Original Article

Establishment of Haematological Reference Intervals for Healthy Adults in Karamsad at Shree Krishna Hospital

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Background : The Complete Blood Count (CBC) is the most frequently ordered diagnostic tests in medicine. Cellular components of peripheral blood can be evaluated by Complete Blood Count examination. In era of evidence-based medicine, interpretation of laboratory result requires Reference Interval (RI) or cut-off values for diagnostic accuracy. While establishing physiologically normal values, inherent variables like Gender, Age, Occupation, Body build, genetic background and environment (altitude) are more problematic. This study aims to establish the normal haematology RI for CBC.

Aims : To establish hematological reference intervals of CBC parameters for healthy adults.

Settings and Design : The cross-sectional study with posteriori sampling carried out at Pathology laboratory.

Methods and Material : Retrospective and prospective study including healthy individuals came for routine health check-up during a period from January, 2018 to March, 2022. Total 592 individuals after applying the inclusion and exclusion criteria are included.

Results : All parameters of CBC show significant P value by Shapiro–Wilks test, except MCHC. Mann-witney U test applied to retrieve the reference interval for Males and Females.

Conclusions : The current study revealed a significant gender-based difference in RI and also differ from currently used Reference Interval. As compare to other studies also shows significant difference. As per CLSI guideline and present study, each laboratory should establish their own reference interval.

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Key words **: Complete Blood Count, Reference Interval.**

Health is necessarily a relative and goal-oriented concept[1]. However, to say that health is relative implies that the condition of individuals must be related to something and for this Reference Interval (RI) is used to compare the value of individuals laboratory results[2]. RI plays a great role in patient diagnosis, management, disease prognosis, monitoring of response to therapy and in monitoring possible adverse reactions to therapy[3]. According to the Clinical and Laboratory Standards Institute (CLSI) guideline each laboratory should establish its own RI, as number of factors affect haematological values in apparently healthy individuals like gender, age, occupation, body build, genetic background and environment (altitude). The Complete Blood Count (CBC) is the most frequently ordered diagnostic tests in medicine. The CBC is used to determine quickly whether a patient is anemic or infected and to estimate the blood's ability to coagulate normally. The concept of reference intervals was introduced by International Federation of Clinical Chemistry (IFCC). According to CLS Iguideline the establishment of a reference interval of a laboratory to be done by testing at least 120 samples from non-diseased individuals for each gender and age group[4,5]. This study aims to establish the normal haematology reference intervals for CBC for healthy adult of Shree Krishna Hospital, Karamsad, Anand, Gujarat in NABL accredited laboratory.

Editor's Comment :

- Each laboratory should establish their own reference interval as suggested by CLSI guideline.

Complete blood count parameters included in this study are:

(1) Total Leucocyte Count (TLC)
(2) Red Blood Cell Count (RBCs)
(3) Haemoglobin Concentration (Hb)
(4) Haematocrit (HCT)
(5) Mean Corpuscular Volume (MCV)
(6) Mean Corpuscular Haemoglobin (MCH)
(7) Mean Corpuscular Haemoglobin Concentration (MCHC)
(8) RBC Distribution width SD (RDW-SD)
(9) Platelet Count (PLT)

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Aims and Objectives

Aim : To establish haematological reference intervals of Complete Blood Count parameters for healthy adults at Shree Krishna Hospital, Karamsad.

Objectives : To determine whether the currently used reference interval do represent the adult population in the city.

Materials and Methods

The cross-sectional study with posteriori sampling was carried out at the NABL accredited Central Diagnostic Laboratory, Department of Pathology, Shree Krishna Hospital and Pramukh Swami Medical college, Karamsad, a tertiary centre in Karamsad, Anand. The present study is a retrospective (152 cases) and prospective study (440 cases) including healthy normal individual, who came for health check-up at Shree Krishna Hospital during a period from January, 2018 to March, 2022. Total of 592 normal healthy individuals were identified after applying the inclusion and exclusion criteria and were included for establishing the RI. The proposed number was above the CLSI guidelines for the establishment of RI, which recommends a minimum of 120 participants.

Inclusion Criteria :

(1) Male and Female
(2) Age group : Above18 years

Exclusion Criteria :

(1) Pathophysiological States - Renal Failure, Cardiac Diseases, Chronic Respiratory Diseases, Liver Diseases, Malabsorption Syndromes, Malignancies and Hematological Disorders which included anaemias.

(2) Systemic Diseases – Hypertension and Diabetes Mellitus.

(3) Replacement or Supplementation Therapy eg, Thyroxine, Insulin.

(4) Modified Physiological States - Pregnancy, Psychological and Mental Disorders.

(5) The paediatric age group (below 18 years) was excluded since the haematological parameters have a different reference range from adults.

A volume of 2 ml blood was collected in EDTA vacutainer and was processed within 2 hours of collection for the CBC in automated haematology analyser Sysmex XN 350 or Sysmex XN 550. Instruments were installed after doing validation tests like IQ (Installation Qualification), OQ (Operational qualification), PQ (Performance Qualification), carry over and calibration on installation. The controls were checked at different concentrations (Level 1, Level 2 and Level 3). Daily two level of the total three levels controls were run in the machine.

Statistical Analysis :

The analysis was informed entirely by CLSI guidelines which recommend the use of 97.5 percentile and 2.5 percentile formed the upper and lower limit of reference range respectively of the population and associated group comparisons based either on parametric or non-parametric statistics depending on whether the distribution of the data is Gaussian or non-Gaussian. Shapiro–Wilks test was used to evaluate data distribution. The mean, median, Standard Deviation (SD), range, 2.5 and 97.5 percentile were subsequently evaluated. P value of less than 0.05 was considered statistically significant (null hypothesis is rejected), it indicates that the population does not show a “Normal distribution and there is significant gender-based difference in RI. Differences between males and females were evaluated using the Mann–Whitney U test.

Observation and Results

Total 592 individuals (144 Females and 448 Males) were evaluated and gender wise partitioning of subjects was done. All nine parameters of Complete Blood Count were evaluated for their distribution by Shapiro-Wilk test and significant P value of <0.05 was applied. When parameters distributed normally than reference range is decided by mean ± 2 Standard Deviation (SD). In case of skewed distribution median, Interquartile range (IQR) and 2.5-97.5 percentile were used as reference range. The non-parametric Mann-Whitney U test was applied to all parameters to check for any significant difference between Male and Female subgroups.

The Table 1 and 2 shows that all CBC parameters have significant P value <0.05, except MCHC (P value >0.38). All parameters reject null hypothesis except MCHC, suggesting that all parameters except MCHC have significant gender-based difference.

Discussion

The present study has comparable parameters and has mild variation in Reference Interval with currently used Reference Interval for Hb, RBC and HCT. Rest all parameters like MCV, MCH, MCHC, RDW, TLC and PLT have significant difference in reference interval in comparison to currently used Reference Interval. In present study derived value for PLT count has lower limit value on higher side for both Male and Females (Table 3).

The present study shows the significant difference in RI of Platelet Count in comparison to other study. The values for HB, HCT, MCHC and RBC count of

Table 1 — *Reference interval for different parameters*

Parameter	CV%	N	Shapiro-Wilk test: Significant P value	Min	Max	Mean	Standard deviation	Mean ± 2SD	Median	IQR	Reference range 2.5 percentile and 97.5 percentile
Haemoglobin (g/dl)	0.90	592	P<0.00	12	19.3	14.44	1.32	11.8-17.08	14.5	1.9	12.1-16.8
RBC count (million/cumm)	0.80	592	P<0.00	3.44	7.12	5.13	0.59	3.95-6.31	5.13	0.78	4.07-6.39
Haematocrit (%)	1.20	592	P<0.00	34.9	57.1	44.25	3.64	36.97-51.53	44.5	5.425	37.3-50.5
Mean Corpuscular Volume (fl)	0.80	592	P<0.00	57.2	114.2	86.64	6.65	73.34-99.94	86.85	6.7	71.40-102
Mean Corpuscular Hb (picogram)	0.80	592	P<0.00	18	39	28.31	2.69	22.93-33.69	28.5	2.8	21.6-34.1
Mean Corpuscular Hb Concentration (g%)	1.10	592	P>0.38	29.5	35.7	32.64	1.13	30.38-34.9	32.7	1.5	30.2-34.8
Red Cell Distribution width (fl)	0.70	592	P<0.00	32.6	60.1	41.65	3.93	33.79-49.51	41	4.2	35.8-52.1
Total Leucocyte Count (1000/uL)	2.70	592	P <0.00	3.2	15.8	7.32	1.87	3.58-11.06	7.1	2.3	4.4-11.7
Platelet Count (1000/ul)	4.10	592	P<0.00	128	585	300.17	64.70	170.77-429.57	292.5	84	194-451

Table 2 — *Ranges for parameters with difference in male and female subpopulations*

Parameter	Significant P value	Decision	Mann witney U test: Males Mean	Males Range	Females Mean	Females Range
Haemoglobin (g/dl)	P<0.00	Reject null hypothesis	14.93	12.8-17.0	12.94	12-14.9
RBC count (million/cumm)	P<0.00	Reject null hypothesis	5.32	4.25-6.51	4.98	3.76-5.2
Haematocrit (%)	P<0.00	Reject null hypothesis	45.63	40.2-51.1	39.96	36.6-44
Mean Corpuscular Volume (fl)	P<0.00	Reject null hypothesis	86.34	70.2-102	87.58	76-98.9
Mean Corpuscular Hb (Picogram)	P<0.00	Reject null hypothesis	28.29	21.2-34.1	28.39	24.5-32.7
Mean Corpuscular Hb Concentration (g%)	P>0.38	Retain null hypothesis	32.72	30.1-34.9	32.40	30.7-34.4
Red Cell Distribution Width (fl)	P<0.00	Reject null hypothesis	41.56	35.5-52.1	41.94	36.9-51.3
Total Leucocyte Count (1000/uL)	P<0.00	Reject null hypothesis	7.13	4.4-11.4	7.93	4.6-14.2
Platelet Count (1000/ul)	P<0.00	Reject null hypothesis	292.85	191-429	322.98	209-473

Table 3 — *Comparison of obtained reference interval with currently used reference interval*

Parameters	Currently used Reference Range from Standard Text Book Dacie 11th edition[6]	Present study reference range
Haemoglobin (Hb) (g/dl)	M: 13-17 F: 12-15	M: 12.8-17.0 F: 12-14.9
RBC Count (million/cumm)	M: 4.5-5.5 F: 3.8-4.8	M: 4.25-6.51 F: 3.76-5.2
Haematocrit (HCT) (%)	M: 40-50 F: 36-46	M: 40.2-51.1 F: 36.6 - 44
Mean Corpuscular Volume (MCV) (fl)	83-101	M: 70.2-102 F: 76-98.9
Mean Corpuscular Hb (MCH) (Picogram)	27-32	M: 21.2-34.1 F: 24.5-32.7
Mean Corpuscular Hb Concentration (MCHC)(g%)	31.5-34.5	M: 30.1-34.9 F: 30.7-34.4
Red Cell Distribution Width (RDW) (fL)	39-46	M: 35.5-52.1 F: 36.9-51.3
Total Leucocyte Count (TLC) (1000/uL)	5-13	M: 4.4-11.4 F: 4.6-14.2
Platelet Count (PLT) (1000/ul)	150-410	M:191-429 F: 209-473

present study is comparable to all three studies Siraj *et al*, Sehgal, *et al* and Rahar, *et al*. MCV shows significant difference in RI. Value of TLC is comparable to Sehgal, *et al* and Rahar, *et al*. MCH also shows significant difference as compare to other study as shown in Table 4. Severalfactors can affect the RI, which include age, sex, weight, environment, race and ethnic origin, biorhythms, pregnancy, nutritional state, lifestyle, medication, tobacco and alcohol consumption[7] (Table 4).

Table 4 — *Comparison of the obtained reference interval with other studies*

Parameters	Present study, 2022, Karamsad, Anand (592 samples) (Sysmex XN 350) or Sysmex XN 550)	Sehgal, *et al,* 2020, Mumbai[8] (100 samples) (Sysmex XE-2100)	Siraj, *et al* 2015, Asmara, Eritrea[9] (591 samples) (Backman coulter : AU 480 chemistry system)	Rahar, *et al* 2022, Delhi[10] (123 samples) (Sysmex XN-1000)
Haemoglobin (g/dl)	M : 12.8-17.0 F : 12-14.9	M : 13.18-17.22 F : 12.1-14.6	M : 12.6-17.8 F : 12.5-17.6	M : 12-16.5 F : 12-15
RBC Count (million/cumm)	M : 4.25-6.51 F : 3.76-5.2	M : 4.56-6.16 F : 4.20-5.39	M : 4.2-6.07 F : 4-5.7	M : 4.14-5.49 F : 4.04-5.43
Haematocrit (%)	M : 40.2-51.1 F : 36.6-44	M : 40.24-53.48 F : 37.33-46.05	M : 40.5-55 F : 37.9-52	M : 36-49.6 F : 36-44.6
Mean Corpuscular Volume (fl)	M : 70.2-102 F : 76-98.9	81.125-93.448	M : 85.7-100 F : 85.5-100	M : 80.5-98.7 F : 77-99.5
Mean Corpuscular Hb (picogram)	M : 21.2-34.1 F : 24.5-32.7	M : 26.12-30.67 F : 25.5-30.2	M : 28-33 F : 26.5-32.6	M : 26-34.2 F : 25.6-33.4
Mean Corpuscular Hb Concentration (g%)	M : 30.1-34.9 F : 30.7-34.4	M : 30.88-34.96 F : 30.47-33.95	M : 30.4-33.7 F : 30-33.7	M : 31.5-35.8 F : 30.4-35.1
Red Cell Distribution width	M : 35.5-52.1 (fL) F : 36.9-51.3 (fL)	12.3-15.14 (Unit is %)	M : 12.3-15.5 F : 12.3-17 (Unit is %)	M : 12.2-16 F : 12.1-16 (Unit is %)
Total Leucocyte Count (1000/uL)	M : 4.4-11.4 F : 4.6-14.2	4.2-10.0	M : 3.7-9.3 F : 3.3-8.9	M : 4.16-10.0 F : 4.5-11.0
Platelet Count (1000/ul)	M :191-429 F : 209-473	M : 153-366 F : 182-409	M : 128.4-318.4 F : 145.4-351.6	M : 150-388 F : 164-420

Conclusion

The present study suggests that there is a significant gender-based difference in CBC parameters and has mild variation from currently used Reference Interval. As compare to other studies, present study shows significant difference between laboratory parameters. This difference suggest that each laboratory should establish their own reference interval as suggested by CLSI guideline.

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