

Anthropometric and hematological tests to diagnose nutritional deficiencies in schoolchildren of indigenous communities living in the Andean region of Ecuador

Análise antropométrica e hematológica para o diagnóstico de deficiências nutricionais em escolares de comunidades indígenas que vivem na cordilheira dos Andes no Equador

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ABSTRACT

Objective

To carry out the anthropometric and biometric-hematological assessments in schoolchildren of the Andean region of Ecuador, in order to improve the diagnosis of nutritional deficiencies.

Methods

The study has been carried out in the *San Juan School (Chimborazo, Ecuador)*, located at 3,240m of altitude, to 36 children of 5 and 6 years old. Anthropometric analyses (weight, height and body mass index), and hematocrit and hemoglobin concentrations were measured. The hemoglobin measurement was evaluated considering the normal value and the one adapted to the altitude of the area.

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Results

The schoolchildren showed high prevalence of stunting (44%). The values of hematocrit ($p=0.001$) and hemoglobin ($p=0.003$) were higher in girls. It should be highlighted that using the normal value of hemoglobin, anemia was not detected. However almost a fifth of the schoolchildren studied were diagnosed with anemia when we applied the correction factors adapted to the altitude.

Conclusion

The use of correction factors adapted to the altitude is considered essential to do the hematology test in populations that live in high altitude in order to avoid a false diagnosis. Moreover, it is necessary to establish the environmental factors related to the stunted growth of this population of the Andean region.

Keywords: Anemia. Anthropometry. Biometry. Child development. Indigenous population.

RESUMO

Objetivo

Realizar avaliações antropométricas e biométrico-hematológicas em escolares da cordilheira dos Andes no Equador, a fim de melhorar o diagnóstico de deficiências nutricionais.

Métodos

O estudo foi realizado na escola San Juan (Chimborazo, Equador), localizada a 3240 metros acima do nível do mar, a 36 crianças de 5 e 6 anos de idade. Foram analisadas as medidas antropométricas (peso, altura e índice de massa corporal) e as concentrações de hemoglobina e hematócrito. A medida da hemoglobina foi avaliada considerando os valores absolutos e os corrigidos pela altura geográfica.

Resultados

A população escolar apresentou alta prevalência de altura baixa (44%). Os valores de hematócrito ($p=0,001$) e hemoglobina ($p=0,003$) foram maiores nas meninas. Deve-se destacar que usando o valor normal da hemoglobina, a anemia não foi detectada. No entanto, quase um quinto dos escolares estudados foram diagnosticados com anemia quando os fatores de correção adaptados à altitude foram aplicados.

Conclusão

O uso de fatores de correção adaptados à altitude é considerado fundamental ao fazer uma biometria a os habitantes da zona para evitar um diagnóstico errôneo. Além disso, é necessário estabelecer os fatores ambientais associados ao retardo do crescimento que existe nesta região dos Andes.

Palavras-chave: Anemia. Antropometria. Biometria. Desenvolvimento infantil. População indígena.

INTRODUCTION

Currently, nutritional changes are a Public Health problem in Ecuador, affecting the school-age population in particular. Undernutrition is one of the major causes of death in children [1]. Infant mortality, difficulty to access resources and education are important problems for the populations living in the Andean region [2-5]. In Ecuador, there are numerous indigenous communities living under these unfavorable conditions.

Investigations on the nutritional status of the Ecuadorian population are scarce. The

ELAN Ecuadorian Hospital Malnutrition study reported an undernutrition rate of 37.1% in 2012 [6]. The nutritional status of hospitalized patients was shown as not included within the therapeutic objectives [7]. When analyzing the diets of 110 Ecuadorian adults, it was noted that their daily needs of carbohydrates, vitamins D, E, and minerals, such as iron and calcium were not met [8]. However, there are no recent studies on the nutritional status of children who are hospitalized or attending an outpatient clinic. Neira-Mosquera *et al.* [9] highlighted the need to recompile nutritional information on the Ecuadorian population.

In Ecuador, there are communities living more than 2,500 meters above the sea level (masl) in the Andean Mountain. In this region, the environmental conditions, such as oxygen pressure, solar radiation, or the weather itself change and affect inhabitants. Thus, it was detected adaptations, such as high-altitude hypoxia, which triggers physiological stress, manifesting in higher hemoglobin levels, among other signs [10]. In addition, the first height census carried out in Ecuador revealed that the schoolchildren living in rural Andean areas had the shortest statures in the country [11].

Hematological biometric analysis is an important laboratory test that assesses the nutritional status, detecting anemia, infections, or vitamin deficiencies. In developing countries, 53% of schoolchildren and 42% of pre-schoolchildren suffer from anemia. The prevalence of iron deficiency anemia among Colombian schoolchildren is 47% [12]. Nevertheless, there are no records of subclinical iron deficiency except a study carried out in Medellin [13]. According to the National Survey of Health carried out in the school-age population, there are no official numbers regarding anemia in Ecuador. Thus, the implementation of this type of studies, especially in rural highlands (*Sierra*), inhabited by indigenous communities, is necessary [14].

According to the United Nations Organization for Food and Agriculture, anemia increased in the last decade, especially among children aged 1–10 years [15]. World Health Organization (WHO) experts reiterated the need to carry out studies providing accurate information on the prevalence of iron deficiency anemia. Subclinical iron deficiency is difficult to diagnose, considering that hyperbaric hypoxia stimulates the erythropoiesis in high altitude areas, low sensitivity of conventional measurement methods, and capacity and percentage of transferrin saturation [12]. The WHO established as a general statement that hemoglobin level increases at high altitudes and proposed that these values should be adjusted

by the altitude of the location to define specific cut-off points for anemia. In such settings, the cut-off point for hemoglobin needs to increase with altitude to define anemia better.

Different models have been created with the intent to correct hemoglobin at high altitudes. It was used regression analysis equations, obtained by comparing the hemoglobin level based on the altitude level. A study carried out by Gonzales & Gonzales [16] related the anemia defined by the hemoglobin value corrected by the altitude, to the anemia defined as per iron deficiency, measured by the body iron content. After correcting the hemoglobin by the altitude effect, 26.6% of the individuals are still anemic; however, when defined as iron deficiency, only 5.7% are considered anemic. Therefore, there is no agreement between the prevalence of anemia when hemoglobin values are corrected by the altitude and the values measured based on the iron content in the body. For this reason, it is important to investigate which parameters need to be considered to diagnose anemia correctly in communities living above 2,500masl.

The population of *San Juan* is in the province of *Chimborazo*, 18km from the city of *Riobamba*, located at an altitude of 3,240masl in the Highlands of the volcano *Chimborazo* [17]. In this district, poverty is 82.75% of the total population; 57.89% of them are considered to live in extreme poverty. Data from the last Census of Population and Housing, carried out in 2010, showed a predominantly young population with the greatest growth delay among the Ecuadorian 6-year-old schoolchildren [11,18]. In this investigation, anthropometric and blood biometric research were carried out among schoolchildren in this population, with the aim of improving the diagnosis of nutritional deficiencies, such as anemia and stunting. It is worth noting that, to date, there have been no similar studies on school-age children, living in Andean regions of Ecuador.

METHODS

The investigation was carried out in the School of *San Juan*, in the *Riobamba* canton of *Chimborazo* province in Ecuador. *San Juan* is located at 3,240 meters above the sea level, and it is a predominant indigenous community in the Ecuador Highlands (*Sierra*).

This is a descriptive cross-sectional study, which included 36 schoolchildren aged 5 and 6 years, who were members of the *Evaluación Alimentaria y Nutricional de Escolares de la Sierra* (EVANES project, Alimentary and Nutritional Evaluation of Highland Schoolchildren). Children with physical or mental disability and those whose parents or guardians had not signed the Informed Consent Form were excluded from this study. Healthy looking school-age children living in rural communities and attending rural schools in the *Riobamba* canton were included in this study.

In order to, determine the sample size, we applied the following formula:

$$n = Z_{\alpha}^2 \frac{N * p * q}{i^2(N - 1) + Z_{\alpha}^2 * p * q}$$

taking into account the total number of children enrolled in the educational unit and their proportion per class. Thus, number equal to 40 schoolchildren was determined as an adequate sample size between the preschool and first year. This includes additional 20% participants, taking into account the possible losses to follow-up. The sample used was selected randomly by using a number generator. In total, 36 children participated because 4 schoolchildren did not meet the inclusion criteria or lacked the consent of their parents.

We selected younger schoolchildren for this study as these are priority groups because of their immense vulnerability from a nutritional point of view. Their rapid growth requires higher nutrients and energy per kg of their body weight as compared to the other age groups at normal weight. An impaired linear growth during the

first years of life can be very difficult to recover, even when dietary supplementation is used [19]. Thus, early diagnosis of nutritional deficits is essential to mitigate the potential consequences.

Anthropometric measures for weight and height were taken to determine the Body Mass Index (BMI=weight/height²). We used an arm weighing scale and a portable measuring rod, with the accuracy of 100g and 0.1cm, respectively. The nutritional status was defined according to the BMI Standard Deviation (SD) Score and height based on sex and age, using the software AnthroPlus (World Health Organization, Geneva, Switzerland, 2011) version 3.2.2. Therefore, the children were classified as: 1) BMI/age: undernutrition (<-2 SD), normal weight (-2 to 1 SD), overweight (>1 to 2) obesity (>2 SD); 2) height/age: stunting (<-2 SD) and normal height (≥-2 SD).

Blood samples were taken at school and transferred to 3mL tubes containing anticoagulant ethylenediamine tetraacetic acid. Total count of leukocytes was carried out using a blood dilution in a tube, dilution buffer, cell-counting chamber, and microscopic reading. We used blood smears colored with wright, which is based on the microscopic observation of different types of leukocytes and their percentage to determine the leukocyte or differential leukocyte counts. The hematocrit or globular volume was processed by loading 7cm long glass capillaries up to three quarters and centrifuging them for 5 minutes at 12,000rpm. Further reading was performed using a microhematocrit card. Hemoglobin concentration was determined by the cyanmethemoglobin method (International Committee for Standardization in Hematology, WHO), which requires diluting the blood with Drabkin reagent. The absorption capacity of the solution was measured in a spectrophotometer at 545nm and was compared with a standard solution or multiplied by a counting factor. Platelets were counted in stained blood smears used for differential leukocyte counts [20].

The data obtained were processed with the Statistical Package for the Social Sciences

(SPSS Inc., Chicago, Illinois, United States), version 20.0. The results were expressed as means \pm SD (quantitative variables) and percentages (qualitative variables). The differences between variables were determined based on sex, using the Univariate General Linear Model, which is applied for quantitative variables, and the Chi-square test, which is used for qualitative variables. For all analyses, a bilateral significance was determined at $p < 0.05$.

The project was conducted in accordance with the ethical standards of the Declaration of Helsinki (Hong Kong review, in September 1989, in Edinburgh in 2000 and in Korea in 2008), while following the guidelines of the Council for International Organizations of Medical Sciences for Latin America (Geneva review, 2002; Translation and Adaptation for Latin America, 2003 [21]), and current laws regulating human clinical investigations in Ecuador. The written Informed Consent was obtained from all children and signed by their parents or guardians. The study has been approved by the

Ethics Committee of the San Francisco de Quito University, Ecuador (code 2015-046E).

RESULTS

The anthropometric characteristics of children are shown in Table 1. Of the 36 schoolchildren, 18 were girls. The average age of these students was 5.19 ± 0.40 years. Their average weight, height, and BMI were 18.30 ± 3.42 kg, 105.18 ± 2.89 cm, and 16.45 ± 2.24 kg/m², respectively. When compared by sex, we determined the average age of the girls and boys as 5.17 years, and 5.22 years, respectively. Average weight for girls and boys was 18.23 kg and 18.36 kg, while average height was 104.90 cm and 105.46 cm, and BMI was 16.48 kg/m² and 16.43 kg/m², respectively. No statistically significant differences were observed in any of these parameters. The prevalence of stunting in these children was high at 44.44%. No statistically significant difference was noted between boys and girls ($p = 0.502$).

Table 1. Anthropometric characteristics and nutritional status of schoolchildren according to sex. *San Juan, Riobamba, Ecuador (2015).*

Parameters	Study population (n=36)	Girls (n=18)	Boys (n=18)	<i>p</i>
Age (years) ¹	5.19 \pm 0.40	5.17 \pm 0.38	5.22 \pm 0.43	0.684
Weight (kg) ¹	18.30 \pm 3.42	18.23 \pm 4.08	18.36 \pm 2.72	0.909
Height (cm) ¹	105.18 \pm 2.89	104.90 \pm 23.44	105.46 \pm 34.06	0.530
BMI (kg/m ²) ¹	16.45 \pm 2.24	16.48 \pm 3.04	16.43 \pm 1.03	0.948
SDS–BMI ¹	0.64 \pm 1.06	0.52 \pm 1.36	0.76 \pm 0.66	0.494
SDS–Height ¹	-1.77 \pm 0.89	-1.70 \pm 0.83	-1.85 \pm 0.97	0.639
<i>Nutritional status according to SDS–BMI²</i>				0.597
Undernutrition	0.00	0.00	0.00	
Normal	91.67	88.89	94.44	
Overweight	5.56	5.56	5.56	
Obesity	2.78	5.56	0.00	
<i>Nutritional status according to SDS–Height²</i>				0.502
Stunting	44.44	38.89	50.00	
Normal height	55.56	61.11	50.00	

Note: Results expressed as ¹mean \pm standard deviation and ²percentages. Statistically significant difference: $p < 0.05$ (¹Univariate general linear model, ²Chi-square).

BMI: Body Mass Index; SDS–BMI: Standard Deviation Score of BMI; SDS–Height: Standard Deviation Score of Height.

Table 2 shows the biometric-hematological characteristics of schoolchildren according to their sex. Hematocrit level in girls was 44.81% and hemoglobin level was 15.03g/dL. The boys had the hematocrit level of 41.88% and hemoglobin level of 14.09g/dL. The leukocyte

Table 2. Biometric-hematological parameters of schoolchildren according to sex. *San Juan, Riobamba, Ecuador (2015).*

Parameters	Study population	n	Girls	n	Boys	n	<i>p</i>	Reference values
Hematocrit (%)	43.34±2.65	32	44.81±2.23	16	41.88±2.22	16	0.001	37–47*
Hemoglobin (g/dL)	14.56±0.92	32	15.03±0.72	16	14.09±0.88	16	0.003	12.2–15.5*
Hemoglobin CA (g/dL)	12.34±0.92	32	12.81±0.72	16	11.87±0.88	16	0.003	11.5 ¹
Leukocytes (x mm ³)	10607.81±2453.07	32	10209.38±2788.32	16	11006.25±2079.17	16	0.367	4000–12000*
Segmented (%)	49.94±13.03	32	47.00±13.40	16	52.88±1.36	16	0.207	55–65*
Monocytes (%)	2.69±1.65	29	2.30±1.30	14	3.29±1.82	15	0.059	2–8*
Lymphocytes (%)	46.03±13.15	32	49.06±12.76	16	43.00±13.23	16	0.197	20–45*
Eosinophils (%)	2.63±2.59	19	2.73±3.26	8	2.50±1.41	11	0.856	1–4*
Basophils (%)	1.00±0.00	2	1.00±0.00	-	-	-	-	0–1*
Platelets (x mm ³)	438437.50±55189.37	32	458125.00±48883.37	16	418750.00±55482.73	16	0.041	200000–450000*

Note: Reference values: *Gonzales *et al.* [16] and ¹World Health Organization [44]. Results expressed as mean ± standard deviation. *p*-values in bold indicate statistically significant difference: *p*<0.05 (Univariate general linear model).

Hemoglobin CA: Hemoglobin Corrected by geographic altitude.

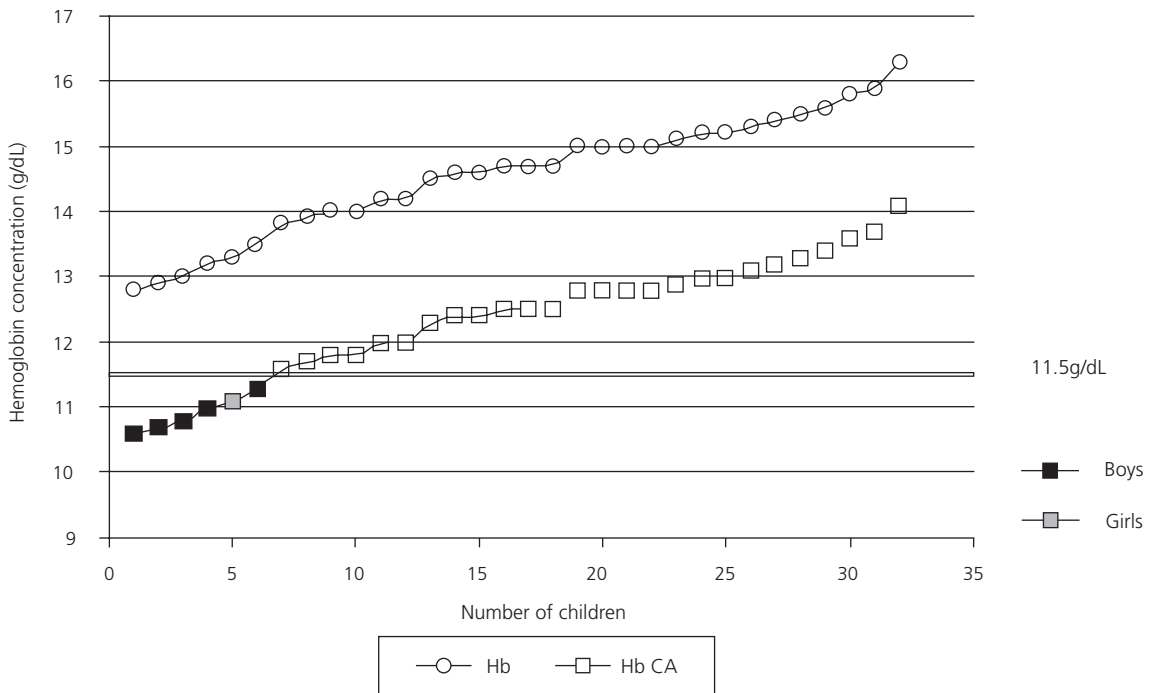


Figure 1. Comparison of absolute and adjusted hemoglobin concentration by geographic altitude. *San Juan, Riobamba, Ecuador (2015; n=32).*

Note: Concentrations below 11.5g/dL indicate anemia.

Hb: Hemoglobin; Hb CA: Hemoglobin Corrected by Geographic Altitude.

counts were $102,09.38 \times \text{mm}^3$ in girls and $11,006.25 \times \text{mm}^3$ in boys. The platelet count in girls was $458,125 \times \text{mm}^3$ and $418,750 \times \text{mm}^3$ in boys. It should be noted that hematocrit values ($p=0.001$), such as hemoglobin ($p=0.003$) and platelets ($p=0.041$) were higher in girls than in boys.

Moreover, we determined the hemoglobin values adjusted by the geographic elevation. The results were adjusted with correction factors for elevation, which was measured in the *San Juan* parish as 3,240masl. Hemodynamic biometrics statisticians showed that corrected hemoglobin values for boys ranged from 10.58g/dL to 14.08g/dL. On the other hand, the values of hemoglobin without correction varied from 12.8g/dL to 16.3g/dL, as shown in Figure 1. However, it is noted that, after adjusting the concentrations, 6 children in the study (18.8%) presented values below 11.5g/dL, which is the reference cut-off value to diagnose anemia, and 5 of these children were boys.

DISCUSSION

The analysis of anthropometric characteristics of the schoolchildren indicated that there were no statistically significant differences between boys and girls. However, in a study carried out in the region of *Aymara*, Bolivia, which is at 3,900masl, there were slight differences in BMI values (*i.e.*, BMI higher in girls) [22]. Nevertheless, in a study carried out in *Funza*, Colombia, at 2,548masl, the prevalence of stunting was higher in boys as compared to girls [4].

It is important to note the high prevalence of stunting among schoolchildren in *San Juan*. According to the first height census carried out in the country, the height of the 6 years-old girls and boys in this area was 106.7cm, and 107.7, respectively [11]. However, although the population size of this study is smaller, the data obtained for height is even lower (104.9 for girls and 105.5 for boys). In a research carried out in *Funza*, Colombia, which included rural

and urban areas without specifying indigenous origin, 9.1% stunting in children aged 2–5 years was detected [4], which is substantially lower than the growth delay found in our study.

It is very important to identify risk factors capable of triggering this health problem, as has been done in similar studies conducted in the rural populations of Chile and Argentina. In these works, stunting was found to be related to maternal illiteracy, low birth weight, lack of maternal feeding, lack of hygiene at home, malnutrition, and low socioeconomic status of the families [23-25]. In addition, undernutrition has been associated with delayed psychomotor development in Ecuadorian [26] and Colombian [27] children. Furthermore, experimental animal studies associated brain growth retardation with undernutrition, which could not be reversed with nutritional supplements [28]. Thus, child nutrition is essential for maintaining adequate psychomotor and cognitive development.

An investigation on children aged 0–2 years, which was carried out in regions close to the study area revealed that risk factors for stunting are age, male sex, low socioeconomic status, having more brothers, and illiterate mothers [29]. Karra *et al.* [30] agree that the lack of maternal education, lack of health coverage, and poor living conditions in general result in short stature in children. Notably, a study in Tibet showed that after socioeconomic and sanitary improvements in the area, children grew taller when compared to their previous height [31]. A meticulous investigation carried out in Nepal with children under 2 years old found other risk factors for short stature, such as living in rural areas and/or mountains at high altitude, mothers that smoke in addition to those mentioned above (*i.e.*, illiterate mothers, poor living conditions, and multiple births) [32]. Also, it was observed that being Buddhist, breastfeeding, and having received medical care at least 4 times after birth could be considered protective factors for height. It is important to point out the positive correlation between data

referring to children's stunting and living at high-altitudes and ancestral regions, which appear in a study carried out in areas at 3,700masl [33].

In a study conducted by Ferreira *et al.* [34], the birth weight and height of indigenous children in Brazil were similar to the average WHO reference standards. However, shortly after birth, a change in growth was observed in both parameters. The authors suggested that the growth alterations in this population might be related to the precarious sanitary conditions and food insecurity, especially during the first years of life. This highlights the importance of environmental aspects in indigenous communities as the growth-influencing factors. This is supported by the notion that these children presented with values similar to the other populations at birth. The province of *Chimborazo*, where the community of *San Juan* is located, presents the greatest poverty in the country with indigenous populations being the most affected and deprived of basic necessities [18].

Although it does not refer specifically to indigenous communities, a study conducted in healthy children from low- and middle-resource countries reinforced the previous concept; the authors concluded that the large gaps in children's linear growth were probably because of exposure to an unfavorable environment instead of any innate or genetic differences [30].

The parameters of blood count, total and differential white blood cell counts were within the reference values for children aged 2–12 years. A slight increase in platelets was observed only in girls.

The biometric-hematological analyses of hematocrit and hemoglobin demonstrated that the levels in the schoolchildren were within the admitted reference range [16] when the correction factor for height is not applied. Other studies carried out in Bolivia, at 3,700–4,000m elevation, set the cut-off value of anemia diagnosis at 14.4–15.8g/dL [35,36]. However, the corrected hemoglobin value gives us lower

results when compared to the WHO reference range [2], where anemia is considered when values are lower than 11.5g/dL. In this study, we found values of 10.5g/dL and detected anemia among children at the School of *San Juan* in *Riobamba*, Ecuador.

When comparing the values by gender, boys presented with lower levels of hemoglobin and hematocrit than girls did. Another study carried out in children living in the Himalayas, at 3,200m and 3,800m elevation had similar findings [37]. This is an important point that should be taken into account because the reference values shown in the bibliography are based on age only [38] and gender was not considered.

The average values of hemoglobin found in different studies that were carried out at high altitude areas vary according to the altitude, age, and the gender of the population. Several good examples are as follows: 16.1g/dL for adult men living in Nepal, at 3,300masl [39], 18.8g/dL in adult men living in *La Paz*, Bolivia at 3,700masl [29], 17.5g/dL in adult Peruvians living at 3,680masl [40], 14.5g/dL in Bolivian adolescent girls living at 3,600masl, and 16.6g/dL in Peruvian adolescent girls living at 4,300masl [41]. In this study, the average value corresponds to 14.5g/dL for schoolchildren (boys and girls) who live at 3,240masl. Thus, reference values depending on age, sex, and altitude of residence are required to establish a common national and international pattern.

There are numerous studies comparing hemoglobin values among inhabitants of high altitude areas, above 3,000m elevation, and the residents of areas at sea level. Most of the time, these values are higher in areas of high altitude [5]. Erzurum *et al.* [42] demonstrated this when comparing Tibetan women, living at 4,200masl with women in the United States, living at 206masl. Fiori *et al.* [43] compared three groups of inhabitants from Central Asia, living at 900, 2,100 and 3,200masl, and showed that residents at 3,200masl had higher hematocrit and hemoglobin values than the others. EVANES

project aims to carry out a comparative study with students from other communities living at lower altitudes in the province of *Chimborazo* in Ecuador.

It should be noted that 6 schoolchildren in our study had anemia and 5 of them were boys. Velásquez-Hurtado *et al.* [5] also found that anemia is usually prevalent in boys. In our study, 18.8% of the schoolchildren participated had anemia which is a significant percentage of the population. Such anemia occurs when reference limits are applied to WHO-corrected hemoglobin values instead of the uncorrected values. Therefore, it is fundamental to correct hemoglobin values when working with inhabitants living in a region of high altitude, due to possible anemia cases might be masked. However, the determination of serum iron, ferritin, and transferrin is required to reach conclusive results. Thus, the next goal of the EVANES project is to analyze these metabolites, with the aim of defining the parameters to be considered when diagnosing anemia in indigenous populations, living in the Andean region.

Therefore, we conclude that the rural indigenous schoolchildren population in the province of *Chimborazo*, Ecuador had high prevalence of stunting. In addition, approximately one-fifth of the study population had anemia when WHO correction factors for height were applied. Anemia was mainly detected in boys, suggesting that schoolboys from the rural Highlands are more susceptible to develop anemia than girls. Given the differences identified with the use of the absolute hemoglobin concentration and the adjusted values, it is fundamental to apply correction factors for altitude when diagnosing this pathology. It should be noted that the authors will perform complementary tests, such as serum iron, ferritin and transferrin proteinogram, with the intent to confirm the results in schoolchildren presenting with anemia and residing at high altitude regions. In addition, environmental factors that predispose

schoolchildren to stunting and anemia will be studied. These aspects are critical in addressing the health policies considering the real needs of the rural indigenous populations of Ecuador.

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CONTRIBUTORS

All authors participated in the development of the manuscript, approved the final version, sent to the journal. They all agree with authorship criteria and professional affiliation. XR ROBALINO FLORES and AM BALLADARES SALTOS carried out the fieldwork, analyzed the biological samples, and collected the data presented in the manuscript. ME GUERENDIAIN MARGNI carried out the analysis, interpreted the data obtained, organized the work, decided the design of the manuscript, and reviewed the final version, while F MORALES MARÍN elaborated the final draft of the manuscript presented herein.

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