



ESTABLISHING REFERENCE INTERVALS FOR HAEMATOLOGY LABORATORY TEST RESULTS IN HEALTHY ADULTS

Pathology

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ABSTRACT

Background-Reference intervals are essential for clinical laboratory test interpretation and patient care. A test result by itself is of little value unless it is reported with the appropriate information for its interpretation. Typically, this information is provided in the form of a reference interval (RI). Several studies have shown significant differences between and within populations, emphasizing the need for population-specific reference ranges. **Objective**-This study was carried out to establish the haematological reference intervals in apparently healthy individuals in the Marathwada region of Maharashtra. **Materials and Methods** - A total of 246 healthy adults were recruited consisting of 123 males and 123 females. Median for haematological parameters were calculated and reference intervals were determined by calculating 2nd and 97th percentiles using statistical package for social science (SPSS). **Results**- Red blood cell (RBC) count, haemoglobin, and haematocrit (HCT) were higher in males compared to females while platelet count was higher in female. **Conclusion** - The reference interval established by our study were slightly different from the currently used reference values. So there is a need to establish its-specific reference intervals, if accurate diagnosis and concise clinical decisions are to be made.

KEYWORDS

Haematology parameters, Reference range, Reference values

INTRODUCTION:

Complete blood count (CBC) is the most commonly ordered investigation. It is most versatile yet most economical of all investigations. It has wide diagnostic applications. The CBC measures several components of blood including Red blood cells, White blood cells, Haemoglobin, Haematocrit, Platelets. The present study was carried out to establish reference intervals for various elements of the CBC i.e. haemoglobin(Hb), total leucocyte count(TLC), differential leucocyte count(DLC), platelet count, red cell indices etc.

The interpretation of any haematological investigation depends upon reference interval (RI). RI is also termed as reference range or reference value. RI is term where values that fall inside the range are considered normal and those occurring outside the range are considered abnormal.¹ A reference interval is defined by threshold values between which the test results of a specified percentage (usually 95%) of apparently healthy individuals would fall.² RIs are very useful to provide medical information that ensures correct medical decisions. As laboratory results are interpreted in comparison with these intervals, the reliability of the RI play major role in result interpretation and as a measure of quality of the result itself.^{3,4} The Clinical and Laboratory Standards Institute (CLSI) recommends that RIs should be established for each region and specific populations.^{5,6}

Reference values differ on many socio-demographic factors such as age, gender, dietary patterns, ethnic differences and altitude.⁶ The haematological reference ranges have intra- and inter-population variation, differing from one population set to another and from one country to another.

Clinical laboratory tests are useful for diagnosing health disorders, disease staging and monitoring of response to treatment. Interpretation of laboratory test results with appropriate diagnostic accuracy requires reference or cutoff values. So this study was conducted to establish reference intervals of different haematological parameters in healthy adult population, in the Marathwada region of Maharashtra.

MATERIALS AND METHODS:

This prospective study was carried out in department of pathology in government medical college & hospital Aurangabad over the period 2 years. The reference intervals of different haematological parameters were determined in male and female population by automated haematology analyzer- Sysmex XS1000i five part differential cell counter.

Methodology:

Total study population was 246 healthy adults consisted of 123 male and 123 female. They were selected from doctors, paramedical staff, undergraduate students, postgraduate students of this locality and volunteers visiting to blood bank for blood donation.

a) Selection criteria -

Selection was based on apparently healthy people who fulfilled the following criteria:

- Age between 18–65 years.
- No history of recent of blood loss or blood transfusions.
- non pregnant and non lactating female
- woman not menstruating at the time of blood collection.

b) Collection of blood -

The blood sample was collected using all aseptic precautions. The 2.5 ml of blood was collected from anti-cubital vein in an ethylene diamine tetra-acetic acid, tri-potassium (K3 EDTA) anticoagulant tube. The sample was processed within 4 hours of collection using Sysmex XS1000i haematology analyzer. The haematology analyzer was calibrated by standardized commercially prepared calibrators. The relevant information such as age, sex, any complain, personal history were noted into a specially designed proforma.

c) Statistical Analysis-

Data were statistically analyzed using statistical package for social science (SPSS, version 20). By using SPSS median, 2nd and 97th percentiles were determined. This was according to the CLSI/IFCC guidelines on defining, establishing and verifying reference intervals in the clinical laboratory.⁸ Reference values were determined separately for male and female population.

RESULTS:

The present study was performed on 246 healthy adults with male to female ratio (M:F) of 1:1. The mean age was 36 year with range of 19-62 year. Median, 2nd percentile & 97th percentiles were calculated separately for male shown in Table no. 1 and for female population shown in table no. 2 using SPSS version 20.

Table No.1 – Median, 2nd and 97th percentile of different haematological parameters in Male population (n= 123)

Parameter	Median	2 nd percentile	97 th percentile	Unit
WBC count	7.04	4.9	11.24	X 10 ³ /μL
RBC count	5.15	4.2	6.02	X 10 ⁶ /μL
Hb	14.9	11.91	16.7	g/dL
HCT	43.1	37.33	47.7	%
MCV	83.8	71.37	99.8	fL
MCH	28.9	22.825	33.8	Pg
MCHC	34.4	31.9	36.4	g/dL
Platelet count	279	199	411.2	X 10 ³ /μL
RDW-SD	41.1	35.94	49.87	fL
RDW-CV	13.8	12.7	16.8	%

PDW	11.9	9.2	16.35	fL
MPV	10.2	8.8	12.105	fL
P-LCR	25.8	16.5	40.5	%
PCT%	0.29	0.2	0.41	%
Neutrophil %	53	35.4	71.02	%
Lymphocyte %	36.2	18.5	50.2	%
Monocyte %	8.3	5.13	12.9	%
Eosinophil %	2.8	0.5	7.4	%
Basophil %	0.3	0.1	0.8	%
ANC	3.4	1.8	7.58	X 10 ³ /μL
ALC	2.55	1.53	3.5	X 10 ³ /μL
AMC	0.6	0.34	0.9	X 10 ³ /μL
AEC	0.21	0.05	0.54	X 10 ³ /μL
ABC	0.02	0.01	0.05	X 10 ³ /μL

(WBC- white blood cell, RBC- red blood cell, Hb- haemoglobin, HCT- Haematocrit, MCV- mean cell volume, MCH- mean cell haemoglobin, MCHC- mean cell haemoglobin concentration, RDW-SD- red cell distribution width standard deviation, RDW-CV- coefficient of variation, PDW-Platelet distribution width, MPV-Mean platelet volume, P-LCR-Platelet large cell ratio, PCT%-Plateletocrit, ANC- Absolute Neutrophil Count, ALC- Absolute Lymphocyte Count, AMC- Absolute Monocyte Count, AEC- Absolute Eosinophil Count, ABC- Absolute Basophil Count)

Table No.2 – Median, 2.5th and 97.5th percentile of different haematological parameters in Female population (n= 123)

Parameter	Median	2.5 th percentile	97.5 th percentile	Unit
WBC count	7.92	4.88	10.95	X 10 ³ /μL
RBC count	4.44	3.493	5.268	X 10 ⁶ /μL
Hb	12.5	10.5	14.475	g/dL
HCT	37.2	31.92	42.6	%
MCV	85.6	67.5	99.73	fL
MCH	25.4	23.005	31.77	Pg
MCHC	33.6	30.8	35.995	g/dL
Platelet	318	220	457.8	X 10 ³ /μL
RDW-SD	39.25	36.40	53.52	fL
RDW-CV	14.4	12.7	17.6	%
PDW	12.3	9.4	17.94	fL
MPV	10.4	8.9	13.05	fL
P-LCR	27.4	16.3	44.9	%
PCT%	0.33	0.23	0.49	%
Neutrophils %	54.4	38.36	69	%
Lymphocytes %	35.2	21.53	47.82	%
Monocytes %	7.5	4.82	12	%
Eosinophils %	1.8	0.4	8.3	%
Basophils %	0.3	0.1	1.5	%
ANC	4.09	2.12	7.4	X 10 ³ /μL
ALC	2.64	1.38	4.11	X 10 ³ /μL
AMC	0.6	0.37	0.96	X 10 ³ /μL
AEC	0.14	0.03	0.65	X 10 ³ /μL
ABC	0.02	0.01	0.06	X 10 ³ /μL

2.5th percentile represents lower reference range while 97.5th percentile represents upper reference range for that particular population. Accordingly reference intervals of different haematological parameters were estimated in male and female population. Table no. 3 shows reference intervals for different haematological parameters in male and female population.

Table No.3 - Reference Intervals of various haematological parameters

Parameter	Reference Intervals for Male (n=123)	Reference Intervals for Female (n=123)	Unit
WBC count	4.9 - 11.24	4.88 - 10.95	X 10 ³ /μL
RBC count	4.2 - 6.02	3.493 - 5.27	X 10 ⁶ /μL
Hb	11.9 - 16.7	10.5 - 14.475	g/dL
HCT	37.33 - 47.7	31.92 - 42.6	%
MCV	71.37 - 99.8	67.5 - 99.73	fL
MCH	22.825 - 33.8	23.005 - 31.77	Pg
MCHC	31.9 - 36.4	30.8 - 35.995	g/dL
Platelet	199 - 411.2	220 - 457.8	X 10 ³ /μL
RDW-SD	35.94 - 49.87	36.40 - 53.52	fL

RDW-CV	12.7 - 16.8	12.7 - 17.6	%
PDW	9.2 - 16.35	9.4 - 17.94	fL
MPV	8.8 - 12.105	8.9 - 13.05	fL
P-LCR	16.5 - 40.5	16.3 - 44.9	%
PCT%	0.2 - 0.41	0.23 - 0.49	%
Neutrophils %	35.4 - 71.02	38.36 - 69	%
Lymphocytes %	18.5 - 50.2	21.53 - 47.82	%
Monocytes %	5.13 - 12.9	4.82 - 12	%
Eosinophils %	0.5 - 7.4	0.4 - 8.3	%
Basophils %	0.1 - 0.8	0.1 - 1.5	%
ANC	1.8 - 7.58	2.12 - 7.4	X 10 ³ /μL
ALC	1.53 - 3.5	1.38 - 4.11	X 10 ³ /μL
AMC	0.34 - 0.9	0.37 - 0.96	X 10 ³ /μL
AEC	0.05 - 0.54	0.03 - 0.65	X 10 ³ /μL
ABC	0.01 - 0.05	0.01 - 0.06	X 10 ³ /μL

RBC parameters including RBC count, Hb, PCV, MCV, MCH, MCHC were lower in females than in males while RDW-SD and RDW-CV higher in females than in males.

Reference range for Platelet count was higher in females than in males. The present study establishes reference intervals for new parameters like RDW-SD and P-LCR.

The reference interval established by our study slightly differed from the currently used reference values⁸. The 2.5th percentile i.e. lower reference limit was comparatively lower for haemoglobin, haematocrit, MCV, MCH, MCHC, RBC count, neutrophil%, lymphocyte %, absolute neutrophil count. The 97.5th percentile i.e. upper reference interval was comparatively higher for WBC, RDW, platelet count, lymphocyte %, monocyte %.

DISCUSSION:

Clinical laboratory reference intervals are an important tool to identify abnormal laboratory test results. The generation of haematological parameters reference intervals for local population is very crucial to improve quality of health care, which otherwise may lead to unnecessary expenditure or denying care for the needy. There are no well-established reference intervals in this region.

Table no.4 shows comparison of reference intervals of haemoglobin, white blood cell count(WBC) and platelet count with other studies.

Table No.4 - Comparison of studies for reference interval of Hb, WBC and platelet count

Study	Hb g/dl		WBC count X10 ³ /μl		Platelet count X10 ⁵ /μl	
	Male	Female	Male	Female	Male	Female
Ashavaid T.F. et al. ¹⁰	14 - 16	12 - 15	4 - 10	4 - 10	1.5 - 4.5	1.5 - 4.5
Balasubramaniam U. et al. ¹¹	12.3 - 17	9.9 - 14.3	4.2 - 9.8	4 - 10.4	1.3 - 3.8	1.31 - 4.24
Subhashree A.R. et al. ¹²	11.13 - 17.4	9.22 - 14.67	4.63 - 13.53	4.6 - 12.95	1.48 - 4.04	1.46 - 4.08
Dosoo D.K. et al. ¹³	11.3 - 16.4	8.8 - 14.4	3.5 - 9.2	3.4 - 9.3	0.88 - 3.52	0.89 - 4.03
Sehgal K.K. et al. ¹⁴	13.18 - 17.22	12.1 - 14.6	4.2 - 10.4	4.2 - 10.4	1.53 - 3.66	1.82 - 4.09
Present study	11.9 - 16.7	10.5 - 14.47	4.9 - 11.24	4.88 - 10.95	1.99 - 4.11	2.2 - 4.57

Ashavaid T.F. et al.¹⁰ and Sehgal K.K. et al.¹⁴ shows that reference interval for WBC count is same for male and female. Subhashree A.R. et al.¹² shows that, WBC count was slightly higher in males than females which was concordant with present study. The environment may influence the leucocyte count and even genetics likely to play a part.⁹

Ashavaid T.F. et al.¹⁰ shows that reference range for platelet count was same in both males and females. Balasubramaniam U. et al.¹¹, Dosoo D.K. et al.¹³ shows that, reference range for platelet count were slightly higher in females than males which was comparable with our study.

We compared reference intervals of red blood cell count(RBC) and haematocrit (HCT%) with studies done by Balasubramaniam U. et al.¹¹, Subhashree A.R. et al.¹² etc shown in table no.5.

Table No.5 - Comparison of studies for reference interval of RBC count and haematocrit (HCT%)

Study	RBC count X10 ⁶ /μl		HCT %	
	Male	Female	Male	Female
Balasubramaniam U. et al ¹¹	4.5 – 5.5	3.5 – 5.2	37 – 51	30 – 43
Subhashree A.R. et al ¹²	4.01 – 6.04	3.44 – 5.3	33.07 – 49.97	29 – 42.78
Dosoo D. K. et al ¹³	3.79 – 5.96	3.09 – 5.3	32.2 – 50.5	26.4 – 45
Sehgal K.K. et al ¹⁴	4.56 – 6.16	4.2 – 5.39	40.24 – 53.48	37.33 – 46.08
Present study	4.2 – 6.02	3.49 – 5.27	37.33 – 47.7	31.92 – 42.6

The aforementioned studies shows that, there is gender difference in reference values of RBC count, haemoglobin level and hematocrit. All studies shows that, RBC parameters were higher in males than in females. The current study shows similar result. The gender-wise differences in these reference ranges may be attributed to the variations in the types of hormones produced and their corresponding concentrations in the different sex as well as the effect of erythropoietin release in response to regular menstruation and cross-stimulating megakaryopoiesis.^{15,16}

We found slight difference in MCV value in males and females with slightly lower value of MCV in females. Lower reference range for MCH was lower in males than females while upper reference range was higher in males than females. While both lower and upper reference range of MCHC were lower for females than males. Reference range for RDW was higher in females than males.

There was higher reference range for Neutrophil% in males than females. Balasubramaniam U. et al.¹¹ shows that there was no difference in reference range of Lymphocyte% in males and females. Dosoo D.K. et al.¹³ shows that, lower reference range for males was lower than females which was concordant with present study. Balasubramaniam U. et al.¹¹ shows that, eosinophil% were same in both males and females. Present study shows eosinophil% is slightly higher in females than in males. The present study shows slightly higher values of monocyte% in males than females.

The central role of the laboratory scientist is to aid the clinician, in interpreting observed values, by providing relevant reference intervals in a convenient and practical form. In India, reference intervals used in laboratories have been established in the western population. But these can be questioned due to differences in genetic load, lifestyle, and diet. This study highlights the approach for establishing reference intervals in our population using the IFCC guidelines.¹⁰

CONCLUSION:

Reference interval of different haematological parameters varies with age, sex, geographic location, altitude, ethnic origin, dietary habits. Therefore there is a need to establish databank of reference values that are specific to a particular population instead of applying reference values determined for one population on another population. The reference intervals established by our study were slightly different from the currently used reference values.

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Ethical considerations:

Ethical clearance was obtained from Institutional Ethics committee of government medical college and Hospital, Aurangabad. A written informed consent was obtained from the study participants. The data were kept confidential through anonymity. The samples collected from the participants were analyzed only for the intended purpose

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