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ORIGINAL RESEARCH

Heterogeneity in Hematological Parameters of High and Low Altitude Tibetan Populations

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Introduction: High altitude hypoxia is believed to be experienced at elevations of more than 2500 meters above sea level. Several studies have shed light on the biochemical aspects of high altitude acclimatization, where participants were sojourners to the high altitude from low altitude areas. However, information regarding the difference between the high altitude adapted Tibetans living at high altitude and their counterparts who reside at low altitude are lacking. To understand this, we have measured various hematological parameters in the Tibetan populations, who are residing in both high and low altitudes in India.

Methods: A total of 168 individuals (79 from high altitude (≥4500 meters) and 89 from low altitude (~850 meters) were recruited for this study. Hematological parameters such as red blood cells (RBC) count, hematocrit (HCT), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were measured from the individuals from high and low altitudes. Serum erythropoietin (EPO) was measured by ELISA. Statistical analyses were performed to compare data from both of the altitudes. Gender-wise comparison of data was reported. Correlation analysis was performed within relevant parameters.

Results: Highly significant differences ($p < 0.0001$) between high and low altitude Tibetans were detected in RBC count, HCT, Hb, MCHC in both males and females and in MCV in females. In the case of MCHC, however, age and BMI were potential confounders. Nominally significant differences ($p < 0.05$) were detected in MCV and MCH within males. No significant difference in serum EPO level was found between altitude groups, in any gender. No significant correlation was found between serum EPO with Hb as well as serum EPO with HCT.

Discussion: Our study explores significantly lower RBC count, HCT, Hb, MCH, MCHC and higher MCV in long-term Tibetan residents living at low altitude compared to their high altitude counterparts, which is likely due to the outcome of hematological adaptation to a relatively hyperoxic environment in low altitude areas.

Keywords: high altitude, low altitude, Tibetans, hematological parameters, erythropoietin

Introduction

High altitude is clinically defined as altitudes ≥2500 meters above sea level, where physiological changes start appearing in vulnerable people.[1] The distinct environmental features of the high altitude area, that are, extreme cold and hypobaric hypoxia, solar radiation and aridity, make it difficult for sea-level residents to cope, at least for a few days after arrival until they acclimatize sufficiently. Often, travelers develop acute mountain sickness (AMS), high-altitude pulmonary edema (HAPE) or high-altitude cerebral edema (HACE); in contrast, native high altitude

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dwellers undergo adaptation and natural selection that give them survival benefits in the harsh environment.[2] Tibetans, Andeans and Ethiopians are among the most studied native highlanders; among them, Tibetans are the oldest. Initial colonization of the high altitude Tibetan plateau dates back 25,000–30,000 years.[1,3,4] Prolonged exposure allows the populations to experience physiological and genetic adaptations, as revealed by multiple studies.[5–15] Compared to the Andeans, Tibetans have lower hemoglobin concentration at high altitude.[16] The Tibetans have lower hemoglobin concentration and hematocrit compared to ethnically non-highlander, acclimatized Han migrants at high altitude.[17,18]

Several studies have looked at the genetic aspects of high altitude adaptation and physiological changes.[5–15] However, studies on physiological aspects of high altitude acclimatization or adaptation performed to date have focused on individuals traveling to a high altitude from a low altitude or compared high altitude natives with non-native residents of high altitude areas to a large extent.[11,17,19–22] Studies on ethnically high altitude adapted populations living at low altitude for a long period are also very limited.[23,24] Adaptation is a slow process and might take thousands of years to integrate genetic changes, whereas acclimatization can happen in hours/days to combat the stress in an urgent manner. Therefore, if high altitude adapted populations reside at low altitude for several years, it is likely that they will experience the impact of this environmental shift as the partial pressure of oxygen is higher at low altitude compared to at high altitude.

According to Gustavo Zubieta-Calleja, high altitude residents experience "relative hyperoxia" at sea level, which is an apparent aggressive environment to the high altitude hypoxia-adapted people.[25] This results in their experiencing lower ventilation, probably to combat "relative hyperoxia". Zubieta-Calleja et al reported lower hematocrit after descent from high altitude to low altitude.[26] Decreased hemoglobin has been reported in the Andean natives after arrival at sea level.[27] Studies of people belonging to the same ethnic group but inhabiting high and low altitudes could be interesting as those are rare and could be insightful to understand the impact of the environmental shift. In this study, hence, we have included Tibetan people from high and low altitudes from India. Migration of ethnically Tibetan populations in India can be classified into two distinct categories: (1) migration of the Tibetans to Ladakh (sometimes described as "India's little Tibet") that started apparently during the 9th–10th centuries and continued for centuries thereafter (known as native Ladakhis also), and (2) migration of the Tibetans taking shelter in India during or after 1959 following subdual of the Tibetan uprising in 1959 by the People's Liberation Army of China. The later migrants reside in various Tibetan settlements across India, including places at high altitude, Ladakh, and low altitude, Karnataka.[28–31] Therefore, in our study, the high altitude group comes from different regions of Ladakh (altitude ≥4500 meters), and the low altitude group comes from Karnataka state (altitude ~850 meters). In the current study, we aimed to investigate how hematological parameters change in response to an extreme environmental shift (altitude) in the Tibetan population.

Materials and Methods
Participants and Study Design
Our study includes a total of 79 high altitude Tibetans from Samad Rakchan village and adjacent pastures (Norchen, Dipling, Kumlung), having an average elevation of 4500–4900 meters above sea level (barometric pressure <430 mmHg) in the Rupsho valley (part of the Changtang region of the Tibetan plateau, extending to Southeastern Ladakh) of the union territory of Ladakh, India. Another set of samples includes 89 low altitude Tibetans from a small town, Bylakuppe, having an average elevation of ~850 meters above sea level (barometric pressure ~690 mmHg), in Karnataka state, India, (Figure 1). People of non-Tibetan descent were excluded from our study.

High altitude Tibetans in our study belong to semi-nomadic and pastoral communities and low altitude Tibetans are dependent on agriculture, handicraft and trade.[32] In Ladakh, we organized health camps during July, 2017 and August, 2018 at the above-mentioned sites. In Karnataka, camps were organized during February−April, 2018. The current study was conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from all participants after explaining the purpose of the study. Consent was obtained from the father of a lone participant who was under the age of 18 years. The Institutional Ethical Committee (IEC) of the CSIR - Centre for Cellular and Molecular Biology, Hyderabad, the Institutional Review Board of the Ladakh Institute of Prevention and the District Ethical Committee, Leh, Ladakh approved the study.

About 3.0 mL of venous blood was collected in EDTA vacutainers (BD, New Jersey, USA) for the

Figure 1 India map showing sampled area. The distance between Samad Rakchan and Bylakuppe is ~2320 km.
Abbreviation: masl, meters above sea level.

assessment of hematological parameters. About 3.0 mL of blood was also collected in vacutainer serum tubes (BD, New Jersey, USA) and were kept at room temperature for half an hour. Clots were removed by centrifuging at 2000 x g for 10 minutes. Clear serum was immediately transferred into clean polypropylene tubes, which were kept in dry ice temporarily and stored at −80 °C for further experiments.

Anthropometric and Hematological Data

Age and gender data were collected from all partici-pants. Height and weight were measured. Body mass index (BMI) was calculated by dividing the weight in kilograms of the individuals by their height, in meters squared. Hematological data were acquired using ADVIA 2120i automated hematology analyzer (Siemens AG, Erlangen, Germany) for the low altitude participants. For high altitude participants, hematologi-cal data were obtained using manual techniques, where WBC and RBC were counted by microscopic examina-tion of Leishman-stained blood smears within 3 days of sample collection. HCT was measured using Wintrobe hematocrit tubes.[33] Hemoglobin was measured using Sahli's method on the spot.[34] RBC cellular indices were calculated accordingly.[35] We analyzed WBC, RBC, HCT, MCV, MCH, MCHC and Hb parameters. A quantikine human erythropoietin kit from R & D Systems (Minneapolis, MN, USA) was used to mea-sure serum EPO levels in high and low altitude Tibetans.

Data Analysis

GraphPad Prism trial version 8.4.3 (San Diego, CA) was utilized for statistical analysis. The distribution of the variables was checked using the D'Agostino-Pearson omnibus normality test. For comparison between the two groups, unpaired *t*-tests with Welch's correction were per-formed where residuals followed Gaussian distribution and Mann–Whitney tests were performed where residuals did not follow Gaussian distribution. Spearman correlation coefficients were calculated to assess the correlation between parameters. Univariate and multiple linear regres-sion analyses were performed in R (version 3.6.1). P-values of less than 0.05 were considered to be significant.

Results
Anthropometric and Hematological Parameters

Our study includes a total of 168 individuals, of whom 79 were from high altitude and 89 were from low altitude areas, with ages ranging from 24 to 80 years and 17 to 92 years, respectively. The median age was 58 years. The age distribution of participants is provided in Figures 2A and B. BMI of the participants was in the range 14.24–34.48 kg/m^2 and median BMI was 23.82 kg/m^2. Table 1 shows that high

altitude participants had lower mean age and BMI com-pared to low altitude participants.

Highly significant differences (p <0.0001) in RBC, HCT and hemoglobin concentration were detected between the high and low altitude Tibetans (Figures 3A–C). Mean values of RBC and HCT were higher in high altitude males and females compared to low altitude males and females, respectively. Mean values of RBC in males from high altitude and low altitude were 6.21 ± 0.74 and 4.45 ± 0.49 million cells/μL, respectively. In females, these were 5.4 ± 0.40 and 4.19 ± 0.40 million cells/μL, respectively. Mean values of HCT in high altitude males, low altitude males, high altitude females and low altitude females were 59.83 ± 6.89, 44.57 ± 4.56, 50.09 ± 3.6, 42.5 ± 4.06%, respectively. Hemoglobin concentration was significantly (p <0.0001) higher in high altitude indivi-duals, irrespective of gender, compared to low altitude indivi-duals. Mean hemoglobin concentrations in males from high and low altitudes were 20.01 ± 1.91 and 13.42 ± 1.75 g/dL, respectively. In females, these were 16.97 ± 1.74 and 12.56 ± 1.19 g/dL, respectively. MCV was significantly (p <0.05 in males and p <0.0001 in females) lower in both genders at high altitude compared to their low altitude counterparts, with mean values of 96.6 ± 8.06 and 100.5 ± 6.97 fL in males and 92.7 ± 6.17 and 101.5 ± 4.67 fL in females from high and low altitudes, respectively (Figure 3D). MCH showed a nominally significant difference (p <0.05) only in males, with mean values of 32.29 ± 2.51 and 30.27 ± 3.40 pg/cell in high altitude and low altitude males, respectively (Figure 3E). A significant difference was also detected in MCHC in indi-viduals of both genders; however, age and gender were poten-tial confounders (Figure 3F). Details of the tests performed and statistical parameters are given in Tables 2 and 3.

Univariate linear regression analysis was performed separately with age, gender, altitude and BMI for each blood parameter. Out of these (age, gender, altitude and BMI), whichever showed significant association with the blood parameter, were considered for multiple linear regression analysis as potential confounding factors. Complete details of univariate and multiple linear regres-sion analyses are provided in Supplementary Tables S1–S6. In multiple regression models, age and BMI did not show significant associations with most of the blood parameters (p >0.05) except MCHC and hemo-globin concentration. Both age and BMI showed a significant association with MCHC (p-values 0.0200 and 0.0232, respectively). However, only BMI (but not age) was significantly associated with hemoglobin con-centration (p-value 0.0386) in the model and had a very
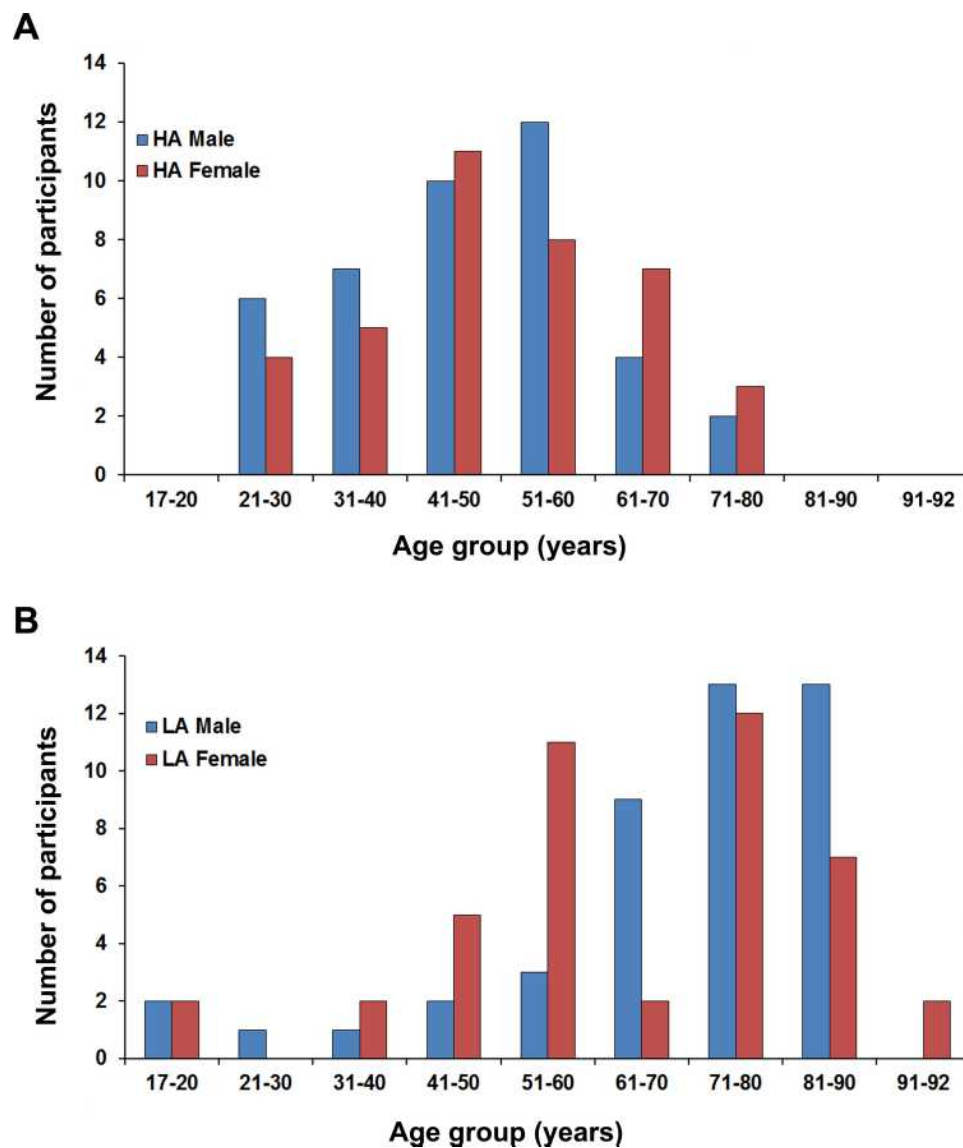
Figure 2 Age distribution among (**A**) high altitude and (**B**) low altitude participants.
Abbreviations: HA, high altitude; LA, low altitude.

small regression coefficient (0.0723), after correcting for confounding factors.

Additionally, we categorized our participants (up to 80 years, maximum age of high altitude participants) into three age categories, and checked the differences in hemoglobin concentrations between them. Details of BMI in these age categories are provided in Table 4. Hemoglobin concentration was significantly different in high altitude

Table 1 Description of Anthropometric Parameters of the Subjects (n=168); Data is Expressed in Mean ± SD Format

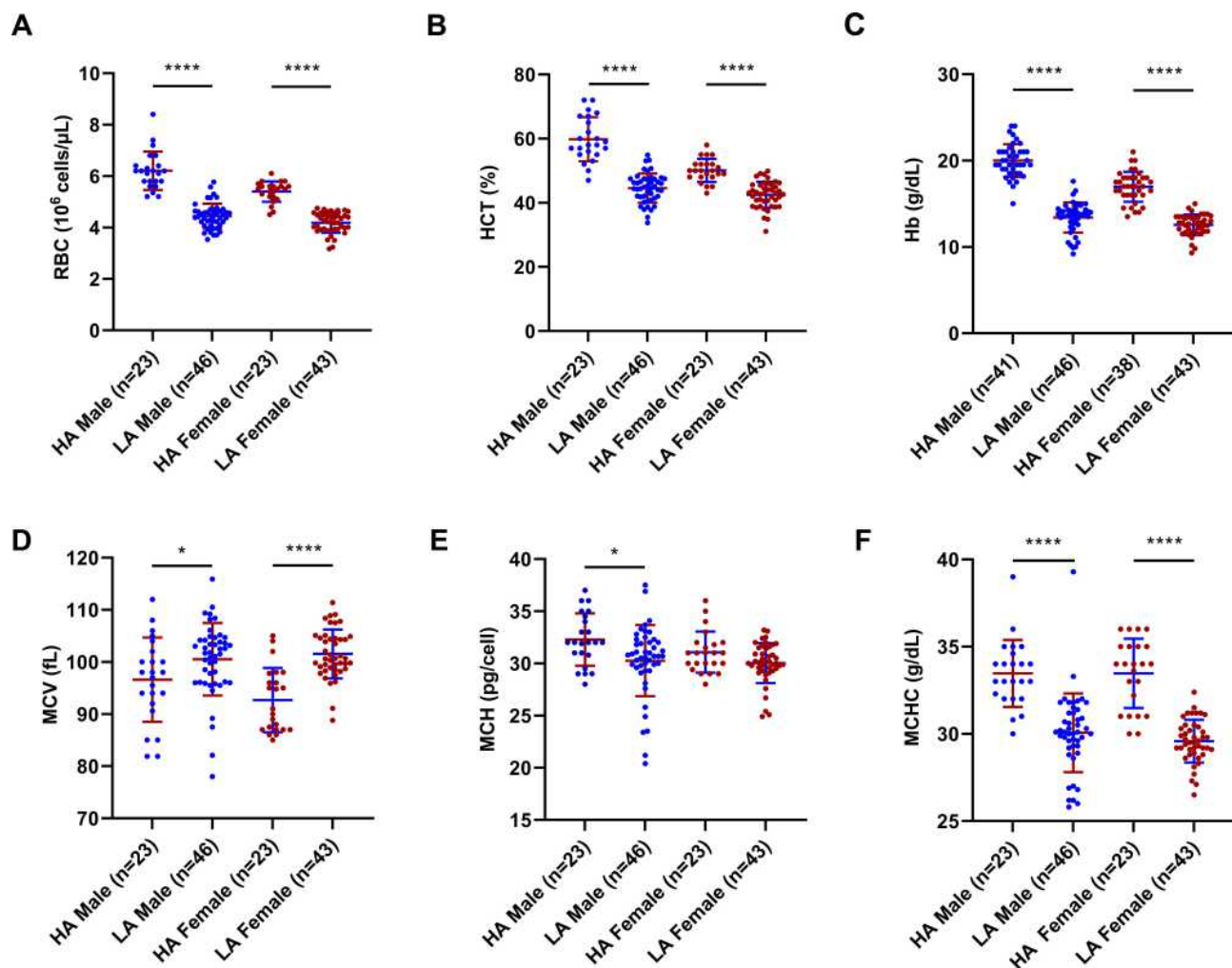| Parameter	High Altitude	Low Altitude	Test, p-value
Age (years)	49.32 ± 13.70	67.15 ± 18.42	Mann-Whitney test, <0.0001
BMI (kg/m^2)	22.75 ± 3.67	25.28 ± 4.78	Unpaired *t*-test with Welch's correction, 0.0004

Figure 3 Various hematological parameters of high and low altitude Tibetan males and females: (**A**) RBC, (**B**) HCT, (**C**) Hb, (**D**) MCV, (**E**) MCH and (**F**) MCHC. Blue and maroon horizontal lines represent mean ± SD. ****p <0.0001, *p = 0.01–0.05.

Abbreviations: HA, high altitude; LA, low altitude; RBC, red blood cell; HCT, hematocrit; Hb, hemoglobin concentration; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

and low altitude participants, after categorization of age as well (Figures 4A–C; Table 5). For other parameters, age categorization could not be performed, as the number of individuals in each age category was very low or absent.

Serum EPO

Serum EPO is one of the prominent outcomes of altitude-induced hypoxia that becomes elevated in people traveling to high altitudes. We checked serum EPO levels within high and low altitude Tibetan males and females. No significant difference was observed in either males or females, with mean values of 13.3 ± 11.61, 11.32 ± 7.77 mIU/mL in males and 13.21 ± 6.98 and 11.31 ± 7.63 mIU/mL in females from high and low altitudes, respectively (Figure 5; Table 3).

In an earlier study, serum EPO was shown to be correlated with HCT and hemoglobin concentration in children

and young adults from Ladakh by Yanamandra et al.[36] Since one group of our study participants is also from Ladakh, we checked the correlation of serum EPO with hemoglobin concentration and HCT of the participants. Both serum EPO and hemoglobin concentration data were available from 130 participants and both serum EPO and HCT data were available from 107 participants. Interestingly, none of these parameters showed any significant correlation (Spearman r = −0.03984, p-value = 0.6526 for serum EPO and Hb) with serum EPO in our study (Spearman r = 0.1562, p-value = 0.1081 for serum EPO and HCT).

Discussion

Change of hematological parameters has been shown to be associated with various pathological conditions like myocardial infarction, leukemia, coronary heart disease,

Table 2 Description of Various Hematological Parameters in the Subjects; Data is Expressed in Mean ± SD Format

Parameter	HA Male (n=23)	LA Male (n=46)	HA Female (n=23)	LA Female (n=43)	Test, p-value (HA Male vs LA Male); Test, p-value (HA Female vs LA Female)
WBC (cells/µL)	6957 ± 1788	6744 ± 1682	6743 ± 1938	7277 ± 1472	Mann-Whitney test, 0.7022; Unpaired *t*-test with Welch's correction, 0.2565
RBC (10^6 cells/µL)	6.21 ± 0.74	4.45 ± 0.49	5.4 ± 0.40	4.19 ± 0.40	Mann-Whitney test, <0.0001; Unpaired *t*-test with Welch's correction, <0.0001
HCT (%)	59.83 ± 6.89	44.57 ± 4.56	50.09 ± 3.6	42.5 ± 4.06	Unpaired *t*-test with Welch's correction, <0.0001; Unpaired *t*-test with Welch's correction, <0.0001
MCV (fL)	96.6 ± 8.06	100.5 ± 6.97	92.7 ± 6.17	101.5 ± 4.67	Mann-Whitney test, 0.0280; Unpaired *t*-test with Welch's correction, <0.0001
MCH (pg/cell)	32.29 ± 2.51	30.27 ± 3.40	31.07 ± 1.97	30.04 ± 1.92	Mann-Whitney test, 0.0212; Mann Whitney test, 0.1347
MCHC (g/dL)	33.47 ± 1.92	30.07 ± 2.26	33.47 ± 1.99	29.58 ± 1.23	Mann-Whitney test, <0.0001; Unpaired *t*-test with Welch's correction, <0.0001

cardiovascular disease and so on.[37–40] Our study shows differences in hematological parameters between Tibetans residing at high and low altitudes for the first time, to the best of our knowledge. We observed significantly higher RBC, HCT and hemoglobin concentration and significantly lower MCV in high altitude males and females compared to their low altitude counterparts. Though BMI showed a significant association with hemoglobin concentration (p-value = 0.0386) after adjusting for age, gender, BMI and altitude, the coefficient of its association was too small (0.0723) to be considered a strong confounder, considering apparently small mean BMI differences (2.53 kg/m^2) and apparently high mean hemoglobin concentration differences between high and low altitude

male (6.59 g/dl) and female (4.41 g/dl) Tibetans. In our regression model for hemoglobin concentration (Hemoglobin concentration + Age + Gender + BMI + Altitude), the coefficient of altitude (low) was −5.5356 (p-value <2.25×10^{-7}), which suggests that, on average, hemoglobin concentration is ~5.5 g/dl lower in low altitude individuals compared to high altitude individuals. Our observed values of hemoglobin concentration were a little higher than some studies conducted with high altitude Tibetans; however, it was reported that, at an elevation of more than 4500 meters, hemoglobin concentration in Tibetans also showed higher mean values.[16,18,41–43] The hemoglobin concentration observed in our study for the low altitude Tibetans was on a par with another study with Tibetans at sea
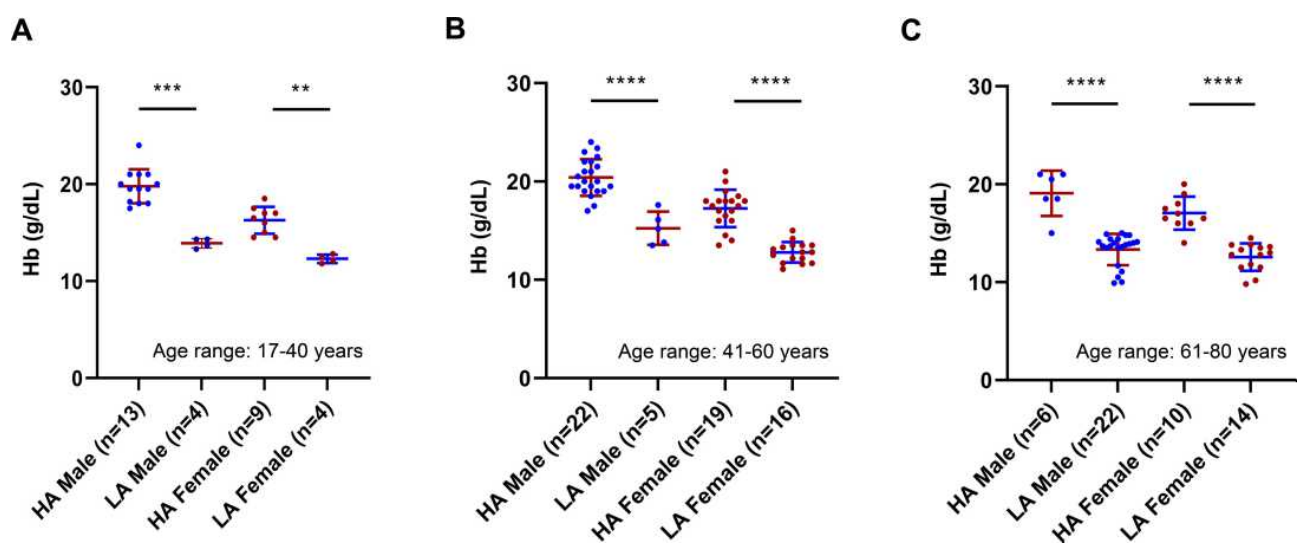

Figure 4 Hemoglobin concentration (Hb) among age-categorized participants: (**A**) in the age range of 17–40 years, (**B**) 41–60 years, (**C**) 61–80 years. ****p <0.0001, ***p = 0.0001–0.001, **p = 0.001–0.01. Blue and maroon horizontal lines represent mean ± SD.
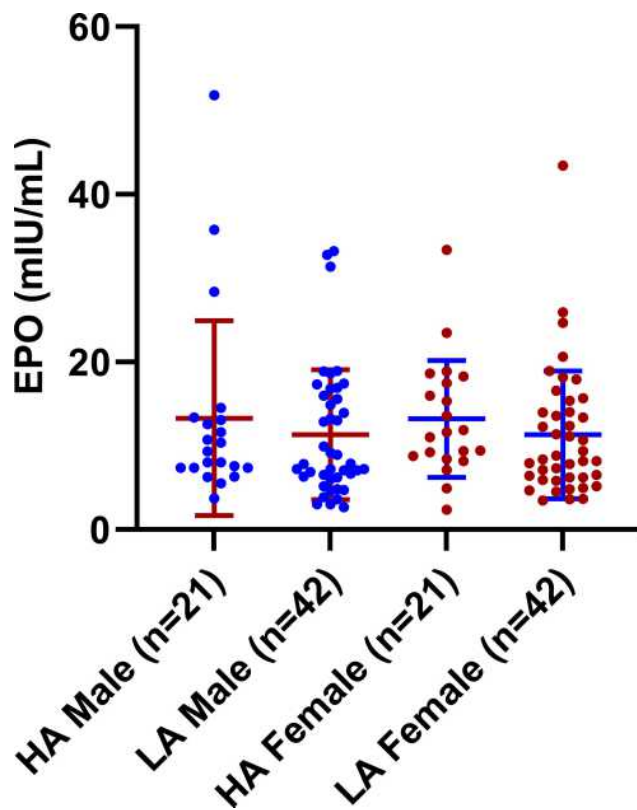**Abbreviations:** HA, high altitude; LA, low altitude.

Figure 5 Distribution of serum erythropoietin (EPO) level among participants.
Abbreviations: HA, high altitude; LA, low altitude.

level.[44] Since mean hemoglobin concentration was apparently high in our high altitude participants, we checked their chronic mountain sickness (CMS) scores, which had been recorded during the current health camp or a previous health camp organized in similar places. Data were available for the CMS scores from 41 male and 36 female participants. Among them, 12 males and 5 females had mild CMS, having scores of between 6 to 10 as per the Qinghai scoring system, resulting in a 22.07% CMS prevalence (unpublished data, Basak/ Norboo et al).[45] Earlier studies have reported a CMS prevalence of 13.73–28.7% in high altitude natives of the neighboring state, Himachal Pradesh.[46,47] The Tibetans are known to have a lower prevalence of CMS compared to the

Andeans.[48–50] Though our participants had a comparatively higher prevalence of CMS, all of them had mild CMS. Though RBC, HCT and hemoglobin were significantly higher in the high altitude Tibetans, interestingly, serum EPO was only insignificantly higher in high altitude individuals; this might be because the normal concentration of serum erythropoietin has an apparently broad range: 3.3–16.6 mIU/mL.[51] A similar observation was reported by Winslow et al, whereby HCT and hemoglobin were higher in Andean natives compared to Sherpas from Nepal living at the same altitude, yet serum erythropoietin was not significantly different.[52] Moreover, serum EPO becomes elevated upon acute hypoxic exposure, when low altitude residents visit high altitude areas. Within a few days, it returns to almost the pre-exposure level. Both our high altitude and low altitude groups comprise long-term residents of the respective altitudes. Our observation of an almost similar level of serum EPO in both groups is consistent with that as well. A recent study showed that blood cell phenotypes are ancestry-dependent and selective pressure can give rise to different blood cell traits.[53] Our study additionally shows that different exposure of the same population to different environments can also alter blood cell traits. These changes of hematological parameters are possibly part of the hematological adaptation mechanism, to cope with the "relative hyperoxia" that is evidently an aggressive environment to the high altitude hypoxia-adapted Tibetans when they migrate to low altitude.

It has previously been hypothesized that hemoglobin concentration might be modulated by environmental context.[54] Our study reveals differences in various hematological parameters including hemoglobin concentration between the high and low altitude Tibetans, which fits the hypothesis well. Hemoglobin concentration has been shown to play an important role in reproductive fitness among Tibetan women, where lower hemoglobin favored reproductive fitness.[55] Similarly, Tibetan men, having lower hemoglobin concentrations are known to show better exercise capacity at high altitude.[41] It would be interesting to

Table 3 Description of Serum EPO and Hb in the Subjects; Data is Expressed in Mean ± SD Format

Parameter	HA Male	LA Male	HA Female	LA Female	Test, p-value (HA Male vs LA Male); Test, p-value (HA Female vs LA Female)
EPO (mIU/mL)	13.3 ± 11.61 (n=21)	11.32 ± 7.77 (n=42)	13.21 ± 6.98 (n=21)	11.31 ± 7.63 (n=42)	Mann-Whitney test, 0.4826; Mann-Whitney test, 0.1283
Hb (g/dL)	20.01 ± 1.91 (n=41)	13.42 ± 1.75 (n=46)	16.97 ± 1.74 (n=38)	12.56 ± 1.19 (n=43)	Unpaired t-test with Welch's correction, <0.0001; Unpaired t-test with Welch's correction, <0.0001

Table 4 Description of BMI Among Participants Belonging to Three Different Age Categories; Data is Expressed in Mean ± SD Format

Age Category	BMI				Test, p-value (HA Male vs LA Male); Test, p-value (HA Female vs LA Female)
	HA Male	**LA Male**	**HA Female**	**LA Female**	
17–40 years	20.46 ± 2.18 (n=13)	23.83 ± 7.25 (n=3)	24.34 ± 3.74 (n=9)	28.08 ± 5.35 (n=4)	Mann-Whitney test, 0.5643; Mann-Whitney test, 0.3119
41–60 years	21.66 ± 3.01 (n=22)	24.44 ± 5.18 (n=5)	23.83 ± 3.78 (n=19)	25.98 ± 3.86 (n=10)	Mann-Whitney test, 0.1413; Mann-Whitney test, 0.0915
61–80 years	24.33 ± 4.89 (n=6)	23.35 ± 4.50 (n=22)	23.7 ± 4.08 (n=10)	28.88 ± 4.24 (n=8)	Mann-Whitney test, 0.7745; Mann-Whitney test, 0.0143

Table 5 Description of Hemoglobin Concentration Among Participants Belonging to Three Different Age Categories; Data is Expressed in Mean ± SD Format

Age Category	Hemoglobin Concentration (g/dl)				Test, p-value (HA Male vs LA Male); Test, p-value (HA Female vs LA Female)
	HA Male	**LA Male**	**HA Female**	**LA Female**	
17–40 years	19.78 ± 1.7 (n=13)	13.9 ± 0.49 (n=4)	16.28 ± 1.39 (n=9)	12.3 ± 0.44 (n=4)	Mann-Whitney test, 0.0008; Mann-Whitney test, 0.0028
41–60 years	20.4 ± 1.87 (n=22)	15.24 ± 1.69 (n=5)	17.26 ± 1.90 (n=19)	12.79 ± 1.04 (n=16)	Mann-Whitney test, <0.0001; Mann-Whitney test, <0.0001
61–80 years	19.08 ± 2.30 (n=6)	13.34 ± 1.59 (n=22)	17.05 ± 1.69 (10)	12.56 ± 1.41 (14)	Mann-Whitney test, <0.0001; Mann-Whitney test, <0.0001

investigate those events separately in the low altitude Tibetans to check whether the trend is true since hemoglobin concentration was strikingly lower in the Tibetans from low altitude areas in our study.

John B. West discussed "Barcroft's bold assertion" that assumes "All dwellers at high altitudes are persons of impaired physical and mental powers." This is a provocative statement; nonetheless, it does provide scope to study native highlanders at low altitude and to assess their health status.[56] Our study is a step in that direction, which would help to understand the physiological aspects better when native Tibetans are not present in their native high altitude environment. Simultaneously, these kinds of studies would help to understand the impact of environmental change, knowledge of which could be important for tourists and soldiers traveling to high altitude areas. It would be interesting to explore the molecular driving forces behind these kinds of differences. Epigenetic studies, important tools for exploring gene–environment interaction, could be fruitful in this context.

A limitation of our study is that we could not measure complete blood count (CBC) profiles of the high altitude and low altitude individuals using the same method because of geographical differences between the sampling sites, remote locations and limited resources (at high altitude). CBC profiles for the high altitude samples were obtained using manual techniques; while for the low altitude samples, these were obtained using an automated hematology analyzer. However, both of the methods are known to be well-correlated.[33,57,58] Harris et al reported good correlation between ADVIA 2120 (previous version of ADVIA 2120i) and the manual method in an international, multicenter clinical trial. Values reported for WBC differentials measured by the ADVIA 2120 analyzer and the manual method in that study were close. The within-run precision of CBC on the platform was also very good.[59] We used Sahli's method, one of the most commonly used methods for hemoglobin estimation where resources are limited, for estimating hemoglobin concentration in remote high altitude areas. This method has been proven to be efficient, resulting in an absolute difference between two measurements of less than 1 g/dl. Another study reported that Sahli's method provides lower values of hemoglobin concentration of 0.62gms/dl in capillary

blood and 1.1gms/dl in venous blood compared to a hemiglobincyanide (HiCN)-based reference method. Our observed differences in mean hemoglobin concentration between high and low altitude male and female Tibetans were 6.59 g/dl and 4.41 g/dl, respectively, which are far above these values. Moreover, Sahli's method was used to measure hemoglobin concentration in high altitude individuals, in whom we observed higher values than in low altitude individuals. On the other hand, technical specifications of ADVIA 2120i show that the precision of the instrument is good, with a coefficient of variation for MCV, Hb, RBC, WBC in the range of 0.78–2.7.[60]

Considering all these facts, it is highly unlikely that our observed differences in hematological parameters in high and low altitude individuals are due to artifacts. We needed to be a bit careful while selecting the parameters for analysis considering the gap between sample collection and hematological data collection. As the samples were collected from remote areas, due to logistic issues, hematological data collection (except hemoglobin measurement by Sahli's method) of the samples could be done within 48–72 hours after sample collection. A recent meta-analysis and systematic review reports that Hb, WBC, RBC count, HCT, MCH, MCHC are quite stable within the time range in which we collected and measured them.[61]

Conclusion

In conclusion, our study reveals that prolonged residence of native Tibetans at a low altitude (over 50 years) results in significantly lower RBC count, HCT, Hb, MCH, MCHC and higher MCV compared to their high altitude counterparts, which is likely due to the outcome of hematological adaptation to the relatively hyperoxic environment in low altitude areas.

Acknowledgments

Disclosure

The authors declared no conflicts of interest for this work.

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