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Effect of Antimalarial Intermittent Preventive Treatment with Sulfadoxine-pyrimethamine on Plasma levels of ions (Na+, K+ and Cl-) in Pregnant Women of Libreville city

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ABSTRACT

Introduction: *Malaria remains a serious global health problem. Despite implementation of intermittent preventive treatment in pregnancy with sulfadoxine–pyrimethamine (IPTp-SP), pregnant women in endemic areas remain highly susceptible to Plasmodium falciparum.*

This study aims to evaluate the effect of IPTp-SP on plasma electrolyte concentrations (Na^+, K^+, Cl^-) in pregnant women from Libreville using plasma samples from infected pregnant women (IPW) and non-infected pregnant women (NIPW).

Method: *A retrospective cross-sectional survey was conducted between March and September 2011 at ANC and delivery units of Libreville. All IPTp-SP users have received at most 2 doses of IPTp-SP and electrolytes levels were performed during the second and third trimesters of gestation.*

Results: *Among 313 pregnant women studied (88.5% NIPW, 11.5% IPW; 66.4% primigravidae; 52.7% IPTp-SP users), the NIPW control group exhibited predominantly hypernatremia (148.8[145-152] mmol/L), normokalemia (3.9[3.69-4.66] mmol/L), and normochloremia (106[103-109] mmol/L). Malaria infection was significantly associated with hyponatremia (IPW, 132.7[128-137] mmol/L). Submicroscopic infections were associated with hyponatremia and hypokalaemia. Among IPTp-SP users, the malaria-associated decline in natremia (IPW 143.7[132-154] mmol/L) was less pronounced than in infected women who did not receive IPTp-SP (IPW 134[129-138] mmol/L).*

Conclusion: *Plasmodial infection was associated with reductions in both Na^+ and K^+ levels, placing pregnancy at increased risk of hyponatremia and hypokalemia, particularly in submicroscopic infections. IPTp-SP use attenuated malaria-related plasma electrolyte disturbances in both infected and non-infected pregnant women, suggesting a protective effect of IPTp-SP against hyponatremia and hypokalaemia in pregnant women in endemic settings.*

Keywords
Malaria, Pregnancy, Electrolytes, Hyponatremia, IPTp-SP.

Introduction
With an estimated 282 million cases and 610,000 deaths worldwide in 2024, malaria remains the leading endemic parasitic disease [1]. Most malaria cases and nearly 93% of global malaria deaths occur in the Sub-Saharan Africa (SSA) region [1,2].

Malaria is a serious illness that exposes more than 32 million

pregnant women in SSA each year [3]. Plasmodium falciparum is the predominant species and is associated with maternal anemia, increased risk of low birth weight, and elevated maternal and neonatal mortality [4-6]. Clinically, pregnant women more often experience severe manifestations of malaria including hypovolemia and renal complications compared with nonpregnant adults [3,7-9].

Several studies have reported hyponatremia, hypokalemia, and hypovolemia as complications observed in many adult and pediatric patients with severe malaria [10,11]. However, data on changes in these physiological parameters in pregnant women living in endemic SSA regions are scarce. Pregnant women are not only at higher risk of severe disease but are also exposed to antimalarial drugs that may induce plasma electrolyte disturbances and thereby disrupt cardiac conduction [12,13]. and renal function [14,15].

To protect women in areas of moderate to high malaria transmission, the World Health Organization (WHO) recommends intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine–pyrimethamine (SP) as a preventive strategy. Because of widespread chloroquine resistance in Plasmodium falciparum, artemisinin-based combination therapies (ACTs) are the current first-line treatment for malaria in most endemic countries [4,16].

The impact of antimalarial drugs on physiological parameters during pregnancy remains incompletely understood. This study is the first to investigate the effect of IPTp-SP on plasma electrolyte concentrations during pregnancy.

Gabonese pregnant women, who reside in an endemic area, remain more susceptible to malaria than nonpregnant women [17], despite IPTp-SP implementation beginning in 2005 [18]. Therefore, we investigated the effect of IPTp-SP on plasma concentrations of Na^+, K^+, and Cl^- in pregnant women from Libreville.

Materials and Methods

Methods

Study sites

This ancillary study used samples from pregnant women delivering or attending routine antenatal care (ANC) at the primary sentinel sites for malaria surveillance in Libreville, Gabon. Approximately 45% of the Gabonese population resides in Libreville, where the prevalence of P. falciparum infection among nonpregnant febrile individuals is currently around 23-30% [19]. Malaria is a leading cause of fever and anemia (52.6%) in obstetric wards [20]. Coverage with two to three doses of IPTp-SP is estimated at 59% [21].

Study design and population

Blood samples were obtained during a cross-sectional, observational survey conducted between March and September 2011 at ANC and delivery units. Available, properly labeled samples stored at -80°C in the Parasitology-Mycology Department of Université des Sciences de la Santé were selected according to the following criteria: age, area of residence, gestational age, singleton pregnancy, uncomplicated delivery, HIV-negative status, gravidity, IPTp-SP uptake and number of SP doses, malaria diagnosis result, and hemoglobin level. Details of the questionnaire are described elsewhere [21].

Malaria diagnosis

Malaria diagnosis was performed by microscopy according to the Lambéréné method [22]. Parasitemia was expressed as parasites per microliter (p/µL), and species identification was performed on matched thin blood smears. Slide reading quality control followed standard procedures [18,21].

Molecular Detection of P. falciparum Infections: DNA was extracted from the stored blood samples of all women with a negative microscopy result using the QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. Nested PCR amplification of the merozoite surface protein 1 (msp1) and merozoite surface protein 2 (msp2) genes was used for molecular detection of P. falciparum [23].

Electrolytes quantification

Plasma concentrations of sodium (Na^+), potassium (K^+), and chloride (Cl^-) were measured using a SPOTLYTE ionometer (Spotlyte Analysers, Menarini Diagnostics). For each assay, 20 µL of plasma was diluted into 180 µL of diluent (Spotlyte Ref B1172, Menarini Diagnostics) in a 1 mL tube, mixed for 1 minute to homogenize, and then analyzed according to the manufacturer's instructions.

Reference ranges and definitions
Hypernatremia: serum $Na^+ > 145$ mmol/L.
Hyponatremia: serum $Na^+ < 135$ mmol/L.
Hyperkalemia: serum $K^+ > 5.5$ mmol/L.
Hypokalemia: serum $K^+ < 3.0$ mmol/L.

Ethical considerations

The primary and ancillary studies were approved by the Gabonese Ministry of Health, which authorized data publication. Participants in the main study were informed about the ancillary analyses and provided signed informed consent prior to blood collection.

Data analysis

Data are presented as medians (range) unless otherwise stated; population characteristics are presented as percentages. Differences in plasma electrolyte levels between groups were assessed by one-way ANOVA with Tukey's multiple comparisons test; baseline characteristics were compared using t tests. Statistical significance was defined as <0.05. All analyses were performed with GraphPad Prism v7.04 (GraphPad Software, San Diego, CA).

Results

Characteristics of the study population

Of 1,035 women enrolled in the main survey, peripheral blood samples from 313 pregnant women were analyzed. Median age was 23 years (range 14-40). Most participants were microscopy-negative

for Plasmodium (88.5%, 277/313); 36(11.5%) had microscopic infections with a median parasite density of 39(7-2100) p/µL (Table 1). Among the 277 microscopy-negative women, 29(9.3%) were PCR-positive, indicating submicroscopic P. falciparum infections; P. falciparum was the sole species identified. Primigravidae comprised 66.4% (208/313) of the cohort.

Overall, 37.4% (117/313) received two IPTp-SP doses, 15.3% (48/313) received one dose, and 47.3%(148/313) received no IPTp-SP. In the infected group (n=36), infection frequencies were higher among women who did not receive IPTp-SP (7.7%, 24/313) compared with those who received one (1.3%, 4/313) or two (2.6%, 8/313) doses. Seventy-five percent of women were seen in the third trimester, consistent with multiple ANC visits and expected IPTp-SP administration.

Baseline plasma levels of Na^+, K^+ and Cl^- in the study population

Overall, the cohort was divided into non-infected pregnant women (NIPW) and infected pregnant women (IPW). NIPW displayed an elevated median plasma sodium concentration of 148.8(145-152) mmol/L, above the stated normal range (138-143 mmol/L) (Figure 1A). In contrast, IPW had a significantly lower median sodium concentration of 132. (128-137) mmol/L ($p<0.01$), consistent with malaria-associated hyponatremia. Median plasma potassium concentrations were within the reference interval (3.5-5.0 mmol/L) for both NIPW (3.97[3.69-4.66] mmol/L) and IPW (3.53[3.23-3.83] mmol/L); the reduction in IPW versus NIPW was not statistically significant (Figure 1B). Chloride concentrations were similar between groups and within the normal range (98-108 mmol/L): NIPW 106(103-109) mmol/L and IPW 107.1(101-112) mmol/L ($p=0.7817$). Electrolyte concentrations did not vary by gestational age.

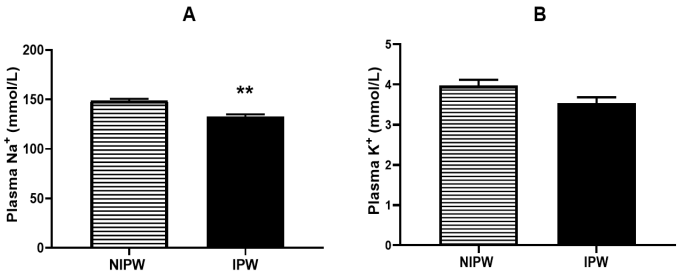

Figure 1: Baseline plasma levels of sodium (A) and potassium (B) measured in NIPW and IPW groups. NIPW (non-infected pregnant women) and IPW (infected pregnant women). Women were hypernatremic and normokalaemic. Plasmodia infection was associated with hyponatremia state in pregnant women.

Plasma electrolyte concentrations by P. falciparum infection category

Median plasma Na^+ was lowest in submicroscopic infections (IPW-Sub: 132[126-137] mmol/L) compared with microscopic infections (IPW-Mic: 142[132-151] mmol/L) and uninfected women (NIPW: 148.7[145-152] mmol/L) ($p<0.01$) (Figure 2A). Na^+ did not differ significantly between IPW-Mic and NIPW.

Median plasma K^+ was significantly lower in submicroscopic infections (IPW-Sub: 3.2[3.01-3.51] mmol/L) than in NIPW (3.97[3.69-4.66] mmol/L) ($p<0.05$); IPW-Mic patients were normokalemic (3.67[3.26-4.26] mmol/L) (Figure 2B). Thus, submicroscopic P. falciparum infection was associated with both hyponatremia and hypokalemia.

In contrast, plasma Cl^- concentrations did not differ by infection status: NIPW 106(103-109) mmol/L, IPW-Sub 107.8(100-114)

Table 1: Characteristics of the study population.

	NIPW (n=277)		IPW (n=36)	
Gravidity	**n**	**%**	**n**	**%**
Primigravidae	187	59.7%	21	6.7%
Multigravidae	90	28.8%	15	4.8%
IPTp- SP	**n**	**%**	**n**	**%**
No IPTp- SP	124	39.6%	24	7.7%
1 SP dose	44	14%	4	1.3%
2 SP dose	109	34.8%	8	2.6%
Gestational age	**n**	**%**	**n**	**%**
1st Trimester	12	3.8%	0	0
2nd Trimester	56	17.9%	10	3.2%
3rd Trimester	209	66.8%	26	8.3%
Microscopic infection			36	11.5%
Median parasitaemia (p/µL)			39 [7- 2100]	
Median age (year)	23 [15- 40]		22 [14- 32]	

mmol/L, IPW-Mic 106.1(97-115) mmol/L (p=0.9232).

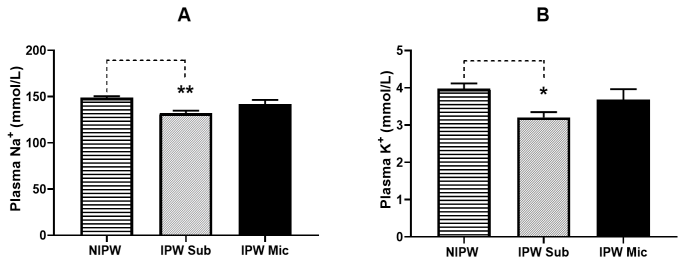

Figure 2: Effects of P. falciparum infections on natremia (A) and kalaemia (B) in pregnant women with microscopic (IPW Mic) or submicroscopic (IPW Sub) parasitic infections. Submicroscopic (IPW Sub) infection was associated with hyponatremia and hypokalaemia occurrence in pregnant women.

Plasma levels of electrolytes according to IPTp-SP uptake

In this part of study, plasma levels of ions from infected and non-infected IPTp-SP users were compared to those coming from non-users infected pregnant women. In the users group (NIPW 149.5[145-153] *vs* IPW 143.7[132-154] mmol/L, p=0.2516), the decrease of natremia associated to plasmodial infection was beneficially more attenuated than that observed in the non-users group (NIPW 149.5[145-153] *vs* IPW 134[129-138] mmol/L, p<0.01), Figure 3A. In the same way, any change of kalaemia was noted in IPTp-SP users, even in the presence of plasmodia infection (NIPW 3.80[3.45-4.15] *vs* IPW 3.84[3.22-4.46], p=0.9267). Whereas, a non-significant decrease of kalaemia was displayed in infected IPTp-SP non-users (IPW 3.36[3.04-3.68] mmol/L, p=0.1622), when compared to NIPW group, (Figure 3B).

In contrast, chloride levels did not differ by IPTp-SP use: NIPW users 108.4(105-111) mmol/L, IPW users 107.2(102-111) mmol/L, and IPW nonusers 109(106-112) mmol/L (p=0.7308).

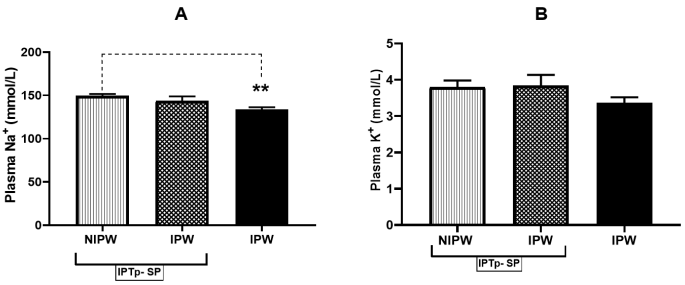

Figure 3: Plasma levels of electrolytes accoding to IPTp-SP uptake: natremia (A) and kalaemia (B). IPTp-SP therapy attenuates beneficially the decline of hyponatremia and kalaemia in pregnant women.

Discussion

Findings from the present study indicate that pregnant women in Libreville were, overall, hypernatremic. This observed hypernatremia may be partly attributable to pregnancy-related alterations in renal physiology, systemic hemodynamics, and the regulation of water and electrolytes. Indeed, one of the most pronounced changes of pregnancy is retention of salt and water. Unexpectedly, pregnant women in this cohort exhibited hypernatremia, whereas clinical reference ranges for pregnancy typically describe relative hyponatremia or, at most, normal natremia [24,25]. Pregnancy is a physiological state characterized by volume expansion, sodium retention, vasodilation, and augmented glomerular filtration, changes that occur under the coordinated influence of multiple hormones [26,27]. Several mechanisms could account for the observed hypernatremia, including upregulation of the renin-angiotensin-aldosterone system [27], gestationally regulated expression of Na^+/K^+-ATPase [28], and fluctuations in hormones such as antidiuretic hormone, which plays a critical role in body fluid homeostasis via renal handling [29].

Second, kalaemia in these patients remained within normal limits, despite the multifactorial influences that govern potassium balance namely, intracellular translocation of potassium and urinary potassium losses. The combination of pregnancy-associated perturbations and local dietary practices may explain the coexistence of hypernatremia and normokalaemia; Libreville, as a coastal city, is characterized by a diet often high in salt and spices.

However, infection with P. falciparum was associated with a significant reduction in natremia, shifting from hypernatremia to hyponatremia. This decline in natremia aligns with previous evidence identifying hyponatremia as a common consequence of plasmodial infection and is frequently associated with hypovolemia and hypokalemia [10,11,30].

Additionally, our data show that 11.5% of pregnant women had microscopically detectable parasitemia, while 9.3% harbored submicroscopic infections detectable only by polymerase chain reaction (PCR). Paradoxically, pronounced hyponatremia and hypokalemia were observed in cases of submicroscopic infection (IPW Sub) but not in women with microscopic parasitemia (IPW Mic). These unexpected results suggest that submicroscopic infections may produce greater perturbations of plasma electrolytes than microscopic infections. The basis for this discrepancy is not immediately clear; possible explanations include chronic low-level parasitemia below the threshold of microscopy, the poorly elucidated contribution of submicroscopic malaria to the infectious reservoir and transmission, or sequestration of infected erythrocytes in the placenta, as previously reported [31,32].

Several studies have suggested that submicroscopic malaria is associated with higher risks of anaemia during pregnancy, placental infection, and low birth weight [31,33,34]. Viewed as a potential reservoir for gametocyte transmission [35], pregnant women are particularly susceptible to malaria; in the absence of immunity, severe malaria can necessitate emergency treatment and may result in pregnancy loss [36].

Furthermore, this study is the first, to our knowledge, to evaluate the effect of intermittent preventive treatment with sulfadoxine–pyrimethamine (IPTp-SP) on plasma sodium, potassium, and chloride concentrations in pregnant women exposed to malaria.

Notably, IPTp-SP use did not alter plasma chloride levels when comparing users to nonusers among infected pregnant women. In contrast, IPTp-SP mitigated the decline in sodium and potassium associated with malaria infection; these electrolyte disturbances, particularly hyponatremia, were markedly present in infected pregnant women who did not receive IPTp-SP. This observation suggests that IPTp-SP confers a protective effect against malaria-associated hyponatremia and hypokalemia in pregnant women. Recent work has reinforced strong associations between malaria in pregnancy and adverse outcomes such as small-for-gestational-age infants and preterm birth during the third trimester despite treatment [37], and has identified primigravidity as a major risk factor for both malaria and anaemia [38,39]. Primigravidae, especially younger women, are the most vulnerable to anaemia early in pregnancy and are at greatest risk for malaria and its adverse consequences; thus they are a primary target for malaria prevention [39].

Although the principal objective of IPTp-SP administration is to prevent poor maternal and birth outcomes, our study did not assess dose–response effects across three SP doses. Recent investigations have demonstrated that adding a third SP dose significantly reduces peripheral parasitemia and the incidence of low birth weight across all gravidities [39,40].

While the mechanisms by which IPTp-SP protects against malaria-associated hyponatremia and hypokalaemia remain to be elucidated, it is important that IPTp-SP not only clears peripheral parasitemia but also attenuates electrolyte disturbances and thereby mitigates the systemic effects correlated with P. falciparum infection.

Conclusion

Although pregnant women in Libreville were generally hypernatremic and normokalemic, P. falciparum infection produced a significant decline in both serum sodium and potassium, increasing the risk of hyponatremia and hypokalaemia, particularly in submicroscopic infections. IPTp-SP administration, in both infected and uninfected pregnant women, limited the electrolyte disturbances associated with P. falciparum infection, suggesting a protective effect of IPTp-SP against hyponatremia and hypokalaemia in pregnant women exposed to malaria in endemic regions.

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