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RESEARCH ARTICLE

Hematological Profile of Pregnant Women Co-Infected with Malaria and HIV in Buea, South West Region of Cameroon

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Abstract

Background: Malaria and HIV coinfection (MHC) pose significant problems to pregnant women and their unborn babies in sub-Saharan Africa (SSA). Studies reporting the haematological profile of pregnant women with MHC are not readily available in SSA. This study was designed to determine the hematological profile of pregnant women co-infected with malaria and HIV in Buea, South West region of Cameroon.

Methods: This was a comparative cross-sectional study. Pregnant women were enrolled from the ANC unit of the Regional Hospital of Buea and categorized into four groups of 15 participants each, namely: malaria and HIV coinfected (MHC+) group, Malaria monoinfected (M+) group, HIV monoinfected (H+) group and negative controls. Malaria parasitaemia was detected and quantified using Giemsa microscopy. Screening for HIV was done using immunochromatographic rapid strip test. And complete blood count (CBC) was performed using an automated haematological analyzer. The student T-test, ANOVA and multivariate regression analysis were performed as part of the statistical analysis.

Results: Overall, multivariate analysis revealed that there were significant differences in the red blood cell (RBC) count ($p < 0.0001$), white blood cell (WBC) count ($p = 0.023$), haemoglobin concentration (Hb) ($p < 0.0001$) and the mean cell haemoglobin concentration (MCHC) ($p = 0.008$) between the study groups adjusting for gestation age. Compared to negative controls, the MHC+ group had statistically significant low values of RBC count [3.0×10^6/µL vs. 4.29×10^6/µL ($p = 0.012$)], Hb [7.6 g/dl vs. 11.17 g/dl ($p < 0.0001$)], WBC count [3.33×10^3/µL vs. 4.83×10^3/µL ($p = 0.011$)] and higher MCHC [35.83 g/dl vs. 28.83 g/dl ($p = 0.031$)]. Furthermore, the H+ group had significant low values of RBC [2.33×10^6/µL vs. 4.29×10^6/µL ($p < 0.0001$)] and Hb [6.17 g/dl vs. 11.17 g/dl ($p < 0.0001$)] compared to negative controls.

Conclusion: This study revealed that MHC has a great impact on the haematological parameters of pregnant women in the study area demonstrated by significant decreases in the values of the RBC, WBC, Hb, and increases in the MCHC.

Keywords

HIV, Malaria, Haematological profile, Coinfection, Comparative study, Pregnant women

Introduction

Malaria and HIV, two of the most deadly diseases of our time, are disproportionately more common in sub-Saharan Africa (SSA). Malaria, a protozoan disease caused by parasites of the genus *Plasmodium*, accounted for approximately 216 million cases and 445,000 deaths worldwide in 2016 [1]. About 90% of cases and death attributed to malaria occurs in SSA [1]. On-the-other-hand, there were 36.9 million people

living with HIV and 940,000 people died from AIDS-related illnesses in 2017 [2]. Like malaria, a majority (70%) of cases and deaths attributed to HIV infection occurs in SSA [3].

Due to the overlapping distribution of malaria and HIV in SSA, coinfection with malaria and HIV (MHC) is therefore common in the region. Prevalence of MHC in SSA ranges between 0.7% - 72% [4]. Pregnant women and children are at greatest risk of malaria morbidity and death [5,6], meanwhile, women and adolescent girls are at greatest risk of HIV infection [7]. Pregnant women and their unborn babies are at particular risk of co-infection with malaria and HIV. In SSA, approximately 1 million pregnancies are complicated by MHC every year [8], putting the life of the pregnant woman and her infant at risk. Complications associated with MHC in pregnancy include low birth weight, preterm delivery, higher rates of neonatal mortality, placental malaria, severe anaemia, slow gestational development, reduced transfer of maternal antibodies from mother to child, and in some cases increased the risk of mother-to-child transmission of HIV [4,9,10]. HIV associated risk of maternal malaria affects women of all gravidities, thus attenuating or even eliminating the decrease in malaria parasitaemia normally seen in HIV negative multigravida [11].

Like most countries in SSA, MHC is common in Cameroon. Epidemiological studies report prevalence ranging between 1.2% and 29.4% [12-16]. One study conducted on pregnant women in Cameroon reported the prevalence of 17.3% for MHC [14]. Studies on the haematological profile of pregnant women with MHC are not readily available in SSA. This study was therefore designed to determine the haematological profile of pregnant women coinfected with malaria and HIV in Buea, in order to generate baseline data that may be of clinico-epidemiological relevance.

Materials and Methods

Study area

Participants were enrolled at the Buea Regional hospital in Buea, South West Region of Cameroon. The study area has been described in great detail elsewhere [17].

Study design

This study was a comparative cross-sectional study performed between May and June 2018. The participants were categorized into four groups: malaria and HIV coinfected (MHC+) pregnant women (MHC+), malaria monoinfected (M+) pregnant women, HIV monoinfected (H+) pregnant women and negative controls.

Study population

Pregnant women were enrolled from the ANC unit of the Buea Regional Hospital. The participants were not to be on any malaria treatment one week prior to the study. Pregnant women who were currently on iron supplementation and HIV positive women who were on antiretroviral therapy (ARV) were also excluded. Participants were required to provide a signed informed consent form which was duly explained to them.

Sample size estimation

Using an effect size of 0.45 deduced from Njunda, et al. [18], power of 80%, $\alpha = 0.05$, and the ANOVA function in G-power with 4 groups, a sample size of 15 participants per group was obtained, giving a total of 60 (15 × 4 groups) participants.

Ethical consideration

Ethical approval was obtained from the Faculty of Health Sciences Institutional Review Board (IRB) of the University of Buea. Administrative authorization was obtained from the Delegation of Public Health, Buea and the Director of the Regional Hospital of Buea.

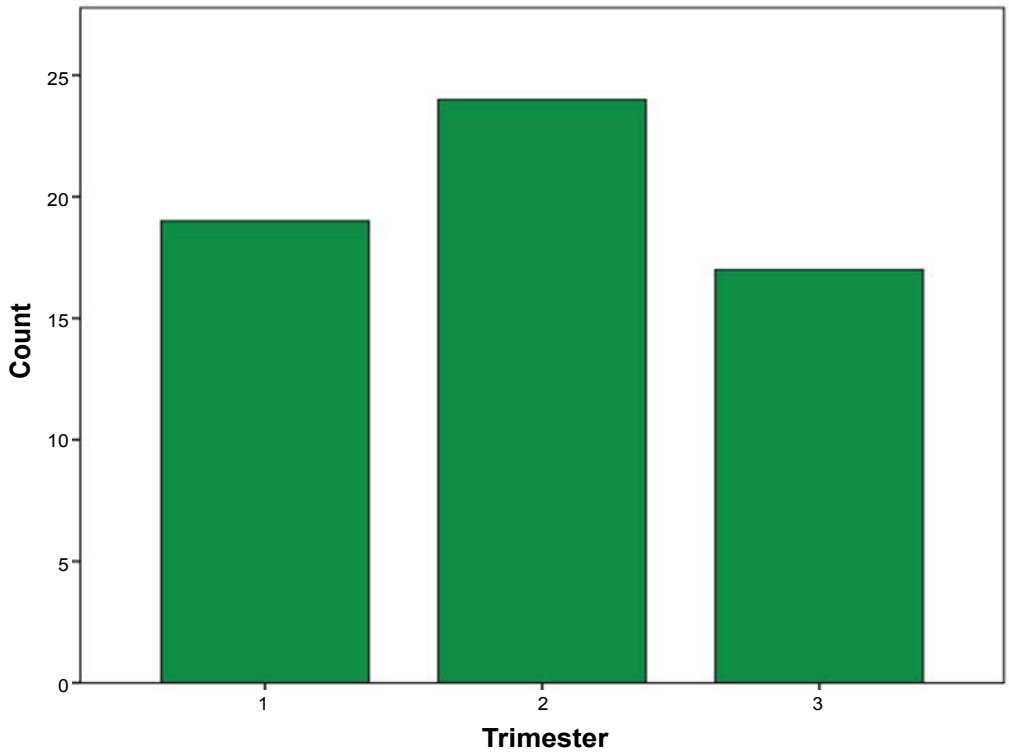

Figure 1: Repartition of the participants according to the trimester of pregnancy.

Data collection

A semi-structured questionnaire was used to collect demographic characteristics (age, gestational age, etc). The questionnaire was administered to the pregnant women by members of the research team.

Sample collection and laboratory analysis

About 3 ml of blood was collected from pregnant women into EDTA anticoagulated tubes following antiseptic techniques. The blood was used to perform the complete blood count, preparation of blood films as well as screening for HIV.

Determination malaria parasite: The prepared blood films were air-dried and stained with 10% Giemsa (1 in 20 dilutions) for 25-30 minutes [19]. The blood films were read by two expert microscopists who were blinded from the results of the other. In the case of any discrepancy with the results obtained by the two microscopists, a third was bought in and the results he gave was considered as final. At least 200 fields were screened for malaria parasite using the 100x (oil immersion) objective and where parasites were seen, they were counted until 500 WBC was reached. The slides were only declared negative after counting to 2500 WBC. Malaria parasite density was estimated by dividing the parasites counted by 500 WBC and then multiplied by the actual WBC count of the participant to give numbers in parasite per µl [20].

Screening for HIV: HIV screening was done in accordance with Cameroon's national algorithm for HIV screening by detecting anti-HIV antibodies [21]. Briefly, a first rapid test was used and if positive, a second rapid test was used to confirm the result. In this study, the first rapid and second rapid tests were Determine™ HIV (Abbott Laboratories, Abbott Park, IL, USA) and First Response HIV- 1-2 kits (Premier Medical Corporation Ltd., Kachigam, India) respectively.

Full (complete) blood count (CBC): CBC was performed using Mindray Auto haemology analyzer (BC-2800, Shenzien Mindray Bio-Medical Electronics CO., Ltd). The white blood cell counts were obtained from the CBC results and used in the estimation of the parasite density.

Statistical analysis

Data obtained was entered into excel spreadsheet and analyzed using SPSS v. 20. The statistical tests performed included ANOVA for comparison of group means and multivariate regression analysis was used for the determination of the association between groups adjusting for gestational age. The cut-off for statistical significance was set at $p \leq 0.05$.

Results

Description of the study population

In all, 60 participants were enrolled, 15 for MHC+, M+, H+ and negative controls each. The ages of the participants ranged between 18 and 38 years, with mean ± SD = 25.95 ± 4.53. The mean (± SD) gestational age was 5.04 ± 2.31. The majority of the participants were in their second trimester (Figure 1). The participants in the different groups did not differ with respect to their age ($p = 0.535$) and gestational age ($p = 0.625$). *Plasmodium falciparum* was the only species identified in malaria cases.

Comparison of the hematological profile in the different study groups

Comparison of the blood cell count: Overall, the mean RBC count was lowest in the H+ group and highest in the negative controls (Table 1). The differences in the RBC count between the different groups was significant ($p < 0.0001$). Multivariate regression analysis also revealed this difference to be significant ($p < 0.0001$) adjusting for gestational age. The group-specific comparison revealed that compared to the negative controls, the RBC count was significantly lower in the H+ group ($p < 0.0001$) and the MHC+ group ($p = 0.012$). Comparisons between the other groups were not significant.

Generally, the mean WBC count was lowest in the MHC+ group and highest in the negative controls (Table 1). The differences in the WBC counts was significant ($p = 0.011$). Multivariate analysis adjusting for gestational age revealed the differences in the WBC count between the four groups to be significant ($p = 0.023$). Group-specific comparisons revealed that compared to the control, the WBC count of MHC+ group was significantly lower ($p = 0.022$), meanwhile no differences were observed with the other groups.

In general, the mean lymphocyte count was highest in the MHC+ group and lowest in the H+ group (Table 1). However, the overall differences were not observed to be significant ($p = 0.055$). Multivariate regression analysis also revealed that the difference in the lymphocyte count between the four groups was not significant ($p = 0.061$) adjusting for gestational age. Similarly, group-specific comparisons yielded no significant differences either.

Overall, the mean neutrophils count was highest in the H+ group and lowest in the MHC+ group (Table 1). However, the differences were not observed to be significant ($p = 0.070$). Multivariate regression analysis adjusting for gestational age revealed no significant differences in the mean neutrophil counts between the four groups ($p = 0.062$). Similarly, group-specific comparisons yielded no significant differences.

Generally, the mean monocyte count was highest in the MHC+ group and lowest in the negative controls. However, the overall differences were not observed to be significant ($p = 0.184$). Multivariate regression analysis adjusting for gestational age did not reveal any significant differences in the monocyte count between the four groups ($p = 0.171$). Similarly, group-specific comparisons yielded no significant differences.

Overall, the mean eosinophil count was highest in the MHC+ group and lowest in the H+ group (Table 1). However, the overall differences were not observed to be significant ($p = 0.055$). Multivariate regression analysis adjusting for gestational age also did not reveal any significant differences in the eosinophil count between the four groups ($p = 0.101$). Similarly, group-specific comparisons yielded no significant differences.

Table 1: Blood cell counts by study groups.

Study group		Red cells (x 10^6/µL	Total white cells (x 10^3/µL)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Basophils (%)	Platelets (x 10^3/µL)
Controls	Mean	4.29	4.83	43.71	51.91	1.54	0.51	188.37
	Min	3	3	30	42	1	0	91
	Max	6	8	50	60	5	2	366
	95% CI	4.1-4.5	4.4-5.3	42.3-45.1	50.4-53.4	1.2-1.9	0.3-0.7	167.2-209.5
HIV monoinfected	Mean	2.33	4.33	49.83	45.5	2.33	0.67	221.0
	Min	1	3	37	34	1	0	103
	Max	5	5	60	56	3	1	521
	95% CI	0.6-4.1	3.3-5.4	41.7-58.0	37.6-53.4	1.3-3.4	0.1-1.2	55.8-386.2
Malaria + HIV coinfected	Mean	3.0	3.33	41.33	54.67	2.17	0.33	120.67
	Min	1	2	32	44	1	0	70
	Max	5	4	50	64	5	1	251
	95% CI	1.2-4.8	2.5-4.2	33.4-49.3	46.6-62.8	0.5-3.9	0.2-0.9	51-190.4
Malaria monoinfected	Mean	3.8	4.0	44.85	52.29	1.85	0.46	176.3
	Min	3	2	30	41	1	0	78
	Max	5	5	55	67	3	1	532
	95% CI	3.3-4.2	3.5-4.6	40.1-49.6	47.4-57.1	1.4-2.3	0.2-0.8	100.7-252
	p-value	< 0.0001	0.011	0.070	0.055	0.184	0.751	0.267

CI: Confidence Interval; HIV: Human Immunodeficiency Virus.

Table 2: Red cell indices by study groups.

Study group		Hb (g/dl)	MCH (pg)	MCHC (g/dl)	MCV (fL)
Controls	Mean	11.17	26.8	28.83	93.51
	Min	8	23	21	70
	Max	14	30	34	114
	95% CI	10.7-11.6	26.2-27.4	27.8-29.9	90.5-96.6
HIV monoinfected	Mean	6.17	28.67	32.83	87.33
	Min	2	27	29	77
	Max	12	30	38	96
	95% CI	1.8-10.5	27.2-30.1	29.2-36.4	79.7-95.0
Malaria + HIV coinfected	Mean	7.67	30.5	35.83	84.5
	Min	4	19	26	73
	Max	11	61	67	91
	95% CI	4.8-10.5	14.5-46.5	19.6-52.1	77.9-91.1
Malaria monoinfected	Mean	10.15	26.3	29.23	90.54
	Min	9	22	22	79
	Max	12	31	33	110
	95% CI	9.5-10.8	24.8-27.9	27.6-30.9	85.0-96.1
	p-value	< 0.0001	0.289	0.022	0.071

CI: Confidence Interval; HIV: Human Immunodeficiency Virus; Hb: Hemoglobin Concentration; MCH: Mean Cell Hemoglobin; MCHC: Mean Cell Hemoglobin Concentration; MCV: Mean Cell Volume.

Generally, the mean basophils count was highest in the H+ group and lowest in the MHC+ group (Table 1). However, the overall differences were not observed to be significant (p = 0.751). Multivariate regression analysis adjusting for gestational age also did not reveal any significant differences in the basophil counts between the different groups (p = 0.688). Similarly, group-specific comparisons yielded no significant differences.

Globally, the mean platelet count was highest in the H+ group and lowest in the MHC+ group (Table 1). However, the overall differences were not observed to be significant (p = 0.267). Multivariate regression analysis adjusting for gestational age revealed that the differences in the platelet count were not significant (p = 0.302). Similarly, group-specific comparisons yielded no significant differences.

Comparison of the red blood cell indices: Overall, the mean haemoglobin concentration (Hb) was highest in the negative controls and lowest in the H+ group (Table 2), and this difference was significant (p < 0.0001). Multivariate regression analysis adjusting for gestational age revealed that the differences in the Hb between

the different groups were significant ($p < 0.0001$). Group-specific comparisons revealed that compared to the negative controls, the mean Hb was lowest in the MHC+ group ($p < 0.0001$) and H+ group ($p < 0.0001$), meanwhile the others were not significant.

Generally, the mean cell hemoglobin (MCH) was highest in the MHC+ group and lowest in the M+ group. However, the difference was not observed to be significant ($p = 0.289$). Multivariate regression analysis adjusting for gestational age did not reveal any significant differences in the MCH between the four groups ($p = 0.170$). Similarly, group-specific comparisons yielded no significant differences.

In general, the mean cell volume (MCV) was highest in the controls and lowest in the MHC+ group (Table 2). However, the difference was not observed to be significant ($p = 0.071$). Multivariate regression analysis adjusting for gestational age revealed that the difference in the MCV between the four groups was not significant ($p = 0.100$). Similarly, group-specific comparisons yielded no significant differences.

Overall, the mean cell hemoglobin concentration (MCHC) was lowest in the MHC+ group and highest in the negative controls (Table 2). This difference was significant ($p = 0.022$). Multivariate regression analysis adjusting for gestational age, also revealed that the difference in the MCHC between the four groups was significant ($p = 0.008$). Group-specific comparisons revealed that when compared to the negative controls, the MCHC was significantly lower only in the MHC+ group ($p = 0.031$), meanwhile, the others were not significant.

Discussion

Separately, malaria and HIV are known to have a profound impact on the haematological parameters of infected patients [19,22,23]. In malaria and HIV coinfection (MHC), the effect is expected to be exponential. But in co-endemic areas, studies reporting the haematological profile of malaria and HIV coinfected patients are not readily available. This, to the best of our knowledge, is one of the first studies to have been conducted in SSA, aimed at determining the haematological profile of pregnant women coinfected with malaria and HIV. Buea (where this study was conducted) is known to be highly endemic for malaria [18,24] and HIV [25]. *Plasmodium falciparum* is the predominant species causing malaria in Buea and Cameroon in general [17,26,27].

In this study, HIV monoinfection was found to significantly decrease the mean haemoglobin concentration (Hb) and the red blood cell (RBC) count of infected patients when compared to negative controls, meanwhile malaria monoinfection did not have any effect on the haematological parameters of infected patients. The finding of decreased Hb and RBC count in HIV monoinfected patients is in line with the study by Abdulgadir, et al. [23] and could be attributed to either decreased production or increased destruction of RBC, which contributes to the development of anaemia in pregnancy. The finding of malaria not having an impact on the haematological parameters of infected patients is contrary to studies that have shown a significant decrease in the Hb of pregnant women infected with malaria [22,28]. The failure to observe any significant impact of malaria on the haematological parameters of infected patients could be attributed to the fact that in Buea, pregnant women are routinely placed on iron supplements (to boost their Hb) and prophylaxis with sulfadoxine-pyrimethamine.

On the contrary, MHC significantly decreased the Hb, RBC count, WBC count and the mean cell haemoglobin concentration (MCHC), which is substantially a higher effect on the haematological parameter compared to separate infections of malaria and HIV. MHC has been shown to exponentially increase the adverse effects of one infection on the other, has a negative impact on the prognosis of both diseases and complicates the treatment of both infections [4]. The decrease in the Hb, RBC, and MCHC in MHC could be attributed to the aforementioned reason. This, therefore, implies that MHC exacerbates the risk of anaemia in the target population

compared to separate infection of malaria and HIV. Anaemia during pregnancy is a major concern because it is associated with low birth weight, premature birth and maternal mortality. Unfortunately, in this study, the type of anaemia resulting from MHC was not determined. Iron deficiency is generally regarded as the most common cause of anaemia in pregnancy [29]. Further research will be needed to identify the type of anaemia in pregnant women co-infected with malaria and HIV. On-the-other-hand, the decreased value of the WBC count (leukopenia) could be attributed to myelosuppression from HIV itself or altered cytokine response. Proinflammatory cytokines are known to play an important role in both the control and pathogenesis of both diseases. Available data show conclusively that malaria and HIV interact to bring about dysregulation of the immune system [4]. Alternatively, the decreased WBC count could be a consequence of the depletion of CD4+ T cell count, neutropenia, or a combination of these [23]. The decreased WBC count in pregnant women with MHC may have deleterious consequences as this could hamper their ability to find infections and increases their chances to acquire opportunistic infections associated with HIV. Further research is also needed to shed more light on the mechanism surrounding the depletion of Hb, RBC and WBC in patients coinfected with malaria and HIV.

This study which has demonstrated the negative impact of MHC on some haematological parameters of pregnant women may have significant implications in the management of pregnant women co-infected with malaria and HIV in the area. The study is however limited in that participants were enrolled only from an urban setting and in just one region in Cameroon. The findings may not be generalizable to pregnant women in rural areas and in other regions of Cameroon. Further research will be required in pregnant women in other areas of Cameroon and sub-Saharan Africa, to fully elucidate the impact of MHC on the haematological parameters.

Conclusion

This study shows that malaria and HIV coinfection (MHC) has a great impact on the haematological parameters of pregnant women in the study area. The impact of MHC was higher compared to separate infections of malaria and HIV. Pregnant women coinfected with malaria and HIV tend to have a significant decrease in the values of RBC count, WBC count and red blood cell indices (Hb and MCHC). Further research is needed in pregnant women in other areas to confirm this, as well as determine the factors associated with the depletion of the RBC and WBC in pregnant women coinfected with malaria and HIV.

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