Validation Hematological Analyzer for Assay of Hemoglobin in Hodeidah City , Yemen

Abstract

The hematological indices namely hemoglobin is that important parameter for the evaluation of human physiological status. Their changes depend on the human species (Asian, Arabian, European … etc), sex, age, and health condition. To the best of our knowledge, because of the lack of research, and the absence of constant values in this area, this is the first study, which aimed to identify the reference values of hemoglobin of adults in Hodeidah, Yemen. The hematological analyzer (Sysmex KX-21) was validated by assessment of the linearity, accuracy, precision and quantification limit of different blood samples and was used for samples research analysis. Participated volunteers in the study were provided by informed consent, according to university of Hodiedah and Office of Health and Public declaration. The hemoglobin and related parameters namely RBC), packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), of two groups (500 Males and 500 females) was investigated. Obtained data was analyzed by descriptive statistic and Z-test based on Excel 2010 and SPSS. The results of validated method were precise to each analyte with percent relative standard deviation (RSD %) of intra-assay and inter – assay (< 5.0 %). Furthermore, the accuracy of validated method exhibited well recovery value of ± 5 % and the coefficient correlation (R^2) value was more than 0.9995 as a good linear method . On the other hand, the results showed the level of Hb (g/dl), RBC ($\times 10^{12}$/L), PCV (%) and PLT (10^3/L) to be significantly different between the males and the females ($p < 0.05$). The haematological parameters means of this study were constant with the international normal range except for the MCH (pg/cell) both groups, where Hb reduction was found. These variations might be related to the incidence of infectious diseases, and low in nutrition and the seaside location of Hodeidah city in Yemen.

Keywords: Validation, Hemoglobin , Hodeidah, Yemen

Introduction

Blood consists of three types of cells: white blood cells (WBCs), red blood cells (RBCs), and platelets (PLTs). They are produced and mature primarily in the bone marrow [1]. These parameters available in human body in limit values that called blood indices which are important parameters for the evaluation of human physiological status. Their changes depend on the human species (Asian, Arabian, European … etc) , sex , age, the cycle of sexual maturity and health condition [2].

The Complete Blood Count (CBC) is a test that evaluates the cells that circulate in blood. A CBC is typically performed using an automated instrument that measures various parameters. The results of a CBC can provide information about not only the number of cell types but also can give an indication of the physical characteristics of some of the cells. Also, these parameters plays important role to help doctors know these values in a true way so that they can come to a sound diagnosis [3].

Many people have a CBC performed when they have a routine health examination. Also it may be ordered when a person has any symptoms or signs that may be related to blood disorders. When an individual has fatigue or weakness or has an infection, inflammation, bruising, or bleeding, a doctor can order a CBC to help diagnose the cause and/or determine its severity. When a person has been diagnosed with a disease known to affect blood cells, a CBC will often be ordered on a regular basis to monitor their condition. Likewise, if someone is receiving treatment for a blood-related disorder, then a CBC may be performed frequently to determine if the treatment is effective. Some therapies, such as chemotherapy, can affect bone marrow production of cells. A CBC should be ordered on a regular basis to monitor these drug treatments [4].

On the other hand, this study will help us to put programs and true clear values at the health office in the province which we can refer to and generalize at centers, laboratories and hospital, and consider as reference values for the area. And it can also be used in epidemic and survey studies in the area.

This study was aimed to identify the hemoglobin values and related parameters namely packed cell volume (PCV), the Red Blood Cells (RBC) indices namely; mean cell volume (MCV), mean concentration hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) of adults in Hodeidah, Yemen because of the lack of researches done in this field and the absence of a constant reference for the normal values of every area that can be referred. Also the difference in the climates of the areas in the one country may affect the normal values from one person to the other. Also , to make a constant reference for the normal values of the CBC that is true and sound for Hodeidah city, Yemen. To compare the practical normal values with the values credited by the companies and to know the error ratio.

Materials:

Study design:

This is a prospective descriptive cross sectional study about Reference Values for hematological parameters in adults of Hodeidah City, Yemen. The study was conducted during the period June 2013 to May 2014.

Study population:

The personal data was collected through structured questionnaire, including age , situation condition, sex, Body Mass Index (BMI), heart rate and blood pressure (BP) .

Study area

This study was conducted in Hodeidah city selected Yemen country that is tropical region Hodeidah Governorate borders the Red Sea and is part of the narrow Tihama region. It serves as an important local port city. With a population of 2,687,674 and an area of 17,509 km2. It contains 26 districts, three of them in the urban (AL-AL-Hali, Hawak and AL-Meena districts), the remaining districts are in the rural areas. Hodeidah climate is semi tropical (warm and humid in the summer and moderate in winter). The highest temperature reaches 40 °C during the summer and the temperature in winter amounts to 24 °C [33].

Sample technique and size:

Representative sample size was calculated using the computer package Epi info 6 according to the registered number of Hodeidah secondary schools students for the teaching year 2014-2015. The sample was divided equally on the randomly chosen schools and the randomly chosen students was studied as members of study sample.

Inclusion and exclusion criteria:

Inclusion criteria:

Healthy volunteers. Age between 18 – 40 year old , accommodation in Hodeidah city , Yemen .

Exclusion criteria:

Volunteers with chronic diseases (anemia, renal failure, hepatitis, HIV, malaria, bilharzias, heart disease and psychiatric problem).

Sample collection

Whole blood namely 1000 (500 male and 500 female) samples were collected into EDTA anticoagulated tubes and mixed well with the anticoagulant.

Ethical issues

The study was integrated within the clinical practice. Volunteers received simple explanation for the aim of the study. If agreed to participate verbally, blood sample was collected and interview was conducted. Volunteers were reassured that this will be treated as usual. Confidentiality of the collected data was achieved by keeping data record in a locked room with limited access to the research team only.

Methods:

The materials of our study included solutions of hematological analyzer of Sysmex – version 3 (Germany), reagent of hematological analyzer (Diluent CELLPACK), WBC/ RBC lyse reagent (STROMATOLOGYSER – WH), Cell clean detergent, tube with EDTA, microscope (Olyompus, Japan), slides, cover slide, platelets count chamber (Brand , Germany), sphygmomanometer, Balance, Meter (China Brand), ethanol 70 %, sterile syringes, tourniquet and Gaemsa´s stain.

After collecting the blood samples, they were immediately examined by using Sysmex hematological analyzer for assaying of hemoglobin (Hb), white blood cell (WBC), red blood cell (RBC), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and platelets (PLT). On the other hand, differential count namely neutrophil, basophil, eosinophil, monocyte and lymphocyte were reported by using manual method because the hematological analyzer cannot differentiate between different types of granulocytes [5 ,6].

Validation of Hematological Analyzer Method

Calibration was performed to compensate for any inaccuracies of the pneumatic, hydraulic, and electric systems which will affect analysis results. This is very important in maintaining the system accuracy, precision and linearity. For calibration, five samples of fresh normal blood were used which meets the following condition:

1) blood of a healthy person who was not taking any medicines,

2) added with EDTA anticoagulant,

3) per – sample whole blood volume to exceed 2 mL,

4) Hb value to exceed 10.0 g/dl,

5) HCT (PCV) value to be within 35.5 % - 55.5 %.

Reference values of calibration was used as the following: five normal blood samples were prepared for calibration of Hb and HCT and were accurately analyzed three times each in accordance with the reference method. The measurements thus obtained were used as reference values (Hb values: Cyanmrethemoglobin and HCT value: Microhematocrit method). The samples were gently mixed sufficiently and analyzed in the whole blood mode [5].

Assay of Hematological Parameters

Procedure of hematological analyzer:

a) **Collection and preparing samples**: A specified 3 ml amount of venous sample, corresponding to 3 ml of EDTA anticoagulant, was collected.

b) **Selecting whole blood mode**: When the line on the side of the system status area on the LCD screen, the pre – diluted (PD) mode was in use for analysis

c) **Inputting sample number:** The sample number was set by the incremented value for each analysis and input the sample number when changing.

d) **Analyzing samples**: The sample was mixed sufficiently, remove the plug while taking care not to allow blood scatter, the tube was set to the sample probe, and in that condition, start switch.

3.2.2.2. Principle of hematological analyzer

This instrument works in two modes: whole blood mode for adult and pre-diluted mode for child's blood. Whole blood mode was used in analyzing collected blood samples in the whole blood status. The tube cap was opened and the sample was aspirated through the sample probe one after another. It employs three detectors blocks and two kinds of reagents for blood analysis. The WBC count was measured by the WBC detector block using the DC detection method. The RBC count and platelets took the RBC detector

block, also using the DC detection method. The Hb detector block measures the hemoglobin concentration using the non – cyanide hemoglobin method [5].

Statistical Data Analysis

The differences between the females and males groups were analysed by using Excel 2010 and SPSS version 15 to calculate the descriptive analysis and Z-test at $\alpha = 0.05$ that were used to explore the reference haematological values in Hodeidah city, Yemen.

Results

Characteristics of Volunteers

The background information of personal data on the1000 samples in the adult group in Hodeidah city, Yemen was summarized in Table 1. The personal data namely age, sex, blood pressure (BP), Body Mass Index (BMI), family situation and risk factors namely (Khat chewing, smoking, coffee drinking, tea drinking, chronic disease associated) were recorded and the results showed that the age of the male included in this study between 18 to 40 years with mean of BMI 20 while in the female between 18 –40 years with mean of BMI 22.00. The BP was found to be within normal values. The persons with risk factors namely chronic diseases were excluded. The volunteers with abuse substances namely smoking, coffee drinking, and tea were within normal international range for blood indices.

Table 1. Mean of Personal Data

Parameters	Male (n= 500)	Female (n = 500)
Age (Year)	18 – 40	18 – 40
BMI (Kg/mm)	20	22
BP (mm/Hg)	120/80	120/80
Normal Range of BP : 120/80 (mm/Hg)		

Validation of haematological analyzer method

Linearity

Linearity was performed for RBC count, Hb (g/dl), PCV (%), WBC count, PLT count and the coefficient correlation (R^2) value was more than 0.9995 as a good linear method for all parameters (Table 2).

Precision

The percent of relative standard deviation RSD (RSD %) for repeatability of the blood indices namely RBC count, Hb (g/dl), PCV (%), WBC count, PLT count was presented in Table 2 . The maximum RSD was 10 % for PLT count in intra – assay, and the minimum RSD was 0.1 % for the PCV (%) in intra – assay.

Accuracy

The accuracy (represented by recovery) of hematological analyzer method was determined at the target concentration levels used to construct the hematological profile in Table 2. All recoveries are within acceptable

limits (± 5), indicating that the method was suited for the analysis of blood indices in adult in Hodeidah, Yemen.

Table 2. Validation of haematological analyzer method

Parameters	Linearity		Precision (RSD %)		Accuracy (%)
	Range	R^2	Intra – assay	Inter – assay	
RBC ($\times 10^{12}$/L)	0.3 – 7.0	> 0.995	< 0.3	< 3.0	2
Hb (g/dl)	0.1 – 25	> 0.995	< 0.2	< 2.0	2
PCV (%)	10 – 60	> 0.995	< 0.1	< 3.0	4

Hemoglobin and related parameters

The RBC count, Hb, PCV, MCV, MCH and MCHC levels of adults in Hodeidah, Yemen were determined by hematological analyzer and summarized in Table 3. The results showed that all the means of RBC count and related indices were within normal range of international references. In addition, the results presented showed the level of RBC count, Hb and PCV to be significantly different ($p > 0.05$) between males and females. Also, all RBC count and related indices in male were more than female except MCV and MCHC were similar.

Table 3. Results of RBC count and Related Parameters

Parameters	Male (n= 500) Mean ± SD	Female (n = 500) Mean ± SD	*p* value
RBC ($\times 10^{12}$/L)	5.1±0.6	4.4± 0.4	
Median	5.1	4.4	$p > 0.05$
Rang	3.1-7.9	3.2-6.0	
Normal values	5.5±1.0	4.8±1.0	
Hb(g/dl)	13.5 ± 1.5	11.5 ± 1.1	
Median	13.7	11.3	$p > 0.05$
Rang	09.4 -17.0	08.2-13.9	
Normal values	15.5 ± 2.5	14.0 ± 2.5	
PCV(%)	42.4± 4.4	36.3± 3.2	
Median	43	36	$p > 0.05$
Rang	27.0 -67.0	28.0-46.0	
Normal values	47± 7	42±05	
MCH (pg/cell)	26 ± 3	25.5 ± 2.8	
Median	26	25.9	$p > 0.05$
Rang	16.0-34.0	17.0-33.2	
Normal values	29.5 ± 2.5	29.5 ± 02.5	
MCV(fl)	82.3 ± 8.2	82.5± 7.2	
Median	83	83.1	$p > 0.05$
Rang	60 -102	62-101	
Normal values	85 ± 8	85± 8	
MCHC (g/dl)	31.4± 01.6	30.7±01.7	
Median	31.4	31	$p > 0.05$
Rang	26-36	24-46	
Normal values	33±02	33±02	

pg: pigogram ; fl: femtoliter ; g/dl : grams/deciliter ; µl: microliter

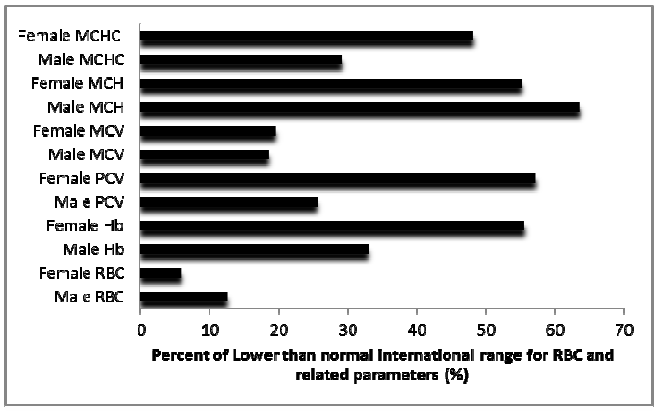

Figure 1. Percent of Lower than normal international range for RBC and Related Parameters (%)

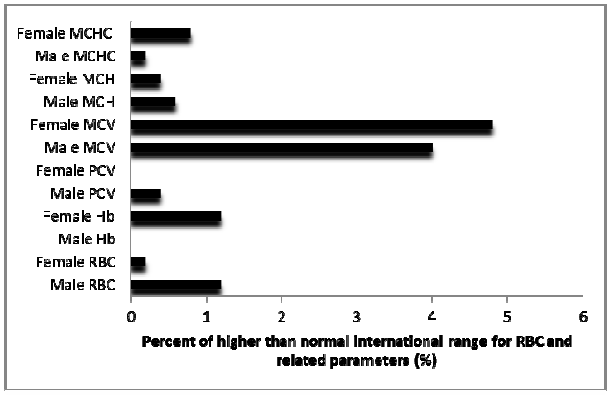

Figure 2. Percent of higher than normal international range for RBC and related parameters (%)

Discussion:

Hemoglobin parameter is very important in diagnosing and following up anemia. This test helps doctors to relate any symptoms, such as weakness, fatigue, or bruising to diagnose certain conditions, such as anemia . Also, it is very important in monitoring of therapy response such as anti – anemia [7].

On the other hand , these values are affected by a number of factors even in apparently healthy populations which include age, sex, ethnic origin, body build, social, genetic disease, nutritional and environmental factors, especially altitude [8 - 11] .

This study was conducted in Hodieidah city that is the fourth-largest city in Yemen, during the period June 2013 to May 2014. The study was carried out to identify the hemoglobin value in adults of Hodeidah city, Yemen because of the lack of research done in this field and recorded decrease in hemoglobin in our routine work .

Findings of the present study showed that almost all the mean of blood indices of adults sampled from Hodeidah Yemen (collected randomly from healthy volunteers) were found to be within international references. These results were estimated according to direct hematological analyzer that was validated using a classical approach for the assay of hemoglobin. This approach gives enough guarantees for the results that will be generated by this method during blood analysis [12].

In addition, 33.00 % and 55.6 % showed lower levels in Hb (g/dl) of the males and females, respectively that were not consisted with international references. These variations might be related to the incidence of infectious diseases and less food security that causes decrease in body nutrition in Hodeidah city in Yemen.

In comparison with previous studies, there are similar results were recorded with comparing with our study. Previous study in Arabic countries Saudi Arabia were carried out, both studies were within normal range of international references [13]. Also, our study was compared with developed countries "the study in United State America (USA) was carried out ", the results were within normal range as the same of our study [14].

On the other hand, in our study, the mean values for Hb , RBC , PCV , MCV , MCH and MCHC were higher in males than females except PLT of males less than female . These results similar with previous studies from Asian countries such as healthy adult of Western Rajasthan, and Southern India [15,16], and the same results were recorded in Pakistan [17].

In addition, previous study was recorded in African countries such as Togo which similar to our study [18]. Other previous study in Ghana for healthy adults of blood component in the Middle Belt of Ghana were determined , this study, most hematological values in males were higher than females [19].

Also , hematological reference values for healthy adults in Port Harcourt, Nigeria were studied , all parameter of this study similar for our study except MCHC was different namely in female higher than male [20] .

Finally, the results of Hematology out of range (OOR) values based on comparison with international values was compared with previous study was carried out in on Healthy adults in the Middle Belt of Ghana [19]. The similarity between both results available in Hb and related indices of both gender.

5.2. Conclusion:

Hematological analyzer method was validated using a classical approach based on the Sysmex guideline with aid international conference harmonization (ICH Q 2) for validation of analytical method for the determination of reference values of blood indices in Hodeidah city, Yemen. This approach gives enough guarantees for the future results that will be generated by this method during blood analysis. In addition, the study concluded that most the means of blood indices were within normal international range and the mean values were similar to the ones found in other studies performed in Africa and even in northern countries. On the other hand, three parameters namely Hb, MCH and lymphocytes in more volunteers are out of

hematological range that are usually due to the factors influencing hematological values namely malnutrition and infectous disease.

References

1. Hoffbrand AV, P.A.H. Moss, and J.E. Pettit, Essential Haematology, Blackwell Publishing; 5th edition, 2006.
2. Peck Palmer OM. Effect of age, gender, diet, exercise and ethnicity on laboratory test results. In: Accurate results in the clinical laboratory: a guide to error detection and correction. London ; Waltham, MA: Elsevier, 2013. 9–17.
3. Petra S and Aryeh S., Basics of Blood Management, Blackwell Publishing, 1st edition, 2008.
4. Hillman, Robert S.; Ault, Kenneth A.; Rinder, Henry M., Hematology in Clinical Practice: A Guide to Diagnosis and Management, McGraw-Hill Professional. 4th edition , 2005.
5. Operator Manual Automated Hematology Analyzer KX_ 21N Sysmex Corporation Kope Japan . 2003 .
6. Renu Saxena HP Pati , Laboratory Techniques in Hematology VP Choudhry , 1st edition , 2008.
7. Bunn HF. Approach to the anemias. In: Goldman L, Schafer AI, eds. Goldman's Cecil Medicine. 24th edition . Philadelphia, Pa: Elsevier Saunders; 24th edition , 2011.
8. Hutchison RE, McPherson RA, and Schexneider KI. Basic examination of blood and bone marrow. In: McPherson RA, Pincus MR, eds. Henry's Clinical Diagnosis and Management by Laboratory Methods. Philadelphia, Pa: Elsevier Saunders , 22nd edition , 2011.
9. Bain B. , Normal ranges in Blood cells—A Practical Guide. Blackwell Scientific Publications, Oxford, UK, 3rd edition, **2002**.
10. Lewis SM , Bain BJ , and Bates I. . Reference ranges and normal values," in Dacie and Lewis—Practical Haematology.Eds Churchill Levingstone, New York, NY, USA, 3rd edition, 2006 .
11. Cheng CKW, Chan J , Cembrowski GS, and van Assendelft OW,. Complete blood count reference interval diagrams derived from NHANES III: stratification by age, sex, and race. *Laboratory Hematology*, 10 (1): 42–53 , 2004.
12. Hubert, Ph., Nguyen-Huu, J., Boulanger, B., Chapuzet, E., Chiap, P., Cohen, N., Compagnon, P., Dewe,W., Feinberg, M., Lallier, M., Laurentie, M., Mercier, N., Muzard, G., Nivet, C., and Valat, L., Validation of quantitative analytical procedures. Harmonization of approaches. *STP Pharma Pratiques*. 13: 101-138 , 2003.
13. Scott GT. , A pilot study of the reference values for the commoner haematological and biochemical parameters in Saudi nationals , *J Clin Pathol*. 1982, 35 (1) : 69-73 .

14. Kratz A, Ferraro M, Sluss PM, and Lewandrowski KB., Case records of the Massachusetts General Hospital. Weekly clinic pathological exercises. Laboratory reference values. *N Engl J Med.* 2004, 351 (15) : 1548–1563.

15. Kumar R, Soni ND , Choudhary R, and Khan I., Reference range values of haematological parameters of healthy adults in western Rajasthan. *Int J Biol Med Res* . 2013 . 4 (3) : 3383-3387.

16. Subhashree AR , Parameaswari PJ , Shanthi B , Revathy C , and Parijatham BO. , The Reference Intervals for the Haematological Parameters in Healthy Adult Population of Chennai, Southern India . *J Clin Diagn Res*. 2012. (10) : 1675–1680 .

17. Usman K , Syed AZ , and Rao AA. , Reference Range Values of Haematological Parameters in Healthy Pakistani Adults , *Pak J Physiol* . 2007. 3(1): 19 – 22 .

18. Irenee MK , Akuete YS , Helene J , Ahoefa V , and Michele I., Hematological Reference Values for Healthy Adults in Togo , *International Scholarly Research Network ISRN Hematology* , 2011 (736062) : 1 – 5 .

19. D. K. Dosoo, K. Kayan, D. Adu-Gyasi, E Kwara, J. Ocran, K. Osei-Kwakye, E. Mahama, S. Amenga-Etego, P. Bilson, K. P. Asante, K. A. Koram, and S. Owusu-Agyei1, Haematological and Biochemical Reference Values for Healthy Adults in the Middle Belt of Ghana , *PLoS One*. 2012. 7(4): e36308.

20. Dapper DV , Nwauche CA , and Didia BC. , Haematological reference values for healthy adults in Port Harcourt, Nigeria , *Port Harcourt Medical Journal*. 2006, 1(1) , 25-28 .