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Differential Correlation between Foetal Haemoglobin and Full Blood Count Based on Inherited Haemoglobin Type; A Cross-Sectional Study in Cape Coast, Ghana

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Abstract

Background: Foetal haemoglobin (Hb F) has been shown to modulate the severity of sickle cell anaemia (SCA). However, there is scarcity of data on the impact of Hb F levels in other inherited haemoglobin variants. This cross-sectional study sought to investigate the relationship between Hb F and full blood count parameters in participants based on their inherited haemoglobin type. Materials and methods: Four milliliters of venous blood were drawn from 170 consecutively consented participants (aged 10 -55 years) into EDTA-anticoagulated tubes. Full blood counts (FBC) were estimated using Horiba ABX Pentra XL80 analyzer, whereas haemoglobin variants were determined using cellulose acetate electrophoresis. Hb F was estimated using the modified Bekte-alkali denaturation method. Data was analysed using SPSS (version 25 for Windows). Relationship between Hb F and FBC parameters were explored using Pearson correlation coefficient analyses. Statistical significance was established at $p < 0.05$ level.

Results: Whereas majority (61.8%) were females, there was no significant differences in age among the participants based on gender. Participants with inherited haemoglobin variants comprised 20% of the study population. Total WBC was significantly higher in participants with inherited haemoglobin variants ($p = 0.011$). Hb F levels were also significantly elevated in participants with inherited haemoglobin variants ($p < 0.001$). Additionally, whereas Hb F was inversely correlated with RBC ($p = 0.226$), Hb ($p = 0.021$), HCT (0.031), MCV (0.266), MCH (0.15) and MCHC (0.231) in those with no haemoglobin variants, it was positively correlated with RBC (0.409) Hb ($p = 0.006$), HCT ($p = 0.003$), MCV ($p = 0.074$), MCH (0.047) and MCHC ($p = 0.583$) in those with inherited haemoglobin variants. Moreover, there was inverse correlation between Hb F and total WBCor platelet counts in participants with inherited haemoglobin variants.

Conclusions: Leukocytosis and inverse relationship between Hb F and WBC or platelet count in those with haemoglobin variants might predispose them to severe manifestations of haemoglobinopathy.

Keywords: Haemoglobinopathy; Sickle cell disease; Bekte-alkali denaturation; Cellulose acetate electrophoresis; Foetal haemoglobin; Full blood count

Abbreviations: Hb F: Foetal haemoglobin; SCA: Sickle Cell Anaemia; FBC: Full Blood Count; SPSS: Statistical Package for Social Sciences

Introduction

Globally, haemoglobinopathies are one of the most common genetic inherited disorders. Previous study by Kohn estimated prevalence of 5 – 30% in Africans, 5 – 40% in Arabians, 5 – 20% Central Americans, and 0.5 – 1% in Europeans [1]. Although, it has been stated that selective pressures due to severe malaria infection may partly account for the high prevalence of haemoglobinopathies in sub-Saharan Africa, it is still important to mention that effective premarital genetic counselling can significantly reduce the disease burden [2]. Sickle cell anaemia (SCA) is perhaps the most studied of the haemoglobin variants probably because of the severe vaso-occlusive crises that it causes to those with homozygous Hb SS inheritance [3]. The other haemoglobin variants like Hb C, D, E etc. are not as well studied even though the inheritance of any of these may impact varying rheological properties to the red cells.

Foetal haemoglobin (Hb F) has been demonstrated both experimentally and clinically to modulate severity of SCA [4-6]. The relationship between the various haemoglobin variants and some blood components have been established by other studies. For instance, the prevalence and influence of HbS, HbC and Hb-Thalassemia on red blood cell parameters has been reported previously [7]. The interaction of the various Hb variants with white blood cells remains unclear [8]. The role Hb F levels play in modulating the FBC parameters in other haemoglobin variants have also not been clearly delineated. We thus sought to interrogate the relationship between the Hb F levels and full blood count parameters in participants with inherited haemoglobin variants as well as those without any inherited haemoglobin variants. The aim was to investigate whether the relationship established for individuals with SCA extends to the other haemoglobin variants considering that the inheritance of any of these different haemoglobin variants may impact different rheological properties to the red blood cells.

Materials and Methods

Study Design/Population

This cross-sectional study consecutively recruited 170 consenting participants aged 10 – 55 years. The participants attended the out-patient department (OPD) section of the University of Cape Coast Hospital, Ghana, between January 2017 – May 2017.

Data Collection

Four (4) milliliters of venous blood was drawn from each participant into EDTA-anticoagulated tubes following standard protocols. The blood was used for haemoglobin electrophoresis, %Hb F and and full blood count (FBC).

Ethical Consideration

All protocols for the study were approved by University of Cape Coast Institutional Review Board (IRB number, UCCIRB/CHAS/2017/76), Department of Medical Laboratory Technology and the University of Cape Coast hospital.

Full Blood Count (FBC)

FBC for each sample was estimated using Horiba ABX Pentra XL80 (Horiba medical, Japan) automated haematology analyser. The RBC count, red cell indices, haemoglobin (Hb) concentration, Platelet count and WBC count and WBC absolute differential counts were recorded.

Estimation of Fetal Hemoglobin (Hb F)

Concentration of Hb F was estimated for each sample according to previously described protocols Bekte, et al. Samples were centrifuged at 2000 rpm for 10 minutes to remove plasma before haemolysates were prepared.

The proportion of Hb F was calculated from the absorbance ratio between Hb F and total Hb after

correcting for the dilution factor as: Hb F (%) = [100*Absorbance of test/ (Absorbance of Ref*20)].

Cellulose Acetate Haemoglobin Electrophoresis

Hb variant of each participant was determined by electrophoresis on cellulose acetate using whole blood in accordance with previously published protocols [9]. A control sample containing haemoglobin (Hb) A, C, S, and F was run with each sample to ensure validity of results interpretation.

Data Analysis

Data collected was entered into Microsoft Office Excel 2016 and analysed using Statistical Package for Social Sciences (SPSS) version 25.0 for Windows (IBM, USA). A summary was presented using descriptive statistics such as frequencies, percentages, mean and standard deviations. Chi square analysis was used to establish association between Hb variants and gender. Pearson correlation coefficient analysis was used to explore the relationship between %Hb F levels and FBC parameters. One-Way ANOVA (with Dunnett's multiple comparison) was used to compare the absolute white blood cell differential counts between Hb AA participants and those with inherited haemoglobin variants. A p value of <0.05 was considered statistically significant.

Results

Table 1 describes the general characteristics of the study participants. Whereas majority belonged to the 21 – 30 years age group, 61.2% were females. There was no significant difference in participant age based on gender. Also, 20% inherited some form of hemoglobin variant. Leukocytosis was observed in 10.0% of the participants with 2.9% demonstrating thrombocytosis. Additionally, 17.1% of the participants had %Hb F ≥2.5 (7.7% males vs 22.9% females).

Parameter	Total	Males	Females	p-value
	N (%)	N = 65	N = 105	
Age (years)				0.971
10 – 20	45 (26.5)	17 (26.2)	28 (26.7)	
21 – 30	59 (34.7)	22 (37.3)	37 (62.7)	
31 – 40	30 (17.6)	11 (16.9)	19 (18.1)	
>40	36 (21.2)	15 (23.1)	21 (20.0)	
Hb variants				
A	136 (80.0)	52 (80.0)	84 (80.0)	0.761
AS	18 (10.6)	6 (9.2)	12 (11.4)	
AF	5 (2.9)	3 (4.6)	2 (1.9)	
SS	2 (1.2)	0 (0.0)	2 (1.9)	
AC	7 (4.1)	3 (4.6)	4 (3.8)	
SC/CC	2 (1.2)	1 (1.5)	1 (1.0)	
WBC (*10^9/L)				0.148
<3.0	5 (2.9)	4 (6.2)	1 (1.0)	
3.0 – 11.0	148 (87.1)	55 (86.2)	93 (88.6)	
>11.0	17 (10.0)	1 (1.5)	11 (10.5)	
Platelet (*10^9/L)				0.083
<150	12 (7.1)	8 (12.3)	4 (3.8)	
150 – 450	153 (90)	56 (86.2)	97 (92.4)	
>450	5 (2.9)	1 (1.5)	4 (3.8)	
%Hb F				**0.012**
<2.5	141 (82.9)	60 (92.3)	81 (77.1)	
≥2.5	29 (17.1)	5 (7.7)	24 (22.9)	

Table 1: General characteristics of study participants.

Table 2 compares the mean FBC parameters in relation to the type of inherited hemoglobin variant. The RBC count did not significantly differ across the different inherited haemoglobin types. The WBC count was significantly higher in participants with Hb AS, AF, SS, or AC compared to those with haemoglobin A (p = 0.011). Also, the %Hb F significantly differed across participants with different haemoglobin types. However, platelet count did not significantly differ across the different haemoglobin types.

Parameter	HB Variants	Mean (±Std)	P-Value
RBC			0.242
	AA (136)	4.529(±0.666)	
	AS (18)	4.488(±0.784)	
	AF (5)	4.666(±0.561)	
	SS (2)	4.825(±0.926)	
	AC (7)	4.980(±0.746)	
	SC/CC (2)	5.430(±0.566)	
WBC			**0.011**
	AA (136)	6.542(±2.633)	
	AS (18)	10.395(±11.465)	
	AF (5)	10.060(±4.111)	
	SS (2)	11.350(±1.061)	
	AC (7)	11.186(±16.137)	
	SC/CC (2)	3.950(±1.768)	
HbF			**<0.0001**
	AA (136)	0.728(±0.465)	
	AS (18)	1.206(±0.768)	
	AF (5)	16.044(±11.412)	
	SS (2)	1.625(±0.997)	
	AC (7)	0.940(±0.927)	
	SC/CC (2)	2.150(±0.636)	
PLT			0.567
	AA (136)	268.934(±90.885)	
	AS (18)	274.167(±67.116)	
	AF (5)	274.400(±74.614)	
	SS (2)	172.500(±132.229)	
	AC (7)	262.000(±49.608)	
	SC/CC (2)	195.000(±74.953)	

Table 2: Comparison of mean HB, WBC, HBF and PLTs with the hemoglobin variants of participants.

Participants differential WBC were compared based on their inherited haemoglobin type (Figure 1). Although neutrophil counts differed across the haemoglobin types, only participants with Hb SS had significantly higher neutrophil count compared to Hb AA participants. Also, monocyte counts were significantly higher in Hb AF participants compared to those with Hb A. Although basophil, lymphocyte, and lymphocyte counts differed across the haemoglobin types, these did not reach statistical significance.

This study also investigated the relationship between Hb F levels and red cell indices (Tables 3). Whereas Hb F was inversely related to all red cell indices (reaching significance in Hb and HCT) in participants with no haemoglobin variants, it was directly related to all red cell indices (reaching significance in Hb, HCT, and MCH) in participants with inherited haemoglobin variants. Additionally, whereas Hb F was directly related to platelet counts in participants with Hb A, it was inversely related with platelet count in participant with inherited haemoglobin variants.

Parameter	Hb A		Hb variants	
	r	p	r	p
Platelet	0.234	0.006	-0.108	0.544
RBC	-0.104	0.226	0.146	0.409
Hb	-0.198	**0.021**	0.458	**0.006**
HCT	-0.185	**0.031**	0.497	**0.003**
MCV	-0.096	0.266	0.31	0.074
MCH	-0.124	0.15	0.342	**0.047**
MCHC	-0.103	0.231	0.098	0.583

Table 3: Pearson correlation between Hb F and red cell indices among participants.

Parameter	Hb A		Hb variants	
	r	p	r	p
WBC	0.081	0.35	-0.032	0.857
Neutrophil	-0.111	0.198	-0.331	0.056
Lymphocyte	0.102	0.235	0.226	0.198
Monocyte	-0.006	0.948	0.491	**0.003**
Eosinophil	0.066	0.446	0.196	0.266
Basophil	-0.01	0.908	-0.174	0.325

Table 4: Pearson correlation between Hb F and WBC differentials in study participants.

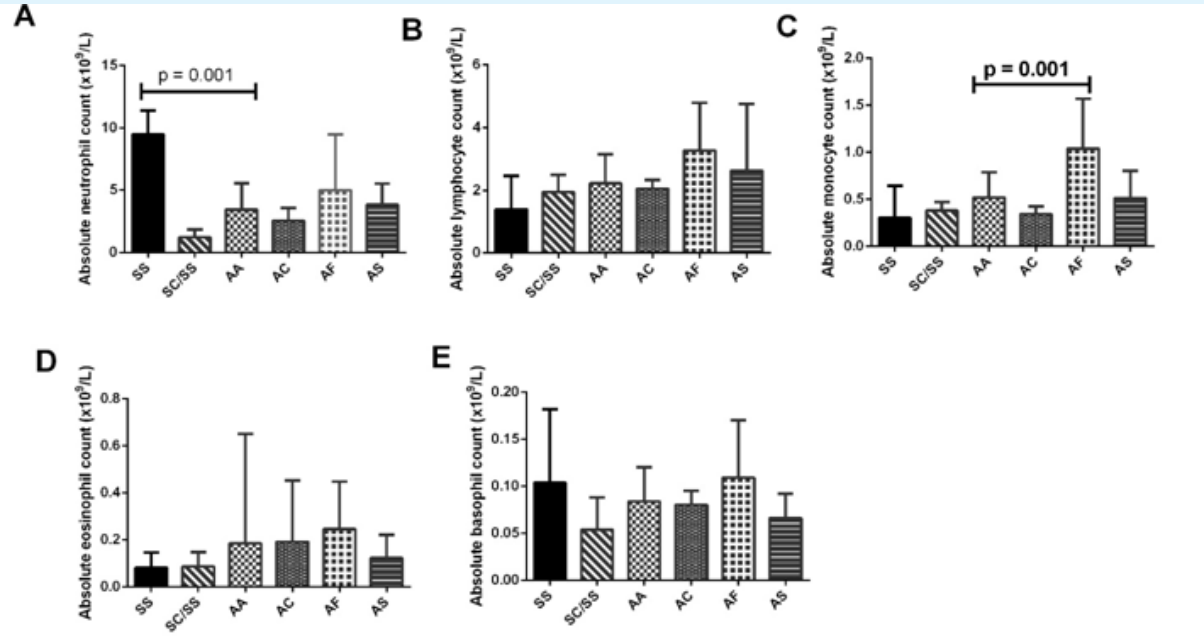

Figure 1: Comparison of mean absolute WBC differentials of participant. Participants were stratified based on inherited Hb variants and absolute (A) neutrophil (B) lymphocyte (C) monocyte (D) eosinophil (E) basophil counts of participants with Hb variants compared to respective counts in those with Hb AA. Statistical significance was estimated using One-Way ANOVA with Dunnett's multiple comparison.

The correlations of Hb F and white cell differential count is presented in table 4. Whereas Hb F level was significantly related with monocyte count in participants with inherited Hb variants, it was inversely, but non-significantly related to monocyte in participants with Hb A type. There were however, differential correlation between the other white cell differentials and Hb F among the participants.

Discussion

In sub-Saharan Africa, selective pressure due to malaria has led to balanced polymorphism that selects for some genetically inherited traits that gives survival advantages. Thus, evolution has preserved genetically inherited diseases such as sickle cell disease in sub-Saharan Africa. Research has demonstrated that increased Hb F expression has co-evolved with SCD to mitigate the severity of this disease. In this study, we showed that 20% of the participants inherited some form of variant haemoglobin, with 17.1% demonstrating elevated %Hb F levels. Additionally, whereas those with inherited haemoglobin variants demonstrated inverse correlation between % Hb F and WBC or platelet count, those with no inherited variant haemoglobin generally showed direct correlation between Hb F and WBC or platelet count.

Within the limits of the cellulose acetate electrophoresis technique, we report that a wide spectrum of variant haemoglobin (Hb S, C, F) were detected in the study population. The limitations associated with cellulose acetate electrophoresis is well documented in the literature particularly the co-migration of some haemoglobin variants [10,11]. Therefore, it is possible that this study underestimated the spectrum of variant haemoglobin in the study population. The prevalence of haemoglobin variant inheritance in sub-Saharan Africa has been stated at 5% - 30% [1]. In Ghana specifically, previous cross-sectional studies have found 25 – 30% prevalence of haemoglobin variant inheritance [12,13]. This cross-sectional study which used convenience sampling design found a prevalence of 20% among the study population which is comparable to the estimated prevalence for the sub-region. Considering that premarital genetic counselling could significantly reduce the incidence of haemoglobinopathies [2], this high prevalence is indicative that premarital counselling is probably not being given adequate consideration in our study

population. Sickle cell trait was the predominant haemoglobin variant detected in the study population. Elevated Hb F levels in individuals with SCA have been extensively reported in the literature [5,14,15]. This study demonstrated that increased Hb F production is not limited to SCA, but also other variant haemoglobin as well. This study employed the Bekte-alkali denaturation method to report elevated Hb F levels comparable to that of other reports in the sub-region that used the same technique in similar study designs [14,16].

Total WBC counts were significantly elevated in participants with inherited haemoglobin variants compared to those without. This is in agreement with previous findings by Akinlosotu, et al. that reported significantly elevated WBC in sickle cell anaemia children in South-Western Nigeria [17]. However, whereas Akinlosotu also reported significant differences in the differential WBC parameters, we could only establish significant increases in monocytes and neutrophil counts in Hb AF and AS respectively. The slightly different study designs might have accounted for this. Whereas the study by Akinsolotu compared the differential WBC between Hb AA individuals with that of individuals inheriting only Hb SS in children (50% cases against 50% controls), this study largely recruited adult population and with those inheriting haemoglobin variants comprising only 20% of the participants. Individual with SCA comprised only 2.4% of the study population in the present study (compared to 50% in the Akinlosotu study). It is interesting to point out that leukocytosis reported herein has also been demonstrated in several multi-center studies as a risk-factor for severe sickle cell disease-related complications including death [18-20]. This poor prognostication of leukocytosis is supported in the present study by the inverse relationship between Hb F and leucocyte count established among participants with inherited haemoglobin variants.

This study also found significant differential Hb F correlations with red cell indices or platelet count between those with inherited haemoglobin variants and those without. The direct associations between red cell indices and Hb F recorded among the participants with inherited Hb variants has been previously reported in another cross-sectional study in the sub-region [17]. Indeed, it has been demonstrated by others that elevated Hb F levels are protective in SCA patients [3,6,21,22]. The fact that high leucocyte count has been identified in multivariate analyses as powerful predictor of favourable response to hydroxyurea therapy may be an indication that the driving force behind this evolved mechanism may not be red cell indices. This is buttressed by the direct correlation between Hb F and red cell indices in those with inherited Hb variants contrary to the observation in Hb AA clients. On the other hand, since those with no haemoglobin variants are not under the same selective pressures as those with inherited haemoglobin variants, different mechanisms might be at play to regulate Hb F expression. The inverse association between Hb F and platelet count, although reported in another study [17], requires further studies to clearly delineate the role platelets play in the pathogenesis of haemoglobinopathies.

Conclusion

Leukocytosis and inverse relationship between Hb F and WBC or platelet count in those with haemoglobin variants might predispose these individuals to severe manifestations of haemoglobinopathy.

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Author contributions: PA conceived, designed, supervised all the experimental work in the research and revised the manuscript. FA, DB, and RA were involved in participant recruitment, sampling, and experimental work. BKSD analysed the data and wrote the initial draft. RPS was involved in literature search, initial manuscript editing and experimental work. All authors read and approved the final manuscript prior to submission.

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