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Establishment of Hematological Reference intervals for healthy Children in Elobied City, Sudan

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Abstract

Hemogram Reference intervals are established since a healthy population is critical to accurately interpret laboratory tests, which include Hemoglobin estimation, Red blood cells count and indices, White blood cells count and differential in addition to Platelets count. This study aims to establish the reference interval of the complete hemogram amongst healthy Sudanese children in Elobied city, Sudan. A descriptive cross-sectional study included 354 healthy children, aged between 3 to 17 years, who were categorized into three groups according to age. A questionnaire was fulfilled, EDTA anti-coagulated venous blood sample was collected from each child then the complete hemogram was performed automated hematological analyzer (Sysmex Xp 300), Finally, Data was analyzed by a software program (SPSS version 21). The hematological reference intervals for healthy children [Hb g/dl, HCT%, RBCs count x106µL, MCV/fL ,MCH /pg., MCHC g/dl ,WBCs count x10³/µL, Neutrophil count%, Eosinophil count %, Basophile count %, lymphocyte count ,Monocytes count%, RDW CV and PLTs count×10³/µL] are [(12.4±1.2),(37±4),(5.0 ±0.4), (82±5.0), (26±3.0), (32±3.0) ,(7.0±2.0) (47±10), (1±0.1) ,(0±0),(45± 10),(7 ± 4), (13±1.8) &(227±91)] respectively. The hematological RI for healthy children in Elobied was established in this study to be representative of this population, there was a significant gender-based difference in all the evaluated hematological parameters, they were found to be higher in males than in females except for basophil (%). Finally, the results of this study would shed a light on the importance of establishing RI for the children population in Elobied.

Introduction

Reference intervals or 'normative values' are defined as the central 95% of values from a healthy reference population and established since a healthy population is critical to accurately interpret laboratory tests. The results that lie outside this range are considered abnormal, and often indicate that the patient requires additional testing and/or medical treatment (Jung & Adeli, 2009).The process of establishing reliable reference intervals is quite complex (Shaw et

al.,2013). Children respond differently to Infections compared to adults and are also exposed to unique infections as a result of their 'immunologic state(Gomez et al.,1984), (Lugada et al.,2004). Appropriate local reference values for hematological parameters are essential for screening, follow-up, interpreting laboratory data, and detecting hematological abnormalities that aid physicians in differentiating between healthy patients and guide them to take appropriate clinical actions.(Karita et al, 2009; Kabir et al.,2011, and Kaspir et al., 2008) Reference intervals for hemoglobin, red blood cell count, hematocrit, mean corpuscular volume, platelet, and neutrophils, and higher monocytes and eosinophils level for African population compared to their western counterparts and African of European descent (Zeh et al.,2010, and Mathur et al.,2011).

The International Federation for Clinical Chemistry (IFCC) and the Clinical and Laboratory Standards Institute (CLSI) recommended that reference ranges should be established for each region (Hoffbrand et al., 2003; Dacie & Lewis, 2006). The inappropriate reference range can cause unnecessary follow-up investigations, treatment, and mismanagement of patients. (Gomella et al.,2013). The CBC test determines, as a proportion or absolute number, the quantity of two or more of the following cells present in whole blood: erythrocytes(red blood cell, RBC), leukocyte (WBC), platelet (thrombocytes), (Bruce et al.,2002) as well as measuring the hemoglobin concentration in addition, the CBC determined the ratio of erythrocyte in whole blood (HCT), the average red blood cell size (MCV), the amount of hemoglobin per red blood cell (MCH), and the amount of hemoglobin relative to the size of the cell (hemoglobin concentration) per red blood cell (MCHC) as well as performing what is known as a leukocyte differential (Costanzo, 2007). The reference value in children was not established in Sudan, this study may fill this gap. Recently, several authors tried to establish reference values in hematology for African countries. (Quintó et al.,2006). However, there are some discrepancies from one study to another which may be related to different factors such as. Age, ethnicity, gender, and environment including altitude can influence the hematological profiles in different populations. (Akunov et al., 2018) some behavioral patterns have been also shown to influence the number of hematological parameters (Petra et al.,2014). There are many studies conducted to ass the hematological change among pregnancy ,hypertension, diabetes and other disorder which can lead to significant change among those people (Mohamed et al., 2016, Mustfa et al., 2015). The current study is first study conducted among heathy people for the necessity of estimating the reference interval for CBC according to gender and age group, of healthy children, involved 354 children and carried out in a hematological Reference Laboratory of Elobied City

Methods

This descriptive cross-sectional study was conducted in Elobied city, western Sudan, from August to November 2016.It included 354 healthy male and female children, aged between 3-17 years. The number of participants was taken according to CLSI guidelines concerning RI establishment, which recommend 120 participants minimum to be representative to each category for stratification. Moreover, those who complain of any infection or disease that may affect the hemogram parameters were excluded.

Data Collection

This study was approved by the ministry of health and informed consent was taken from parents of each Child thenceforth; the data was collected using a Structured Interview questionnaire that comprised of no questions, most of which were closed-ended. The questionnaire involves inquiries into the demographics of those children: age, gender. The rest of the questions were about their health status, that ensuring they have neither Chronic, Genetic diseases, Hepatitis nor HIV, or never been infected with malaria during the last month. In addition, they would

have not undergone surgical operations during the last 12 months. Finally, they should not be under Medication.

Sample Collection

The tourniquet was applied to make the veins more prominent afterward a local antiseptic (70% ethanol) was used to clean the skin. A 3ml venous blood sample was collected from each participant by clean venipuncture in K3EDTA containers, the parameters of the complete hemogram were measured using an automated hematology analyzer (Sysmex XP 300) (Agabeldour et al., 2015). All measurements were imperiled to quality control of the analyzer, in the hematological Reference Laboratory of Elobied

Statistical analysis

Data were analyzed statistically using the Package for Social Science (SPSS version 20) software program. The mean and standard deviation were considered to calculate the reference interval of each parameter.[Hb g/dl, HCT%, RBCs count x106µL, MCV/fL ,MCH /pg., MCHC g/dl ,WBCs count x10^3/µL, Neutrophil count%, Eosinophil count%, Basophile count%, lymphocyte count% , Monocytes %, RDW-CV and PLTs count×10^3/µL]

Result and Discussion

The current study includes 354 healthy children, amongst them 209(59.03%) are males and 145 (40.96%) are females, aged [3-17] years, amidst them the mean reference intervals of the hemogram parameters [Hbg/dl,HCT%, RBCs count x106µL, MCV/fL ,MCH /pg., MCHC g/dl ,WBCs count x10^3/µL, Neutrophil count%, Eosinophil count %, Basophile count %, lymphocyte count ,Monocytes count%, RDW CV and PLTs count×10^3/µL] is [(12.4±1.2),(37±4),(5.0 ±0.4), (82±5.0), (26±3.0), (32±3.0) ,(7.0±2.0) (47±10), (1±0.1) ,(0±0),(45± 10),(7 ± 4), (13±1.8) &(227±91)] respectively. the present study revealed that the reference intervals of hematological parameters were statistically established from mean and ranged within two unit's standard deviation, RI [Reference interval= mean ± 2SD] .for children RI are varied according to their age. RI for children aged from [3- 17] years are shown in table (1), while RI for children belongs [3-7] years old are demonstrated in table (2), as RI for [8 - 12] years old are noted in table (3). Moreover, RI for [13-17] years children presented in table (4).

Finally, hematological parameters amongst children are varied by gender, a comparison of means for hematological parameters between males & females in the study population is shown in table (5).

Table 1. Reference intervals of the hematological parameters amongst healthy Children aged [3-17] years in Elobied City, Sudan

Total N =354		
Parameter	***Mean±SD***	***R-value=mean ± 2SD***
RBCs(10^{12})	5 ± 0.4	(4.2 – 5.8)
Hb (gldl)	12.4 ± 1.2	(10 –14.8)
HCT (%)	37 ± 4	(31 – 45)
MCV (fL)	82 ± 5	(72 – 92)
MCH (pg)	26 ± 3	(20 – 32)
MCHC (g/dl)	32 ± 3	(26 – 38)
TWBCs(10^9)	7 ± 2	(3– 11)
Neutrophil (%)	47 ±10	(27 – 67)
Eosinophils (%)	1 ± 0.1	(0– 1)
Basophils (%)	0 ± 0	(0)
Lymphocytes (%)	45 ±10	(27 – 67)

Monocytes (%)	7 ± 4	(0– 15)
Platelets(10^9)	327 ± 91	(145 – 509)
RDW-CV (%)	13 ±1.8	(9- 17)

Table 2. Reference intervals of the hematological parameters amongst healthy Children aged [3-7] years in Elobied City, Sudan

Hematological parameter	***Mean ±SD***	***R-value± 2SD***
RBCs(10^{12})	4.6 ± 0.4	(3.8 – 5.4)
Hb (g/dl)	12 ± 0.9	(10.2 – 13.8)
HCT (%)	37 ± 3.4	(30 – 44)
MCV (fl)	81 ± 3.6	(74 – 88)
MCH (pg.)	26 ± 3.5	(19 – 33)
MCHC (g/dl)	32 ± 2.4	(26 – 38)
TWBCs(10^9)	7.7 ± 1.9	(4 – 11.5)
Neutrophil (%)	45 ± 11	(23 – 67)
Eosinophils (%)	1± 0.2	(0 – 1)
Basophils (%)	0	(0)
Lymphocytes (%)	47 ± 12	(23 -71)
Monocytes (%)	6 ± 4	(2 – 14)
Platelets(10^9)	330 ± 93	(145- 506)
RDW-CV (%)	13 ± 1	(11 – 15)

Table 3. Reference intervals of the hematological parameters amongst healthy Children aged [8-12] years in Elobied City, Sudan

Hematological parameter	***Mean ±SD***	***R-value± 2SD***
RBCs(10^{12})	4.6 ± 0.4	(3.8 – 5.4)
Hb (g/dl)	12.5 ± 1	(10.5 – 14.5)
HCT (%)	38 ± 4	(30 – 46)
MCV (fl)	82 ± 5	(72 – 92)
MCH (pg)	26 ± 2.5	(21 – 31)
MCHC (g/dl)	33 ± 2.5	(27 – 38)
TWBCs(10^9)	7 ± 2	(3 – 11)
Neutrophil (%)	47 ± 10	(27 – 67)
Eosinophils (%)	1 ± 0.2	(0 – 1)
Basophils (%)	0	(0)
Lymphocytes (%)	46 ± 9	(28 – 64)
Monocytes (%)	6 ± 3.6	(0 – 13)
Platelets (10^9)	338 ± 83	(162 – 504)
RDW-CV (%)	13 ± 1	(11 – 15)

Table 4. Reference intervals of the hematological parameters amongst healthy Children aged [13-17] years in Elobied City, Sudan

Hematological parameter	***Mean ±SD***	***R-value± 2SD***
RBCs(10^{12})	4.7 ± 0.7	3.3 - 6
Hb (g/dl)	12.4 ±1.3	9.8 - 15
HCT (%)	38 ±4	30 - 46
MCV (fl)	84 ± 5.4	73 - 95
MCH (pg)	26 ±3.5	19 - 33

MCHC (g/dl)	31 ±3.6	24 - 38
TWBCs(10^9)	7 ±1.7	4 - 10
Neutrophil (%)	48 ±10	28 - 68
Eosinophils (%)	1 ± 0.1	0 – 1
Basophils (%)	0	0
Lymphocytes (%)	43 ± 9	25 -61
Monocytes (%)	8 ± 3	2 – 14
Platelets (10^9)	304 ± 92	120 - 488
RDW-CV (%)	13 ± 3	17 – 19

Table 5. Comparison between hematological RI of male & female healthy children in Elobied City, Sudan

Parameters	***Male N = 177***		***Female N =223***		***P-value***
	Mean ±SD	***R. value***	***Mean ±SD***	***R. value***	
RBCs(10^{12})	4.7 ± 0.4	3.8 – 5.5	4.5 ± 0.3	3.9 - 5.1	0.01
Hb (g/dl)	12.3 ± 1.2	10 – 14.6	12.4 ± 0.9	10.6-14.2	0.6
HCT (%)	37.7 ± 4.4	29 – 46	37.5 ± 3	31 – 43	0.5
MCV (fl)	82.3 ± 5.3	72 – 93	81.5 ± 5	71 – 91	0.2
MCH (pg.)	26 ± 3.4	19 – 33	27 ± 2.4	22 – 32	0.001
MCHC (g/dl)	31.7 ± 3.3	25 -38	33.3 ± 2	29 - 37	000
TWBCs(10^9)	7 ± 2	3 – 11	7 ± 2	3 – 11	0.2
Neutrophil (%)	47 ± 10	27 – 67	46 ± 10	26 – 66	0.2
Eosinophils (%)	1 ± o.1	0 – 1	1 ± 0.1	0 – 1	0.08
Basophils (%)	0 ± 0	0	0 ± 0	0	0.3
Lymphocytes (%)	44 ± 10	24 – 64	47 ± 10	27 – 67	0.01
Monocytes (%)	7 ± 4	0 -15	6 ± 4	0 – 14	0.001
Platelets(10^9)	314 ± 95	145–504	347 ± 81	185 - 509	0.000
RDW-CV (%)	13.4 ± 2	9-17	12.8±1	10.8-14.8	0.003

Current results represent hematological RI for healthy children population in Elobied, Sudan. RI are useful for comparing the laboratory test results with locally generated reference ranges, also to succeed in accurate diagnosis and appropriate treatment. Which can reduce the children morbidity and mortality rates . several studies revealed significant differences in hematology reference range amongst children of different ages, as well as in populations, seasons, racial-ethnic groups, and gender subgroups. (Sahoo et al.,2015, Béavogui et al. 2020). Current findings revealed that the mean of Hb concentration regarding different age groups (3-7, 8-12, 13-17) is (12.4, 12, 12.5, 12.4) respectively, this agrees with Al-Jafars' study (Al-Jafar, 2016). which reported that the mean of Hb among age group(3-6, 7-12, 13- 17) is (12.6,12, 12.8). Another study done by (Osama,2015) noted that the mean of Hb is increased in all age groups and it does not support the current study. The present study found that the mean of RBCs count among age group (3-7, 8-12, 13-17) is (4.6, 4.6, 4.7) this parallel with a study conducted by Nahounou et al.,2012 who conveyed that the mean of RBCs count among the age group (4-8,9-12,13-18) is (4.8,4.7 and 5) respectively. On another hand study done by Buchanan et al. (2010). in Tanzania, show a slight decrease in RBCs count and this disagrees with the current study (Saathoff et al.,2008). Also ,the present findings revealed that the mean of PCV level in age group (3- 7, 8-12, 13-17) is (37, 38, 38), this parallel with a study done by (Hsaan ,2016) who found that the mean of PCV among a different aged group (3-6, 7-12, 13-17) is (36, 38,

39) respectively. Another study was done by Buchanan et al. (2010) notice that the mean of PCV is lower than the current study this disagrees with our finding. Furthermore, this study shows that the mean of MCV among the study age group above is (81, 82, 84) this supports the previous study done by AlJafer (Hsaan ,2016) reported that the mean of MCV among age group (3-6,7-12, 13-17) is (80, 82, 85).respectively. Another study was done by Nahounou et al., 2012 and Osam (Osama,2015) noted that the mean of MCV is decreased, this is inconsistent with the present findings. Moreover, the present study shows that the mean of MCH among above age group is(32, 26, 26) consistent with a study of Nahounou et al.,2012 and other done by (Osama,2015) who found that similar findings in the same group (28.4,26.6 and 26.9). This study reported Platelets count was higher in females than the male . Sex-related differences in platelet count were described early in 1977 for the first time in a study that analyzed 868 blood donors. Several studies confirmed that females have a slightly higher platelet count (Segal & Moliterno,2006; Balduini & Noris, 2014). Another comparative study conducted by Bain (1985) he compared the platelet count of healthy males to the healthy females. A higher platelet count in women was confirmed. The slightly higher MPV in men was insufficient to compensate for the lower platelet count so that the plateletcrit, which measures total volume of platelets in each volume of blood, was also significantly higher in women (Bain,1985).

RDW-CV indicate slightly significant differences between age group and no significant differences among different sex. This finding is supported by study conducted among newborn concluded that during the newborn period, the normal range of RDW differs among newborns in terms of gestational age, and RDW values is not different among the groups according to gender. Additionally, our results suggest that RDW values should be evaluated according to these specific results for diagnosis of newborn blood disease without respect to adult or child values (Tonbul et al.,2011). Also, our finding is disagreed with study conducted in Macedonia aimed to assess the basic red blood cell variables and hematological indices in children and adolescents and analyze the differences regarding age and sex and concluded that RBC variables in male group showed high statistical level of significance between age different groups for all studied parameters except MCHC and RDW (Jasmina et al,2019). Generally, there is much variation in complete hemogram parameters in Sudanese people when compared to other nationalities and till now the reference value for sundaes people are not confirmed.

The major strength of our current study is the careful recruitment of healthy children with an even distribution of age and sex, because in children we excluded all inherited disease of adult people like diabetes , hypertension and heart disease and the other physiological change like pregnancy which can change in some hematological parameters.

One of the limitations of this study was that sample size is (354 children) usually for assessing the reference values it must be conducted among large population size. Another limitation is that we conducted the study among the population in Elobeid city only. It's better to cover all Sudan states and enroll all ethnics groups in the study.

Conclusion

The hematological RI for healthy children in Elobied was established in this study to be representative of this population according to their demographics and genetics, moreover, there was a significant gender-based difference in the RBCs count, HCT, MHCT, Lymphocytes (%), Monocytes (%), Platelets count, RDW-CV (%). All the evaluated hematological parameters were revealed to be higher in males than in females except for basophil (%). Furthermore, the results of this study would shed a light on the importance of establishing RI for the children population in Elobied, therefore this would be useful in essential for screening, follow up, interpreting laboratory data, and detecting hematological abnormalities that aid in differentiating between healthy patients and guide them in taking appropriate clinical actions.

Source of Funding

This study is self-funded.

Conflict of Interest

The authors report no conflicts of interest.

Ethical Approval

The study was approved by the ministry of health and Informed consent was taken from children's parents before enrollment in the study.

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