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REFERENCE RANGES OF PLATELET VARIABLES AMONGST APPARENTLY HEALTHY ADULTS IN PORT HARCOURT, RIVERS STATE, NIGERIA

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ABSTRACT

In clinical medicine, laboratory testing results are of great value and reference ranges play vital role in assisting health professionals to interpret the results obtained. Since age, gender, ethnicity, social differences and environmental factors have been reported as factors which influence haematological blood parameters in different populations, this study was designed to establish baseline reference ranges for platelet count and platelet variables (MPV, PDM, and PCT) in the Niger Delta area of Nigeria. Fasting blood samples were collected from 300 apparently healthy 150 male and 150 female subjects whose ages ranged between the ages of 18-50 years. Platelet count and platelet variables were determined using Sysmex KX 21. Results shows that both lower and higher limits of the reference range (142-358 $x10^9$/L) obtained in the overall population were lower than the reference range (150-400$x10^9$/L) currently used by most laboratories. We also observed that female subjects had higher values compared to the males. Platelet count showed significant difference ($p<0.05$) in males and females, whereas platelet variables showed no significant difference $p>0.05$. This study also demonstrated similarity in results of PLT, MPV, PDW and PCT with values reported in previous studies using sysmex KX 21. Data from the present study further showed that reference ranges of platelet count and platelet variables varies due to factors such as gender and age. Further studies on platelets to ascertain the underlying causes of the difference within this population in the Niger Delta, Nigeria is thus suggested.

KEYWORDS: Platelet count, Platelet variables, and reference range.

INTRODUCTION

Platelets are the smallest cells among all the blood cells (red blood cells, white blood cells, and platelets).[1] In the field of medicine, the quantification of these blood cells is a well recognized tool for effective management of patients.[2] The evaluation of the range of platelet variables otherwise known as platelet indices is essential for the management of patients. Platelets play a crucial role in the regulation of haemostasis.[3]

Platelets interact with each other and even with leucocytes and endothelial cells in managing vascular integrity.[4] They have also been found useful in diagnosing various clinical conditions,[2] such as in inflammatory diseases, microbial host defence, cardiovascular disorders and cerebrovascular disease.[4,5]

The functional activity of platelets is a reflection of the changes in the size and function of the different platelet variables. With the availability of blood cell auto analyzers, these new platelet variables alongside platelet count can be estimated easily. Most important of the variables are mean platelet volume used to predict the

rate of production or destruction of platelets in the bone marrow[6] and platelet distribution width (PDW),used for the evaluation of the size of circulating platelets and changes in platelet activation. Plateletcrit (PCT) is used to evaluate the percentage volume occupied by platelet in the blood. It is the product of platelet count and MPV.[4]

It is well documented that baseline values for haematological blood cells vary according to different population groups and in different geographical areas due to certain factors such as age, sex, attitude, ethnicity, social differences, and environmental factors.[7-9] Infact, most hospitals and public health laboratories rely on reference values found in the literatures, textbooks, reagent inserts that accompany the reagent kits or instrument manuals of companies involved in the production and manufacture of reagents and equipments.[10] It is a known fact most of the reference ranges published in literatures were derived studies done in developed contries and especially on whites and these have also been shown to differ significantly from what is obtainable in most African countries including Nigeria.[7,8,10] The existing reference ranges used by

most laboratories for platelet count is 150-400x10^9/L, mean platelet volume (MPV); 7.2-11.7/fl, platelet distribution width (PWD); 10-17.9/fl and plateletcrit (PCT) 0.22-0.24%.[10]

The guidelines by International Federation of Clinical Chemistry (IFCC)[11] has long recommended that every country develop and establish reference ranges for biochemical and haematological parameters used in the diagnosis, monitoring and management of health.[11] Although health practitioners reocognise and acknowledged that ethnic and racial differences exist for most laboratory parameters, no efforts have been made to develop reference ranges that are specific for use in a multiethnic country like Nigeria as a whole and in various parts of the country like Rivers State in the Niger Delta. Due to paucity of studies on baseline haematological values in Nigeria, this study was designed to establish baseline reference ranges for platelet count and platelet variables (Platelet count, MPV, PDW and PCT) in the Niger Delta area of Nigeria.

MATERIALS AND METHODS
Subjects' selection
This study was carried out on a total of 300 apparently healthy adult (comprising of 150 males and 150 females) undergraduates and postgraduate students of the Rivers State University of Science and Technology, Port Harcourt, located in the Niger Delta region of Nigeria.

Ethical approval
Ethical approval was sought from the Research Ethics Committee, Rivers State University of Science and Technology, Port Harcourt. Blood samples were strictly and randomly collected from the subjects who willingly gave their consent to participate in the study.

Inclusion and exclusion criteria
Subjects were recruited into the study based on the following criteria:
- ➤ Those who were between the ages of 18-50 years, without any medical history of bleeding disorder, diabetes mellitus, and cardiovascular disease.
- ➤ Subjects who were not involved in too much alcohol, tobacco and cigarette consumption,
- ➤ Those who were not currently on drugs such as vitamins, iron drugs or even antibiotics at the time of the study.

- ➤ Subjects who have not donated or received blood for the past one year and
- ➤ Those who were had no surgery within the last six months.

Menstruating females, pregnant women and lactating mothers at the time of blood collection were obviously excluded.

Sample collection and analysis
A total of 300 fasting blood samples were collected from (150) males and (150) females within the hours of 8.00-11.00am in the morning. Five millilitres (5mls) of venous blood was taken from a prominent peripheral vein on the arm of each subject and immediately transferred into sterile K_2 EDTA anticoagulant bottles and was mixed properly according to the procedure described by Dacie and Lewis.[12]

Laboratory determinations
Within 3 hours of sample collection, platelet count, mean platelet volume, platelet distribution width and plateletcrit (PC, MPV, PDW) were analyzed using an automated machine SYSMEX, manufactured by KOBE, Japan, model no: KX-2IN. Platelet count was confirmed manually using the improved Neubauer counting chamber.

Statistical Analysis.
Data analysis was performed using the GraphPad Instat Version 3.10, 12 bit for Windows. The analysis includes the computation of the means and standard deviation. Comparison of the means was done using the one way analysis of variance (ANOVA). The Turkey-Kramer comparison test was used to verify significant difference between the age classes in the male and female subjects. Comparison between the means of the parameters in the male and female subjects was done using student t-test. Mean values were considered significant at $p<0.05$.

RESULTS
The comprehensive result consisting Mean ±SD and reference values of platelet variables which are platelet count, PCT, MPV and PDW obtained from all the analysis for both males and females with their age differences is shown in the tables below.

Table1: The mean ±SD and reference values of PLT, PDW, MPV and PCT in the overall population (males and females combined).

Variables	N	Mean	SD	Min	Max	Reference Range
Platelets X 10^9/L	300	250	53.8	134	399	142 -358
MPV/fl	300	9.9	0.84	6.7	10.2	8.2 -12.0
PDW/fl	300	13.1	1.85	3.6	17.8	9.4 -16.8
PCT%	300	0.25	0.04	0.05	0.51	0.17 -0.33

The mean ±SD of platelet count obtained in this study was (250±53.8x10^9/L) and the reference range was (142-358 x10^9/L).It was observed that both the lower and

higher limits of the reference values obtained in the study (150-400 x 10^9 /L) are lower than the reference ranges that is currently used by most laboratories as their

standard reference values. The reference range of MPV corresponds with the existing reference range currently in use. The result of the study also showed that PWD and plateletcrit had values which did not correspond with the reference ranges currently existing in most local laboratories in River State, Niger Delta region of Nigeria. The reference ranges of PDW in this study was 9.4- 16.8/fl, compared to the existing reference range of 10-17.9/fl. Similarly, PCT had a lower value 0.17-0.33% at the lower limit and also a higher value at the upper limit of the reference range compared to the existing reference range of 0.22-0.24% which is currently used by most local laboratories.

The reference values for PLT, PDW, MPV and PCT in the females are 157 -383 x 10^9/L, 8.9-14.5/fl, 8.2-12.1/fl and 0.13 -0.47%, while that of the males are 120 -326 x 10^9/L, 9.70 -15.9 fl, 7.32 -11.7 fl, 1.00 -0.38% respectively (table 2). The comparison of the means of the platelet count (PLT) and plateletcrit (PCT) between the male and female populations showed significant difference ($p<0.05$) respectively. Similarly, the comparison of the means of MPV and PDWshowed no significant difference ($p>0.05$) respectively.

The reference values of PLT, PDW, MPV and PCT in the males of 18-24 years were 123-347 x 10^9/L, 8.0-13.1/fl, 7.4 - 9.3/fl, and 0.14-0.26 % respectively. For 25-29 years, the reference values of PLT, PDW, MPV and PCT were 117-277x10^9/L, 10.3-16.0/fl, 6.0 - 8.4/fl and 0.10-0.18% respectively. The reference values of PLT, PDW, MPV and PCT for 30-35 years were 146-346 x 10^9/L, 10.4-16.8/fl, 7.2-10.4/fl and 0.14-0.30 respectively. Lastly for the 36-50 years age class, the reference values of PLT, PDW, MPV and PCT were 138-384 x10^9/L, 12.0-16.4/fl, 7.3-10.3/fl and 0.13 – 0.33%. Comparison of the means using one-way analysis of variance shows that there is a significant difference ($p<0.05$) in the platelet count in all the age classes, and no significant difference ($p>0.05$) was seen in the other variables in all the age classes (table 3).

Table: 2 The mean ±SD and the reference values of PLT, PDW, MPV and PCT of the male and female subjects.

Variables	N/300	Mean	SD	Mean-2SD	Mean+2SD	Reference Range	t –value	p-value
Platelet X 10^9/L	F	270	56.7	157	383	157 -383	t=3.870	P<0.05,*
	M	223	51.3	120	326	120 -326		
MPV/fl	F	10.2	0.98	8.2	12.2	8.2-12.1	t=1.528	P>0.05
	M	9.71	1.00	7.3	14.5	7.32 -11.7		
PDW/fl	F	11.7	1.40	8.9	10.7	8.9-14.5	t =1.585	P>0.05
	M	10.8	0.95	8.9	0.47	8.9 -12.7		
PCT %	F	0.28	0.05	0.13	0.38	0.13 -0.47	t=2.851	P<0.05*
	M	0.21	0.05	0.10		0.10 -0.38		

TABLE 3: REFERENCE VALUES OF PLATELETS COUNT/PLATELET VARIABLES OF THE MALE SUBJECTS IN ALL AGE CLASSES (18-50 YEARS)

Age classes	Platelet count/platelet Variables											
	PlateletX10^9/L			MPV/fl			PDW/fl			PCT (%)		
	Mean	SD	Ref Range	Mean	SD	Ref. Range	Mean	SD	Ref. Range	Mean	SD	Ref. Range
18-24(n=25)	235	56.1	123-347	8.3	0.48	7.4 - 9.3	10.3	1.4	8.0-13.1	0.20	0.03	0.14-0.26
25-29(n=28)	197	40.0	117-277	7.2	0.61	6.0 - 8.4	13.1	1.4	10.3-16.0	0.14	0.02	0.10-0.18
30-35(n=6)	246	50.2	146-346	8.8	0.82	7.2-10.4	13.6	1.6	10.4-16.8	0.22	0.04	0.14-0.30
36-50(n=8)	261	61.3	138-384	8.8	0.74	7.3-10.3	14.2	1.1	12.0-16.4	0.23	0.05	0.13-0.33
P=Value	P<0.05*, F=2.413			P.>0.05, F=1.878			P>0.05, F=1.902			P>0.05, F=1.975		

Key:* significance, Mean-2SD to Mean+2SD = Reference Range

TABLE 4: REFERENCE VALUES OF PLATELETS COUNT/PLATELET VARIABLES OFTHE FEMALE SUBJECTS IN ALL AGE CLASSES (18-50 YEARS)

Age classes	Platelet count/Platelet Variables											
	PlateletX10^9/L			MPV/fl			PDW/fl			PCT (%)		
	Mean	SD	Ref. Range	Mean	SD	Ref. Range	Mean	SD	Ref. Range	Mean	SD	Ref. Range
18-24 (n=25)	298	78.8	140-456	8.2	0.68	7.0-9.6	13.3	1.8	9.7-16.9	0.24	0.05	0.14-0.34
25-29 (n=28)	274	70.3	133-414	9.0	0.71	7.6-10.4	11.8	2.1	7.6-16.0	0.25	0.05	0.15-0..35
30-35 (n=6)	245	66.2	134-356	9.8	0.82	8.1-11.4	12.6	1.9	8.8-16.4	0.24	0.05	0.14-0.34
36-50 (n=8)	258	61.9	134-382	8.8	0.74	7.3-10.3	12.2	1.7	8.8-15.6	0.23	0.05	0.13-0.33
p-value	p>0.05, F=1.846			p>0.05, F=1.589			p>0.05, F=1.940			p>0.05, F=1.893		

Key   Mean-2SD to Mean+2SD = Reference Range

TABLE 5: COMPARISON OF PLATELET VARIABLES WITH RESULTS FROM OTHER PUBLISHED STUDIES

Population	Instrument	Sex	N	PLT X 10^9/L	MPV/fl	PDW/fl	PCT%	Author
Iranian	Technicon H*2	M/F	993	145-356	7.4-10.7	*40-57%	0.13-0.32	Adibi (et al.,2006)
German	Sysmex XE-5000	M/F	309	166-390	9.1-12.1	9.9-16.1	0.17-0.39	Pekelharing (et al.,2010)
South African	Sysmex XE-2010	M/F	60	178-429	8.8-12.5	9.3-16.0	0.19-0.40	Botma (et al., 2012)
Sudanese	Sysmex KX-21	M/F	300	146-378	8.2-11.6	8.3-15.9	0.13-0.34	Abass (et al.,2016)
Nigerian	Sysmex KX-21	M/F	300	142-358	8.2-12.0	9.4-16.8	0.17-0.33	Present study.

The reference values of PLT, PDW, MPV and PCT in the females of 18-24 years were 140-456 x 10^9/L, 9.7-16.9/fl, 7.0-9.6/fl, and 0.14-0.34% respectively. For 25-29 years, the reference values of PLT, PDW, MPV and PCT are 133-414 x10^9/L, 7.6-16.0/fl, 7.6-10.4/fl and 0.15-0.35% respectively. The reference values of PLT, PDW, MPV and PCT for 30-35 years shows 134-356 x10^9/L, 8.8-16.4 /fl, 8.1-11.4/fl and 0.14-0.34% respectively. Lastly for the 36-50 years age class, the reference value of PLT, PDW, MPV and PCT are 134-382 x10^9/L, 8.8-15.6/fl, 7.3-10.3/fl and 0.13-0.33%. Comparison of the means using one-way analysis of variance shows that there is a significant difference at $p<0.05$ for the PLT, PDW and MPV in all the age classes and no significant difference ($p>0.05$) seen in the PCT in all the age classes (table 4).

The comparison of platelet variables measured with Sysmex KX-21 in this study could not be effectively done with those measured with other autoanalyzers such as Technicon H*2, Sysmex XE-5000 and Sysmex 2010 because with these different instruments, the results were obtained in different units. While the Sysmex autoanalyzers measured the platelet variables in femtoliters, the Technicon autoanalyzer measure these variables in percentage (table 5).

DISCUSSION
In clinical medicine, laboratory testing results are of great value and reference ranges play vital role in assisting health professionals to interpret the results obtained. Therefore, reference ranges are considered the most authoritative tools in laboratory science. The word reference ranges are defined as the set of values which consist of 95% of the normal healthy population.[13] From the analysis made in this study which consist of female and male populations between the age of 18-50 years, it was observed that both the lower and higher limits of the reference values (142-358 x10^9/L) obtained for platelet count were lower than the reference values (150-400 x10^9/L) which is currently used by most laboratories in Rivers State as their standard reference ranges. However, the lower limit of the MPV value (8.2-12.0/fl) obtained in this study is slightly higher than the lower limit of reference range curently in use (7.2-11.7/fl) currently in use. Similarly, the values for PWD and plateletcrit (9.4-16.8/fl and 0.17-0.33%) respectively is slightly different from the existing reference ranges of 10-17.9/fl and 0.22-0.24% respectively being used in most local laboratories in River State, Niger Delta region of Nigeria.

It has been reported that the baseline values for haematological blood cells vary according to different population groups and in different geographical areas due to certain factors such as age, sex, attitude, ethnicity, social differences and environmental factors.[7-9] The lower ranges recorded in this study could be associated to this differences in age, sex, attitude ethnicity, social differences, environmental factors and even dietary factors and influences. The study showed a significant difference ($p<0.05$) in platelet count and plateletcrit between male and female subjects. Females had higher platelet count and plateletcrit reference ranges (157-383 x10^9/L and 0.13-0.47%) respectively than the males (120-326 x10^9/L and 0.10-0.38%) respectively. Their mean platelet volume and platelet distribution width showed no significant difference at $p>0.05$. Our results demonstrate that there is gender difference between healthy males and females and this supports the report of previous study that females have higher platelet count than the males.[14]

Comparing the reference values of platelet counts and platelet variables, MPV, PWD and PCT in the different age classes studied, it is interesting to note that among the age classes in the females there was a significant difference ($p<0.05$) for platelet count. The difference in reference ranges for these age classes were observed in the ages between 30-50 years which had lower reference ranges compared to ages 18-29. Physiologically, the age class of 30-50 is a very active age class when child birth and other multi-tasking activities which could affect the hormonal profile of individuals takes place. Abass et al.,[15] reported that differences in hormonal profile could be responsible for the gender differences in platelet count and platelet variables. This is also in line with the report of Kadikoylu et al.[16] on gender-related differences in platelet variables. They further stated that platelet counts increase in women with iron deficiency anaemia and that iron saturation is an important factor that affects platelet counts.

Results of the reference ranges obtained by the age classes for the males, shows significant difference ($p<0.05$) in the platelet count (117-277 x10^9/L) among the age group of 25 -29. The values of the the other variables; MPV, PWD and PCT at the same age 25-29 were low compared to that of the other age classes and showed no significant difference ($p>0.05$). Balduini et al.[16] had reported that ethnicity affects platelet counts as much as age and sex. Thus, the result of this study suggest that different populations should generate their

reference ranges instead of a single reference value being used by all laboratories in the whole world. Another interesting observation from this study is that in both males and females and in all the age classes, the lower limits of PCT showed a shift to the left from the currently used values. Thus, geographical location and ethnicity as reported by Horn *et al*[8]. could have contributed in influencing the result obtained in this study.

A comparison of the reference ranges of all the platelet count and platelet variables with works done by Pekelharing *et al.*[18] using Sysmex XE-5000, Botma *et al.*[19] with Sysmex XE -2010 and Abass *et al.*[15] who used Sysmex KX-21 showed that the reference ranges obtained in this study compared favourably with values obtained with Sysmex KX-21. It was observed in our study that except in PCT where the lower limit of the reference range showed a little high value, platelet count, MPV and PWD compared very well with the study done by Abass *et al.*[15] It may seem reasonable to state that same type of equipments also give similar results in haematological determinations especially if the equipments are used within the same location.

However, it was observed that some of the results could not be compared with others because different instruments were used for the analysis and the results obtained are in different units. While the sysmex autoanalyzers measures the platelet variables in femtoliters, the Technicon autoanalyzer measure these variables in percentages. Furthermore, it is worthy to note that the different results obtained are from different continents and countries though from male and female subjects, and that accounts for the lower and higher limits of the values compared to the existing reference ranges currently in use by local laboratories.

CONCLUSION AND RECOMMENDATION.

Data from the present study shows that reference ranges of platelet count and platelet variables varies due to factors such as gender and age. The fact that the reference values of platelet count and plateletcrit obtained in this study were higher in the female subjects than their male counterparts suggest that different reference ranges of these parameters should be used for male and female patients in our medical and health care delivery systems in the interpretation of laboratory results relating to these parameters. The reason for the difference in the value of the platelet counts in the male subjects between the age classes of 25-29 in this population is unknown, however, it is pertinent and timely to suggest further studies on platelets to ascertain the underlying causes of the difference within this population in the Niger Delta, Nigeria.

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