

Hematological Profile and Risk Factors of Anemia in Pregnant Women: A Cross Sectional Descriptive and Analytical Study in Douala Cameroon

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Abstract

During pregnancy, the hematological system undergoes numerous changes so as to meet up with the demands of the developing fetus and placenta, with major alterations in blood volume and this differs with women from different regions. The aim of this study was therefore to assess the hematological parameters and risk factors for anemia among pregnant women according to different trimesters of pregnancy in Douala, Cameroon. **Methods:** A cross-sectional study was conducted from February to May 2017, and all pregnant women who attended antenatal visits during our study period and who suited our inclusion criteria were recruited. The study was carried out in the antenatal care Unit of the Douala Laquintine Hospital (DLH). A pretested questionnaire was used for the necessary data collection. Venous blood was collected from each of these women to perform a Complete Blood Count (CBC) test using an automated hematological analyzer (URIT 3010). Data were analyzed using XLSTAT 2007 and Stata version 11 software. **Results:** The mean age of the participants was 28 (SD = 5 years). The prevalence of anemia among pregnant women was 22% with a majority (18.4%) of these women being mildly anemic. Mean Hemoglobin values were significantly higher among women in first trimester compared to the third (12.1 ± 0.9 g/dl vs 11.8 ± 1.3 g/dl; $p = 0.043$). There was also a significant change in mean hematocrit (HCT) values between the first and second trimester ($32.8\% \pm 2.5\%$ vs $31.4\% \pm 2.9\%$, $p =$

0.004) and between the first and third trimester ($32.8\% \pm 2.5\%$ vs $30.8\% \pm 3.5\%$, $p < 10^{-4}$). RBC count value was higher in the first trimester than in the second trimester ($3.7 \pm 0.3 \times 10^{12}/L$ vs $3.5 \pm 0.4 \times 10^{12}/L$, $p < 10^{-4}$) and in the third trimester ($3.7 \pm 0.3 \times 10^{12}/L$ vs $3.5 \pm 0.4 \times 10^{12}/L$, $p = 0.001$). After a multivariate analysis, the following categories of women had more odds of developing anemia; women between the age range of 30 - 35 (OR = 2.81, 95%CI: 1.16 - 6.81, $p = 0.023$), women in the second trimester of pregnancy (OR = 2.20, 95%CI: 0.88 - 5.48, $p = 0.024$), women with blood group O (OR = 3.57, 95%CI: 1.41 - 16.66, $p = 0.012$). **Conclusion:** This study confirms significant variations in hematological parameters. The findings reinforce the need for supplementation and provide additional information on hematological reference values in pregnancy in Cameroon. It also helps us understand that, third trimester, age range 30 - 35, and blood group may be potential risk factors associated with anemia in pregnancy though a cohort study would be necessary to ascertain this hypothesis.

Keywords

Hematological Profile, Pregnant Women, Anemia, Risk Factors, Cameroon

1. Introduction

During pregnancy, a lot of changes and or adaptations occur in almost all body systems. The body undergoes remarkable changes in the cardiovascular, respiratory, renal and gastrointestinal physiology and many studies have identified the hematological profile of the pregnant woman as one of the factors affecting pregnancy and its outcome [1] [2] [3].

According to the World Health Organization (WHO), the hematological profile of an individual reflects their general health state to a large extent and variations should therefore not be ignored [4]. Changes can be observed in hematological indices such as: red blood cell (RBC) count, hemoglobin (Hb) concentration, platelet (PLT) count, and white blood cell (WBC) count. Plasma volume increases steadily as early as 4 weeks of pregnancy to a maximum of 30% - 45% above non-pregnant levels at 28 - 32 weeks of gestation. RBC count increases generally with little or no changes in Hb levels in iron supplemented pregnant women [5] [6]. A greater increase in plasma volume compared to an increase in RBC mass leads to physiological anemia [7]. There is a small increase in mean corpuscular volume (MCV) of an average of 4 fl in an iron-replete woman, which reaches a maximum at 30 - 35 weeks of gestation and does not suggest any deficiency of vitamin B12 and folate. Increased production of RBCs to meet the demands of pregnancy, reasonably explains why there is an increased MCV (due to a higher proportion of young RBCs which are larger in size). However, MCV does not change significantly during pregnancy [5]. WBC increases during pregnancy; meanwhile platelet count decreases particularly in the third trimester termed as “gestational thrombocytopenia” partly due to hemodilu-

tion and accelerated platelet destruction [8]. The most significant hematological changes are physiological anemia, neutrophilia, mild thrombocytopenia, increased pro-coagulant factors and diminished fibrinolysis. Anemia is considered as the most frequent hematological abnormality during pregnancy and it is associated with adverse pregnancy outcome, followed by thrombocytopenia which generally normalizes a few weeks postpartum [9] [10].

Some studies have identified the hematological indices of the pregnant woman as one of the factors affecting pregnancy. In India, a study was done on hematological profile of normal pregnant women and results of the study may be used as reference values in the assessment of the health status of normal pregnant women [11]. Another study was carried on the hematological profile of pregnant women in Umuahia in Nigeria which showed serious changes in most of the trimesters in the hematological parameters which could affect the pregnancy outcome adversely if not effectively managed [12].

A study was carried out in Cameroon to determine the prevalence and factors associated to anemia during pregnancy at the Douala General Hospital (DGH) which showed an increased prevalence in anemia during pregnancy [13]. This study was carried out on pregnant women who had carried out a complete blood count at the DGH but the hematological profile of these women was not assessed according to trimester.

Very few studies have been carried out to assess the hematological profile and risk factors for anemia among pregnant women according to trimesters of pregnancy in Cameroon generally and Douala in particular. Moreover, there are no specific reference values for hematological parameters during pregnancy in Cameroon. Our study was therefore designed to assess the hematological profile and the associated risk factors of anemia among pregnant women in the Douala Laquintinie Hospital given that a proper understanding of hematological changes during pregnancy will enable us to set up proper strategies for the control of pregnancy complications thereby encouraging the optimal management of pregnancies. This study also gives room for further research on the establishment of local hematological reference values for pregnant women in Cameroon and will serve as database for future studies on pregnant women at the DLH.

2. Methodology

A cross-sectional study targeting pregnant women who came for antenatal visits in the Douala Laquintinie Hospital (DLH) was conducted to access the hematological profile and the associated risk factors for anemia within this cohort. The study was conducted from the 20th February 2017 to the 01st June 2017. Recruitment of participants was done at the Obstetrics unit of the DLH and complete blood count test was done at the Central laboratory of the DLH. This study was carried out on all consenting pregnant women attending antenatal consultations at the Douala Laquintinie Hospital and who had registered for ANC at the obstetric unit during the period of the study. Excluded from this study were pregnant women with previous hematological abnormalities, such as hemolytic prob-

lems, clotting/bleeding disorders, sickle cell patients and pregnant women with a history of any chronic disease. The questionnaire used was designed by the first 3 authors and was then given to the other coauthors for review. It was tested and modified in a nearby health center. The reliability and validity was ascertained by limiting the recruiters to 2 and they did a cross-examination of the work done.

Douala Laquintinie Hospital is a teaching hospital affiliated to the University of Douala. It is a tertiary reference hospital with the highest bed capacity and patient entry in the country. It is located in Douala the economic capital of Cameroon and it is at the reach of every citizen.

The minimum sampling size for the representation of the population of pregnant women with hematological abnormalities was determined using Lorentz formula as seen below:

$$N = \varepsilon^2 \times p \times (1-p) / l^2$$
, where: N = Minimal size of the sample, ε = constant whose value depends on the statistical risk which is equal to 1.96 for a risk of 5%, p = Prevalence of women with anemia (considered here as hematological abnormality) in Cameroon estimated at 31.8% in the Littoral region [14]. $1 - p$ which is an estimation of variance and l = Precision level fixed at 10%, margin of error acceptable for the estimated proportion.

Therefore, the minimum sample size of pregnant women with abnormalities to be representative for an entire population is $N = 92$ women.

For our study, we included all women who will have given their signed consent form and for minors the consent form of their parents/legal guardians during our presence in DLH.

Patients were identified and all those eligible received a brief explanation on the study. After obtaining consent, we used a pretested questionnaire to obtain data on socio-demographic variables, obstetric history and other relevant possible risk factors.

For the collection of blood samples and for the full blood count test we used EDTA tubes, tourniquet, disposable gloves, sterile disposable needles, alcohol (90%), cotton and needle holders.

Concerning specimen collection and processing, three milliliters of venous blood was collected from each pregnant woman into an EDTA anticoagulant tube under sterile conditions to perform Complete Blood Count (CBC). CBC was done using an automated hematological analyzer (Urit 3010 plus). The cell counter performs a Complete Blood Count (CBC), Platelet Count, and a Three-Part Differential. Whole blood is aspirated, diluted, and then divided into two samples. One sample is used to analyze the red blood cells and platelets while the second sample is used to analyze the white blood cells and hemoglobin. Electrical impedance is used to count the white blood cells, red blood cells, and platelets as they pass through an aperture. As each cell is drawn through the aperture, a change in electrical resistance occurs generating a voltage pulse. The number of pulses during a cycle corresponds to the number of cells counted. The amplitude of each pulse is directly proportional to the cell volume. Lyse reagent is added to

the diluted sample and used to count the white blood cells. After the white blood cells have been counted and sized, the remainder of the lysed dilution is transferred to the Hb Flow Cell to measure Hemoglobin concentration. The automated cell counter uses electronic sizing to determine a three-part automated differential. The parameters assessed include: Hemoglobin (Hb) Concentration, Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cell Count (WBC), Red Blood Cell Count (RBC) and Platelet Count (PLT).

3. Data Analysis

Data was recorded and processed using the Microsoft Excel 2007 software and was analyzed using the XLSTAT 2007 and Stata version 11 software. The quantitative variables were presented as mean with standard deviation and the qualitative variables in frequencies and percentages. For bivariate analysis, the comparison between the qualitative variables was performed using the Chi squared test and the Fisher exact test for probabilities was determined for dichotomous variables. The ANOVA test was used to compare the hematological parameters between the trimesters of pregnancy. Fisher's post hoc test allowed for comparison of the groups two by two. To adjust for potential confounders, a multivariate analysis using a logistic regression test was used to investigate the association between anemia and the different variables in the study. The analysis was adjusted for other risk factors for anemia. The odd ratio and confidence interval at 95% were determined. The results were considered significant for $p < 0.05$.

4. Results

Two hundred and eighty-four pregnant women were recruited, 250 fulfilled the inclusion criteria. 24 of the women did not fulfill the inclusion criteria and 10 refused to participate in our study. Mean age of the participants was 28 (SD = 5 years) and the ages ranged from 15 - 40 years. Majority of the study group were in the age range of 25 - 29 years. Sixty seven (26.8%) women were in the first trimester, 102 (40.8%) in the second trimester and 81 (32.4%) in the third trimester.

More than half of the pregnant women had two or more children and 20.4% of the women experienced abortions in their previous pregnancies. Very few of the women experienced still births in their previous pregnancies and almost all of the women had singleton pregnancies.

Majority of the study participants were in the second trimester of pregnancy, and almost all of them were on iron supplements. One-tenth of the women suffered from malaria in the present pregnancy and only 10.2% of women in the third trimester received the complete dose (4 doses) of Intermittent Preventive Treatment (IPT) for malaria.

Table 1 shows the hematological profile of pregnant women according to trimesters. There was a statistically significant difference between mean Hb values in the 1st and 3rd trimester ($p < 0.05$). The mean HCT value of pregnant women

Table 1. Haematological profile of pregnant women according to trimester in the DLH.

Haematological parameters	Category				<i>P</i>		
	Overall	1 st trimester	2 nd trimester	3 rd trimester	1 st vs 2 nd	1 st vs 3 rd	2 nd vs 3 rd
Hb mean \pm SD g/dl	11.9 \pm 1.1	12.1 \pm 0.9	11.8 \pm 1.0	11.8 \pm 1.3	0.054	0.043	0.830
HCT	31.6 \pm 3.1	32.8 \pm 2.5	31.4 \pm 2.9	30.8 \pm 3.5	0.004	<10⁴	0.166
RBC $\times 10^{12}/l$	3.6 \pm 0.4	3.7 \pm 0.3	3.5 \pm 0.4	3.5 \pm 0.4	<10⁴	0.001	0.739
MCV fl	88.9 \pm 7.0	88.3 \pm 6.6	90.1 \pm 7.0	87.7 \pm 7.2	0.096	0.596	0.019
MCH pg	33.4 \pm 2.9	32.9 \pm 2.7	33.7 \pm 3.1	33.4 \pm 2.8	0.060	0.258	0.462
MCHC g/dl	37.5 \pm 1.5	37.2 \pm 1.6	37.4 \pm 1.4	38.0 \pm 1.3	0.060	0.258	0.462
WBC $\times 10^9/l$	5.5 \pm 1.5	5.6 \pm 1.3	5.7 \pm 1.6	5.3 \pm 1.4	0.680	0.265	0.095
Platelet $\times 10^9/l$	193.8 \pm 46.2	199 \pm 40	192 \pm 48	192 \pm 49	0.353	0.381	0.992

in the 1st trimester was higher compared to the values in the 2nd trimester and in the 3rd trimester; with a significant difference between values in the 1st and 2nd trimester ($p < 0.05$) and in the 1st and 3rd trimester ($p < 0.05$). The mean RBC values follow the same trend as those of the mean HCT values. The mean MCV value in pregnant women in the 2nd trimester was significantly higher compared to the value in the 3rd trimester ($p < 0.05$). The mean values of the rest of the parameters (MCH, MCHC, WBC and PLT) did not differ significantly between pregnant women in the three trimesters.

Using the WHO criteria of Hb < 11.0 g/dl in pregnant women as indicative of anemia, 22% of the women in our study were anemic amongst which 18.4% were mildly anemic and 3.6% moderately anemic (**Table 2**).

To determine if these variables were independently associated to anemia, we performed a multivariate analysis using only variables that were associated to anemia in univariate analysis (**Table 3**). Age, gestational age, blood group and abortion were significantly associated with anemia. Pregnant women within the age range of 30 - 35 years were two times more likely to be anemic when compared to those less than 25 years of age. Pregnant women of blood group O were three times more likely to be anemic compared to those of blood group A, and pregnant women in the second trimester were two times more likely to be anemic compared to those in the first and third trimester. Women who had 1 - 3 abortions were four times more likely to be anemic compared to those who never experienced abortion

5. Discussion

In this study we noticed a progressive decline in the hemoglobin levels from first to third trimester with a significant drop between the first and third trimester. HCT values also decreased significantly from the second to third trimester. These findings are consistent with those of a similar study carried out by Akinbami *et al.* [5] in Lagos Nigeria. Meanwhile it contradicts with studies conducted in Nigeria by Ifeanyi *et al.* [12] and Osonugu *et al.* [3] which showed low Hb and

Table 2. Classification of haematological status among pregnant women at DLH according to trimesters of pregnancy and WHO reference values.

Variables	Pregnancy Trimesters			Total	<i>p</i>
	1 st trimester	2 nd trimester	3 rd trimester		
Hb status					
Normal	59 (88.1)	77 (75.5)	59 (72.8)	195 (78.0)	0.013
Mild anaemia	8 (11.9)	23 (22.5)	15 (18.5)	46 (18.4)	
Moderate anaemia	0	2 (2.0)	7 (8.6)	9 (3.6)	
HCT status					
Normal	57 (85.1)	74 (72.5)	54 (66.7)	185 (74.0)	0.036
Low	10 (14.9)	28 (27.5)	27 (33.3)	65 (26.0)	
RBC status					
Normal	58 (86.6)	76 (74.5)	72 (88.9)	206 (82.4)	0.023
Low	9 (13.4)	26 (25.5)	9 (11.1)	44 (17.6)	
MCV status					
Normal	54 (80.6)	80 (78.4)	68 (84.0)	202 (80.8)	0.013
High	0	8 (7.8)	0	8 (3.2)	
Low	13 (19.4)	14 (13.7)	13 (16.0)	40 (16.0)	
MCH status					
Normal	24 (35.8)	26 (25.5)	31 (38.3)	81 (32.4)	0.190
High	35 (52.2)	64 (62.7)	46 (56.8)	145 (58.0)	
Low	8 (11.9)	12 (11.8)	4 (4.9)	24 (9.6)	
MCHC status					
High	43 (64.2)	83 (81.4)	75 (92.6)	201 (80.4)	<0.0001
Normal	24 (35.8)	19 (18.6)	6 (7.4)	49 (19.6)	
WBC status					
Low	33 (49.3)	56 (54.9)	47 (58.0)	136 (54.4)	0.561
Normal	34 (50.7)	46 (45.1)	34 (42.0)	114 (45.6)	
Platelet status					
Low	16 (23.9)	31 (30.4)	24 (29.6)	71 (28.4)	0.627
Normal	51 (76.1)	71 (69.6)	57 (70.4)	179 (71.6)	

HCT values in the first trimester, highest in the second trimester and a drop in the third trimester. The decrease in hemoglobine may be due to an increased demand for iron and nutrients. As pregnancy progresses, more iron is required to meet the expansion of maternal Hb mass and the needs for fetal growth. Another physiological cause of the decrease in Hb and HCT values from first to second trimester may be due to hemodilution, increased iron demand and hormonal changes that increase fluid retention leading to an increase in plasma volume at a higher rate than in the red cell mass causing physiological anemia [5] [9] [14].

Table 3. Multivariate analysis of risk factors for anaemia.

Variables	N	OR	95%CI	p
Age, years				
<25	69	1		
25 - 30	88	0.78	0.32 - 1.89	0.579
30 - 35	57	2.81	1.16 - 6.81	0.023
≥35	36	0.08	0.25 - 2.58	0.708
Blood groups				
A	71	1		
AB	7	0.39	0.04 - 4.33	0.446
B	55	0.50	0.20 - 1.25	0.138
O	117	3.57	1.61 - 7.69	0.002
Gestational age				
1 st trimester	67	1		
2 nd trimester	102	2.20	0.88 - 5.48	0.024
3 rd trimester	81	1.90	0.73 - 4.92	0.186
Abortions				
0	199	1		
1 - 3	42	4.7	1.40 - 16.67	0.012
≥3	9	NA		

RBC count significantly decreased from first to third trimester. This might be due to increased nutritional demands for the fetal growth process which is managed by an increased stimulation of erythropoietin [15]. This finding is contrary to those of Akinbami *et al.* [5] and Elgari [16]. This difference may be attributed to a variation in supportive supplementation during pregnancy and/or nutritional habits.

MCV values significantly decreased from second to third trimester which correlates with findings in studies carried out by Akinbami *et al.* [5]; meanwhile it contradicts a study carried out by Snehalata *et al.* [8] which showed no significant changes in MCV values. This significant decrease in MCV may reflect iron deficiency anemia. It may also be attributed to changes in the growth and development of the fetus with increased requirement for hematinics [12].

No statistically significant difference was observed in MCH, MCHC, WBC and PLT values between pregnant women at different trimesters. These findings correlate with those of Snehalata *et al.* [8].

The prevalence of anemia in the present study was 22% which is lower than that in another study conducted in Cameroon at the Douala General Hospital (DGH) by Tchente *et al.* in 2016 [13] which showed a prevalence of 39.8%. This difference could be due to the fact that majority of the women in our study were on hematinic supplements.

Regarding the severity of anemia in our study, 18.4% of the participants had mild anemia, 3.6% had moderate anemia and none had severe anemia. This finding is similar to the studies carried out by Olatunbosun *et al.* [17] at the Uyo teaching hospital in Nigeria in 2014, Angesom in Ethiopia in 2015 [18] and Mariebb Hoehn [15]. This could be due to the fact that majority of the women took their hematinic supplements. However, our result is deviated from the findings obtained in the study conducted in Karnataka India, west Algeria and Jima which showed a high percentage of moderate anemia [19] [20] [21].

There was a statistically significant association of age, blood group, gestational age (trimester) and abortion to anemia. Women within the age range of 30 - 35 years had twice a higher risk of developing anemia compared to women < 25 yrs. This finding is contrary to those in the study carried out by Uche-Nwachi *et al.* [22] which revealed that pregnant women of the age range 15 - 20 years were more liable to develop anemia. This could be due to the growing demand of the teenager and also because of financial instability which may influence the proper acquisition of iron rich foods and supplements.

Pregnant women of blood group O were three times more prone to develop anemia than women of blood group A. This finding contradicts that of Basak *et al.* [23] in India which revealed that there is a strong correlation between blood group and anemia and individuals with blood group antigen alpha and beta are comparatively more prone to be anemic, whereas the individuals devoid of these antigens are resistant to anemia. This could be explained by the difference in geographical location of participants and the difference in the study population.

Pregnant women who had 1 - 3 abortions were four times more disposed to develop anemia compared to those with no abortions. These results contradict the findings in the study carried out by Uche-Nwachi *et al.* [22] in Nigeria which revealed that abortion did not affect the prevalence of anemia during pregnancy. This variation could be as a result of the social stigma associated to abortion thus limiting the number and/or types of abortions reported.

Women who were in their second trimester of pregnancy were two times more prone to develop anemia compared to those in the first and third trimester. This might be due to the higher maternal plasma volume increments (40% - 50%) relative to red cell mass (20% - 30%) and accounts for the fall in hemoglobin concentration [11] or the fact that as gestational age increases, the pregnant woman becomes weaker and the iron in the blood is shared with the fetus in the womb thereby decreasing the iron binding capacity of the mother's blood.

6. Limitations

The results of this study should be interpreted with care. The cross-sectional design of this study does not permit a direct establishment of cause-effect relationships. The reliability of the information given by the study participants is questionable. Moreover, we cannot with certainty determine risk factors of anemia in pregnancy due to the fact that our study is a cross sectional study. A pros-

pective cohort study would be of valid importance. However, the results obtained following norms of scientific research and with adequate methodology are in absolute conformity with previous research findings.

7. Conclusions

This study confirms significant variations in hematological parameters. The findings reinforce the need for supplementation and provide additional information on hematological reference values in pregnancy in Cameroon.

It also helps us understand that, third trimester, age range 30 - 35, and blood group may be potential risk factors associated with anemia in pregnancy though cohort study with perhaps a larger sample size would be necessary to ascertain this hypothesis.

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Availability of Data and Materials

The datasets used and/or analyzed during the current study are non-public but however available from the corresponding author on reasonable request.

Authors' Contributions

EH conceived the idea, helped in the review of literature, data collection, analysis and discussion. MKV, TG, CM helped in the review of literature, data collection and data analysis. EEL, CIP, SE helped in data analysis, review of literature and discussion. CE helped in overviewing the work.

Ethical Approval

Ethical clearance was obtained from the institutional ethical review board of the Faculty of Medicine and Pharmaceutical Sciences of the University of Douala (reference number CEI-UDo/922/16/2017). In addition, administrative authorization was obtained from the director of the Douala Laquintinie Hospital. All participants provided written informed consent. For participants below 18 years of age, consent to participate was obtained from their parents or their legal guardian and an assent signed by the participants.

Conflicts of Interest

There is no competing interest.

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Abbreviations

ANC: Antenatal Consultation

CBC: Complete Blood Count

DLH: Douala Laquintinie Hospital

EDTA: Ethylene Diamine Tetra Acetic Acid

fl: Femtolitre

Hb: Hemoglobine

HCT: Hematocrit

IPT: Intermittent Preventive Treatment

MCH: Mean Cell Hemoglobin

MCHC: Mean Cell Hemoglobin Concentration

MCV: Mean Corpuscular Volume

PLT: Platelet

RBC: Red Blood Cell

WBC: White Blood Cell

WHO: World Health Organization