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CLSI-Derived Hematology Reference Intervals for Healthy Males in Eastern India

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ABSTRACT

Haematological reference ranges are often influenced by individual variables, such as race, age, gender and dietary habits. In addition, ecological factors such as climate and altitudemight affect the parameters, while variations in instrumentation techniques and laboratory personnel involved also contribute to the measurements. Therefore, the currently used reference ranges, which were originally adopted from other countries and mainly refer to European subjects, might be misleading in some cases. This study is an attempt to establish haematological reference ranges for male subjectsfrom Eastern India. Haematological tests, using an automated haematology analyzer, were carried out on 528blood samples from healthy male donors. The population was found to exhibit lowerhaemoglobin (HGB) and platelet (PLT) contents as compared to the standard reference values, although the difference was statistically significant only for the platelet count. Nevertheless, the digression from the international range of data was clinically significant, except for the white blood cells (WBC) and red blood cells (RBC) counts. Finally, the respective reference values for HGB, RBC and hematocrit (HCT) were found to vary significantly with the age group of the subjects. It is expected that the study will facilitate the interpretation and reporting of haematological parameters in Eastern India.

Keywords: Haematology, Reference Ranges, Variables, Parameters.

Abbreviations: CLSI (Clinical and Laboratory Standards Institute; formerly known as NCCLS i.e. National Committee for Clinical Laboratory Standards), ELISA: Enzyme linked immunosorbent assay, HbsAg: Hepatitis B surface antigen, HCT: Haematocrit, HCV: hepatitis C virus, HGB: Haemoglobin, HIV: Human immunodeficiency virus, K_2 – EDTA: Di-potassium ethylene diaminetetra acetic acid, MCHC: Mean Corpuscular Haemoglobin Concentration, MCH: Mean Corpuscular Hemoglobin, MCV: Mean Corpuscular Volume, PLT: Platelets, RBC: Red Blood Cells, SD: Standard Deviation, VDRL: Venereal Disease Research Laboratory, WBC: White Blood Cells

INTRODUCTION

In India, the haematology parameters are interpreted as normal or abnormal based on the reference ranges obtained from the international data base[1-5]. However, a variety of factorscould influence these values due todemographicvariables including gender, ethnic origin, dietary habitsand geographical location[6-9]. In addition, laboratory methods, instrumentation, personnel involved, and type of container used for specimen collection could be implicated in this regard[5, 10-13]. Thus, indigenous reference values established in consideration of these factors would help to improve the quality of reporting haematological

parameters. Attempts to establish more accurate reference ranges for clinical haematological assessments of specific populations were made in several countries[14-18].

Thus, the aim of present study is to establish a set of standard reference ranges for the haematological assessment of male population of different age groups, and offer therecommendationto the local hospitals and community laboratories in Eastern India.

MATERIALS AND METHODS

A prospective cross-sectional study was carried out in Kolkata on 528 male individual to establish reference haematological ranges for male population of ages between 20 to 59[19]. These individuals were mainly from West Bengal, and other States in Eastern India.

Di-potassium ethylene diaminetetraacetic acid (K_2– EDTA) blood samples were collected from antecubital vein of blood donorsrepresenting a healthy population. The donors wereselected by conducting a rigorous pre-donation screening through interviews and hemoglobin measurement. Further routine screening was conducted after donation, when the blood was tested for Human immunodeficiency virus (HIV)- I and II (4^{th} Generation), hepatitis C virus(HCV),hepatitis B surface antigen (HbsAg), and malaria parasites by enzyme linked immunosorbentassay (ELISA), and the presence of reagin antibody was detected by flocculation method (Venereal Disease Research Laboratory, VDRL).

Blood samples were analyzed using automated haematology analyzer (Wipro LAB LIFE) working on Coulter principle[20]for counting of the WBC, RBC and PLT, and cyanmet hemoglobin determination of HGB[21-22]. The blood samples were analyzed soon after collectionin order to reduce the storage variables[11].

The reference range intervals were obtainedfor HGB, HCT, RBC, WBC, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH), and PLT by calculating the values within 2.5 to 97.5 percentile limit[23]. Age group specific variation of haematological parameters was evaluated by one-way analysis of variance (ANOVA) for independent samples, and variation was shown by box-and-whisker plots. Statistical difference between the obtained mean and international data[1] for each parameter was compared by Chi-square test. Finally, clinical acceptability or bias percentage[24] was checked between the obtained mean of hematological parameters with international data for male subjects[1].

RESULTS

A reference range was obtained following standard guidelines[23], showing the 2.5-97.5 percentile intervals and median values for each of the haematological parameters determined for 528 male subjects (Table 1). Next, the above data were classified (Table 2) into three age-groups of 20-29 (n=193), 30-39 (n=201), and 40-59 years (n=134) as per NCCLS specification which requires at least 120 subjects to construct a reference range. Here, the variation among the data sets for haemoglobin, RBC and HCT of the three age groups was found to be statistically significant (Table 2). This age group specific variation was depicted by box-and-whisker plot in Figure 1. In Table 3, an attempt was made to compare the mean values of the 'obtained range' with the 'international range' as given in Dacie and Lewis Practical Haematology, 2006[1]. The comparison showed that the reference values for haemoglobin and platelet were lower than the 'international range', although this difference was statistically significant only for the platelet counts (Table 3). However, calculation of clinical bias showed that this difference was clinically significant for all the parameters except for WBC and RBC[24] (Table 3).

Table 1 Overall male (n = 528) specific median value and reference interval of the haematological parameters [23]

Parameter	Median	Reference interval (2.5 – 97.5 percentile)
WBC ($x10^9$/L)	7.10	4.42 – 11.10
RBC($x10^{12}$/L)	4.70	3.88 – 5.71
HGB(g/dL)	13.60	11.00 – 16.38
HCT(%)	40.40	32.51 – 47.50
MCV(fL)	86.50	72.32 – 96.70
MCH(pg)	29.10	23.00 – 34.00
MCHC(g/dL)	33.60	30.02 – 37.25
PLT($x10^9$/L)	149.00	73.35 – 273.48

Table 3 Statistical and clinical significance of the comparison between obtained mean of haematological parameters with international data[1] for male subjects (n = 528)

Parameters	Obtained Range ($Mean_1 \pm 2SD$)	International Range[1] ($Mean_2 \pm 2SD$)	Chi-square value [a]	*Bias %	Acceptable limit of Bias%
WBC ($x10^9$/L)	7.21 ± 3.43 [b]	7.0 ± 3.0	0.01	3.00	5.6
RBC ($x10^{12}$/L)	4.73 ± 0.97 [b]	5.0 ± 0.5	0.01	5.40	7.8
HGB (g/dL)	13.64 ± 2.60 [b]	15.0 ± 2.0	0.12	**9.07**	**1.8**
HCT (%)	40.53 ± 7.52 [b]	45.0 ± 5.0	0.44	**9.93**	**1.7**
MCV (fL)	86.13 ± 11.92 [b]	92.0 ± 9.0	0.37	**6.38**	**1.2**
MCH (pg)	28.94 ± 5.50 [b]	29.5 ± 2.5	0.01	**1.90**	**1.4**
MCHC (g/dL)	33.63 ± 3.87 [b]	33.0 ± 1.5	0.01	**1.91**	**0.8**
PLT ($x10^9$/L)	152.92 ± 104.34 [c]	280.0 ± 130.0	57.68	**45.39**	**5.9**

[a] Chi-square value was calculated to compare between the mean values of 'Obtained Range' and 'International Range'.
[b] Chi-square value < 3.84 was considered as statistically non-significant at 95% confidence interval.
[c] Chi-square value > 3.84 was considered as statistically significant at 95% confidence interval.
** Bias% = mod [($Mean_2$ – $Mean_1$)/ $Mean_2$] x 100, if greater than the acceptable limit[24] was considered as clinically significant difference between two mean values.*

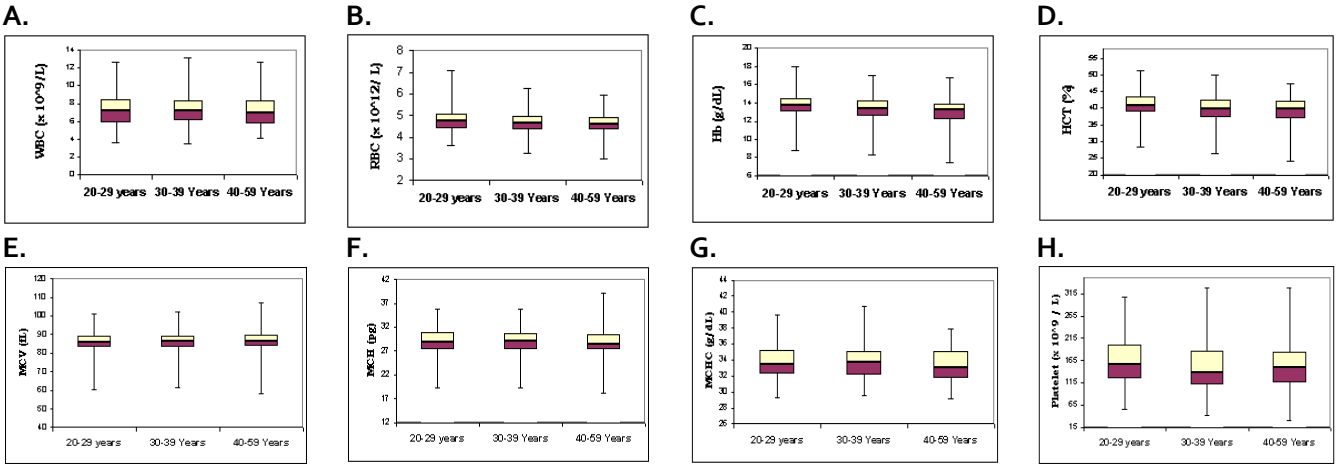

Figure 1 Box-and-whisker plot

(graphical representation of the smallest observation, lower quartile, median, upper quartile, and largest observation)showing comparison of variations among three age groups for (A) WBC ($x10^9$/L), (B) RBC ($x10^{12}$/L), (C) HGB (g/dL), (D) HCT (%), (E) MCV (fL), (F) MCH (pg), (G) MCHC (g/dL) and (H) PLT ($x10^9$/L)

Table 2Mean, median, standard deviation and reference range intervals of the haematological parameters, obtained for three different age groups of male subjects (n = 528)

Parameter	Age 20-29 (n=193)					Age 30-39 (n=201)					Age 40-59 (n=134)					*p value in ANOVA
	Mean	Median	SD	Percentile		Mean	Median	SD	Percentile		Mean	Median	SD	Percentile		
				2.5	97.5				2.5	97.5				2.5	97.5	
WBC ($x10^9$/L)	7.25	7.20	1.77	4.46	11.40	7.28	7.20	1.65	4.60	10.50	7.03	6.80	1.73	4.40	10.27	0.372[a]
RBC ($x10^{12}$/L)	4.80	4.80	0.51	3.94	5.81	4.70	4.65	0.46	3.93	5.66	4.67	4.66	0.47	3.70	5.57	0.024[b]
HGB (g/dL)	13.90	13.90	1.25	11.08	16.20	13.58	13.50	1.33	11.20	16.50	13.36	13.50	1.28	10.80	15.84	0.0008[b]
HCT (%)	41.15	40.90	3.69	35.06	48.32	40.25	40.10	3.79	32.40	47.50	40.03	40.10	3.72	32.39	46.70	0.012[b]
MCV (fL)	86.12	86.40	6.06	71.60	96.70	86.05	86.50	6.10	68.00	96.70	86.26	86.80	5.64	72.83	94.44	0.951[a]
MCH (pg)	29.06	29.00	2.88	23.48	34.60	28.98	29.20	2.78	22.30	34.10	28.71	28.70	2.51	23.00	32.80	0.512[a]
MCHC (g/dL)	33.76	33.60	1.89	30.30	37.30	33.71	33.80	1.97	29.90	37.40	33.34	33.20	1.94	29.53	36.61	0.115[a]
PLT ($x10^9$/L)	159.88	155.00	49.36	80.80	255.00	149.33	138.00	52.93	73.00	277.00	148.28	145.50	54.25	59.95	281.32	0.065[a]

*$p<0.05$ was considered as statistically significant

[a]Variance was statistically non-significant by One-Way Analysis of Variance (ANOVA) for independent samples

[b]Variance was statistically significant by One-Way Analysis of Variance (ANOVA) for independent samples

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DISCUSSION

The determination of reference range for haemoglobin, HCT and red cell indices would be crucial for the individuals suffering from iron deficiency and/ or nutritional anaemia, whichcouldbe re-confirmed by serum ferritin, Vitamin B_{12} and folate examination[18]. The reference ranges wouldindicate whether the values for the hematological parameters of an individual differed from the reference population.

The internationalreference range of platelet count was considered to be 150 – 410 ($x10^9$/L)[1], whereas this range was calculated to be 73.35 – 273.48 ($x10^9$/L), based on the data collected in Eastern India(Table:1). However, it is to be noted that in case of platelets, the readings given by the auto analyzers may not be sometimes reliable,hence, need to be confirmed by manual counting method and smear review[25].

Our study also indicated that the variation among the data sets for haemoglobin, RBC and HCT of the three age groups was statistically significant (Table 2). Therefore, there is a definite need to introduce age specific reference ranges for males.

While checking for clinical bias (Table 3), a significant difference was observed between the mean value obtained and the mean of the international data[1] for all parameters, except for WBC and RBC[24]. This indicatedthe necessity of the regional reference range, although this difference was not statistically significant (Table 3). Further study to establish independent reference range for adult female population should be undertaken separately.

CONCLUSION

A group of male population in Eastern India was found to exhibit lower haemoglobin and platelet contents as compared to the international reference values, although the difference was statistically significant only for the platelet count. Nevertheless, the digression from the international range of data was clinically significant, except for the WBC and RBC counts. Finally, the respective reference values for HGB, RBC and hematocrit were found to vary significantly with the age group of the subjects. The study would facilitate the interpretation and reporting of haematological parameters in Eastern India.

Authors' disclaimer

The opinions expressed in this paper are those of the authors and may not reflect the position of their employing organizations.

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