Reference Value of Platelets Count and Indices in Sudanese Using Sysmex KX-21

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Abstract: **Platelets indices have been recently reported as clinically useful biomarkers. In most clinical laboratories in Sudan; platelet indices are not reported/or evaluated, this could be of lack of knowledge about their availability, importance, and local laboratory reference values. The study aimed to determine the Sudanese reference value for platelet count and indices, to investigate whether a significant age/gender difference are exists. A total of 300 apparently healthy Sudanese; 150 males and 150 females, their ages range between 10 and 60 years; categorized in 5 groups. A full blood count was performed using Sysmex KX-21 blood analyzer and the platelet count and indices were recorded. The following reference values were obtained for platelet count – males $130\text{-}357 \times 10^3$ /µl , females $146\text{-}378 \times 10^3$/µl; mean platelets volume (MPV) – males 8.4-11.4 fl, females 8.0-11.6 fl; platelets distribution width (PDW) – males 8.7-15.7 fl, females 7.8-16.2 fl; platelets -large cell ratio (P-LCR) – males 12.7-36.1%, females 10.9-38.8%; plateletcrit (PCT)–males 0.14-0.24%, females 0.13-0.33%. A significant difference between males/females and ages below/over 35 years were obtained for platelet count (p: 0.0001, 0.0001) and PCT (p: < 0.0001, 0.0006); respectively, while other indices did not show significant differences. This study illustrated the importance of determining local reference values, because populations, reagents and instruments may differ from published reference values. These reference values pave the way for the clinicians to apply these indices in the investigations of disease states.**

Keywords: **Normal, range, Platelet, volume, indices, Sudan.**

I. INTRODUCTION

Automated hematology analyzers have contributed to more accurate, precise, and faster results. [1].They also made it possible to determine other parameters that had not previously been measured [2]. Sysmex KX-21 one of these blood analyzers is wide commonly used in most of clinical laboratories in Khartoum, it is used to determine full blood counts including platelet counts and its volume indices. Platelets indices affected by a wide range of variables that interfere with its value [3]. Evidence exists that reference values indicated by some manufactures may be unsuitable for every laboratory and therefore local laboratory reference values for platelet indices should be established [1], [4], [5].The importance of determining local references values stems from the observation that laboratory machines may be using different measuring principles and population dynamics vary [5].

Platelet indices including mean platelet volume (MPV), platelet distributing width(PDW), platelet large cell ratio (P-LCR) and plateletcrit (PCT) offer valuable information about the morphology and maturity of platelets. MPV is a measurement of the average size of platelets in blood [5].The higher MPV is a larger platelets size. PDW reflects the variability in the platelets size [6] and it's therefore increased in the presence of platelets anisocytosis. PCT is an expression of a percentage that reflects the volume occupied by platelets in blood [7]. PCT is directly related to the platelet count and the size of the platelets [8]. It has been suggested that platelet indices are potentially useful markers for the early diagnosis of many types of diseases, can play a role in the rapid evaluation of bone marrow activity of patients with platelet-associated disorders [9,] [10], [11], [12], [13], [14], [15]. MPV reflects an atherosclerothrombic tendency in the human body [16,] [17], [18]. MPV and P-LCR are indicators of active bone marrow [19], [20]. On the other hand, PCT and PDW can be used to differentiate reactive thrombocytosis from myeloproliferative disorders [11], [13], [21], [22].

These parameters are now available, but they are not reported in most clinical laboratories in Sudan because clinicians are not aware that they are available, and the reference ranges with which the patients' results should be interpreted are not known. Thus, the present study aimed to establish Sudanese reference ranges for platelet count and its volume indices, to investigate whether there is a significant gender and age differences.

II.   MATERIAL AND METHOD

Ethical approval was obtained from the Research Ethics Committee at FMLS-Khartoum University. A cross sectional study was carried out in Khartoum state among apparently healthy Sudanese during a period of January to April 2014. A written informed consent was obtained; demographic data were recorded in a questionnaire. Individual with any condition affecting platelets number or function was excluded. A total of 300 subjects were included from different schools/universities, private/governmental institutions, and different companies, 150 males and 150 females with different age groups, their ages ranged between 10 and 60 years old. Multistage sampling method was followed; study population was categorized in 5 age groups, each group consists of equal numbers of subjects for both genders.

From each enrolled subject EDTA- K3 venous blood sample (3 ml) was collected, mixed gently, and was delivered within 30 minutes hour of collection for laboratory analysis. Platelets count including platelets indices was performed as part of full blood count by automated procedure using Sysmex KX-21 blood cell analyzer. The whole blood mode was selected to analyze blood sample without pre-dilution .

The results data was collected analyzed using Microsoft office Excell (2007) and SPSS program and presented in tables and figures. Reference value was calculated as mean-2SD to mean+2SD, simple T-test was used to compare the reference value of platelets count and platelet indices between both genders and different age groups.

III.   RESULTS

The full blood counts of 300 individuals (150 male and 150 female) between the ages of 10 and 60 year were analyzed on the Sysmex KX-21 blood cell analyzer to obtain the platelet count and platelet indices (PDW, MPV, PCT, and P-LCR).

A. Platelet count and platelet indices:

Means, standard deviations and reference values of platelet counts and indices of the entire study population is presented in TABLE 1. It should be noted that the study population's reference value for platelet count (146 – 378 x 10^9/L) is approximately similar to the reference value that is in current use (150 – 450 x 10^9/L).

B. Platelet count and platelet indices in regard to gender:

In TABLE II, the reference values are listed separately for males and females and the p-value is noted to indicate whether the difference is significant ($p \leq 0.05$) between male and females. It can be noted that the females' value (161-399 x 10^9/ L) for platelet count is perfectly within the reference value currently in use at local laboratories, while in the platelet count's reference value of male participants (130-357 x 10^9/ L) the upper and lower limits were below than that in current use. The platelet count and PCT reference values between males and females were both significant with p-values of 0.0001 and 0.0001, respectively. MPV, PDW, and P-LCR did not show a significant difference.

C. Platelet count and platelet indices in regard to age:

In TABLE III, the reference values are listed separately for subject below 35 year of age and over 35 year of age and the p-value is noted to indicate whether the difference is significant ($p \leq 0.05$) between subjects below/over 35 years old age. It can be noted that the subject <35 year of age the range (150-401 x 10^9/ L) for platelet count is perfectly within the reference value currently in use at local laboratories, while in the platelet count's reference value of the subject >35 year of age participants (146-358 x 10^9/ L) the upper limit was below than that in current use. The platelet count and the PCT reference values between subjects below/over 35 years old age were both significant with p-values of 0.0001 and 0.0006, respectively. MPV, PDW, and P-LCR did not show a significant difference.

D. Comparison of platelet indices results with other published results:

The findings of present study compared well with those of others published in the literature though there are slight differences, TABLE IV. It must be considered that the different instruments give different results. Technicon blood analyzer measures PDW as percentage and not as femtoliter as other instruments, thus in some cases the results cannot be compared with other instruments' measurements. Results are from different continents of the world and the study population varies from one study to the next.

IV.   DISCUSSION

The study populations' ages ranged from 10 to 60 year and comprised both males and females. Sudanese have genetic mixure of afro-arab race. We categorized the study population into groups according to their ages range. We analyzed the platelets count and indices by using automated blood analyzer Symex KX-21 and compared the results of platelets count and indices in both genders and different age groups to find any statistical significant differences, TABLE II, III, and Fig. 1.

The significant difference between the genders for platelet count and PCT results is in line with what has been published in the literature [23], [24]. A study done by Pekelharing et al [25] found significant difference between females and males with $p < 0.0001$. The difference can possibly be ascribed to the different hormonal profiles amongst the gender [26]. Another study done by Botma et al [2] found significant difference between males and females for platelet count and PCT (p- value <0.05). The present study also revealed a significant differences of platelet count and PCT in ages below and over 35 year old; which in line with a study of Giacomini et al [23] who divided their study subjects into groups according to age, for example 1-10, 18-45 and 45-65, and found a difference among the platelets and different platelet indices during different life stages. Platelets count and PCT decrease from childhood but remain steady afterwards.

In this study the MPV reference value of 8.4-11.4 fl was found for males and 8.0-11.6 fl for females. These reference values compared well with other studies[4], [23].Wakeman et al [4] determined reference values for MPV separately for males and females on the Sysmex XE-2100 and concluded that there was no significant difference between males and females ($p = 0.426$), which confirms this study's findings as declared in TABLE II. In a study done on the Sysmex XE-5000 which is similar to the Sysmex KX-21, the difference in MPV reference value for males and females was found to be significantly different with a p-value of 0.0006 [25].This may be due to the genetic differences among the populations, as the instruments used similar measuring principle.

The reference values of PDW in both male 8.7-15.7 fl and female 7.8-16.2 fl compared well with those published in the literature[15], [25]. Botma et al [2] have reference values for PDW, males 9.3-14.3fl and females 9.8-16.0 fl, that closely resemble that of this study's population. They found no significant difference in PDW between males and females as in our study results. The literature describes several other studies that were performed to determine PDW reference values, but the results are expressed as percentages, probably due to different analyzers being used (e.g. Technicon H*2) and can thus not be compared to results found in this study [27].

In the present study the P-LCR reference value of 12.7 - 36.1% was found for males and 10.9 - 38.8%; with no significant difference. Pekelharing et al [25] determined reference ranges for P-LCR separately for males and females on the Sysmex XE-5000 and concluded that there was significant difference between males and females ($p = 0.0004$), this could be of the genetic differences among the populations studied and the difference in measuring principle of the instrument used.

V.   CONCLUSION

The reference values obtained in this study compared well with the results in the literature. There was a significant difference for platelet count/PCT between males/females and ages below/over 35 year. However, gender and age have no significant effects on platelet indices. These established local reference values will enable the laboratory to effectively use the blood cell analyzer and provide a better service to clinicians who could use these results to provide better care to patients.

REFERENCES

[1]   Farias MG, Schunck EG, Dal Bó S, de Castro SM (2010) Definition of reference ranges for the platelet distribution width (pdw): a local need. Clin Chem Lab Med; 48(2):255-7.

[2]   Botma J Mogongoa LF Jaftha AD, Janse van Rensburg W (2012) Reference ranges for platelet indices using Sysmex XE -2100 blood analyser. Med Tech S Afr; 26(2): 17-21.

[3]   Wendland AE, Farias MG, Manfroi WC (2009) Volume plaquetario medio e doenca cardiovascular.J Bras Patol Med Lab; 45(5):371-278.

[4]   Wakeman L, Al-Ismail S, Benton A, Beddall et al (2007) routine haematology reference ranges for healthy adults. Inter J Lab Haem; 29:279-283.

[5] Lewis SM, Bain BJ & Bates I, "Dacie and Lewis Practical Haematology" 10th ed., Philadelphia: Churchill Livingstone, 2006.

[6] Amin MA, Amin AP & Kulkarnite HR (2004) Platelet distribution width (PDW) is increased in vaso-oclusive crisis in sickle cell disease. Anna Hem; 83(6):331-335.

[7] Beyan C, Kaptan K & Ifran A (2006) Platelet count, mean platelet volume, platelet distribution width, and plateletcrit do not correlate with optical platelet aggregation responses in healthy volunteers. J Thrombo & Thrombol; 22:161-164.

[8] Wiwanitkit V (2004) Plateletcrit, Mean platelet volume, Platelet distribution width: Its expected Values and Correlation with Parallel Red Blood Cell Parameters. Clin Appl Throm/ Hemos; 10(2):175-178.

[9] Briggs C, Harrison P & Machin SJ (2007) Continuing developments with automated platelet count. Inter J Lab Hem; 29:77-91.

[10] Chandra H, Chandra S, Rawat A& Verma SK (2010) Role of mean platelet volume as discriminating guide for bone marrow disease in patients with thrombocytopenia. Inter J Haem lab; 32(5):498-505.

[11] Numbenjapon T, Mahapo N, Pornvipavee R, et al (2008) A prospective evaluation of normal mean platelet volume in discriminating hyperdestructive thrombocytopenia from hypoproductive thrombocytopenia. Inter J Lab Hem;30(5):408-414.

[12] Bowels KM, Cooke LJ, Richards EM & Baglin TP (2005) Platelet size has diagnostic predictive value in patients with thrombocytopenia. Clin Lab Haem; 27:370-373.

[13] Song YH, Park SH, Kim JE, et al (2009) Evaluation of platelet indices for differential diagnosis of thrombocytosis by ADVIA 120. Kor J Lab Med; 29(6):505-509

[14] Cremer M, Paetzold J, Schmalisch G, Hammer H, Loui A, Dame C & Weiman A (2009) Immature platelet fraction as novel laboratory parameter predicting the course of neonatal thrombocytopenia. Brit J Haem; 144(4):619-621.

[15] Kaito K, Otsubu H, Usui N, et al (2005) Platelet size deviation with, platelet large cell ratio, and mean platelet volume have sufficient sensitivity and specificity in the diagnosis of immune thrombocytopenia. Brit J Haem; 128:698-702.

[16] Khandekar MM , Khurana AS, Deshmukh SD, Kakrani AL, Katdare AD, and Inamdar AK (2006) Platelet volume indices in patients with coronary artery disease and acute myocardial infarction: an Indian scenario. J Clin Path; 59.2: 146-149.

[17] Huczek Z, Kochman J, Filipiak KJ, et al (2005) Mean platelet volume on admission predicts impaired reperfusion and long-term mortality in acute myocardial infarction treated with primary percutaneous coronary intervention. J Am Coll Cardiol; 46:284–90

[18] Khode V, Sindhur J, Kanbur D, Ruikar K, and Nallulwar (2012) Mean platelet volume and other platelet volume indices in patients with stable coronary artery disease and acute myocardial infarction: A case control study. Journal of Cardiol Dis Res; 3(4): 273.

[19] Briggs C, Kunka S, Hart D, Oguni S & Machin SJ (2004) Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia. Bri J Haem; 126:93-99.

[20] Threatte GA (1993) Usefulness of the mean platelet volume. Clin Lab Med; 13 (4):937-950.

[21] Mohr R, Martinowitz U, Ayala L, Goor DA & Ramot B (1986) Platelet size and mass as an indicator for platelet transfusion after cardiopulmonary bypass. Circulation; 74(5Pt 2):153-158.

[22] Saigo K, Takenokuchi M, Imai J, et al (2009) Usefulness of immature platelet fraction for the clinical evaluation of myelodysplastic syndromes. Lab Haem; 15(2):13-16.

[23] Giacomini A, Legovivi P, Gessoni G, et al (2001) Platelet count and parameters determined by the Bayer ADVIA TM 120 in reference subjects and patients. Clin Lab Haem; 23:181-186.

[24] Lawrie D, Coetzee LM, Becker P, Mahlangu J, Stevens W &Glencross DK (2009) Local reference ranges for full blood count and CD4 lymphocyte count testing. S Afr Med J; 99:243-248.

[25] Pekelharing JM, Hauss O, De Jonge R, et al (2010) Haematology reference intervals for established and novel parameters in healthy adults. Sysmex Diagnostic Perspectives; 1:1-11.

[26] Bain BJ (1985) Platelet count and platelet size in males and females. Scand J Haem; 35:77-79.

[27] Adibi P, Faghih Imani E, Talaei M & Ghanei M (2006) Population-based platelet reference values for an Iranian population. Inter J Lab Hem; 29:195-199.

APPENDIX – A

List of Figures:

	10_20	21_30	31_40	41_50	51_60
PLC%	0.27	0.26	0.24	0.24	0.24
PLCR%	23.4	25.3	25	24	25.3
PDW	11.8	12.3	11.8	12.3	12.2
MPV	9.8	10	9.9	9.8	10
PLt	287	272	252	248	244
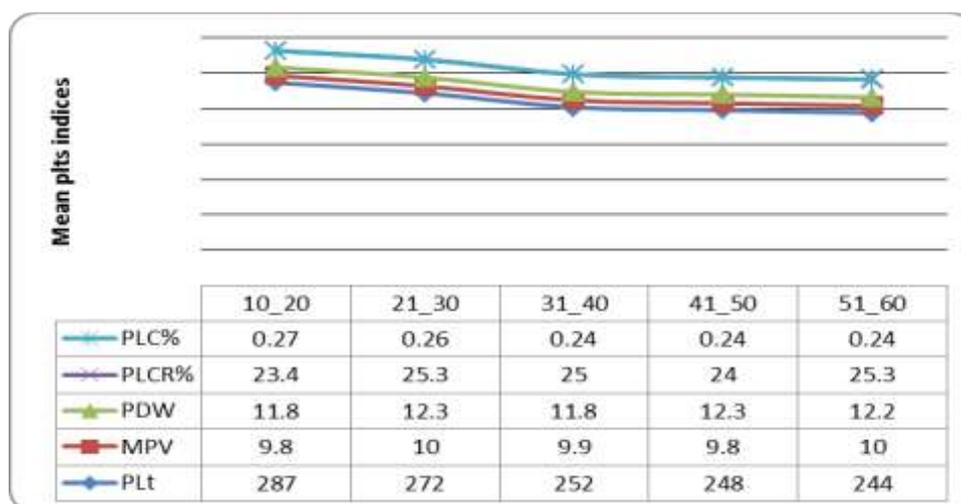
Figure I: MEA OF PLATELET INDICES AMONG DIFFERENT STUDIED AGE GROUPS

List of Tables:

TABLE I: MEAN, S.D AND REFERENCE VALUES OF PLATELETS COUNT AND INDICES

Variable	N	Mean	SD	Min	Max	Reference value
Platelets$X10^9$/L	300	261	57.9	140	439	146 – 378
MPV/fl	300	9.9	0.85	6.7	12.6	8.2 - 11.6
PDW/fl	300	12.1	1.8	1.4	19.3	8.3 - 15.9
P-LCR%	300	24.6	6.4	8.3	44.2	11.8 - 37.4
PLC%	300	0.23	0.05	0.11	0.40	0.13 - 0.34

TABLE II: REFERENCE VALUES FOR PLATELETS COUN/INDICES IN MALES/FEMALE

Variable	N/150	Mean	SD	Mean-2SD	Mean+2SD	Reference Range	P- value
Platelets$x10^9$/L	F	280	59.5	161	399	161 - 399	0.0001
	M	243	56.6	130	356	130 - 357	
MPV/fl	F	9.9	0.96	8.0	11.8	8.0 - 11.6	0.7
	M	9.8	0.75	8.3	11.3	8.4 - 11.4	
PDW/fl	F	11.9	2.1	7.7	16.2	7.8 - 16.2	0.3
	M	12.1	1.6	8.6	15.7	8.7 - 15.7	
P-LCR%	F	24.8	6.9	10.9	38.7	10.9 - 38.8	0.5
	M	24.4	5.8	12.7	36.1	12.7 - 36.1	
PLC%	F	0.23	0.05	0.12	0.33	0.13 - 0.33	0.0001
	M	0.24	0.05	0.14	0.34	0.14 - 0.24	

TABLE III: REFERENCE VALUES OF PLATELETS COUN/INDICES IN AGE BELOW/OVER 35 Y

Variable	N/150	Mean	SD	Mean-2SD	Mean+2SD	Reference Range	P- value
Plateletsx10^9/L	<35	275	62.8	150	401	150 - 401	0.0001*
	>35	247	55.3	146	357	146 - 358	
MPV/fl	<35	9.8	0.86	8.1	11.5	8.1 – 11.6	0.4
	>35	9.9	0.87	8.2	11.6	8.2 – 11.7	
PDW/fl	<35	12	1.6	8.7	15.3	7.8 – 15.4	0.5
	>35	12.1	1.9	8.3	16.0	8.3 – 16.1	
P-LCR%	<35	24.2	6.3	11.6	36.9	11.7 - 37	0.5
	>35	24.8	6.5	11.7	37.8	11.8-37.9	
PLC%	<35	0.26	0.05	0.15	0.37	0.15 – 0.37	0.0006**
	>35	0.24	0.05	0.13	0.34	0.14 – 0.34	

TABLE IV: COMPARING PLATELET IDICES RESULTS WITH OTHER PUBLISHED STUDIES

population	Instrument	sex	N	MPV/fl	PDW/fl	PCT%	Author
Iranian	Technicon H*2	M/F	993	7.4-10.7	40-57%	0.13-0.32	Adibi [24]
German	Sysmex XE -5000	M/F	309	9.1-12.1	9.9-16.1	0.17-0.39	Pekelharing [22]
S. African	Sysmex XE-2010	M/F	60	8.8-12.5	9.3-16.0	0.19-0.40	Botma (2)
Sudanese	Sysmex KX -21	M/F	300	8.2-11.6	8.3-15.9	0.13-0.34	Present study