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HEMATOLOGICAL REFERENCE RANGES FOR HEALTHY ADULTS IN THE NORTHWEST REGION OF CAMEROON[i]

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Abstract:
The interpretation of laboratory test results requires reference or cutoff values. An improved diagnosis with accurate, reliable laboratory reference ranges saves valuable healthcare resources essential for effective clinical evaluation and monitoring. This benefit provides improved cost containment by eliminating unnecessary testing, treatment changes, and use of other healthcare resources during the episode of care. Based on the above fact, this study was designed to establish reference ranges for hematological parameters in apparently healthy voluntary non-remunerated blood donors. The study was a hospital based cross-sectional experimental study conducted between February and October 2020. Blood samples were taken from 356 healthy adults (18 to 60 years) 211 (59.3%) males and 145 (40.7%) females and routine hematology analysis performed. Patients were assessed as healthy on the basis of a medical history and medical examinations. Venous blood from the antecubital fossa was collected in 2-3mL of K-ethylenediamine tetra acetic acid (EDTA) for complete blood counts. A total of nineteen hematological parameters were tested in this study and showed significant differences ($p<0.001$) among males and females with the former showing higher CBC values, except WBC, GRAN# and platelet. More than 10% of the female population presented results that were out of that of the accompanying manual of the hematology analyzer used in the study. The reference range for LYM % for the female was above the upper limit of the manufacturers' manual reference and for GRAN% in the males was below the lower limit of the accompanying manual of the hematology analyzer. Several

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differences were also observed when compared to previously established values from Yaounde (Cameroon), most notably in platelets. Our findings for CBC parameters, are in general agreement with previously published data from more limited trials undertaken in other African countries. In spite of the uncontrolled factors influencing hematological values, this study permitted to establish new hematological reference values for use in the North West region of Cameroon. In the absence of previously detailed and more comprehensive investigated hematological reference values for the region we offered to use these results for clinical management of patients from this region and interpretations of laboratory data.

Keywords: complete blood count, hematology, reference range, North West region, Cameroon

Abstrait:
L'interprétation des résultats des tests de laboratoire nécessite des valeurs de référence ou de coupure. Un diagnostic amélioré avec des plages de référence de laboratoire précises et fiables permet d'économiser de précieuses ressources de santé essentielles pour une évaluation et un suivi cliniques efficaces. Cet avantage permet une meilleure maîtrise des coûts en éliminant les tests inutiles, les changements de traitement et l'utilisation d'autres ressources de soins de santé pendant l'épisode de soins. Sur la base de ce qui précède, cette étude a été conçue pour établir des plages de référence pour les paramètres hématologiques chez des donneurs de sang volontaires non rémunérés apparemment en bonne santé. L'étude était une étude expérimentale transversale en milieu hospitalier menée entre février et octobre 2020. Des échantillons de sang ont été prélevés sur 356 adultes en bonne santé (18 à 60 ans) 211 (59,3%) hommes et 145 (40,7%) femmes et une analyse hématologique de routine a été réalisée. Les patients ont été jugés sains sur la base des antécédents médicaux et des examens médicaux. Le sang veineux de la fosse antécubitale a été collecté dans 2-3 ml d'acide K-éthylènediamine tétra acétique (EDTA) pour une numération globulaire complète. Un total de dix-neuf paramètres hématologiques ont été testés dans cette étude et ont montré des différences significatives ($p < 0,001$) entre les hommes et les femmes, le premier montrant des valeurs de FSC plus élevées, sauf globules blancs, GRAN # et plaquettes. Plus de 10% de la population féminine a présenté des résultats différents de ceux du manuel d'accompagnement de l'analyseur d'hématologie utilisé dans l'étude. La plage de référence pour LYM% pour la femelle était au-dessus de la limite supérieure de la référence manuelle du fabricant et pour GRAN% chez les mâles était inférieure à la limite inférieure du manuel d'accompagnement de l'analyseur d'hématologie. Plusieurs différences ont également été observées par rapport aux valeurs précédemment établies à Yaoundé (Cameroun), notamment dans les plaquettes. Nos résultats pour les paramètres du FSC, sont en accord général avec les données publiées antérieurement à partir d'essais plus limités entrepris dans d'autres pays africains. Malgré les facteurs incontrôlés influençant les valeurs hématologiques, cette étude a permis d'établir de nouvelles valeurs hématologiques de

référence à utiliser dans la région du Nord-Ouest du Cameroun. En l'absence de valeurs de référence hématologiques étudiées précédemment détaillées et plus complètes pour la région, nous avons proposé d'utiliser ces résultats pour la prise en charge clinique des patients de cette région et l'interprétation des données de laboratoire.

Mots clés: formule sanguine complète (FSC), hématologie, gamme de référence, région du Nord-Ouest, Cameroun

1. Introduction

Over the last decade, there has been a significant increase in the number of clinical trials taking place in sub-Saharan Africa in a concerted effort to identify safe and effective prevention and treatment strategies to combat the heavy burden of infectious diseases in this region [1-3]. This is because numerous viral, parasitic and bacterial diseases are endemic in this region, including: 66% of the global HIV/AIDS infections, 31% of tuberculosis infections, and 86% of malaria cases [3, 4]. Routine capacity for clinical laboratory testing is also increasing in Africa. Clinical trials and clinical care in sub-Saharan Africa require accurate laboratory reference intervals for appropriate assessment of patients/participants, monitoring disease progression, and reporting of possible toxicity and adverse events. A reference range is a range of values of a laboratory test usually based on predetermined test results from a group of apparently healthy individuals and used for diagnostic accuracy [5]. It is critical for medical professionals to have access to an accurate management resource such as reference ranges. They are important for accurate interpretation of laboratory data and provide assistance to the clinician in creating a more comprehensive clinical perspective for diagnosis and management of patients [6]. Of particular importance is the use of reference values as surrogate markers for monitoring disease progression and response to antiretroviral therapy in HIV-infected individuals [7]. Population-based hematological reference ranges have not been established for many healthcare facilities in Cameroon unlike many developing countries [4]. Many of the reference ranges in use are those established in the countries of origin of the hematological analyzers in use. The danger accompanying this is the use of established reference ranges in western settings for populations that are diverse in social status, health, and geographical setting [5]. Studies from literature [6] have revealed that there are inter- and intra-population variation in hematological reference ranges even among populations of the same race and especially so in populations of varying genetics, pathogen sets, nutritional status, and altitude. The variation makes dependence on pre-established hematological reference values from other countries inappropriate, thereby leading to misdiagnoses resulting in wrong treatment and its attendant dire health implications on individuals, families, communities, and the nation at large. It is therefore expected that different parts (regions) of the country will have different reference ranges based on their peculiarities. Out of this, a set of national reference ranges may be established. This study, therefore, sought

to establish the hematological reference values in apparently healthy voluntary non remunerated blood donors from the North West region of Cameroon.

2. Materials and Method

2.1 Study Site

This study was carried out in the Northwest region (Figure 1) of Cameroon which is the third most populated region of Cameroon.

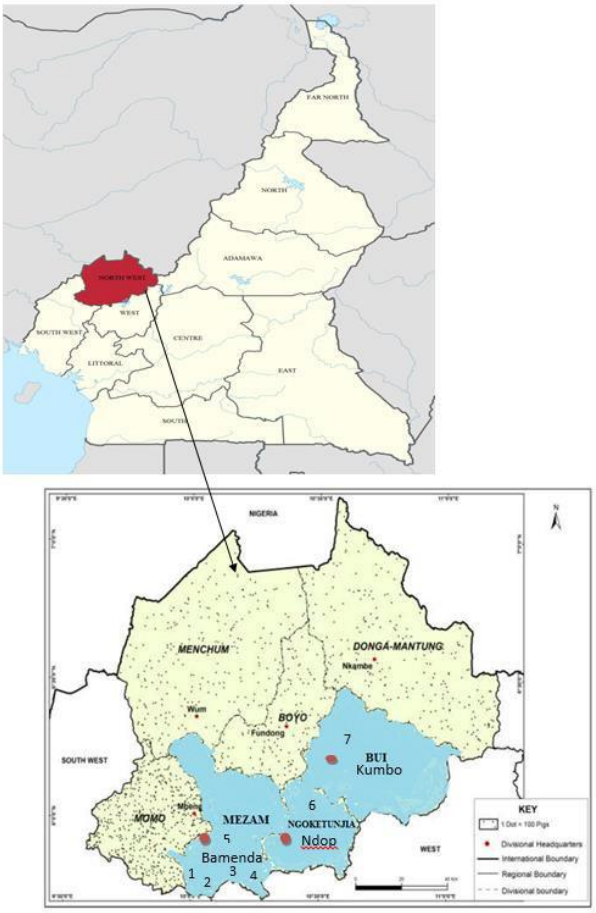

Figure 1: Map of Cameroon including the North West Region

It has one major metropolitan city, Bamenda, located along 10.15 longitude and 5.96 latitude and situated at an average height of 1258 meters above sea level. The region saw an increase in its population from approximately 1.2 million in 1987 to an estimated 1.8 million in 2010 [8]. The main ethnic groups are of Tikar origin: Tikari, Widikum, Fulani, and Moghamo. During the colonial period, administrative boundaries were created which cut across ethnic groups and cultures. As a result, parts of some ethnic groups now lie in different divisions and regions. The region is bordered to the southwest by the Southwest Region, to the south by the West Region, to the east by the Adamawa Region, and to the north by the Federal Republic of Nigeria. The region is made up of

seven administrative divisions Bui, Donga-Mantung, Menchum, Mezam, Momo, Boyo and Ngoketunjia with a population of 1.969 million [8].

The population density of the region is 99.12 people per square kilometer and is higher than the national average of 22.6. The regional urban growth rate is 7.95%, higher than the national average of 5.6%, while the rural growth rate, at 1.16%, is equal to the national rate. The region has two seasons; the dry and the rainy seasons, with a balanced rainfall per year being 2064 mm (and 172 mm per month). The peak of dry season occurs in January. Meanwhile, the peak of the rainy season is in September. This study was carried out in the Bamenda Regional Hospital (BRH), the chief government hospital with the largest blood bank in the North West Region. The BRH is part of the Bamenda Health District (BHD), which is made up of many public, private, and mission health facilities located within 17 health areas. The BRH, therefore, functions as the referral hospital in the region, with an estimated 337,036 inhabitants [9].

2.2 Study Design and Duration

The ethical clearance for this study was obtained from the Ethical Review Committee/Institutional Review Board of The University of Bamenda (2020/0115H/UBa/IRB).

An administrative authorization for research was obtained from the General Supervisor of the Regional Hospital Bamenda, and permission from the chiefs of services of the Regional Hospital Bamenda Laboratory and Blood Transfusion Centre. Only participants who agreed to take part in the study after clear explanation about the research work were asked to fill the questionnaire and sign the consent form.

This study was a hospital based cross-sectional experimental study conducted between February and October 2020.

2.3 Study Population

The study subjects were eligible blood donors at the blood transfusion center of the BRH. Non-remunerated healthy blood donors between the ages of 18 and 60 years, who signed the consent forms, were recruited in this study.

2.4 Sample Size

The recommended method to establish a reference interval which is to collect samples from a sufficient number of qualified reference individuals to yield a minimum of 120 samples for analysis, by non-parametric means was used [10]. For this study therefore, the sample size was based on recommendations made by the CLSI and funding available to the researcher, to achieve a minimum of 356 qualified participants.

2.5 Questionnaire Survey

Informed written consent was provided by interested blood donors based on the national requirements followed by pre-donation information, advice and counselling about the process of blood donation.

A well-structured questionnaire was administered to the potential blood donor in order to collect information on:

- relevant history of the donor covering health, body weight and height, demographic data (age, sex, region and division of origin) and high-risk behavior as well as to screen for habits such as smoking, alcoholism, which were considered unhealthy for the study.
- history of mastectomy, current and recent medications or chronic infections,
- history of prolonged bleeding or a past diagnosis of bleeding disorders,
- history of previous donations, to ensure the waiting period is respected,
- a preliminary physical check-up of the donor including; blood pressure, signs of infection or scarring at potential sites.

The following screening tests were done on the blood samples of participants:

- HIV type 1 and 2, using Alere Determine strips and Oral Quick,
- Haemoglobin electrophoresis was done using the Hospitex Diagnostics (Hospitex Diagnostics Srl, Sesto Fiorentino, Italy) electrophoresis machine,
- Hepatitis B virus using the HBsAg DiaSpot rapid diagnostic test (DIASpot Diagnostics, Jawa Barat, Indonesia),
- Hepatitis C virus with antigen detected using the HCV Ag DiaSpot rapid diagnostic test (DIASpot Diagnostics, Jawa Barat, Indonesia).
- Syphilis using the Rapid Plasma Reagin carbon slide agglutination assay
- Malaria, using the Rapid Diagnostic Test kit method.
- Blood group using ABO typing
- Diabetes (Glucose screening test)
- Pregnancy test (EUROMEDI EQUIP LTD, West Harrow U.K).
- The stool specimen was examined by wet preparation method
- Blood pressure and temperatures were checked using blood pressure monitor and thermometers respectively

Those with positive clinical or laboratory test results were referred for suitable treatment and care.

2.6 Inclusion Criteria

Individuals negative for any of the screening test performed to assess state of health were included in the study.

2.7 Exclusion Criteria

The presence of any disease including: Malaria, Sickle cell disease, Hepatitis B and C, HIV type 1 and 2, anemia, individuals with chronic diseases such as diabetes mellitus (DM) (as revealed by the screening test), individuals who had donated blood in the last 3 months, individuals who had received blood in the last 12 months, and individuals who had undergone surgery in the recent past were all excluded. Participants with blood pressure outside the range of $\frac{120-180mmHg(systole)}{60-100mmHg(diastole)}$ were equally excluded from the study. All pregnant females were excluded in the study.

2.8 External Quality Assessments

The automated counter used for the analysis of participants' samples had already been verified [11, 12]. It also underwent regular external quality assessment (EQA) checks by the South African National Accreditation Society (SANAS). So, it was checked for; Accuracy, Precision, Carry-Over, Linearity as a requirement for establishing reference values.

2.8.1 Laboratory Procedures

For participants who passed the first screening phase, blood samples were collected for screening tests to be carried out. Venous blood from the antecubital fossa was collected in 2-3mL of K_3 ethylenediamine tetra acetic acid (EDTA) blood for hematology analysis with one of the tubes used for screening by the laboratory and the second tube used for full blood count. This was to prevent wrong data from being collected using the hemoanalyzer. Since the blood sample for screening was centrifuged, using it for full blood count could negatively impact the results. The blood collection tubes were labelled with the participant's code, sex, age, date and time of sample collection. Analysis of samples was performed within 8 hours of blood draw as the samples were at room temperature. All samples were analyzed according to the stipulated procedures. Blood was tested for infections by use of test kits as directed by the manufacturers of the kit. All positive samples results to any of these tests were discarded and the samples which were negative were utilized for the study. When the patient's results were negative for the screening tests, the second sample collected was then analyzed. Blood samples for full blood count were transported to the hematology laboratory of the hospital in sample transportation flasks for analysis, to avoid spillages and mechanical damages.

2.8.2 Sample Analysis

Hematological tests were analyzed using automated hematology analyzer (Urit-3000) [13] which performs two independent measurement, which are the impedance method for determining the WBC, RBC, and PLT and their indices and the colorimetric method for determining the Hemoglobin. After testing, blood samples were disposed of according to the hospital's policies for discarding samples and human specimens using their standard operating procedures.

2.8.3 Data Analysis

Demographic data was collected using an investigator-administered questionnaire in a language (Pidgin English, French and English) that could easily be understood. Data was then entered into excel and cleaned prior to analysis. All categorical data was presented as frequencies and percentages and reference ranges were calculated using nonparametric methods. Reference values were determined at $2.5^{th}$and 97.5^{th} percentiles using the non-parametric test [14]. The Dixon method was used to identify outliers within each subgroup, [14] and the extreme values were retained in the distribution if $D/R<0.33$, where D is the absolute difference between the most extreme distribution and the next

value and R is the Range (maximum – minimum). The Kruskal-Wallis test was used to determine the significance of differences between the divisions of the North West Region of Cameroon. The values defined were then used to compare with the recommended reference values (based on a North American population) provided in the Urit 3000 Hematology User Manual [13]. P-value < 0.05 was considered significant. All statistical analyses were performed with Graph pad Prism version 8.2.1.

3. Results

3.1 A Demographic Characteristics of Study Participants

A total of 423 participants of whom 250 (59.1 %) were males and 173(40.1%) were females were enrolled in the study. After applying the exclusion criteria 356 participant were retained for further study and 67 rejected. From this total 145(40.7%) were females and 211(59.3%) were males.

3.2 Distribution of Study Participants by Age Group

The majority of the sampled participants were in the 18-30 (75%) age range for the females (Figure 2) and 18-28 (56%) for the males (Figure 3).

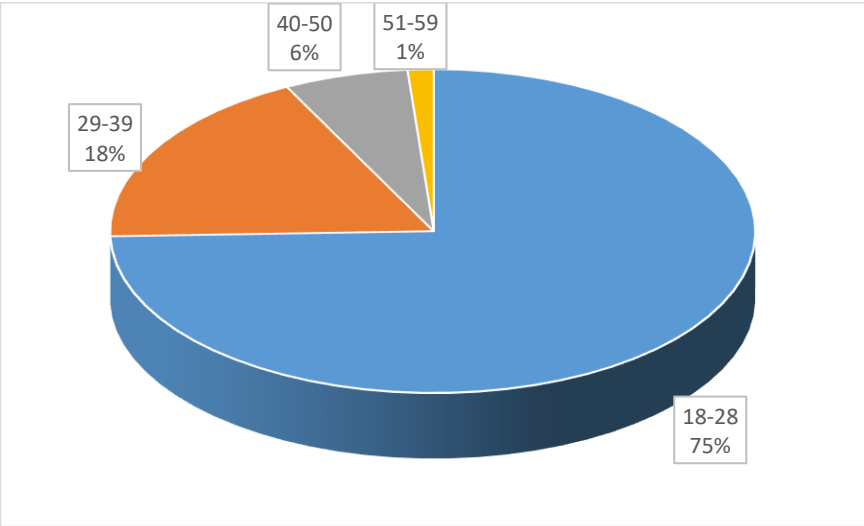

Figure 2: Age Frequency of Female Participant

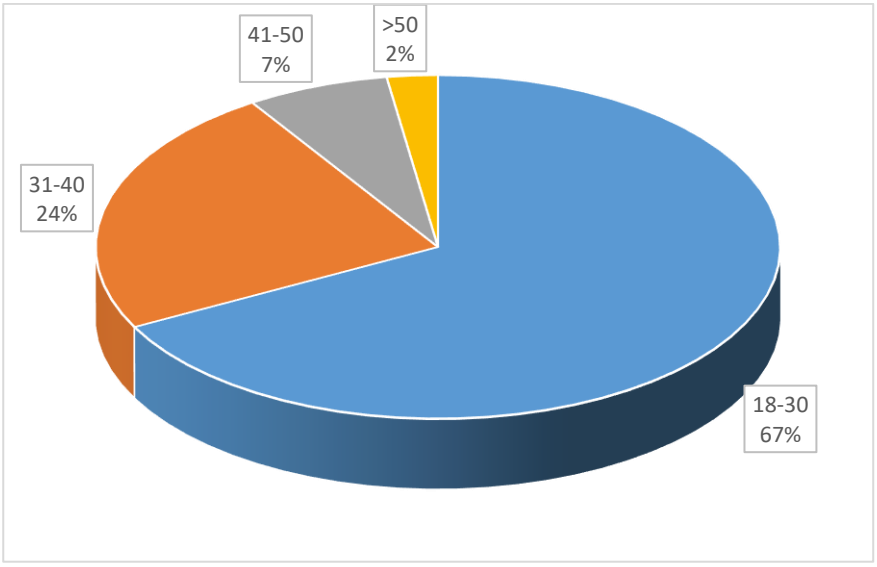

Figure 3: Age Frequency of Male Participant

3.3 Distribution of Study Participants by Division

Most of the female participants came from Mezam 56(38.6%) and the least 2(1.4%) came from Boyo (Figure 4). For the males 96(45.5%) came from Mezam while the least came from Boyo 7(3.3%) (Figure 5).

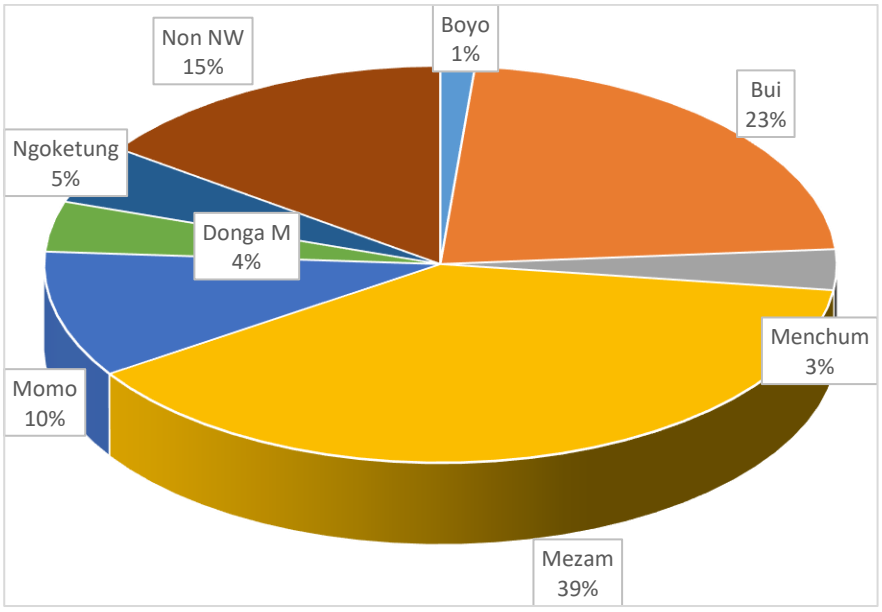

Figure 4: Frequency of Division of Origin for Females

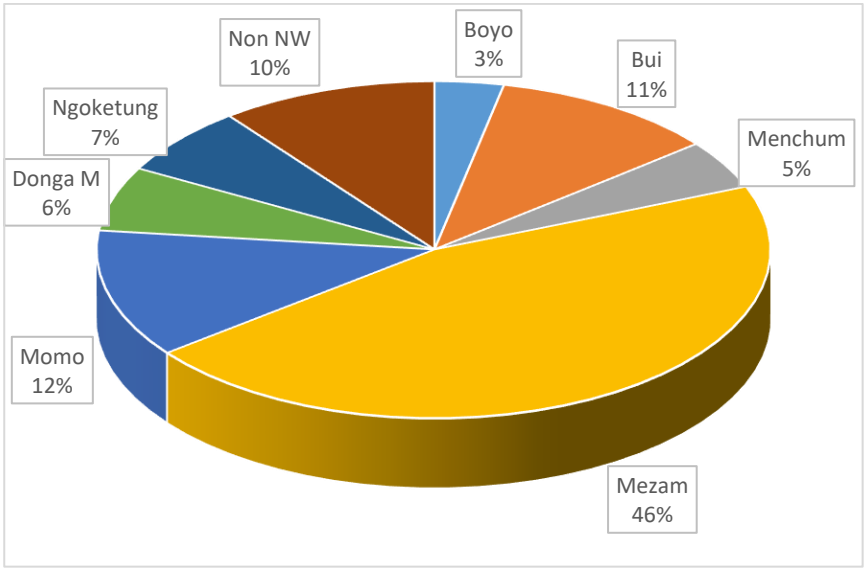

Figure 5: Frequency of Division of Origin for Males

3.4 Blood Group Distribution

ABO blood groupings were determined for the female study subjects with the most common blood type being 'O' 84(58%) and the least group AB 5(3%) (Figure 6). For the male 112 (53%) participants were in blood group 'O' and the least was blood group AB participants 7(3%) (Figure 7)

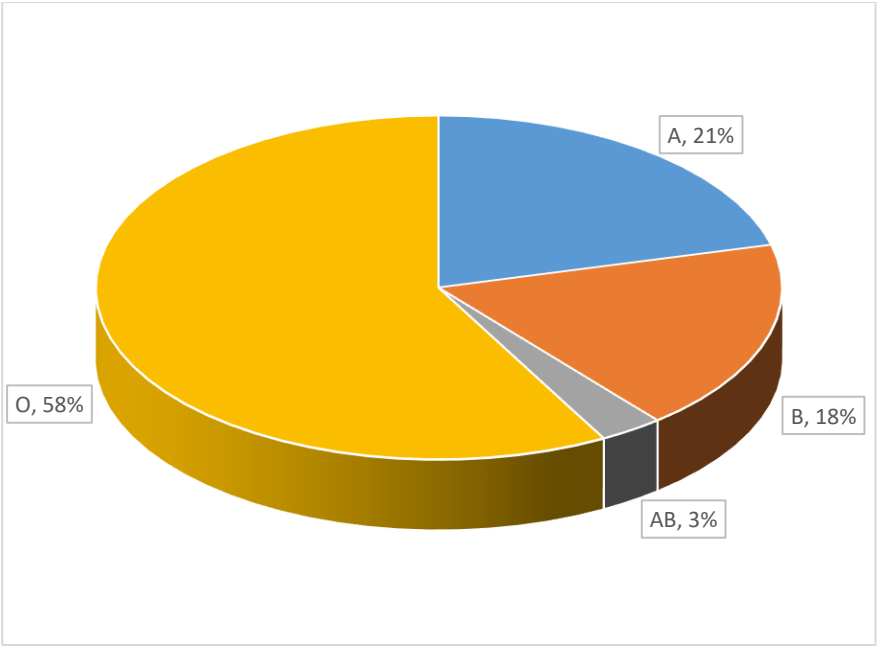

Figure 6: Frequency of Blood Groupings for Females

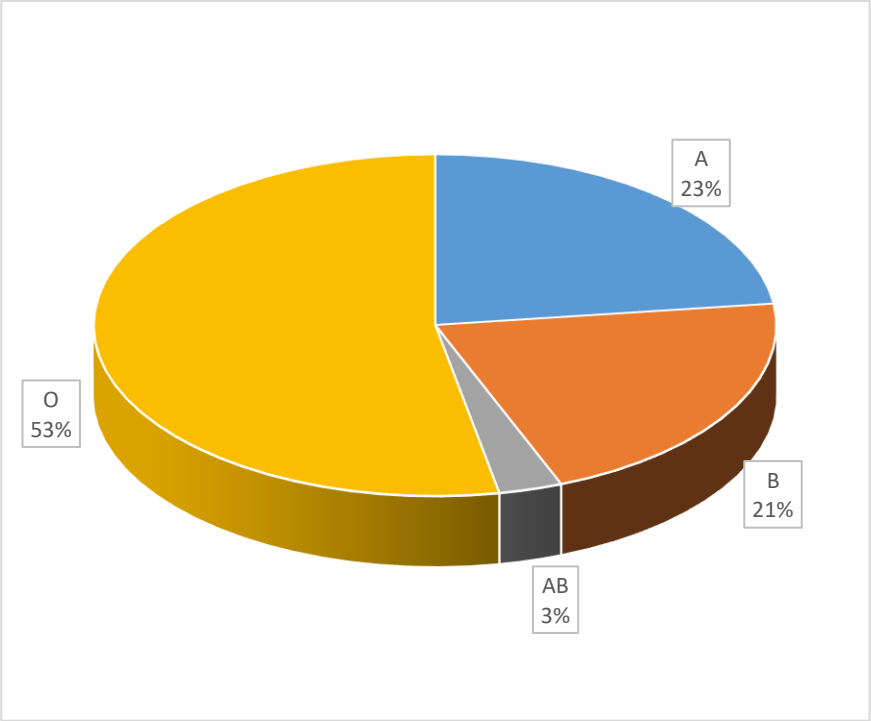

Figure 7: Frequency of Blood Groupings for Male

3.5 Hematological Parameters of the Study Subjects

3.5.1 Verification of the Values of the Manufacturer's Manual of the Hematology Analyzer

Table 1 and 2 shows the median, 95th percentile reference ranges for FBC established for, adult female population (n=145) and adult male population (n=211) compared with the values of the manual of the hematology analyzer currently in use.

For the females it was observed that only three out of the nineteen parameters passed the verification check. The remaining sixteen parameters showed that more than 10% of the population was out of the manufacturer's manual range with up to 80% and above in some parameters. The others especially RDW-SD, MPV, LYM%, HCT were 95%, 82.5%, 75% and 67.5% respectively out of the manufacturer's manual range. For the males the most glaring differences were seen in WBC, LYM%, MID%, GRAN%, RBC, HGB. The other parameters showed differences (though not so pronounced), which were equally of statistical significance.

Table 1: The Median, Local and Manufacturer's Reference Values for Adult Females

Parameter	Unit	Median (95% CI)	Reference values	Range on manual of the hematology analyzer	% out of range of manual of the hematology analyzer
WBC	10^3/uL	5 (4.0-5.0)	3.0-10	4.0-10	30
LYM%	%	43.6 (42.1-44.8)	27.2-62.38	20.0-40.0	75
MID%	%	8.1 (7.8-8.4)	5.05-13.42	1.0-15.0	5
GRAN%	%	47.6 (46.8-49)	31.02-72.74	50.0-70.0	65
LYM#	10^3/uL	2.2 (2.1-2.3)	1.04-4,86	1.0-4.1	7.5
MID#	10^3/uL	0.4 (0.4-0.4)	0.2-1.3	0.1-1.8	0
GRAN#	10^3/uL	2.4 (2.3-2.5)	1.2-5.2	2.0-7.8	30
RBC	10^6/uL	4.7 (4.6-4.8)	3.6-7.3	3.5-5.0	25
HGB	g/dL	12.9 (12.6-13.1)	8.8-19.24	11.0-15	22.50
HCT	%	36.6 (35.6-37.3)	25.1-55.6	37-43	67.50
MCV	fL	78.8 (78-80.3)	57.41-90.1	82-92	50.0
MCH	pg	27.6 (27.3-27.9)	20.09-33.46	27.0-31	50.0
MCHC	g/dL	35.2 (34.9-35.3)	28.6-38.66	32.0-36	22.50
RDW-CV	%	12.4 (12.3-12.6)	10.6-16.51	11.5-14.5	25
RDW-SD	fL	33.9 (29.7-34.7)	24.17-44.3	37.0-54	95
PLT	10^3/uL	281 (263-307)	102-656.9	100-300	37.50
MPV	fL	11.1 (10.7-11.8)	8.1-14.78	7.4-10.4	82.50
PDW	fL	12.2 (11.8-13.6)	8.6-16.96	10.0-14	42.50
PCT	%	0.29 (0.27-0.31)	0.1-0.76	0.1-0.28	47.50

GRAN#: Granulocyte concentration, **GRAN%:** Granulocyte percentage, **HCT**: Hematocrit, **HGB**: Hemoglobin concentration**, LYM#:** Lymphocyte concentration, **LYM%:** Lymphocyte percentage, **MCH:** Mean corpuscular Hemoglobin, **MCHC**: Mean corpuscular Hemoglobin concentration**, MCV**: Mean corpuscular volume, **MID#**: Mid-sized cell concentration, **MID%**: Mid-sized cell percentage, **MPV**: Mean Platelet concentration, **PCT**: Platelet crit, **PDW**: Platelet distribution width, **PLT**: Platelet concentration, **RBC**: Red blood cell ,**RDW-CV**: Red blood cell distribution width coefficient of variation, **RDW-SD**: Red blood cell distribution width standard deviation, **WBC:** White blood cell.

Table 2: The Median, Local and Manufacturer's Reference Values for Adult Males

Parameter	Unit	Median (95% CI)	Reference values	Range on manual of the hematology analyzer	% out of range of manual of the hematology analyzer
WBC	10^3/uL	4.9 (4.8-5.2)	2.86-8.54	4.0-10	18
LYM%	%	44.4 (42.7-45.5)	27.89-62.42	20.0-40.0	73
MID%	%	9.6 (9-10)	6-17.58	1.0-15.0	5
GRAN%	%	45.2 (43.5-47.3)	29.09-59.31	50.0-70.0	77.50
LYM#	10^3/uL	2.1 (2.1-2.2)	1.2-4.5	1.0-4.1	2.50
MID#	10^3/uL	0.5 (0.4-0.5)	0.2-1.1	0.1-1.8	0.0
GRAN#	10^3/uL	2.2 (2.1-2.3)	1.2-4.4	2.0-7.8	37.50
RBC	10^6/uL	5.4 (5.3-5.4)	4.4-7.0	4.0-5.0	80
HGB	g/dL	15.2 (15-15.4)	12.1-18.2	12.0-16	17.50
HCT	%	43.3 (42.4-44)	35.1-51.69	42.0-49.0	45
MCV	fL	80.2 (79.7-82.4)	63.21-89.81	82-92	57.50
MCH	pg	28.2 (28-28.7)	22.4-31.9	27.0-31	30
MCHC	g/dL	35.1 (34.8-35.2)	32.1-37.9	32.0-36	17.50
RDW-CV	%	12.5 (12.2-12.7)	10.4-15.4	11.5-14.5	37.50
RDW-SD	fL	33.9 (33-34.7)	24.8-43.7	37.0-54	80
PLT	10^3/uL	224 (217-231)	104.4-338.8	100-300	12.50
MPV	fL	12.5 (12.2-12.6)	8.33-14.1	7.4-10.4	82.50
PDW	fL	12.9 (12.2-13.3)	9.068-17.04	10.0-14	37.50
PCT	%	0.3 (0.25-0.28)	0.11-0.39	0.1-0.28	30

3.6 Differences in Reference Ranges by Blood Groups

The medians for the measured parameters according to blood groups of participants were determined at 95% confidence interval. There was no statistically significant variation among the observed values for the males ($p>0.05$). With the exception of MCH ($p=0.0372$), all other parameters were statistically insignificant when compared between blood

groups. The distribution of measured parameters among the male participants by blood groups was thus similar, and variations observed were negligible.

The female statistics for the ABO blood group showed that there was a statistically significant higher platelet value recorded in blood group AB compared to blood group A, B and O. The AB blood group also presented higher PCT% values ($p<0.05$) compared to the rest (Table 3).

Table 3: 95% CI Local Adult Female Reference Ranges According to ABO blood Groups

Parameter	Unit	Blood group A	Blood group B	Blood group AB	Blood group O	p-Value
		N=30	N=26	N=5	N=84	
		R.V	R.V	R.V	R.V	
WBC	10^3/uL	3.3-9.3	3.3-9.7	5.6-5.6	2.513-12.31	0.8906
LYM%	%	14.9-70.4	27.2-54.1	53-53	28.33-60.63	0.7239
MID%	%	4.7-12.4	0.2-11.9	6.0-6.0	5.448-15.01	0.3685
GRAN%	%	24.9-88.5	36-63.1	41-41	30.56-64.8	0.3575
LYM#	10^3/uL	0.5-4.7	1.3-5	3.0-3.0	1.4-5.4	0.7273
MID#	10^3/uL	0.2-1.2	0.2-1.2	0.3-0.3	0.2-1.3	0.8605
GRAN#	10^3/uL	1.3-4.5	1.3-4.9	2.3-2.3	0.8375-5.725	0.8165
RBC	10^6/uL	3.35-5.64	3.23-6.55	6.07-6.07	3.853-8.4	0.1838
HGB	g/dL	9.6-15.5	8.8-19.9	16.5-16.5	8.15-19.73	0.2476
HCT	%	26.5-48.9	25.1-55.6	48.8-48.8	22.66-63.65	0.229
MCV	fL	69.1-92	58.2-85.5	80.4-80.4	54.78-90.1	0.8801
MCH	pg	23.9-35.2	20.4-30.5	27.1-27.1	19.54-33.64	0.8645
MCHC	g/dL	31.6-38.3	33.1-36.8	33.8-33.8	7.238-38.98	0.7032
RDW-CV	%	10.7-15.1	10.6-16.3	11.7-11.7	10.44-18.98	0.6383
RDW-SD	fL	24.8-42.2	23.1-45.4	28-28	24.9-44.3	0.62
PLT	10^3/uL	143-579	64-422	733-733	108.5-757.3	0.0003*
MPV	fL	8.1-15.4	7.7-15.6	12.3-12.3	8.2-13.96	0.6459
PDW	fL	8.2-23	8.6-15.8	11.5-11.5	8.65-16.64	0.7357
PCT	%	0.2-0.6	0.09-0.42	0.9-0.9	0.1225-0.8763	<0.0001*

Where, N is the number of samples and R>V the reference values.

Table 4: 95% CI Local Adult Male Reference Ranges According to ABO Blood Groups

Parameter	Unit	A	B	AB	O	P value
WBC	10^3/µL	2.63-9.418	3.01-10.66	3.5-6.6	2.8-8.59	0.7226
LYM%	%	33.05-63.02	22.25-64.57	37.9-61.3	27.23-62.74	0.1311
MID%	%	6.7-20.38	5.24-17.16	8-9.7	5.525-17.99	0.5856
GRAN%	%	14.77-57.66	25.11-69.66	29-54.1	29.39-59.58	0.0968
LYM#	10^3/µL	1.41-4.73	1.305-5.48	1.5-4	1.105-4.19	0.4582
MID#	10^3/µL	0.21-1.09	0.2-0.995	0.3-0.6	0.2-1.1	0.5329
GRAN#	10^3/µL	0.91-4.32	1.11-4.395	1.2-3.2	1.4-4.48	0.7221
RBC	10^6/µL	4.531-6.066	4.544-6.242	5.32--6.98	4.341-7.107	0.1121
HGB	g/dL	12.11-44.44	13.2-16.9	13.9-17.5	12.1-18.4	0.2545
HTC%	%	34.02-53.09	38.02-51.1	41.1-49.6	34.72-52.19	0.9173
MCV	fL	67.41-93.32	37.03-89.87	61.4-80.6	62.29-89.37	0.1294
MCH	Pg	24.11-32.82	23.56-31.39	20.3-28.9	21.43-32.26	0.0372*
MCHC	g/dL	32.72-38.48	31.93-82.81	33.1-35.9	31.91-37.5	0.4076
RDW_CV	(%)	10.62-15.64	10.41-15.1	10.8-13.7	10.12-15.4	0.8277
RDW_SD	fL	25.6-43.76	24.8-45.3	24.8-30.6	23.95-44.26	0.168
PLT	10^3/µL	129.1-335.2	69.2-380.8	126-295	91.4-360.9	0.5864
MPV	fL	8.16-14.26	8.515-14.2	9.2-14	8.205-14.1	0.9378
PDW	fL	9.77-18.19	8.6-15.79	10.8-13.3	9.008-16.5	0.2112
PCT	%	0.1473-0.408	0.0835-0.4275	0.14-0.36	0.0848-0.39	0.8504

3.9 Differences in Reference Ranges by Divisions

The medians for the measured parameters according to divisions of the NW region was determined at 95% confidence interval. For the females the hematological reference ranges stratified by division showed that study subjects from Momo recorded statistically significant higher MCV, MCH, ($p<0.05$) to the rest of the divisions and Non-North Westerners (Table 5).

For the males there was no statistically significant variation among the observed values ($p>0.05$) except MCHC ($p=0.003$).

Table 5: 95% CI Reference Ranges for Hematology Parameters by Divisions Females

Parameter	Unit	Bui	Boyo	Donga-Mantung	Menchum	Mezam	Momo	Ngoketunjia	Non-NW	p-Values
		N=32	N=2	N=6	N=5	N=56	N=15	N=7	N=22	
		R.V	R.V	R.V	R.V	R.V	R.V	R.V	R.V	
WBC	10^3/uL	2.4-10.2	4.9-5.2	4.8-6.2	3.6-9.3	2.38-12.6	3.3-9.7	4.2-5.5	3.3-8.6	0.6221
LYM%	%	27.7-56.5	35.6-42.7	39.1-53	48.9-50.1	27.2-56.76	14.9-59.4	31.2-70.4	28.2-64.3	0.2734
MID%	%	5.6-13.1	9.5-10.5	6-10.0	5.7-12.4	4.925-15.54	5.7-11.9	4.7-6.4	0.2-15.2	0.0563
GRAN%	%	15.7-66.7	46.8-54.6	41-53	37.5-45.6	33.78-63.1	34.4-78.2	34.9-62.4	30.4-88.5	0.539
LYM#	10^3/uL	1.949-2.369	0.7294-3.271	1.964-2.736	1.4-3.4	2.216-2.745	1.869-3.184	1.36-3.04	2.059-2.851	0.5861
MID#	10^3/uL	0.2-1.3	0.5-0.5	0.3-0.6	0.2-1.2	0.2425-1.358	0.2-1.2	0.2-0.4	0.2-0.7	0.3235
GRAN#	10^3/uL	0.8-4.9	2.3-2.8	2.1-3.3	1.6-3.4	0.955-5.4	1.9-5.2	3-5.2	1.1-4.1	0.5143
RBC	10^6/uL	3.99-8.77	4.55-4.78	4.75-6.07	4.6-4.66	3.23-8.232	3.89-5.31	3.35-5.31	3.83-6.23	0.7906
HGB	g/dL	7.8-17.4	12.8-13.1	12.8-16.5	11.2-13.1	8.8-19.96	11.6-15	10.1-13.7	9.6-17.2	0.1401
HCT	%	21.7-49.7	35.7-38.9	36.1-48.8	32.2-39.8	25.1-67.56	30-45.2	28.6-36 8	26.5-48.9	0.1591
MCV	fL	54.5-84.4	74.7-85.5	73.1-84.2	69.1-86.7	65.09-90.5	68.5-90.1	58.2-92	69.3-89.9	0.0019*
MCH	Pg	19.5-30.7	26.7-28.7	25.9-28	24-28.4	22.15-34.33	25.8-31.3	20.4-35.2	23.8-33.2	0.0081*
MCHC	g/dL	4.2-37.9	33.6-35.8	33.3-35.4	24-28.4	28.59-38.92	33.1-38,5	34.2-38.3	32.9-40	0.4422
RDW-CV	%	10.4-19.3	10.2-13.1	11,7-14.2	11.7-14.3	10.6-16.11	10.9-14.2	11.6-16.3	10.7-15.1	0.1333
RDW-SD	fL	24.8-41.1	25.6-37.2	28-45.4	28.9-38	23.6-44.93	25.6-42,2	28.9-38.8	23.1-42.2	0.6196
PLT	10^3/uL	101-542	257-299	294-733	143-366	64-835.2	151-400	182-482	143-382	0.5357
MPV	fL	8.2-13.4	12.7-13.5	8.5-12.3	12.2-14.1	7.7-15.6	8.1-14	8.9-15.4	8.5-14.1	0.2043
PDW	fL	9.3-18	11.8-12.6	11.5-15.4	8.2-15.4	8.37-16.99	9.3-16.2	12.2-23	9.7-15.4	0.1181
PCT	%	0.09-0.5	0.34-0.37	0.249-0.9	0.2-0.4	0.09-0.93	0.2-0.42	0.21-0.428	0.14-0.45	0.8041

Where, N is the number of samples and R.V the reference values

Table 6: 95% CI Reference Ranges for Hematology Parameters by Divisions Males

Parameter	Unit	Bui	Boyo	Donga-Mantung	Menchum	Mezam	Momo	Ngoketunjia	Non-NW	p-Values
		N=23	N=7	N=13	N=10	N=96	N=26	N=14	N=22	
		R.V	R.V	R.V	R.V	R.V	R.V	R.V	R.V	
WBC	10^3/uL	2.8-10.1	2.8-6.1	31.4-63.1	3.7-7	2.828-9.033	4-8.1	3.6-7.6	3.2-6.8	0.8778
LYM%	%	28.3-64.8	36.1-63.7	6.2-18.8	31.4-56.8	23.81-61.75	27.2-59.6	37.6-55.4	35-61.3	0.3276
MID%	%	5.2-24.4	4.3-17.7	26.6-59.4	6-13.8	6.643-16.77	4.4-18	6.4-12.5	5.5-14.8	0.5636
GRAN%	%	24.7-57.7	29.3-59.6	1-4.6	32.4-62.3	30.69-62.68	31-56.8	34.3-51.2	13.1-58.4	0.2666
LYM#	10^3/uL	1.2-5.6	1.2-3.3	0.2-1	1.5-3	1.143-4.545	1.4-4.1	1.4-3.8	1.2-4	0.9845
MID#	10^3/uL	0.2-1.3	0.3-1	1.1-4.4	0.3-0.8	0.3-1.058	0.2-1.2	0.2-0.7	0.2-0.8	0.4032
GRAN#	10^3/uL	1.4-4.5	1.3-3.6	4.54-7.11	1.2-4	1.043-4.458	1.4-3.9	1.5-3.1	1.2-3.3	0.4715
RBC	10^6/uL	4.78-6.98	4.96-7.16	13-20.5	4.67-7.05	4.349-6.729	4.46-7	4.82-5.97	4.29-5.69	0.325
HGB	g/dL	14.2-47.4	13.4-16.7	36.5-54.8	13.7-16.7	12.04-18.15	12.7-18.3	14-17.8	12.1-17.1	0.2154
HCT	%	39.5-49.6	39-49.4	71.1-89.3	38.5-48.2	34.76-52.83	37-51.9	37.5-51.2	33.9-48.7	
MCV	fL	61.4-93.3	65.8-85.3	24.9-30.8	35.4-86.3	63.93-91.96	67.9-88.3	75.6-86.9	67.5-88.8	0.363
MCH	Pg	20.3-30.9	21.9-32.9	32-37.4	20.8-31.2	22.51-32.45	23.5-30.9	25.9-32.3	24.2-31.4	0.7807
MCHC	g/dL	31.9-37.4	33.3-38.6	11.5-14.8	33.9-36.5	32.01-37.06	31.9-37.5	33.9-85.1	33-36.6	0.003*
RDW-CV	%	10.1-15.7	11.3-13.8	27.3-42.2	11.8-15.1	10.23-15.65	10.6-15.4	10.5-14.7	11.2-15.4	0.2341
RDW-SD	fL	24.8-44.3	25.6-42.2	122-336	28.9-45.4	24.28-43.63	25.6-43.4	23.9-41.1	25.6-50.6	0.3352
PLT	10^3/uL	124-328	128-247	8.7-36.6	117-453	76.63-334.9	37-312	129-385	126-390	0.4812
MPV	fL	8-14.1	9.2-14.1	10-16.2	8.6-13.8	8.243-13.9	8.5-14.5	7.8-13.5	8.4-14.2	0.1936
PDW	fL	6.8-16.2	11.8-15.1	0.13-0.39	8.6-14.9	9.828-16.78	9.0-18.0	11.1-15.4	9.3-15.8	0.4738
PCT	%	0.15-0.41	0.13-0.26	0.249-0.9	0.14-0.38	0.0817-0.3858	0.05-0.4	0.156-0.43	0.14-0.39	0.9282

Where, N is the number of samples and R.V the reference value.

4. Discussions

The socio-demographic information of respondents provided the characteristics of the study population where the age range and gender of the sampled participant was between 18-60, coinciding with the expected range for blood donors in Cameroon. Data for this study was collected in Bamenda the headquarters of Mezam division, located in the central part of the region the reason why most of the participants came from Mezam.

A total of nineteen hematological parameters were tested in this study and showed significant differences among males and females as reported in other studies [15, 16, 17, and 18] (Table1 and 2). More than 10% of the female population presented results that were out of that of the accompanying manual of the hematology analyzer used in the study with the RBC parameters RDW-SD and HCT showing 95% and 67.5 % respectively out of range, the WBC parameter LYM%, 75% out of range and the platelets parameter MPV, 82.5% out of range suggesting the need for the establishment of new reference values. For the males the significant differences observed between the manual of the hematology analyzer for WBC, LYM%, MID%, GRAN%, RBC and HGB further suggest the establishment of a new reference range for the population under study.

Reference ranges for RBC count, hemoglobin, and HCT were higher among males compared to females (Table1and 2). This finding is consistent with reports from other studies in Africa [16, 19, 20, 21, 22] .The platelet count was higher in women than in men, consistent with studies by Kibaya et al. in 2008 [16], Mine et al. in 2011 [17], and Miri-Dashe et al. in 2011 [19]. This implies that a percentage of these participants could be wrongly classified as having erythrocytopaenia, anemia, and thrombocytopenia using the reference values that are often quoted. The gender-wise differences in these reference ranges may be attributed to the variations in the types of hormones produced and their corresponding concentrations in the different sexes as well as the effect of erythropoietin release in response to regular menstruation and cross-stimulating megakaryopoiesis [19, 21].

The reference ranges for total white blood cells including some differentials counts (LYM %) for the females that were above the upper limit of the manufacturers' manual reference range may be due to the high prevalence of parasitic infections and its associated leukocytosis and eosinophilia [23, 24].The reference range for GRAN% in the males that was below the lower limit of the accompanying manual of the hematology analyzer indicates that participants could be wrongly classified as neutropenic.

The statistically insignificant variation of reference values between blood groups for the males is consistent with studies by Al-Mawali et al., 2018 [25]. The statistically significant higher PCT% values ($p<0.05$ and Platelet value recorded in blood group AB for the females compared to blood group A, B and O is also consistent with studies by Kuriyan and Wells in 1995 [26].

The distribution of measured parameters among the participants by division was similar, and variations observed were negligible although female study subjects from Momo recorded statistically significant higher MCV, MCH, ($p<0.05$) compared to the rest

of the divisions and for the males there was no statistically significant variation among the observed values except MCHC (p=0.003). These variations of hematological reference ranges have also been observed in studies by Koram et al. in 2007 among participants North district in Ghana [27] and by Dosoo et al. in 2012 [24] among subjects from the middle belt of Ghana [20]. Other factors that could contribute to these differences are environmental and genetic factors or a combination of both or several other factors such as lifestyle differences between the participants from this region

5. Conclusion

This study established the hematological reference values in apparently healthy populations from seven divisions in Cameroon. Diversity in social, health status and geographical setting makes the dependence on pre-established hematological reference ranges from other countries that usually accompanying the hematologic analyzer inappropriate thereby leading to misdiagnoses resulting in wrong treatment and increased cost.

The laboratory reference ranges established in this study are one of the most comprehensive hematology data sets generated in the North West region of Cameroon. It is certain that lifestyle, physical, and genetic factors all affect the normal physiological processes of a population, and hence it is expected that there would be variations in the measurement of “normal” functions among and between populations. In spite of the factors influencing hematological values, this study permitted to establish the hematological reference values for use in the North West region. The median values are similar to the ones found in other studies performed in Africa. In the absence of previously established hematological reference values in the North West region these results could be used for clinical management of patients from the region. Further studies on hematological intervals for all age groups are recommended to ensure appropriate general health assessment, treatment monitoring, and efficient implementation of clinical trials.

5.1 Data Availability

All relevant data are within the article. The original data used to support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest Statement

The authors declare that there are no conflicts of interest.

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