Epidemiology and burden of malaria in pregnancy.** *Lancet Infect Dis.* 2007; 7(2): 93–104.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Elsheikh H, Adam I, Elhassan EM, *et al.*: **Dataset 1 in: Leptin, insulin like growth factor-I levels and histology-diagnosed placental malaria in an area characterized by unstable malaria transmission in central Sudan.** *F1000Research.* 2017.
Data Source
- Falade CO, Tongo OO, Ogunkunle OO, *et al.*: **Effects of malaria in pregnancy on newborn anthropometry.** *J Infect Dev Ctries.* 2010; 4(7): 448–53.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kabyemela ER, Fried M, Kurtis JD, *et al.*: **Fetal responses during placental malaria modify the risk of low birth weight.** *Infect Immun.* 2008b; 76(4): 1527–1534.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kabyemela ER, Muehlenbachs A, Fried M, *et al.*: **Maternal peripheral blood level of IL-10 as a marker for inflammatory placental malaria.** *Malar J.* 2008a; 7: 26.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Loffreda S, Yang SQ, Lin HZ, *et al.*: **Leptin regulates proinflammatory immune responses.** *FASEB J.* 1998; 12(1): 57–65.
[PubMed Abstract](#)
- Malik EM, Atta HY, Weis M, *et al.*: **Sudan Roll Back Malaria Consultative Mission: Essential Actions to Support the Attainment of the Abuja Targets.** Sudan RBM Country Consultative Mission Final Report. Geneva: Roll Back Malaria Partnership; 2004.
Reference Source
- Menendez C, Ordi J, Ismail MR, *et al.*: **The impact of placental malaria on gestational age and birth weight.** *J Infect Dis.* 2000; 181(5): 1740–5.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Mohammed AH, Salih MM, Elhassan EM, *et al.*: **Submicroscopic *Plasmodium falciparum* malaria and low birth weight in an area of unstable malaria transmission in Central Sudan.** *Malar J.* 2013; 12: 172.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Mostafa AG, Bilal NE, Abass AE, *et al.*: **Coagulation and Fibrinolysis Indicators and Placental Malaria Infection in an Area Characterized by Unstable Malaria Transmission in Central Sudan.** *Malar Res Treat.* 2015; 2015: 369237.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ndeserua R, Juma A, Mosha D, *et al.*: **Risk factors for placental malaria and associated adverse pregnancy outcomes in Rufiji, Tanzania: a hospital based cross sectional study.** *Afr Health Sci.* 2015; 15(3): 810–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ojurongbe O, Oyediji SI, Oyibo WA, *et al.*: **Molecular surveillance of drug-resistant *Plasmodium falciparum* in two distinct geographical areas of Nigeria.** *Wien Klin Wochenschr.* 2010; 122(23–24): 681–5.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ong K, Kratzsch J, Kiess W, *et al.*: **Size at birth and cord blood levels of insulin, insulin-like growth factor I (IGF-I), IGF-II, IGF-binding protein-1 (IGFBP-1), IGFBP-3, and the soluble IGF-IR/ mannose-6-phosphate receptor in term human infants. The ALSPAC Study Team.** *Avon Longitudinal Study of Pregnancy and Childhood.* *J Clin Endocrinol Metab.* 2000; 85(11): 4266–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Pacifico L, Di Renzo L, Anania C, *et al.*: **Increased T-helper interferon-gamma-secreting cells in obese children.** *Eur J Endocrinol.* 2006; 154(5): 691–697.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Rinderknecht E, Humbel RE: **The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin.** *J Biol Chem.* 1978; 253(8): 2769–76.
[PubMed Abstract](#)
- Rogerson SJ, Pollina E, Getachew A, *et al.*: **Placental monocyte infiltrates in response to *Plasmodium falciparum* malaria infection and their association with adverse pregnancy outcomes.** *Am J Trop Med Hyg.* 2003; 68(1): 115–9.
[PubMed Abstract](#)
- Salih MM, Mohammed AH, Mohammed AA, *et al.*: **Monocytes and macrophages and placental malaria infections in an area of unstable malaria transmission in eastern Sudan.** *Diagn Pathol.* 2011; 6: 83.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Tako EA, Zhou A, Lohoue J, *et al.*: **Risk factors for placental malaria and its effect on pregnancy outcome in yaounde, Cameroon.** *Am J Trop Med Hyg.* 2005; 72(3): 236–42.
[PubMed Abstract](#)
- Umbers AJ, Boeuf P, Clapham C, *et al.*: **Placental malaria-associated inflammation disturbs the insulin-like growth factor axis of fetal growth regulation.** *J Infect Dis.* 2011; 203(4): 561–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Walker PG, Griffin JT, Cairns M, *et al.*: **A model of parity-dependent immunity to placental malaria.** *Nat Commun.* 2013; 4: 1609.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Yang SW, Yu JS: **Relationship of insulin-like growth factor-I, insulin-like growth factor binding protein-3, insulin, growth hormone in cord blood and maternal factors with birth height and birthweight.** *Pediatr Int.* 2000; 42(1): 31–36.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Zhang Y, Proenca R, Maffei M, *et al.*: **Positional cloning of the mouse obese gene and its human homologue.** *Nature.* 1994; 372(6505): 425–432.
[PubMed Abstract](#) | [Publisher Full Text](#)

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Chloe R. McDonald

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The authors present an analysis of two biomarkers, Leptin and IGF-1, quantified in maternal and umbilical cord samples collected at delivery. The results are analyzed in association with malaria infection (by blood smear and/or histology) in pregnancy, a leading contributor to adverse birth outcomes in malaria endemic regions. I appreciate the authors' focus on this important subject matter and present the comments below for their consideration. My comments namely focus on providing additional detail within the article to support the analysis, result and interpretation presented.

Comments:

Abstract

- The abstract states "Placental malaria and preterm delivery were associated with low birth weight (<2500g)." The authors don't provide any information in the methods around gestational dating (LMP, ultrasound etc.) and don't provide any analysis regarding the association with preterm birth. I would remove the association with and discussion around preterm birth

Introduction

- The reference to Dellicour et al. 2010 and 125 million pregnancies includes geographies outside of sub-Saharan Africa. The first two sentences are a bit confusing and might be amended to read "number of pregnancies at risk, number in areas endemic for p. falciparum" since that's the focus of the study.
- I would also amend "the main cause" to "a main cause" since malaria is an important contributor but other factors (nutrition, lack of access to antenatal care etc.) are also principal contributors to adverse outcomes.
- I think the introduction could be expanded to include more background around the selected biomarkers and the rationale for selecting them. e.g. relationship between Leptin and metabolic function (BMI etc.) and pregnancy, perhaps in relation to the nutritional

status of women in the cohort. The authors state that “the pathogenesis of placental malaria and LBW is not fully understood” and then move into Leptin. It might be useful to provide some rationale around how the authors proposed Leptin and IGF-1 play a role in the pathogenesis of LBW resulting from placental malaria.

- I found the paragraph about vaccine development a bit confusing. I would provide more information around how the results of this study (or research on the effects of placental malaria) might contribute to development of a vaccine or potentially remove that paragraph

Methods

- Provide background on any IPTp or anti-malaria clinical care the women in this cohort received in pregnancy (in particular in light of the relatively low levels of active infection), if women were receiving IPTp the authors should comment on how this may impact their results in the discussion (if not the authors should state why women in this cohort did not receive treatment)
- Provide a rationale for excluding women with hypertension and diabetes mellitus (and how was this diagnosis performed to ensure that the cohort doesn't include any women with hypertension or diabetes)
- Provide information around gestational dating (LMP, ultrasound?)
- Provide additional information around the variable “antenatal care attendance” (e.g. is this number of visits total, number of women reaching 3 or 4 visits, number of women beginning visits in the first or second trimester, was this data collected by questionnaire or from the clinics?)
- All variables presented in Table 2 should have more information (in table and in methods) e.g. maternal age (years), weight (kg), education level (years), residence (type?), hemoglobin (g/dL) use of bed net (yes/no or months during pregnancy?), maternal leptin (ng/mL) etc.)
- Missing data should be reported (e.g. are they missing data on birth weight (175-166 = 9 data points on birth weight are missing?)) If these missing cases are home deliveries and/or missing because of very early delivery that could confound the results. Also in Table 4 where $n = 47$ “infected” but the authors report $n = 48$ cases of PM+ in the results, the missing data is reported in the figure legend but should also be included in the tables/methods.
- Are the authors confident that -20 is sufficient? Depending on how long the samples were stored for that might not be sufficient (compared with -80) to preserve the samples.
- Provide the name/institute of where the pathology took place.
- -Were the samples analyzed (by ELISA) in duplicate or triplicate?
- If the numbers of 2 = active infection, 3 = chronic infection are low ($n = 5$), is it appropriate to analyze that group separately? The authors should provide a rationale for collapsing active, chronic and past infections together in the analyses.
- If the study was powered with a primary outcome of placental malaria do they have sufficient power for the analysis around LBW if only $n = 18$ cases of LBW are reported?
- Authors should provide a rationale for the co-variables selected (a priori based on associations with outcomes or based on analysis-association with outcome of interest)

Results

- Age and parity are likely highly correlated. That should be mentioned in the results (and potentially effects the analysis, for example including co-variables that are highly correlated in a multivariate model can influence the results)
- The authors present the breakdown of their cohort by blood group, they may want to

- provide a rationale as to why they present those results or some interpretation of the results in the discussion (did they examine blood group in relation to infection?)
- Since the results are presented with LBW as an primary outcome (in association with malaria) the authors may want to acknowledge (or examine if they have the relevant data) the relative contribution of preterm birth and small-for-gestational age outcomes to LBW (which will be made up of both PTB and SGA babies)
 - The ELISA results should include the inter-assay co-efficient of variability (CV) and intra-assay.
 - The authors should state “maternal” or “cord blood” (or both) when presenting their results (e.g. in paragraph 1 under the title “Serum levels of leptin and IGF-1” as this is how the results are presented in the tables (they may also want to emphasize in the results that the maternal blood samples were collected at delivery)
 - Under the paragraph entitled “Placental malaria associated with low birth weight”, I was unclear why the reference to Elsheikh et al. was included?
 - All tables, include what statistical tests were performed (e.g. P(t-test) is the result of what analysis?)
 - Table 1, I would avoid the use of the term “lighter” neonates and use the term “delivered neonates with a lower mean weight in comparison with uninfected women”
 - In Table 2 I would avoid presenting results within the table footnotes.

Discussion

- Perhaps provide a discussion around the levels in serum vs. in plasma.
- I would avoid the statement “failed to reach statistical significance” (also stated in results sections) and say instead (e.g.) “The study showed that leptin...., however, the difference was not statistically significant”.
- I’m not sure the authors can say that their results “concur with a study conducted in Malawi” if they’re not reporting statistical significance. Perhaps they could state instead that the study in Malawi also observed lower serum levels of leptin in women with placental malaria infection, but reported a statistically significant difference.
- I would avoid the term “normal weight ones” (last paragraph of the discussion) and say instead “those who delivered at normal birth weight (>2500 g)”.
- The authors may want to comment on the incidence of placental malaria (~27%) and LBW in this cohort (~11 %)...do they think this is low/high? In keeping with previous studies in the same region?
- Depending on how gestational age was measured (e.g. LMP?) the authors may want to acknowledge that the association between birth weight and gestational age (which is to be expected) will be influenced by the method for gestational dating.

Minor comments

- I would recommend reducing the size of the figures and collapsing them into 1 figure with 4 panels (a-d). I would also rename the y-axis titles to read “Leptin in Maternal Blood (ng/mL).” It’s likely also worth noting that “placental malaria” in this case is all cases of malaria (identified by microscopy and histology).
- It might be worth exploring the levels of Leptin and IGF-1 in maternal blood vs. cord blood. E.g. does placental malaria infection lower the ratio of Leptin or IGF-1 in maternal blood vs. cord blood, do women who deliver LBW babies have a lower ratio of IGF-1 in maternal vs. cord blood?

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

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

Reviewer Report 15 June 2017

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Edward Kabyemela

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Despite heightened global efforts to control and possibly eliminated malaria in the foreseeable future, the disease remain a public health problem in many parts of the tropical countries. Since malaria in pregnancy predisposes both mother and infant to adverse outcomes, the research on the pathogenesis of these outcomes is of importance as it can lead us to new interventions.

The merit of the paper is that it has explored both leptin and IGF-I (in the same setting) and their effect on placental malaria (PM) related low birth weight (LBW).

This article reports the following main findings.

1. Primigravidity and young age increases the risk that pregnant women will be infected with malaria

2. Placental malaria and pre-term delivery are associated with low birth-weight (LBW)
3. There is no significant difference in the cord blood levels of leptin or IGF-1 in offspring of infected and non-infected mothers
4. There is no significant difference in the maternal levels of leptin or IGF-1 in offspring of infected and non-infected mothers
5. Cord blood levels of leptin or IGF-1 are not associated with birth weight
6. Maternal blood levels of leptin or IGF-1 are not associated with birth weight

Results 1 and 2 above are largely confirming previous literature from areas where malaria transmission is endemic.

The study is based on a substantial proportion of women (90% of the sample) with past malaria infection (by histology). The results differ from many other studies which were based on substantial proportion of women with active placental malaria infection and this has clearly been mentioned by the authors.

Comments on specific parts of the article:

Introduction

The Introduction contains numerous sentences which I think need slight re-wording to make them convey the intended message.

Examples are:

'there are approximately 125 million pregnant women living in malaria endemic regions' **revise to**
'approximately 125 million women in malaria endemic regions become pregnant each year'
'the pathogenesis of placental malaria and LBW is not fully understood' **revise to** 'the pathogenesis of placental malaria related LBW is not fully understood'

Methods

This section is brief. Some parts of important information are needed to allow readers relate the findings and the methodology. Specifically it will be important to reveal the following:

1. What was the intensity of malaria during the study period? Was it low or high transmission season. If low season this may explain the very low prevalence of active infections reported in the study.
2. What was the basis of 23% of women expected to have placental malaria in the study population?
3. It will be good for authors to expand on how the blood samples from mother and the newborn were collected.
4. Samples were collected in 2014. Were the laboratory analysis of these samples run in 2014 or 2017. Please clarify this since the storage temperature is -20°C and not for example -70°C.
5. How exactly was the gestational age determined?

Statistical analysis

The authors used different statistical approaches to tests for associations between different variables in this study. This is a very commendable approach. However I have a few observations to make:

1. It is not clear how the independent variables for the risk of PM were identified. Specifically, why mother's weight, mother's hemoglobin, use of folic acid supplements, mothers serum

leptin and IGF -I were included as independent variables for placental malaria. It will be useful for readers to know how these variables increase/decrease risk of PM. Any literature relating these to risk of PM is needed. If not available then the analysis may need to be revisited. Actually if they have data they may consider Iron supplementation as an independent variable in their analysis (studies relating Fe supplementation and risk of PM are available)

Were these mothers of SP IPTp? Probably this justifies the inclusion of folic acid supplementation as an independent variable.

2. Similarly is not clear how the independent variables for maternal hemoglobin levels were identified.

Why mother's leptin and IGF-I levels were included as independent variables for maternal hemoglobin levels. It will be useful for readers to know how these variables increase/decrease the risk of anemia. Any literature relating these to risk of anemia needed. If not available then the analysis may need to be revisited.

Results

1. Readers may want to know what were the results of malaria microscopy studies.

2. There are discrepancies in the numbers in the text and tables.

Maternal age: Uninfected: Table (27.9) Text (28); Infected: Table (25.7) Text (26)

Maternal Hemoglobin (ALL women): Table (10.3) text (10.2)

Consistency will be welcome.

3. Some of the reported results are not shown.

It is stated that "Moreover, babies born to women with PM tended to be in the LBW (<2500 g) category more often than those born to non-infected women, but the p-value failed to reach significance level ($p = 0.054$)".

What was the proportion of LBW infants in PM+ versus proportion of LBW infants in the PM- mothers?

4. It is stated that PM was associated with LBW: "LBW (< 2500 g) was significantly associated with placental malaria (N= 172, $p = 0.009$)"

What is this N = 172 ?

5. In table 2 univariate and multivariate analyses are shown. In the univariate analysis none of the variables investigated was associated with placental malaria. In the multivariate analysis Maternal age and parity are associated with PM. Can the authors explain this paradox?

6. Table 3 shows that neither leptin nor IGF-I levels differ between LBW babies of PM + and PM - mothers.

The sample size is now 166 instead of 175. Can the authors indicate why 9 subjects are missing ?

7. It is known that cord blood leptin levels correlate with fetal size. It was previously shown that cord leptin levels are lower in LBW babies born to PM- but not in offspring of PM+ mothers probably indicating that PM disrupts the normal relationship between leptin and fetal growth.

The analysis in Table 3 would benefit from separating LBW infants into PM+ and PM- groups and see what will be the relationship with levels of leptin but also IGF-I.

Discussion

A detailed discussion on why many findings in this area (PM) from Sudan differ from findings elsewhere would be most welcome.

In conclusion:

This study explores an important area of public health in Africa. The study is largely based on past malaria infections in pregnant women. The main results are largely confirming previous findings. The study may be missing important aspects which can become clear if the statistical analysis is revisited.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Immunology, Chemical Pathology

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

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