Noninvasive hemoglobin altitude adjustment in guatemala children

by

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Abstract

Background:

The Anemocheck app, designed for convenient and accessible noninvasive Hb measurement, has tremendous potential in a variety of settings. However, there is little research on its accuracy and utility in different settings like at different altitude levels. Due to the body's natural adaptation of increasing hemoglobin (Hb) levels in response to low oxygen at high altitudes, interpreting Hb test results can be challenging for healthcare professionals, especially when the values fall near the reference range limits. This can make diagnosing anemia or polycythemia difficult.

Objective:

To determine the accuracy and precision of the Anemocheck app after using different altitude adjustments compared to traditional hemoglobin measurement.

Methods:

This study was conducted between May and July 2023 in four locations in Guatemala and involved data from 79 children aged 5 to 83 months. Both the Anemocheck app and traditional hemoglobin measurement methods were available for 39 children, the traditional hemoglobin measurements for 40 children could not obtained. The hemoglobin level was measured traditionally in La Asuncion Laboratorio, a laboratory in Sololá, Guatemala using a Mindray BC-5000 automated hematology analyzer.

We employed hemoglobin (Hb) adjustment formulas proposed by various researchers to establish thresholds for categorizing Hb measurements as anemic or non-anemic. Additionally, adjustment values derived from established formulas were applied to measure individual Hb values continuously. A two-tailed t-test was used to assess the reproducibility of the

Anemocheck app. All statistical analyses throughout this study adhered to a significance level of $p < 0.05$. All data analyses were done using Microsoft Office Excel 2024 (Microsoft, Redmond, WA, USA).

Results:

No significant difference was found between the two app measurements ($p = 0.70$) or between the app's average and the laboratory test ($p = 0.21$), indicating good reproducibility and agreement. The app's sensitivity and specificity at the threshold ≤ 11 were 20% (95% CI, 1% to 70%) and 88% (95% CI, 72% to 96%) respectively. % anemia prevalence increased with altitude (details in Table 2.4). The mean and standard deviation of the unadjusted Hb measurement for the Anemocheck app were 12.35 (1.04g/dL) and 12.34 (0.78g/dL) for elevation <2000m and >2000m respectively. The mean and Standard deviation of the adjusted laboratory measurements were 11.74 (0.73g/dL) and 12.45 (1.08g/dL) for elevation <2000m and >2000m respectively.

Conclusion:

While the app's measurements were reproducible, Hb levels themselves increased with higher altitude thereby increasing the. percentage prevalence of anemia. This aligns with previous research. Interestingly, our findings showed small difference between Hb levels measured by the app and traditional lab tests. This highlights the need for considering altitude and potentially using region-specific Hb thresholds to define anemia accurately. Overall, our study suggests that interpreting Hb levels and defining anemia require us to consider altitude-specific factors even when the noninvasive Hb measurement method is used.

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Chapter 1 - Introduction

1.1 Iron Deficiency and Anemia

The World Health Organization estimates that 1.6 billion people are anemic. As of 2019, the prevalence of anemia in all age groups is estimated to be 22.8% globally [1]. Low and Middle-Income Countries (LMIC) account for 89% of all anemia-related disabilities [2]. Children under the age of five (47.4%) and pregnant women (41.8%) are at high risk of this disease [3]. It is defined as low blood hemoglobin concentration (Hb), the main protein in erythrocytes. Hemoglobin's main function is to take up oxygen from the lungs and to deliver it to the tissues, as well as to transport carbon dioxide from the peripheral tissues back to the lungs. Anemia has multiple adverse effects on human health and a substantial impact on socioeconomic development, in both the short- and long-term [4]. Moreover, anemia is associated with negative effects on children's cognitive, motor, and social-emotional development, which adversely affects school performance [5]. The most common symptoms observed are tiredness, weakness, dizziness, and drowsiness [6;7].

Anemia can have numerous causes, ranging from common nutritional causes, such as iron deficiency to rarer genetic causes which may lead to severe and chronic Anemia that requires frequent monitoring [8]. For the diagnosis of anemia, typically, a blood test is required to measure the amount of hemoglobin, which is a critical protein in a person's red blood cells that carry oxygen which acts as a gold standard for detection of Anemia. To minimize human error and make the problem of visual detection easier, color scale depicting the color spectrum along with the hemoglobin concentration were used by clinicians in past years. The primary constituent in human blood responsible for pigmentation i.e., Hemoglobin reflects the red color predominantly when light falls on its surface as compared to other colors such as green and blue of the RGB spectrum which is a major reason for its deep reddish appearance. The hemoglobin concentration in the human blood thus can be estimated using the red and green components of the RGB spectrum of the region of interest, i.e., Conjunctival Pallor. If a person's hemoglobin is lower than normal, it indicates that the person has Anemia.

1.2 Hemoglobin Measurement

Hb measurement can be accomplished in two ways. The most common way is in a clinical setup with an invasive method, where different chemicals are added to a blood sample, and various optical variations and spectroscopic data are used to calculate the Hb level. In a second way, the image sensor's data, spectroscopic information, and photoplethysmographic sensor’s output are analyzed to calculate the Hb level in a noninvasive estimation process. While invasive majorly and non-invasive (minorly) measurement methods are the most common, there are several images- and spectra-based Hb measurement methods that require a couple of blood drops with minimum blood collection, less pain, and fewer complications, which are named minimally invasive processes.

1.2.1 Non-invasive Process

Non-invasive systems are usually composed of the three main functional components: 1) a data acquisition sensor that captures a raw biological (e.g., image or spectral) signal, 2) a feature engineering unit that preprocesses the signal and calculates features from the signals, and 3) a Hb level estimation system, which generally incorporates different layers of user authentication, data storage, prediction model usage, machine learning, and result validation [9]. The user authentication and Hb estimation phase depend on the device, internet availability, user data, and prediction model [10].

Prior to delving into smartphone-based non-invasive approaches, spectroscopy-based techniques were explored, particularly near-infrared spectroscopy (NIRS), as these methods have garnered significant attention for non-invasive measurement of various blood parameters including hemoglobin (Hb), oxygenation, pH, hematocrit, and glucose levels [11]. The utilization of lighting sources in NIRS methodologies aimed to identify the most effective near-infrared (NIR) lights conducive to non-invasively estimating hemoglobin levels. These light sources, incorporated into the spectral investigation, could potentially enhance the feasibility of obtaining hemoglobin data using a smartphone camera, either as attachable external devices or by directly integrating them. External NIR lights become essential when smartphones lack inherent capabilities to non-invasively sense blood hemoglobin levels within living tissues. Research revealed that analyzing the response of NIR spectrometers, which monitor fluctuations in NIR light absorption in the arm, alongside calculating changes in deoxyhemoglobin and oxyhemoglobin concentrations using six specific wavelengths (797.5, 802.5, 831.2, 848.7, 866.5, and 907.8 nm), could enable a non-invasive method of measuring Hb flow [12].

1.2.2 Smartphone-based Hemoglobin Measurement.

With recent advances in high performance photographic sensors in smartphones, smartphone-based tests have demonstrated significant advantages of being easily accessible and making anemia screening low cost [13;14]. However, the variety of photographic sensors installed in smartphones poses challenges in the standardization of methods and in the development of universal tools for image acquisition and data analysis. The majority of smartphone-based technologies analyze tissue colours using captured images from conjunctiva [15;16;17], fingertip [18;19], nailbed [20], or retinal fundus [Error! Reference source not found.]. Collings et al. determined erythema index calculated from smartphone images of palpebral conjunctiva taken under ambient light conditions can be used to measure Hb levels [17]. Dimauro et al. developed an accessory that consists of a special spacer and a macro lens to acquire images using smartphone camera without impact from ambient light [16]. Park et al. leveraged spectral super-resolution spectroscopy to mathematically reconstruct high-resolution spectra of blood Hb acquired by the built-in camera of a smartphone [22]. Wang et al. developed HemaApp involved white and IR LED for anemia detecting over fingertip [19], which has been improved to require only white LED through hardware configuration [18]. Mannino et al. developed machine learning based smartphone app to measure Hb levels from images of the fingernail bed [20]. Mitani et al. developed deep learning algorithm to measure Hb levels using images of retinal fundus, which can be captured in teleretinal disease screening [21]. This method can be valuable for studying the association of anemia and ocular disease, for which fundus images are already available [21].

1.3 Anemocheck app

The Anemocheck app is an innovative and user-friendly tool designed for the measurement of hemoglobin levels, providing a convenient and accessible solution for healthcare professionals and individuals alike. This noninvasive method of hemoglobin measurement is the world's first equipment-free smart phone app making it a valuable tool in areas with limited access to traditional diagnostic methods [23]. The Anemocheck app has been proposed as an innovative tool, which showed promising results in the first study presented by Mannino and colleagues [20]. In a cohort of 100 patients, comprising both individuals with various diagnoses of anemia and healthy subjects, smartphone hemoglobin (Hgb) levels were measured within ±2.4 g dL−1 of complete blood count (CBC) Hgb levels, with a bias of 0.2 g dL−1, using just a single smartphone image and without any personalized calibration step, highlighting its potential for anemia screening purposes. The App has so far only been tested in a controlled laboratory setting in the United States under optimal test conditions [20] and during health screenings of refugees arriving in the United States [24]. Calibrating the app through user data significantly improved its performance. Before the app was recalibrated, the mean absolute error (MAE) was 0.71 g/dL and the root mean squared error (RMSE) was 1.27 g/dL [20]. Following calibration, these values decreased to an MAE of 0.62 g/dL and an RMSE of 0.85 g/dL, demonstrating a noteworthy improvement in accuracy [20]. This aligns with the positive trends observed in clinical studies. Retraining the algorithm with collected data lead to a considerable improvement in performance [20]. This improvement shows the substantial learning capacity of the App and its ability to be adapted to a different population sample.

1.4 Impact of Residing at High Altitudes on Iron Levels in the Body

More than 500 million people reside at altitudes of 1,500 meters or higher, comprising approximately 6.58% of the global population [25]. The prevalence of anemia continues to be used widely as a surrogate marker of iron deficiency in developing countries. The limitation of this approach is highlighted by estimates of the prevalence of iron deficiency ranging from less than one-fifth to more than two-thirds of the world's population [26]. The problems of relying on isolated hemoglobin measurements to estimate iron deficiency are even greater when surveying populations living at high altitudes. The usual approach has been to apply one of several corrections based on hemoglobin measurements in high-altitude dwellers assumed to have normal iron status. Quantitative measurements of body iron based on the ratio of serum transferrin receptor to ferritin concentration have been described as a means of assessing iron status independently of hemoglobin measurements [27]. A single normal distribution of body iron stores was observed in US men aged 20 to 65 years (mean, 9.82 mg/kg) and in pregnant Jamaican women (mean, 0.09 mg/kg). Two populations were observed in US women of childbearing age; 93% of women had mean iron stores of 5.5 mg/kg, while the remaining 7% had a mean tissue iron deficit of -3.87 mg/kg. It is commonly assumed that the prevalence of iron deficiency is higher in vulnerable groups within these populations due to the increased demand for iron caused by the expansion of the red blood cell mass [28]. Results of anemia surveys to identify this iron deficiency are limited by uncertainty in the criteria of anemia at high altitudes. In a study of Bolivian women living in Atocha (3600 m) and Santa Barbara (4800 m), Berger and colleagues reported a prevalence of 51.7% and 26.5%, respectively, based on the hemoglobin response to 3 months of iron supplementation [29]. However, when the results were compared with mixture distribution model the prevalence was below 10% in both regions. This disparity in estimated prevalence underscores the limitations of hemoglobin measurements for evaluating iron status in the millions of people who reside at high altitudes.

1.5 Hemoglobin Concentration of High-Altitude Residence

The hemoglobin concentrations [Hb] of high-altitude residents increase with altitude [30;31]. Approximately, 5% of the world's population lives at high altitudes (above 1,500 meters). This altitude can trigger chronic anemia, even for those with adequate iron and vitamin intake. This is because the body naturally increases hemoglobin (Hb) production, a process called erythropoiesis, to compensate for the lower oxygen levels at high altitude**s.** Moreover, abnormally high Hb is often related to chronic mountain sickness [32]. Increasing Hb is an effective means to maintain a normal arterial blood O2 content despite decreased oxygen partial pressure (PaO2) in inspiratory air at high altitudes that otherwise compromises tissue oxygen supply by decreasing arterial oxygen saturation (SaO2) [33].

The increase in Hb in high altitude residents is also caused by converse changes in plasma volume and total Hb-mass [34]. Interestingly, at comparable total blood volume, the higher

Hb of Andeans natives compared with Sherpas natives found at high altitude seems to be caused by a reduced plasma volume in the Andeans [34] indicating that besides erythropoiesis also, adjustments of plasma volume significantly affect blood oxygen transport. Because hypoxia increases Hb in high-altitude residents, it is a challenge to define whether an obtained Hb value is abnormal. The World Health Organization (WHO) provides cutoff values defining anemia [35] and polycythemia [36] for sea-level populations and values for adjustment accounting for Hb increments above the sea level [7]. This adjustment is intended for worldwide use but appears inappropriate because of strong evidence for differences in the magnitude of Hb increase among world regions [30]. As an example, Tibetan and Ethiopian altitude populations have considerably lower mean Hb concentrations than Andean populations [30;37].

1.6 Mechanisms Causing Elevated Hb Concentration in High-Altitude Residents

1.6.1 Iron

Reduced iron availability often causes iron deficiency anemia characterized by low Hb concentration and hypochromic anemia. Major causes of iron deficiency anemia are insufficient nutritional intake and reabsorption, impaired release from iron stores, and chronic blood loss, which are situations that need to be considered when one is attempting to diagnose anemia based on the Hb concentration [38].

1.6.2 Plasma Volume

Plasma volume decreases rapidly after lowlanders ascend to high altitude [39]. It remains low even during a one-year's stay [40]. Plasma volume is also decreased in high-altitude residents (Sa´nchez, et al. 1970) and increases when those descend to sea level [40]. Consequently, the Hb concentration is increased in the high-altitude residents [40], which adds to the elevation in Hb by enhanced erythropoiesis. Early studies have been performed on Andean high-altitude natives without further discrimination between Aymaras and Quechuas residents in Bolivia. Plasma and blood volumes of Kenyan elite runners, who lived and trained at 2,000 m above sea level, were comparable to volumes measured in German athletes near sea level [41]. However, measurements had been performed after the Kenyans had traveled to Germany, which may have been sufficient to increase plasma volume. Claydon et al. (2005) reported a larger plasma volume in Ethiopians residing at an altitude of 3,622 m than in Peruvian high-altitude natives at 4,338 m, which seems to account for the lower hematocrit in the Ethiopians [42]. It is unclear whether the difference in the residential altitude accounts for this difference. In 2019, Stembridge et al. (2019) found higher Hb values in Andean highlanders (4,340 m) despite a much higher packed cell volume in Andeans, blood volume was comparable to Sherpa ($P = 0.99$) due to a larger plasma volume in the Sherpa [34].

However, the two populations did not differ in total blood volume, which was higher than the blood volume of lowlanders who spent 5–10 days at 5,050 m in the Himalayans. This result points out the significant role of plasma volume in defining the Hb concentration in high-altitude natives [34].

The mechanisms causing the decrease in plasma volume during hypoxia and the difference among high-altitude populations are not well understood. It has been indicated that a decrease in plasma volume is related to elevated erythropoietin levels because it occurred also in normoxic individuals treated with erythropoietin [43]. A decreased activity of the renin-angiotensin-aldosterone system resulting in decreased aldosterone formation in response to hypoxia also contributes to the decrease in plasma volume upon ascent to high altitude. Decreased aldosterone levels reduce renal sodium reabsorption and subsequently increase water excretion. Elevated catecholamine levels, which block the release of aldosterone from the adrenal glands, might enhance this effect [44]. Indeed, high-altitude Andean natives have decreased aldosterone to renin ratios [45].

The angiotensin-converting enzyme (ACE) controls aldosterone synthesis and release and, thus, Na balance and extracellular fluid volume. It also affects arterial blood oxygenation in many ways [46]. A meta-analysis of the literature on a total of 582 high-altitude residents and 487 lowlanders revealed no difference in the frequency of insertion and deletion (D) alleles among Andean populations [19]. No difference in allele frequencies was found between Andean Quechuas and a lowland native American population from the Canadian West Coast; however, the frequency of the I/I genotype of ACE was much higher in those populations than in Caucasians [47]. There is literature showing a correlation between elevated Hb concentration of the Andean population and increased frequency of the I/I and I/D genotypes of ACE [47;48]. In Chileans, the I/I genotype was associated with decreased ACE activity [48], supposedly explaining the decreased aldosterone plasma levels. Interestingly, the elevated frequency of the I-allele of ACE was related to increased oxygen saturation in Peruvian Quechuas [49]. Moreover, elevated levels of atrial natriuretic peptide (ANP) produced in the heart enhance renal Na and water excretion, a mechanism that most probably further contributes to plasma volume loss at high altitudes. Hypoxia, via hypoxia-inducible factor (HIF)-dependent mechanisms, seems to enhance ANP transcription [50]. However, results on ANP concentration in the plasma of high-altitude residents are divergent. These results indicate that variations in the control of fluid balance among groups of different ancestry might cause variations in the decrease in plasma volume in high-altitude residents. In turn, these different control mechanisms might account for the differences in the Hb concentration among high-altitude residents of different ethnicities.

1.6.3 Genetic Variations in the Oxygen-Sensing System

A variety of polymorphisms has been associated with high altitude acclimatization, some of which correlate with the Hb concentration in blood. A polymorphism resulting in the gain of function of EGLN1, the gene encoding for the oxygen sensor prolyl hydroxylase-2 (PHD-2), in combination with a polymorphism in the EPAS1 gene, encoding for HIF-2a has been found in Tibetan highlanders who show low Hb concentration [51-53]. These polymorphisms in genes coding for key proteins involved in oxygen-sensing mechanisms were not detected in Andean highlanders [30;54]. Accordingly, it has been postulated that these polymorphisms cause the decrease in the PHD2/HIF-2/erythropoietin axis, thereby possibly explaining the blunted response of erythropoiesis to hypoxia in Tibetans. It might, however, also lead to the decreased Hb values of Tibetans living at low altitudes [55]. In Ethiopians living at different altitudes, several candidate genes have been implicated to play a role in high-altitude adaptation, some of which might play a role in controlling the HIF pathway [56].

1.7 Reference Ranges and Interpretation

Hemoglobin and hematocrit values frequently serve as indicators for diminished red blood cell levels, potentially linked to iron deficiency anemia. The diagnosis of iron deficiency anemia occurs when hemoglobin levels drop below two standard deviations from the anticipated mean for a healthy population with similar gender, age, and altitude [57]. Anemia is frequently utilized as an indicator of iron deficiency, leading to the interchangeable use of terms such as Anemia, iron deficiency, and iron deficiency anemia. It is crucial to highlight that even in the absence of anemia, mild to moderate iron deficiency can have adverse effects on tissue function. Anemia, marked by a low hemoglobin (Hb) concentration, presents a notable health concern, particularly for pregnant women and young children. Traditionally, anemia was defined as Hb

<11 g/dL. However, recognizing the limitations of this singular criterion, the World Health Organization (WHO) underscores the measurement of Hb to assess anemia prevalence as a screening tool for iron deficiency (ID), emphasizing that anemia alone does not conclusively indicate ID [58]. To capture the diverse factors impacting hemoglobin levels, WHO recommends tailoring the Hb cutoff for anemia diagnosis in specific groups like high-altitude dwellers, smokers, pregnant women, and individuals across various ethnicities. This acknowledges the intricate interplay of age, sex, personal habits, and altitude on this key diagnostic marker. Over the years, researchers have questioned these cutoffs especially, the altitude adjustment. For instance, Beall in a study conducted suggested that only at altitudes exceeding 3800–4000 meters do Hb concentration of the Tibetans significantly increase therefore concluding that the linear relationship between altitude and Hb doesn't hold for all populations [37]. Similarly, Siques et al. suggested that the hematocrit and hemoglobin concentration of sea-level residents increased upon ascent to 3,550 m altitude for 8 months [59].

Chapter 2 - Noninvasive Hemoglobin Altitude Adjustment in Guatemala Children

Abstract

Background:

The Anemocheck app, designed for convenient and accessible noninvasive Hb measurement, has tremendous potential in a variety of settings. However, there is little research on its accuracy and utility in different settings like at different altitude levels. Due to the body's natural adaptation of increasing hemoglobin (Hb) levels in response to low oxygen at high altitudes, interpreting Hb test results can be challenging for healthcare professionals, especially when the values fall near the reference range limits. This can make diagnosing anemia or polycythemia difficult.

Objective:

To determine the accuracy and precision of the Anemocheck app after using different altitude adjustments compared to traditional hemoglobin measurement.

Methods:

This study was conducted between May and July 2023 in four locations in Guatemala and involved data from 79 children aged 5 to 83 months. Both the Anemocheck app and traditional hemoglobin measurement methods were available for 39 children, the traditional hemoglobin measurements for 40 children could not obtained. The hemoglobin level was measured traditionally in La Asuncion Laboratorio, a laboratory in Sololá, Guatemala using a Mindray BC-5000 automated hematology analyzer.

We employed hemoglobin (Hb) adjustment formulas proposed by various researchers to establish thresholds for categorizing Hb measurements as anemic or non-anemic. Additionally, adjustment values derived from established formulas were applied to measure individual Hb

values continuously. A two-tailed t-test was used to assess the reproducibility of the Anemocheck app. All statistical analyses throughout this study adhered to a significance level of $p < 0.05$. All data analyses were done using Microsoft Office Excel 2024 (Microsoft, Redmond, WA, USA).

Results:

No significant difference was found between the two app measurements ($p = 0.70$) or between the app's average and the laboratory test ($p = 0.21$), indicating good reproducibility and agreement. The app's sensitivity and specificity at the threshold ≤ 11 were 20% (95% CI, 1% to 70%) and 88% (95% CI, 72% to 96%) respectively. % anemia prevalence increased with altitude (details in Table 2.4). The mean and standard deviation of the unadjusted Hb measurement for the Anemocheck app were 12.35 (1.04g/dL) and 12.34 (0.78g/dL) for elevation <2000m and >2000m respectively. The mean and Standard deviation of the adjusted laboratory measurements were 11.74 (0.73g/dL) and 12.45 (1.08g/dL) for elevation <2000m and >2000m respectively.

Conclusion:

While the app's measurements were reproducible, Hb levels themselves increased with higher altitude thereby increasing the. percentage prevalence of anemia. This aligns with previous research. Interestingly, our findings showed small difference between Hb levels measured by the app and traditional lab tests. This highlights the need for considering altitude and potentially using region-specific Hb thresholds to define anemia accurately. Overall, our study suggests that interpreting Hb levels and defining anemia require us to consider altitude-specific factors even when the noninvasive Hb measurement method is used.

2.1 Background

Assessing hemoglobin (Hb) concentration is a widely employed method for screening individuals for anemia. This is often done using invasive methods, however, hemoglobin can now be measured noninvasively with recent advances in high-performance photographic sensors in smartphones, smartphone-based tests have demonstrated significant advantages of being easily accessible and making anemia screening low-cost [13;14]. The Anemocheck app can measure hemoglobin concentrations noninvasively by capturing an image of the user's fingernail beds and calculating the paleness.

Altitude adjustment refers to the process of modifying or calibrating certain physiological parameters or measurements to account for changes in altitude. This adjustment is necessary because altitude can affect various aspects of human physiology, such as oxygen availability, atmospheric pressure, and cardiovascular function. For example, individuals living at higher altitudes may have different hemoglobin levels compared to those at lower altitudes due to adaptations in response to reduced oxygen levels. Altitude adjustment techniques are often used to ensure accurate interpretation of data and appropriate adjustments for altitude-related variables.

Altitude, characterized by a decrease in atmospheric pressure and oxygen tension, poses a unique challenge to the human body's oxygen-carrying capacity. As individuals ascend to higher elevations, the demand for efficient oxygen utilization necessitates adaptations in oxygen-binding molecules, particularly hemoglobin. Failing to adjust hemoglobin concentrations for altitude can prevent individuals from getting a proper iron deficiency diagnosis and the treatment they need. Therefore, it is imperative to find an accurate hemoglobin concentration for people living at high altitudes. There has been debate about the current World Health Organization

(WHO) correction factors used to select the thresholds in classifying individuals as being anemic or not [60;61].

Amidst this scientific landscape, the population of Guatemala as a country stands as a compelling subject for inquiry. Nestled within Central America, Guatemala exhibits a diverse topography, ranging from low coastal areas to elevated plateaus. Despite its geographical intricacies and the potential impact of altitude on physiological parameters, limited comprehensive research has explored the relationship between altitude and hemoglobin levels in this region. Understanding the nuances of altitude-related hemoglobin variations in Guatemala is imperative, not only for advancing the frontiers of scientific knowledge but also for addressing the needs of this population. This study addresses this knowledge gap by evaluating different adjustment parameters using noninvasive hemoglobin measurement methods.

2.2 Methods

Study settings.

The locations where the data were collected are San Jorge La Laguna, Tierra Linda, Peña Blanca and Santa Maria El Tablón with elevations of 1805, 1948, 2124 and 2395 meters respectively. Villages like Tierra Linda, Peña Blanca, Santa Maria El Tablón, and San Jorge La Laguna are situated in the Sololá Department of Guatemala. Sololá, located in the western region of Guatemala, is a department with its capital in the city of Sololá. A notable geographical feature is Lake Atitlan, surrounded by several municipalities in the area. The Sololá Department, with a population of 421,583, is characterized by a blend of indigenous heritage and cultural influences among its residents.

Participants

This study was approved by the Institutional Review Board at Kansas State University (no. 11678). The Guatemala Ministry of Health flagged children for Natun based off anthropometric data collection. Natun followed up with one-on-one interviews at their homes to determine if the child(ren) and family were eligible for support with their programs. The data were collected at each participant's house as part of visits by students from K-state Guatemala Service- Learning program and Natun. K-State Guatemala Service-Learning program is offered by the department of Food, Nutrition, Dietetics, and Health for K-State students. The experience offers a K-State's transformative education abroad programs by equipping students with skills to directly address the needs of underserved populations in Guatemala's Western Highlands. Natun is a Non-government Organization in Panajachel, Guatemala. Their focus is to advance education, nutrition, health and economic development, through community-led initiatives which build on existing resources and knowledge.

The inclusion for the nutrition and health program was some degree of malnutrition while the exclusion from the nutrition and health program was anyone not willing to meet program expectations for involvement including having a blood sample taken for analysis.

Demographics

Secondary variables measured were hematocrit, height, weight, 24- hour dietary recall and BMI. Weight (lb) and height (feet) of the children were measured. Weight was measured and recorded to the nearest lb. A questionnaire was administered by a trained research assistant to assess socio-economic and demographic characteristics of the children in their native language.

Out of the 39 children, 18 are females and 21 are males. The demographic characteristics of the children were summarized in Table 2.1.

This study was conducted between May and July 2023 in four locations in Guatemala. This study involved data from 79 children aged 5 to 83 months. Both the Anemocheck app and traditional hemoglobin measurement methods were utilized for 39 children. Unfortunately, traditional hemoglobin measurements for 40 children could not be obtained, resulting in their exclusion from the study. Table 2.5 provides information about the locations along with their corresponding elevations (measured in meters) and the number of children involved in the study.

Hemoglobin Measurement

Primary variable measured was hemoglobin concentration. The hemoglobin level was measured traditionally in La Asuncion Laboratorio, a laboratory in Sololá, Guatemala using a Mindray BC-5000 automated hematology analyzer. The Anemocheck app was used to measure hemoglobin twice, the two measurements were used to test for reproducibility while the average was used for the mean, sensitivity, and specificity. The Anemocheck app measurement was used twice (consecutively) by alternating both hands of the children. The assessments were done. In the late morning or early afternoon. Although, the Anemocheck app require little to no training, however, the K-State students were trained on how to use the Anemocheck app.

Hemoglobin Adjustment to Altitude

The elevations were generated using the NASA Shuttle Radar Topography Mission (SRTM)(2013) elevations (in meters above sea level). The SRTM data was used to generate the elevation because SRTM data has a 1 arc-second (approximately 30 meter) pixel resolution, and it has a superior spatial resolution when compared with others.

Table 2.2 provides information about the 5 different correction factors that were used.

Anemia was considered present when:

A) the individual hemoglobin value is ≤ 11g/dL as suggested by WHO for low altitude not adjusted for high altitude.

B) when the individual hemoglobin value is ≤ 12.8g/dL, 12.9g/dL, 13.3g/dL and 13.0g/dL for altitude 1805m, 1948m, 2395m and 2124m, respectively using the threshold formula suggested by Dirren H. et al. 1994.

C) When the individual Hb is ≤ 11.9g/dL, 12.0g/dL, 12.25g/dL and 12.07g/dL for altitude 1805m, 1948m, 2395m and 2124m, respectively as suggested by the threshold formula by Silvana Ocas-Cordova et al. 2018.

D) When individual Hb is ≤ 12.35g/dL for altitude 1805m as suggested by Silubonde T. M. et al. 2020.

E) When the individual hemoglobin value is ≤ 10.9g/dL, 11.0g/dL, 12.0g/dL, 13.8g/dL for altitude 1805m, 1948m, 2124m and 2395m respectively as suggested by the threshold formula by Andrea J. Sharma et al. 2019.

The Hb adjustment formulas suggested by Dirren H. et al. 1994, Silubonde, T. M., et al. 2020 (only for San Jorge La Laguna) and Silvana Ocas-Cordova et al. 2018 were used to generate a threshold for Hb measurement, the individual Hb values were then compared with these thresholds hence Hb were measured in a categorical form (anemic or non-anemic). Additionally, the WHO and Andrea J. S et al. 2019 formula were used to obtain an adjustment value hence individual Hb values were adjusted and Hb were measured in a continuous form.

Statistical Analysis

The data from the children were de-identified to remove any personal information before being imported into an Excel datasheet. All data analyses were done using Microsoft Office Excel 2024 (Microsoft, Redmond, WA, USA). The group studied were children of age 5-83 months. Hb concentration was considered the outcome variable, which was analyzed in its continuous as well as categorical form (anemic vs. non anemic).

A two-tailed t-test was used to assess the reproducibility of the Anemocheck app. Mean differences were determined using one- way analysis of variance (ANOVA).

All statistical analyses throughout this study adhered to a significance level of $p < 0.05$. Anemocheck app's reproducibility was evaluated by comparing the Hb measurements from the first and second measurements of the same children. Furthermore, a comparison was conducted between the measurement obtained from the laboratory test and the average of the first and second measurements to evaluate overall reproducibility. Sensitivity and specificity of the Anemocheck app in diagnosing anemia on an individual basis were determined by comparing the average measurement with the laboratory measurement. Mean and standard deviation of hemoglobin concentrations for each location were estimated, along with the mean and standard deviation of age, weight, and height. Additionally, the prevalence of anemia in each location was assessed for each of the adjustment factors.

2.3 Results

The demographic characteristics of the participants, including mean age (46 months), height (2.77 feet), and weight (27.7 lb), were presented, with 21 male and 18 female children included in this study (Table 2.1). Table 2.3 presents the mean and standard deviation (SD) of hemoglobin (Hb) measurements obtained using the Anemocheck app for both the first and second assessments. The table also includes the mean Hb value from the laboratory measurements.

The locations were categorized into elevation <2000m (San Jorge La Laguna and Tierra Linda) and >2000m above sea level (Pena Blanca and Santa Maria El Tablon) in order to assess the mean and standard deviation. The mean and standard deviation of the unadjusted Hb measurement for the Anemocheck app were 12.35 (1.04g/dL) and 12.34 (0.78g/dL) for elevation <2000m and >2000m respectively. The mean and Standard deviation of the adjusted laboratory measurements were 11.74 (0.73g/dL) and 12.45 (1.08g/dL) for elevation <2000m and >2000m respectively. ANOVA analysis was done on the unadjusted Hb measurements of both the Aneemocheck App and the laboratory measurement with a p-value of 0.98 and 0.02 respectively. The reproducibility of the Anemocheck app was evaluated in a total of 39 children using a two-tailed t-test. The first measurement yielded a mean of 12.39 g/dL (SD ± 1.14), while the second measurement had a slightly lower mean of 12.29 g/dL (SD ± 1.19). No statistically significant difference was observed between the two measurements (p = 0.70). Additionally, there was no significant difference (p = 0.21) between the mean Anemocheck app and the laboratory hemoglobin concentration further strengthening the evidence for the app's consistent performance across participants. These results suggest that the Anemocheck app produces reliable and reproducible measurements, supporting its potential replicability under similar conditions. The percentage prevalence of anemia by elevations using different hemoglobin adjustment parameters is presented in table 2.4. The sensitivity and specificity of the Anemocheck app at ≤ 11g/dL were 20% (95% CI, 1% to 70%) and 88% (95% CI, 72% to 96%) respectively (Table 2.7). The table also include the overall sensitivity, specificity, positive predictive value, negative predictive value of the adjustment methods. San Jorge La Laguna has a sensitivity and a specificity of 100% (95%CI, 31% to 100%) and 25% (95%CI, 4% to 64%) respectively, when the correction factor suggested by Silubonde, T. M., et al. 2020 was used. The sensitivity and specificity of each of the locations with the adjustments are presented in table 2.5. The positive and negative predictive values across the locations are presented in table 2.6.

2.4 Discussion

Research has consistently demonstrated that Hb concentrations exhibit an adaptive increase in response to hypoxia, a state of low oxygen availability. Because of this, the health practitioners often face challenges in the accurate interpretation of hemoglobin concentration results for individuals residing at high altitudes, particularly when the results fall on the boundaries of reference intervals. Determining the thresholds for defining anemia and polycythemia in such cases poses a common dilemma [62].

Although, our work showed a close Hb mean at <2000m and >2000m, however, in contrast to some previous studies, the mean Hb didntt increase with altitude. The unadjusted mean hemoglobin level in elevation of <2000 m is higher than the mean Hb at elevation >2000 m [63]. Both WHO and Andre J. S. et al. 2019 adjustments result in lower means, emphasizing the potential impact of altitude on hemoglobin levels in this location. In Tierra Linda, the mean unadjusted hemoglobin is 11.79 g/dL which is lower compared to that of Santa Maria El Tablón. Although, similar to Santa Maria El Tablón, adjustments using both WHO and Andre J. S. et al. 2019 methods result in lower mean hemoglobin values, indicating a reasonable estimation of Hb concentration. Furthermore, San Jorge La Laguna shows the highest unadjusted mean of 12.85 g/dL, with the largest standard deviation. Peña Blanca, with only two observations, exhibits the highest unadjusted mean of 13.53 g/dL, accompanied by a minimal standard deviation.

Adjustments, however, result in lower means, emphasizing the need to consider the small sample size as a limitation.

Substantial variations were observed in percentage anemia prevalence when comparing results obtained from the laboratory measurements, unadjusted Hb obtained from the Anemocheck app and different adjustment methods using the Anemocheck app. Percentage anemia prevalence was higher at elevations >2000m (Pena Blanca and Santa Maria El Tablon) across all the adjustment methods when compared with the elevation <2000 (San Jorge La Laguna and Tierra Linda). The findings of our study corroborate with the results observed in the study conducted by Accinelli, R. A., et al. in 2020, which demonstrated an increase in the prevalence of anemia with altitude [64]. This consistency in findings underscores the significance of altitude-specific factors when interpreting hemoglobin (Hb) levels and defining anemia.

Notably, the prevalence of anemia was lower when employing the uncorrected low-altitude Hb threshold of 11 g/dL (unadjusted for altitude) at high altitude (HA) compared to utilizing Hb values corrected with the altitude adjustment factors (Table 2.4).

The overall sensitivity of the Anemocheck app was lower than the values reported by Mannino, R. G et al. 2018 which is 92% (95% CI, 80–97%) and the specificity of the app was higher than the reported value of 76% (95% CI, 62–87%) [20]. Because the research done on the Anemocheck app is limited, this is further strengthening the need for more work to be done especially at different altitude. While the specificity of the app remained relatively unchanged, the sensitivity of the Anemocheck app increased with increased in altitude when considering the Dirren H et al. 1994 method. This specificity value of 100% across any of the locations indicates that the Anemocheck app used in this study is able to show individuals without anemia as negative. Also, the 100% sensitivity across any of the locations indicates that the Anemocheck app used in this study is able to categorize those with anemia as positive.

Notably, the sensitivity of the WHO threshold and specificity of the unadjusted Hb (app), were 0(CI 95% 0-69) and 0(CI 95% 0-80) app at elevations <2000m and >2000m respectively while the specificities of the Anemocheck app at the altitudes did not show a consistent increase with altitude (Table 2.5). The Dirren H. et al. method showed the best sensitivity result across the altitudes. The unadjusted Hb measurement of the app showed a good specificity as compared to the adjustment methods across the altitudes. For the overall sensitivity, Dirren H. et al. had the highest sensitivity while the unadjusted Hb using the app had the highest specificity (Table 2.7). While the WHO adjustment method showed the lowest positive predictive value, the unadjusted Hb using the app had the highest PPV (Table 2.7). we had variations in the NPV with the Silvana Ocas-Cordova adjustment method having the highest while the WHO adjustment methods had the lowest. Table 2.8 showed a comparison between our work and Young M. F. et al. 2021.

The strength of the study lies in its comprehensive examination of hemoglobin concentrations across multiple locations within Guatemala. The measurement of the hemoglobin concentration twice gave a chance to see the reproducibility of the Anemocheck app. Also, the data used were collected from different altitude within the same ethnic group.

This study has limitations. First, the small participant size may compromise its accuracy in providing robust findings applicable to a larger population.

Other limitations include issues of accessibility of the study site and the inconsistent nature of internet connectivity. These challenges significantly impacted the ability to gather data effectively and comprehensively. Specifically, the geographical location of Pena Blanca, situated on a hill, posed difficulties in reaching the area for conducting visits. Additionally, the remote nature of the locations contributed to limited internet access, which hindered the ability to gather data effectively. The long distance that the participants have to travel to get the traditional Hb measurement done was a major limitation in getting the corresponding measurements of the Anemocheck app.

Furthermore, the intermittent availability of children at home during scheduled visits posed another significant barrier. It was also difficult to get the children to curl their fingers toward their palm. This could cause inaccurate assessment.

2.5 Conclusion

Our research showed that the Anemocheck app's measurements were reproducible at the elevations considered. We also showed that the conventional understanding that hemoglobin (Hb) concentrations increase significantly with altitude may not always hold true for all elevations especially when the noninvasive Hb measurement method is used. Although, our work corroborate previous studies that % anemia prevalence increases with altitude, however, we found that the Hb levels in children residing at various altitudes within Guatemala did not show a consistent pattern of increase with higher elevations. Specifically, our results from different altitudes in Guatemala indicated that there is no difference between the observed mean of Hb levels when using the app and the laboratory measurement. Moreover, we observed population differences in Hb levels, highlighting the need for region-specific threshold values to define anemia accurately. Furthermore, our study underscores the importance of considering altitude-specific factors when interpreting Hb levels and defining anemia. The prevalence of anemia in children at high altitudes appears to be higher than at low and altitudes, indicating a widening gap over time. This trend suggests that interventions aimed at reducing anemia prevalence need to account for altitude variations and may require tailored approaches for populations living at different elevations. Our findings emphasize the complexity of interpreting Hb levels and defining anemia, particularly in populations residing at high altitudes. Addressing these complexities requires not only region-specific threshold values but also a comprehensive understanding of genetic and environmental factors influencing Hb concentrations. This knowledge is crucial for designing effective public health interventions aimed at reducing anemia prevalence, especially in high-altitude regions.

Tables

Table 2.1. Demographic characteristics of the children

Location	Mean Age (m)	Mean Ht (feet)	Mean Wt (lb)	Female (n)	Male (n)
San Jorge La Laguna	32	2.69	23	3	8
Tierra Linda	56	3.08	31	4	6
Peña Blanca	42	2.88	27.3	1	1
Santa Maria El Tablón	47	3.00	28.8	10	6

Table 2.2. Formulas and thresholds proposed by different studies.

Study	Formula for Hb Adjustment
Silvana Ocas-Cordova et al. 2018 [63]	$Hb = 1.19e\text{-}07 \times altitude^2 + 0.0001123 \times altitude + 11.2964$
Dirren H et al. 1994 [65]	$Hb = 3.44 * EXP(0.000633 \times altitude) + 116.9$
Andrea J. Sharma et al. 2019 [31]	Hb adjust altitude (g/L) = $(0.0048108 * altitude) + (0.0000004 * altitude^2)$
Silubonde, T. M., et al. 2020 [66]	Cut off threshold of 12.35g/dl for 1700m
WHO [67]	$Hb = -0.32 \times (altitude\ in\ meters \times 0.0033) + 0.22 \times (altitude\ in\ meters \times 0.0033)^2$

Table 2.3. Hemoglobin measurements

Measurement	Mean (SD)
Anemocheck app Measurement 1	12.39 g/dL (1.15)
Anemocheck app Measurement 2	12.29 g/dL (1.19)
Average Anemocheck app Measurement	12.34 g/dL (0.92)
Laboratory Measurement	12.07g/dL (0.97)

Table 2.4. Prevalence of anemia at different altitudes

Elevations (m)	n	Lab Unadjusted Hb (%)	Unadjusted Hb (app) (%)	WHO (%)	Dirren H. et al. 1994 (%)	Andrea J. S. et al. (%)	Silvana Ocas-Cordova et al. 2018%
<2000	21	14	5	29	57	48	48
>2000	18	6	6	33	89	56	50

Table 2.5. Sensitivity and Specificity of the Elevations

Elevations (m) (n)	Unadjusted Hb (App)	WHO	Dirren H et al.	Andrea J. S. et al.	Silvana Ocas-Cordova et al.
	Sensitivity Specificity	Sensitivity Specificity	Sensitivity Specificity	Sensitivity Specificity	Sensitivity Specificity
<2000 (21)	33(1-88) 83(58-96)	0(0-69) 67(41-86)	67(13-98) 44(22-69)	67(13-98) 56(31-78)	67(13-98) 56(31-78)
>2000 (18)	0(0-80) 94(68-100)	50(2-97) 69(42-88)	100(20-100) 0(0-24)	50(3-97) 44(21-69)	50(3-97) 50(26-74)

Table 2.6. Positive and Negative Predictive Values Across Different Altitudes

Elevations (m) (n)	Unadjusted Hb (App)	WHO	Dirren H et al.	Andrea J. S. et al	Silvana Ocas-Cordova
	PPV NPV	PPV NPV	PPV NPV	PPV NPV	PPV NPV
<2000 **(21)**	25(1-78) 88(62-98)	0(0-48) 80(51-95)	16(3-49) 89(51-99)	20(4-56) 91(57-100)	20(4-56) 91(57-100)
>2000 **(18)**	0(0-95) 88(62-100)	17(0.9-64) 92(60-100)	11(2-36) 0	10(0.5-46) 88(47-99)	11(0.6-49) 89(51-99)

Table 2.7. Overall comparison of unadjusted and adjustment methods

	Unadjusted Hb	WHO	Dirren H. et al.	Andrea J. et al.	Silvana Ocas-Cordova
Sensitivity (%)	20(1-70)	20(1-70)	80(30-99)	60(17-93)	60(17-93)
Specificity (%)	88(72-96)	68(49-82)	24(11-42)	50(33-67)	53(35-70)
PPV (%)	20(1-70)	8(0.4-40)	13(4-32)	15(3-39)	16(4-41)
NPV (%)	88(72-99)	85(65-95)	89(51-99)	89(65-98)	90(67-98)

Table 2.8. Comparison with Young et al. 2021 [24]

	Our work using Anemocheck app	Apple® Phone	Android® Phone	Masimo Pronto®
Sensitivity	20(1-70)	63.9% (0.5, 0.8)	36.1% (0.2, 0.5)	45.7% (0.3, 0.6)
Specificity	88(72-96)	48.2% (0.4, 0.5)	67.6% (0.6, 0.7)	85.3% (0.8, 0.9)
PPV	20(1-70)	15.0% (0.1, 0.2)	13.7% (0.1, 0.2)	30.2% (0.2, 0.4)
NPV	88(72-99)	90.4% (0.9, 1.0)	88.1% (0.8, 0.9)	91.9% (0.9, 1.0)

Chapter 3 - Recommendations

Anemocheck app showed a good device reproducibility and reasonable method reproducibility, as well as good performance in estimating the prevalence of anemia. Nevertheless, the consistency wasn't reasonable enough due the participant size. I would recommend that this work should be carried out on a larger population, this will give a more reliable result because larger population would have smaller margins of error. Also, our inability to know the adjustment done on the laboratory measurements of Hb posed a great limitation to this work, therefore, a more detailed information about the adjustment done on the Hb traditional measurements would help to further position the work for good comparison. Root Mean Squared Error should be done to give an estimation of how well the Anemocheck app is accurate. Also, to give a straightforward interpretation of the model's overall error, making it accessible to people without a solid statistical background. It is advised to use the Anemocheck app for the Hb measurement consistently in a particular time of the day to reduce excess variability in the results. Additionally, including a wider range of demographic groups would enhance the generalizability of the findings of the Anemocheck app.

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Appendix A - List of Abbreviations

ANOVA	Analysis of Variance
App	Application
HA	High. Altitude
Hb	Hemoglobin
LMIC	Low and Middle-Income Countries
NASA	National Aeronautics and Space Administration
NIRS	Near-infrared spectroscopy
NPV	Negative Predictive Value
NR	Near Infrared
PPV	Positive Predictive Value
USDA	United State Department of Agriculture
WHO	World Health Organization