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Effect of Altitude on Hemoglobin and Red Blood Cell Indices in Adults in Different Region of India

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Abstract
One frequently used blood test for health checkups is the complete blood count (CBC). This study compared CBC from populations living at sea level and from two different elevations in an effort to propose a correction factor for the elevation of the hemoglobin and red blood cell indices that is related to altitude. Large datasets of CBCs over a 5-year period were screened for this retrospective lab records study from three different localities of India: the coastal city of Gujarat which is located at 1117 meter above sea level, the city of Kasauli, which is located at 1800 meters above sea level; and the city of Chamoli, which is located at 2270 meters above sea level. The CBC variables were compared using descriptive analysis and significance testing at various altitudes. In the end, 57,059 participants were taken into account for the analysis.
At sea level, the mean hemoglobin (Hb) concentration (g/dL) for men and women was 14.81 and 13.77, respectively, while in Kasauli City, the Hb concentrations for men and women were 15.35 and 14.19, respectively. However 15.40 and 14.71in Chamoli City, for men and women, respectively. With the exception of mean corpuscular volume (MCV), hemoglobin and other red cell indices varied significantly between men and women at different altitudes. When comparing the MCV 95th percentile range to the present reference range, it was lower at both elevations and sea level (76–91 fLvs 81–98 fL). While there was a discernible increase in Hb concentration with altitude in both genders, it was not as high as recommended by the World Health Organization and the Centers for Disease Control. Additionally, new CBC reference intervals for India citizens residing at high altitudes can be derived from the study's results. In the adult population residing at high altitudes in India, a correction factor (ΔHb) of 0.30 g/dL per 1000 m altitude is recommended to be employed. This should assist characterize anemia and polycythemia at altitude more precisely.
Keywords: High altitude, reference interval, CBC

Introduction
One of the most significant blood tests used to assess illness and health status is the complete blood count (CBC). To properly interpret the test results, it is imperative to establish a reference interval for the CBC. In statistical terms, the optimal reference range can be defined as two standard deviations (SD) of the mean value of a representative sample population, or the middle 95% range of results.1. Generally speaking, reference range values come from sizable cohorts of people in good health.

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Both healthy and pathological situations have an impact on hemoglobin concentration (Hb), red cell count, and indices generated from red blood cells (RBCs).

The physiological factors of age, gender, ethnicity, and altitude are well-known determinants of blood components. High altitude causes a decrease in plasma volume whereas hypoxia-induced erythropoietic drive causes erythrocytosis, an increase in hemoglobin concentration, and hematocrit.3. The reference intervals recommended by the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) are primarily based on research done on the Caucasian population.4,5 Additionally, a correction factor for hemoglobin has been proposed, primarily based on research done in South America's highlands, for the definition of anemia at different altitudes.

Nevertheless, a number of studies have demonstrated that the link between hemoglobin and altitude is nonlinear and varies across different racial and geographic contexts, making the application of the unified correction factor difficult. This can result in the overestimation of clinical illnesses such as anemia.6–8 The correction factors for red blood cells (RBCs) and other derived indices, such as mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC), are also not well understood. Thus, extensive epidemiological local investigations should serve as the foundation for determining a reference range.

Residents at high altitudes are predicted to exhibit greater hemoglobin concentrations, a larger red blood cell count, and varying values of red cell-derived indices due to high altitude linked erythrocytosis, which calls for the use of a localized reference range.

It is anticipated that modifications to these reference intervals will coincide with adjustments to the cut off points that characterize anemia and polycythemia, two clinical conditions that are frequently assessed. As far as we are aware, there is less epidemiological research has been done in high-altitude of India to determine the reference intervals for blood counts. Therefore, the purpose of this work was to provide accurate CBC parameter reference intervals for Indiahigh altitude.

Material and Methods

This study involved a comparative retrospective evaluation of lab records in different areas of India.The CBC results for individuals of both sexes, aged 18 to 60, who visited commercial laboratories for blood testing over a 5-year period (2019-2022) were chosen from three geographic locations:Gujarat, Kasauli(1800 m altitude), and Chamoli (2500 m altitude).

At the time of the CBC testing, participants who had access to the entire panel of tests were examined. Tests include the complete blood count (CBC), the thyroid function tests (thyroid stimulating hormone, T4), the kidney profile (urea, creatinine), the liver profile (alanine aminotransferase, aspartate aminotransferase, total bilirubin, direct bilirubin), the lipid profile (cholesterol, high-density lipoprotein, low density lipoprotein, triglyceride), and a fasting blood glucose level (HbA1c).

Exclusion Criteria

Individuals who did not meet the predetermined cutoff values for blood glucose, lipid, kidney, or liver profiles were not included in the analysis. In accordance with the established cutoff, participants were also disqualified if their platelet, Hb, or white blood cell counts were aberrant.

The cutoff points for hemoglobin inclusion were 12 g/dL for men and 12 g/dL for women. Participants from the two altitudes were also assessed using higher cutoff values for women (12.5 g/dL) and men (13.5 g/dL) in order to account for the altitude effect. The difference between the two inclusion procedures was also examined. To reduce the impact of undetected latent iron deficiency or thalassemia trait on the obtained data, MCV values less than 75 fL were also removed. Exclusion of red cell distribution width (RDW) greater than 16 was implemented in order to reduce the potential impact of undetected occult micronutrient deficits.

Blood Collection and Laboratory Analysis

With the aid of automated analyzers made by Sysmex Corporation in Kobe, Japan, specifically the XS-1000i and XS-500i, the lab's processes are standardized across its several divisions. The manufacturer's instructions and accreditation requirements are followed for the analyzers' calibration and upkeep. Every day, controls are examined before patient samples are run.

Data Analysis

Prior to being imported into an Excel datasheet, all personally identifiable information about the participants was removed from the data. Following data extraction, the information was edited, coded, and entered into IBM SPSS version 22, a statistical program (SPSS, Inc. Chicago, IL). The participants' age, gender, and residential area were used in a descriptive analysis based on percent distribution and frequency. All scale parameters were assessed using the normal distribution. Descriptive analyses were utilized to determine the normal population range for all CBC values. The mean, together with its 95% confidence interval, median, range, and percentiles (2.5th and 97.5th) were used.

A greater value winsorization procedure was used to minorize any extreme value. The investigated continuous variables were compared between the various groups using the Student's t-test. Two-tailed tests were used for every statistical analysis, with a significance threshold of p value <0.05.

Results and Discussion

More than 120,000 participants were screened. After applying the age and lab exclusion criteria, 46,012 individuals (F: 27,559 and M:18,453) from Gujarat City, 5831 (F: 3656 and M: 2175) fromChamoli City, and 5216 (F: 3193 and M: 2023) from Kasauli City were included in the final analysis. The 5% trimmed mean Hb (g/dL) concentration was 14.81 ± 1.13 and 13.77 ± 1.32 for men and women at Gujarat City (sea level), 15.35 ± 1.28 and 14.19 ± 1.37 for men and women at KasauliCity (1800 m), and 15.40±1.25 and 14.71 ± 1.50 for men and women at ChamoliCity (2500 m), respectively (Table 1). The effect of using the same cutoff values on the mean Hb concentration was modest and restricted to the male population (Table 2).

Table 1: Descriptive Analysis of CBC Parameters at the Studied Three Geographical Areas Using 12.5 g/dL for Women and 13.5 g/dL for Men as Normal Reference Level#

City Name	Gujarat (Sea Level)		Kasauli(1800m)		Chamoli(2500m)	
Gender	**Men**	**Women**	**Men**	**Women**	**Men**	**Women**
Number	n=18,453	n=27,559	n=2175	n=3510	n=2023	n=2943
Age Years	41±10	39±10	40±10	38±10	40±10	38±10
Hemoglobin g /dL	14.81±1.13	13.77±1.32	15.20±1.36	14.71±1.51	15.13±1.26	14.19±1.37
Hematocrit %	43.48±3.18	40.80±3.58	43.86±3.73	42.27±3.81	43.30±3.52	40.84±3.48

City Name	Gujarat (Sea Level) Kasauli(1800m)		Kasauli(1800m)		Chamoli(2500m)	
Gender	**Men**	**Women**	**Men**	**Women**	**Men**	**Women**
Red cell count x10^{12}/L	5.15±0.43	4.84±0.47	5.37±0.48	5.20±0.50	5.18±0.46	4.88±0.47
MCV fL	84.34±4.21	84.38±4.35	81.30±3.80	81.12±3.93	83.58±3.90	83.61±4.15
MCH pg	28.83±1.62	28.55±1.67	28.37±1.43	28.30±1.45	29.23±1.54	29.08±1.65
MCHC g/dL	34.17±1.28	33.82±1.33	34.85±1.28	34.84±1.35	34.94±1.20	34.74±1.38
RDW R%	13.35±0.84	13.48±0.88	13.60±0.81	13.64±0.84	13.36±0.81	13.48±0.84

Note: #5% trimmed mean and standard deviation reported

CBC Parameters at Sea Level and Both Altitudes Using 12 g/dL for Women and 13 g/dL for Men as Lower Normal Reference Level#

City Name	Gujarat (Sea Level) Kasauli(1800m)		Kasauli(1800m)		Chamoli(2500m)	
Gender	Males	Females	Males	Females	Males	Females
Numbers	18,453	27,559	2396	3656	2262	3193
Age (Years)	41±10	39±10	40±10	38±10	40±10	38±10
Hemoglobin g	14.81±1.13	13.77±1.32	15.20±1.36	14.71±1.51	15.13±1.26	14.19±1.37

/dL						
Hematocrit %	43.48±3.18	40.80±3.58	43.86±3.73	42.27±3.81	43.30±3.52	40.84±3.48
Red cell count x10^{12}/L	5.15±0.43	4.84±0.47	5.37±0.48	5.20±0.50	5.18±0.46	4.88±0.47
MCV fL	84.34±4.21	84.38±4.35	81.30±3.80	81.12±3.93	83.58±3.90	83.61±4.15
MCH pg	28.83±1.62	28.55±1.67	28.37±1.43	28.30±1.45	29.23±1.54	29.08±1.65
MCHC g/dL	34.17±1.28	33.82±1.33	34.85±1.28	34.84±1.35	34.94±1.20	34.74±1.38
RDW %	13.35±0.84	13.48±0.88	13.60±0.81	13.64±0.84	13.36±0.81	13.48±0.84

Note: #5% trimmed mean and standard deviation reported.

While the 2.5th percentile values of Hb concentration were affected by the cutoff value used for inclusion, the 97.5th percentile values of Hb concentration showed higher values at both altitudes as compared to sealevel and to the current reference range, suggesting the need for updating the current reference range at high altitudes (Table 3). At the highest altitude (Chamoli City), the Hb increment (Δ Hb) was more prominent in women (0.94) g /dL than in men (0.59 g/dL). The 2.5th and 95th percentile range (95th percentile range) of Hb concentration using two different cutoff values for inclusion are shown in Supplementary Table 1.

The Proposed Update to the Currently Utilized Range According to Suggestions from Our Study*

Parameters	Men		Women	
	Current	Suggested	Current	Suggested
Hb g /dL	13–17.5	13.5–18.1	12–15.5	12.7–17.90
HCT %	38.8–50	38.5–51.4	34.9–44.5	36.7–50
RBCS x10^{12}/L	4.5–6.2	4.64–6.33	3.8–5.0	4.44–6.30
MCV fL	81.2–95	76–90	81.6–98	76–91
MCH pg	27–32	25.5–31	27–32	26–31
MCHC g /dL	32–35	32.34–37.2	33–37	32–37
RDW %	11.6–14.6	12.2–15.3	11.6–14.6	12.2–15.4

Notes: *The reference range is based on the 95% percentile values. Detailed descriptive analysis of hemoglobin values in both genders among all three cities is provided in Supplementary Table 1

Between genders at the same altitude and between the same gender at various cities, there were significant differences in the Hb concentration, RBCs, and derived indices (p value <0.001 for all comparisons), with the exception of MCV.

In comparison to the present reference range, the 95th percentile range (76–91 fL) and the mean value of MCV were lower (Tables 1-3). There was no discernible difference in the mean MCV levels between male and female respondents or across cities. In contrast to Kasauli and Gujarat, there was a trend toward a significantly lower MCV at chamoli (p value = 0.06). There was no discernible age-related difference between the young (18–40) and middle-aged (41–60) age groups according to the analysis (Supplementary Tables 2 and 3).

For medical professionals, interpreting CBC results of people living in high altitudes can be challenging, particularly when findings are near the edge of reference intervals and there is uncertainty about what constitutes polycythemia and anemia.7. The primary goals of this study were to examine the altitude-associated effect on CBC between people living at sea level and those living at an altitude, and to compare the CBC results with those of other populations and areas worldwide. This study examines the differences between sea level and high altitude Hb, RBC count, and red cell indices. It also suggests a locally generated altitude-associated Hb correction factor and newly established reference intervals for the local population.

In order to properly characterize anemia in the absence of a specific reference range, the CDC and WHO recommend using a correction factor for varying altitude4, 9, 10. Numerous studies have, however, demonstrated that the elevationassociated rise in hemoglobin (Hb) is nonlinear, varies spatially and among racial groups, and is highest in the Andes.6–8 This racial and geographic divide was thought to result from South America's relatively recent human colonization in comparison to Asia, Africa, and the Old World. Generally speaking, the Hb concentration is higher in South American highlanders than in Asian and African highlanders.11

Nevertheless, there isn't a correction factor for polycythemia, which is the top limit, so the only practical approach is the epidemiological assessment of reference intervals. In ChamoliCity, the 95th percentile values for Hb concentration are 18.1 g/dL for men and 17.9 g/dL for women. Therefore, many otherwise normal people will be classified as polycythemic if the existing reference intervals for Hb are used.

According to the WHO diagnostic criteria for polycythemia vera (PV), a case of polycythemia should be considered if the level of Hb surpasses more than 16 g/dL and 16.5 g/dl and the hematocrit values exceed 48% and 49% for women and men, respectively.13 The effects of altitude on Hb are not taken into consideration by this definition. It is therefore anticipated that a sizable proportion of individuals at high altitudes may undergo unnecessary polycythemia vera evaluations. Consequently, it's critical to take the altitude effect into account when interpreting the clinical picture and/or using diagnostic standards that weren't proven to work for people living in high altitudes.

Of the three RBC indices, MCV is the most practical and widely used (MCV, MCH, and MCHC).14 MCV, or mean corpuscular volume, is the primary factor used to classify anemia; MCV and MCH have a nearly linear connection.15 The MCV 95th percentile range in this study is biased toward a lower value. This finding was observed in all three of the cities under study. In the current study, the 2.5th percentile of MCV at sea level and elevations is 76 fL, but in the reference range currently in use, it is 81 fL at both sea level and high altitudes.

A research on the adult Moroccan population revealed an MCV value of 86 fL (75–94).17 Similar MCV ranges and lower 2.5th percentiles, which are typically lower when compared to stated reference intervals from the West, were reported in the majority of Middle Eastern and African studies. This suggests potential racial influences.18–22 Alpha thalassemia has been proposed as a genetic factor that contributes to the observed variation in MCV and Hb between African Americans and Whites.23 When comparing the MCV levels at Kasauli and Gujarat area) to Chamoli, a tendency toward much lower MCV was evident.

There are several restrictions on this study. As no participant health records were available, chronic medical disorders were implicitly ruled out using the available lab tests. Smoking and other habits that are known to lower hemoglobin concentration were not evaluated. It wasn't evaluated how well the micronutrients iron, B12, and folate were doing, which could have an impact on the CBC results. Lastly, while we believe that the great majority of the participants are Indian nationals, the individuals' ethnicity was not officially determined.

Conclusion

This study only found a slight rise in Hb concentration, which may be explained by the inhabited altitude of relatively high. At 0.3 g/dL/1000 m altitude, the increment in Hb concentration (Δ Hb) was calculated. The current CBC reference range can be updated with this modification to more accurately characterize anemia and polycythemia at high altitudes. In accordance with quality standards, the recommended update still has to be verified at the nearby laboratories. To learn more about the nature and potential causes of the observed variation in the MCV range when compared to other populations, more research is required.

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