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Establishment of Reference Ranges for CBC Parameters in Adult Pakistani Population

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Abstract:

***Objective**: To establish a reference ranges of haematological parameters in healthy adult Pakistani population*

***Study Design**: Cross sectional study*

***Place and Duration of Study**: Pathology department, CMH Quetta from December 2020 to November 2021.*

***Methodology**: 300 healthy individuals with an age range of 20-55 years. 2ml venous blood sample from antecubital fossa was taken in EDTA tube. This sample was analysed using SYSMEX XP100. Hb, HCT , RBC, PLT, MCV,MCH,MCHC and TLC were recorded. Data was analysed using SPSS 25.0.*

***Results**: Out of total 300 patients 150 (50%) were male and 150 (50%) were females. Mean age of the patients was 35.1 ± 8.0 years. A statistically significant difference seen among various CBC parameters between male and female population. Hb, RBC count, MCV and MCH were significantly higher among males as compared to females. Whereas platelets, TLC and MCHC were raised in female population.*

***Conclusion**: The haematological parameters in CBC are slightly different as compared to previous international standards used by laboratories in clinical settings. People belonging to Quetta & surroundings (Balochistan) situated at level of 5500 feet above sea level. It is concluded that RBC &Hb of people belonging to high altitude region was high as compared to plain areas.*

Keywords: Complete blood count (CBC), Hemoglobin, Red blood cell, Reference range, Mean cell volume MCV Mean corpuscular haemoglobin MCH, Total leukocyte count TLC

INTRODUCTION:

Fertilization of ovum in the uterus marks the start of a complex biological process known as human development. Many models have been proposed to elaborate the various stages of development a new-born goes through to become an adult. These stages are accompanied with various physical and biological changes. Health status of any individual is a relative term. As it is subjected to change with the changing time, area and environment. so in order to identify any irregularity the subject must be compared to a reference[1]. A value or a scale obtained through wide range of researches,

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studies and observations done on a specific population is termed as a reference range/ value for that particular population. The range between upper and lower reference values is known as the reference interval RI[2].

The significance of reference range is two-fold when it comes to the laboratory parameters. It is the basis of diagnosis and interpretation of various diseases which in turn effects the patient management protocols[3]. Haematological reference values are the most important diagnostic tools. 80% of the physician's decisions are based on these laboratory test results. It is relatively risky to use reference ranges from other populations due to variations in the race, ethnicity, genetics, altitude and other environmental and social factors[4]. In Pakistan we usually rely on standards and references set by the studies done in westerncountries (European and American population). This is due to the scarcity of literature available in healthy adult individuals in Pakistan.

Generalizing the reference range of one population for diagnosing various medical conditions will not yield appropriate results for every case as the value considered normal for one population might be abnormal for another set of people[5]. Various Studies have been conducted in different parts of the world in order to establish their own reference interval for various haematological and chemical parameters[6,7,8]. This study was designed with an aim to establish a reference range of haematological parameters in healthy adult Pakistani population in order to make a correct diagnosis based on correct reference values suited to our population.

METHODOLOGY:

It was across-sectional study, carried out in the Pathology department, CMH Quetta. The total duration for which the study was carried out was one year from December 2020 to November 2021.

According to the clinical and laboratory standards institute Guidance Document C28A2A, minimum sample size of 240 (120 male 120 female) was required to establish a reference interval[9]. A Sample size 300 was used for this study. Non probability and purposive sampling technique was used.

300 healthy individuals with an age range of 20-55 years were included in the study. Those with history of any drug usage, blood transfusion, jaundice, anaemia, hepatitis B,C and any other comorbid conditions (Diabetes Mellitus, Hypertension, Ischemic Heart Disease) were excluded from the study. Pregnant, lactating and menstruating women were also excluded from the study.

Prior Approval was taken from the ethical review committee board (Reference number of ethical review committee form: CMH-QTA-IRB/041). Informed consent was taken from the patients prior to enrolling the patients into the study. Demographic details (ethnicity/ socioeconomic status) and history (drug, anemia, comorbid disease)were recorded.Samples of patients were taken from Combined military hospitals (blood banks) of Rawalpindi, Quetta, Karachi and Lahore in order to establish generalizability of reference range.

2ml venous blood sample from antecubital fossa was taken under aseptic conditions in CP tube containing anticoagulant EDTA. This sample was analysed using automatedhematologyanalyser SYSMEX XP100.

All the samples were analysed within 1 hour of collection and results were recorded. Automated analysers were calibrated and standardized by using

commercially prepared calibrators according to the manufacturer's instruction. Calibration was done to compensate for inaccuracy of electric system which might can affectCBC parameters results. 40 samples were reanalysed in order to check for any errors and to ensure good precision.

STATISTICAL ANALYSIS:

Statistical calculations was analysed using Statistical package for social sciences SPSS version 25.0. Mean, SD and 2.5th and 97.5th percentiles were calculated for variables such as Age,Hemoglobin (Hb), hematocrit (HCT), red blood cell count (RBC), platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin(MCH), mean corpuscular hemoglobin concentration (MCHC), total leukocyte count (TLC). Percentage and Frequency was calculated for variables (categorical) such as gender. Comparison of blood counts was carried out among male and female patients using independent samples T test. 2.5th and 97.5th percentiles were calculated for establishment of reference range. Outliers (10% out of manufacturers range) were identified and removed from the study samples. p value of $\leq$0.05 was considered to be significant.

RESULTS:

Out of total 300 patients 150 (50%) were male and 150 (50%) were females. Mean age of the patients was 35.1± 8.05 years. Age range of the patients included in the study fell between20 – 53 years.Basic characteristics of the study population are listed in table I.A statistically significant difference among various CBC parameters between male and female population was seen. Mean hemoglobin levels, RBC count, MCV and MCH were significantly higher among males as compared to females. Whereas platelets, TLC and MCHC were raised in female population. Table II. Reference range of CBC parameters for male and female were calculated and tabulated using the 2.5th and 97.5th percentile. Table III.

Table I: Basic characteristics of study population (n=246)

Variables	Mean ± SD	Reference intervals	
		Upper limit	Lower limit
Hb (g/dl)	13.074 ± 1.43	10	16.10
TLC (x10^9/l)	8.59 ± 0.98	11	6.10
Platelets (x10^9/l)	239 ± 32	149	319
RBC (x10^{12}/l)	4.71 ± 0.48	3.65	5.90
MCV (fl)	75.05 ± 1.84	71.97	78.45
MCH (pg)	25.64 ± 1.46	27.99	6.93
MCHC (g/dl)	35.20 ± 2.45	39.00	30.32

Table II: Comparison of CBC parameters among Male and Female population (n=300)

Variables	Male (n=150) (Mean ± SD)	Female (n=150) (Mean ± SD)	p value
Hb (g/dl)	14.2 ± 0.8	11.8 ± 0.7	0.001
TLC (x10^9/l)	8.3 ± 1.0	8.7 ± 0.9	0.001
Platelets (x10^9/l)	223.8 ± 26.8	254.1 ± 30.7	0.001
RBC (x10^{12}/l)	4.8 ± 0.5	4.5 ± 0.4	0.001
MCV (fl)	76.7 ± 0.5	73.3 ± 0.8	0.001
MCH (pg)	26.3 ± 1.7	24.9 ± 0.4	0.001
MCHC (g/dl)	32.9 ± 0.9	37.4 ± 0.9	0.001

Table III: Reference range of CBC parameters among Male and Female population (n=300)

Variables	Male (n=150)	Female (n=150)
Reference interval	(2.5^{th} - 97.5^{th} percentile)	(2.5^{th} - 97.5^{th} percentile)
Hb (g/dl)	12.6 – 15.9	10.3 – 13.1
TLC ($x10^9$/l)	6.1 – 10.0	6.9 – 10.6
Platelets ($x10^9$/l)	186.5 – 307.0	201.7 – 302.4
RBC ($x10^{12}$/l)	4.1 – 5.9	3.9 – 5.3
MCV (fl)	76.0 – 77.9	72.1 – 75.5
MCH (pg)	24.1 – 27.8	24.0 – 26.0
MCHC (g/dl)	30.8 – 34.6	34.9 – 38.9

DISCUSSION:

Complete blood count is a very basic, powerful and economical diagnostic modality in daily medical practice to avoid misdiagnosis of any individual. It assists in diagnosing physiological status of various conditions, categorizing severity of illness &monitoring disease response to therapy. This is one of the inexpensive and most frequently ordered laboratory test in the world[10].

Almost all of the laboratories in Pakistan use the international reference values developed by their organizations through conduction of researches on their local populations many years ago. This can be misleading for the physicians as they are based on foreign populations[11].

CBC consists of evaluating three basic components which includes red blood cell (erythrocytes), white blood cell (leukocytes) and platelets. Each of these is an important part of the diagnostic procedure.

Blood cells were counted by Automated analyser (Sysmex) at optimum temperature of 15ºC - 30ºC.It was based on principle of electronic impedance introduced by Wallace Coulter. According to this detection method, blood cells being a poor conductors of electricity were suspended in a conductive diluent (liquid) passed through the aperture, causing direct resistance to change between electrodes. As the direct current resistance changes, the size of blood cells is detected as electric pulses. Number of impulses indicate blood cell count and the amplitude(height) of the impulses indicate volume of cell.

Hemoglobin (Hb), hematocrit (HCT), red blood cell count (RBC), platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin(MCH), mean corpuscular hemoglobin concentration (MCHC), total leukocyte count (TLC) were recorded. TLC,RBCs, WBCs and platelets were directly measured by impedance. Whereas, HCT, MCV,MCH and MCHC were calculated values indirectly by applying formulae.

Three part automated analyser differentiate cells into three types: Granulocytes, Lymphocytes and Mixed cells (Monocytes, eosinophils and basophils). Various maturation stages (Blasts, promyelocytes&metamyelocytes) were not present in peripheral blood film. After appropriate staining time & preparation of good blood films, cells were counted either manually or by electronic key counterand seen under the microscope. Differentiated leukocyte counts was assessed from the Total leukocyte count of each type of cell in the form of percentage or absolute count.

Worldwide the reference range used for haemoglobin among males is 13.2-16.6 grams/dl and females is 11.6-15 grams/dl. Our results revealed a significantly lower levels in female population. This difference may be due to the environmental

factors and dietary habits. Many studies have been conducted in our region to establish our reference ranges for various parameters based on data from local population. Usman et al in his study conducted in Multan and its surrounding areas revealed similar results with significant difference in hematological parameters in our population as compared to world wide standards used. Difference between male and female genders was also reported with increased levels of Hb, RBC, hematocrit in males[12].

Contrary to our finding, Siraj et al concluded that reference intervals for RBC, HB, MCHC, HCT differed significantly among male and female population, whereas no difference was found in TLC and platelet counts[13]. Ramezani et al revealed that mean for Hb and platelets was 15.5g/dl and 209 x10^9/l in population of central province of Iran[14]. This was significantly different from our population with lower Mean Hb (13.07 ± 1.43 g/dl) and higher platelet counts (239 ± 32 x10^9/l). Muhammad Awad et al conducted a study in Sudanese population to establish a reference range for hemoglobin and red cell parameters. He showed that mean Hb was 14.9 g/dl in males and 12.2 g/dl in females. His findings were reportedly closer to our results. However other parameters such as MCV, MCH and RBC count were quite higher as compared to our population[15]. Similar findings were reported by yaseen et al in Shendi locality in Sudan[16].

Many studies have been conducted on infants to establish reference ranges for hematological parameters, but very less work has been carried out for the Adult Pakistani population. The range for platelets count used in our laboratories is 135-317 x10^9/l for males and 157- 371 x10^9/l for females[17]. This was quite contradictory to the findings of our study. The lower limit of platelets count (both in male and females) was much higher as compared to these western standards. Nweke et al reported similar results in adult healthy Nigerian population with platelet counts between 150- 305 x10^9/l[18]. A study conducted by Faya et al in Tanzanian healthy adults reporting for blood donation also revealed significant difference in hematological reference ranges as compared to the western standards[19,20].

Neelam et al in her study on Pakistani population also revealed that there is a massive difference in normal values of male and females in all 4 provinces when compared to the reference standards mentioned in textbooks[21]. It was not entirely possible that all the subjects included were 100% healthy due to a large spectrum of subclinical diseases. More studies with multicenter approach and larger sample size, divided on the basis of age groups, ethnicity and locality must be conducted to establish reference ranges of hematological, biochemical and other laboratory parameters used. This will further strengthen the results of our study and better diagnosis and treatment can be made possible.

CONCLUSION:

The haematological parameters in complete blood picture are slightly different as compared to the previous international standards used by various laboratories in our clinical settings. People belonging to Quetta & surrounding areas (Balochistan) situated at level of 1,679 meters (5500 feet) above sea level. It is concluded that RBC &Hb of people belonging to high altitude region was high as compared to inhabitants of plain areas.

Conflict of interest: The author declares no conflict of interest.

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