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Establishment of Reference Intervals for Hemoglobin Levels Among Healthy Adult Males and Females in Derna, Libya

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تأسيس النطاقات المرجعية لمستويات الهيموجلوبين بين الذكور والإناث الأصحاء في مدينة درنة، ليبيا

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Abstract:
Hemoglobin concentration is one of the most important hematological parameters used in clinical practice for the diagnosis and monitoring of anemia and other hematological disorders. However, the reference intervals currently used in Libya are largely derived from non-Libyan populations and may not accurately reflect the physiological characteristics of the local population. Therefore, the present study aimed to establish population-specific reference intervals for hemoglobin concentration among healthy adults in Derna city, Libya. A cross-sectional study was conducted between November and December 2024 involving 129 apparently healthy adults, including 67 males and 62 females, randomly recruited from different districts of Derna city. Venous blood samples were collected in EDTA tubes and analyzed using an automated hematology analyzer. Statistical analysis was performed using nonparametric methods following assessment of data distribution. The findings demonstrated significant sex-related differences in hemoglobin concentration, with males exhibiting higher values than females ($p < 0.05$). The established reference intervals for hemoglobin were 11.1–16.0 g/dL for males and 10.2–13.5 g/dL for females. Furthermore, the hemoglobin values demonstrated a skewed distribution and did not follow normality according to the Anderson–Darling test. The present findings emphasize the importance of establishing locally derived hematological reference intervals rather than relying exclusively on international reference standards. The implementation of population-specific hemoglobin reference values may improve laboratory interpretation, enhance diagnostic accuracy, and support better clinical management of hematological disorders within the Libyan population.

Keywords: Hemoglobin, reference intervals, hematology, anemia, Libya, Derna, complete blood count, population-specific reference values.

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الملخص
يُعد تركيز الهيموجلوبين أحد أهم المؤشرات الدموية المستخدمة في الممارسة السريرية لتشخيص ومراقبة فقر الدم واضطرابات الدم الأخرى. ومع ذلك، فإن النطاقات المرجعية المستخدمة حالياً في ليبيا مستمدة في الغالب من مجتمعات غير ليبية، وقد لا تعكس بدقة الخصائص الفسيولوجية للسكان المحليين. لذلك، هدفت الدراسة الحالية إلى تحديد نطاقات مرجعية خاصة بالسكان لتركيز الهيموجلوبين بين البالغين الأصحاء في مدينة درنة، ليبيا. أُجريت دراسة مقطعية بين شهري نوفمبر وديسمبر 2024 شملت 129 بالغاً ظاهرياً بالصحة، منهم 67 ذكراً و62 أنثى، تم اختيارهم عشوائياً من مناطق مختلفة في مدينة درنة. جُمعت عينات الدم الوريدي في أنابيب تحتوي على EDTA وحُللت باستخدام جهاز تحليل الدم الآلي. أُجري التحليل الإحصائي باستخدام طرق غير معلمية بعد تقييم توزيع البيانات. أظهرت النتائج فروقاً ذات دلالة إحصائية مرتبطة بالجنس في تركيز الهيموجلوبين، حيث أظهر الذكور قيماً أعلى من الإناث .($p < 0.05$) كانت النطاقات المرجعية المحددة للهيموجلوبين 11.1–16.0 جم/ديسيلتر للذكور و10.2–13.5 جم/ديسيلتر للإناث. علاوة على ذلك، أظهرت قيم الهيموجلوبين توزيعاً ملتوياً ولم تتبع التوزيع الطبيعي وفقاً لاختبار أندرسون-دارلينج. تؤكد النتائج الحالية على أهمية إنشاء نطاقات مرجعية دموية مستمدة محلياً بدلاً من الاعتماد حصراً على المعايير المرجعية الدولية. إن تطبيق قيم مرجعية للهيموجلوبين خاصة بالسكان قد يحسن من التفسير المخبري، ويعزز دقة التشخيص، ويدعم إدارة سريرية أفضل لاضطرابات الدم داخل المجتمع الليبي.

الكلمات المفتاحية: الهيموجلوبين، النطاقات المرجعية، أمراض الدم، فقر الدم، ليبيا، درنة، صورة الدم الكاملة، قيم مرجعية خاصة بالسكان.

Introduction

Hemoglobin (Hb) is an iron-containing metalloprotein located within red blood cells and plays a fundamental role in oxygen transport from the lungs to peripheral tissues, as well as carbon dioxide transport back to the lungs for exhalation (Maton *et al.,* 1993; Mairbäurl, 2013). Structurally, hemoglobin consists of four globin subunits, each associated with a heme group containing ferrous iron, enabling reversible oxygen binding and efficient gas exchange (Perutz *et al.,* 1960). Beyond its physiological importance, hemoglobin concentration is widely recognized as one of the most essential hematological parameters used in clinical medicine for the assessment of general health status, oxygen-carrying capacity, and hematopoietic function (Hardison, 1996; Ahlgrim *et al.,* 2014).

Measurement of hemoglobin concentration is routinely performed as part of the complete blood count (CBC), one of the most frequently requested laboratory investigations worldwide (Karakochuk *et al.,* 2019). Hemoglobin levels provide valuable diagnostic information for numerous pathological conditions, particularly anemia, polycythemia, nutritional deficiencies, chronic inflammatory disorders, and hematological diseases (Sidell *et al.,* 2006; Agnello *et al.,* 2021). Reduced hemoglobin concentrations may compromise tissue oxygenation and are commonly associated with fatigue, pallor, dizziness, weakness, and dyspnea, whereas elevated hemoglobin levels may increase blood viscosity and predispose individuals to thrombotic complications and impaired tissue perfusion (Gilbertson *et al.,* 2008).

Physiological hemoglobin concentrations vary considerably according to sex, age, ethnicity, environmental conditions, altitude, nutritional status, and genetic background (Odhiambo *et al.,* 2015; A R S *et al.,* 2012). In healthy adults, hemoglobin concentrations are generally higher in males than females because of hormonal and physiological differences influencing erythropoiesis (Miao *et al.,* 2004). Consequently, the establishment of accurate and population-specific reference intervals is essential for reliable clinical interpretation and appropriate disease diagnosis.

Reference intervals constitute a cornerstone of laboratory medicine and are indispensable for distinguishing healthy individuals from those with underlying pathological conditions (Cook *et al.,* 2003). However, many currently used hematological reference values were established decades ago using analytical techniques, reagents, and study populations that differ substantially from those used today (Beutler *et al.,* 2006). Moreover, universal application of reference ranges derived primarily from European and North American populations may not accurately reflect the physiological characteristics of other ethnic or regional populations (Chernoff *et al.,* 1956; Roshan *et al.,* 2009). It has become increasingly evident that hematological parameters exhibit substantial inter-population variability influenced by race, genetic diversity, environmental exposure, climate, lifestyle, and geographical factors (Timzing *et al.,* 2014).

Several international studies have emphasized the necessity of establishing local hematological reference intervals. In Egypt, (Radwan *et al.,* 2022) demonstrated significant gender-related differences in hemoglobin levels and reported lower hemoglobin values compared with internationally adopted standards. Similarly, (Awad *et al.,* 2019) established hematological reference values for healthy Sudanese adults and found that hemoglobin concentrations in the Khartoum population were lower than commonly used international reference ranges. Comparable findings were also reported in Pakistan, Turkey, Brazil, and Korea, where investigators concluded that hematological reference intervals vary significantly according to demographic and regional characteristics and therefore should be determined locally rather than adopted universally (Nah *et al.,* 2017; Tekkeşin *et al.,* 2014; Mazhar *et al.,* 2017; de Sá *et al.,* 2023).

The importance of population-specific hematological reference intervals has become even more relevant in the context of global anemia assessment. According to the Global Burden of Disease Study, approximately 1.9 billion anemia cases were reported worldwide in 2021, affecting nearly one-quarter of the global population (GBD 2021 Anaemia Collaborators, 2023). Hemoglobin thresholds used for anemia diagnosis have historically been defined by the World Health Organization (WHO), with periodic revisions attempting to account for variables such as age, pregnancy, and altitude (WHO, 1959; WHO, 1968; WHO, 2024). Nevertheless, several investigators have questioned the universal applicability of these thresholds, particularly in populations residing in geographically and genetically distinct regions (Gonzales *et al.,* 2018; Sarna *et al.,* 2020; Kanu *et al.,* 2023).

Despite the growing recognition of the importance of locally derived hematological reference values, Libya still lacks nationally established reference intervals for hemoglobin concentration. Current clinical practice in Libya continues to rely predominantly on reference ranges adopted from Caucasian and non-Libyan populations, which may not accurately represent the physiological and demographic characteristics of the Libyan population. This limitation may potentially affect diagnostic accuracy, disease classification, and clinical decision-making.

Therefore, the present study was conducted to establish reference intervals for hemoglobin levels among healthy adult males and females in Derna, Libya. The findings of this study are expected to contribute to the development of population-specific hematological standards that may improve laboratory interpretation, enhance diagnostic precision, and support future nationwide efforts to establish comprehensive hematological reference values for the Libyan population.

Materials and Methods

A cross-sectional study was conducted between November and December 2024 to establish reference intervals for hemoglobin levels among healthy adults in Derna city, Libya. The study was performed at Sheha Hospital and Tabebak Laboratory. Participants were randomly

recruited from several districts within Derna city, including (Sahal al sharqi - Bab Tobruk – Saiha - Sahal al garbi - Al Blad).
The study included 129 apparently healthy adults aged 18 years and above, comprising 67 males and 62 females. Eligibility criteria included permanent residence in Derna city and absence of chronic illnesses or hematological disorders. Individuals with hypertension, diabetes mellitus, obesity (BMI ≥ 30 kg/m²), smoking history, pregnancy, malignancy, previous chemotherapy treatment, or use of weight-gain supplements were excluded from the study. Athletes with excessive muscle mass were also excluded to minimize physiological variations affecting hemoglobin levels.
Venous blood samples were collected in EDTA anticoagulant tubes under standardized conditions to reduce pre-analytical variability. Samples were gently mixed and analyzed within approximately 10 minutes of collection while maintained at room temperature. Hemoglobin concentrations were measured using an automated hematology analyzer (Celltac, NIHON KOHDEN, three-part differential analyzer). The analyzer was calibrated daily according to the manufacturer's instructions, and all measurements were performed in duplicate to ensure analytical accuracy and reliability.
Statistical analysis was conducted using IBM SPSS Statistics. Descriptive statistics, including mean, standard deviation, and reference ranges, were calculated for hemoglobin levels. Data normality was assessed using the Anderson–Darling test, while comparisons between groups were performed using the Mann–Whitney U test. A p-value of less than 0.05 was considered statistically significant. Ethical approval was obtained from the relevant biomedical ethics committee, and informed consent was secured from all participants prior to enrollment in the study.

Results

A total of 129 apparently healthy adults were enrolled in the present study, including 67 males and 62 females. The age of participants ranged from 18 to 85 years. The mean age was 43.56 years among males and 37.60 years among females. In addition, the mean body mass index (BMI) was 24.3 ± 4.6 kg/m² for males and 23.8 ± 4.7 kg/m² for females. Detailed descriptive statistics for age are presented in (Table 1), while the distribution of age-related variables among male and female participants is illustrated in (Figure 1).

Table 1 Descriptive statistics of age among study participants.

	StDev	**Mean**	**Max**	**Min**
Male	18.5	43.56	85	18
Female	14.15	37.6	74	18

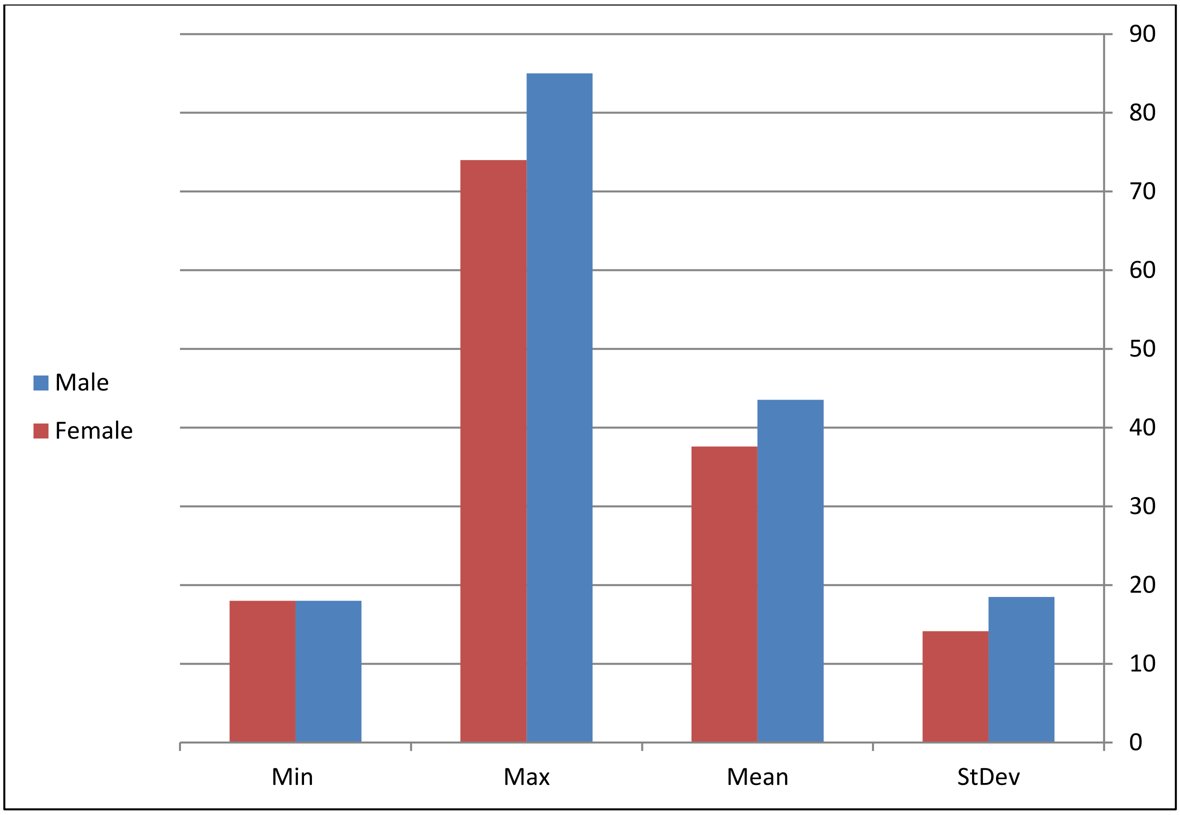

Figure 1 Distribution of age-related statistical parameters among male and female participants.

Descriptive statistical analysis of hemoglobin concentration demonstrated noticeable variability between male and female participants. The highest recorded hemoglobin concentration was 16.1 g/dL in males and 13.5 g/dL in females, whereas the lowest recorded values were 11.1 g/dL and 10.2 g/dL, respectively. The calculated standard deviation and coefficient of variation indicated a moderate dispersion of hemoglobin values within the study population. Detailed descriptive statistics for hemoglobin concentration are summarized in (Table 2), and the distribution of minimum and maximum hemoglobin values is presented in (Figure 2).

Table 2 Descriptive statistics of hemoglobin concentration among study participants.

	CV%	**StDev**	**Mean±SD**	**Max**	**Min**
Male	10.73	1.39	12.95±1.39	16.1	11.1
Female	6.59	0.74	11.35±0.74	13.5	10.2

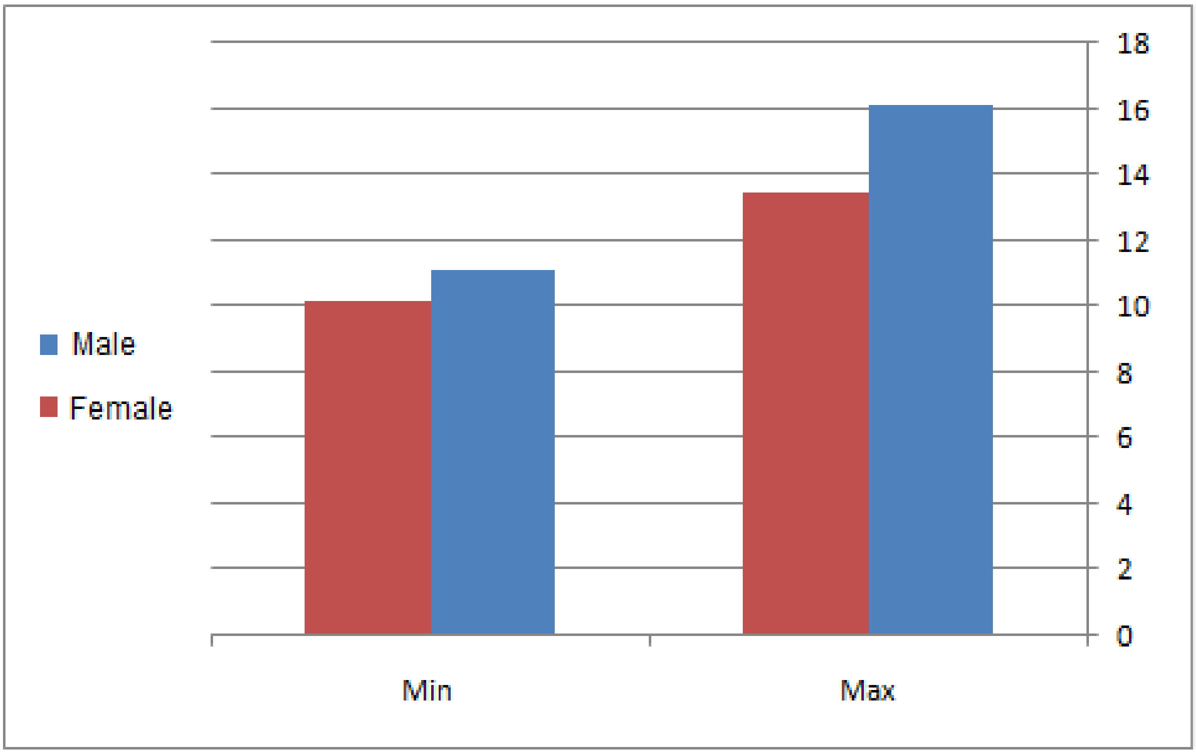

Figure 2 Distribution of minimum and maximum hemoglobin concentrations among male and female participants.

Assessment of data normality using the Anderson–Darling test demonstrated that hemoglobin values were not normally distributed (W = 3.3569, p = 0.00000002). Since the p-value was below the significance threshold of 0.05, the null hypothesis of normal distribution was rejected. The observed discrepancy between the mean and median values further indicated skewness within the dataset, with a tendency toward right-skewed distribution, as illustrated in (Figure 3).

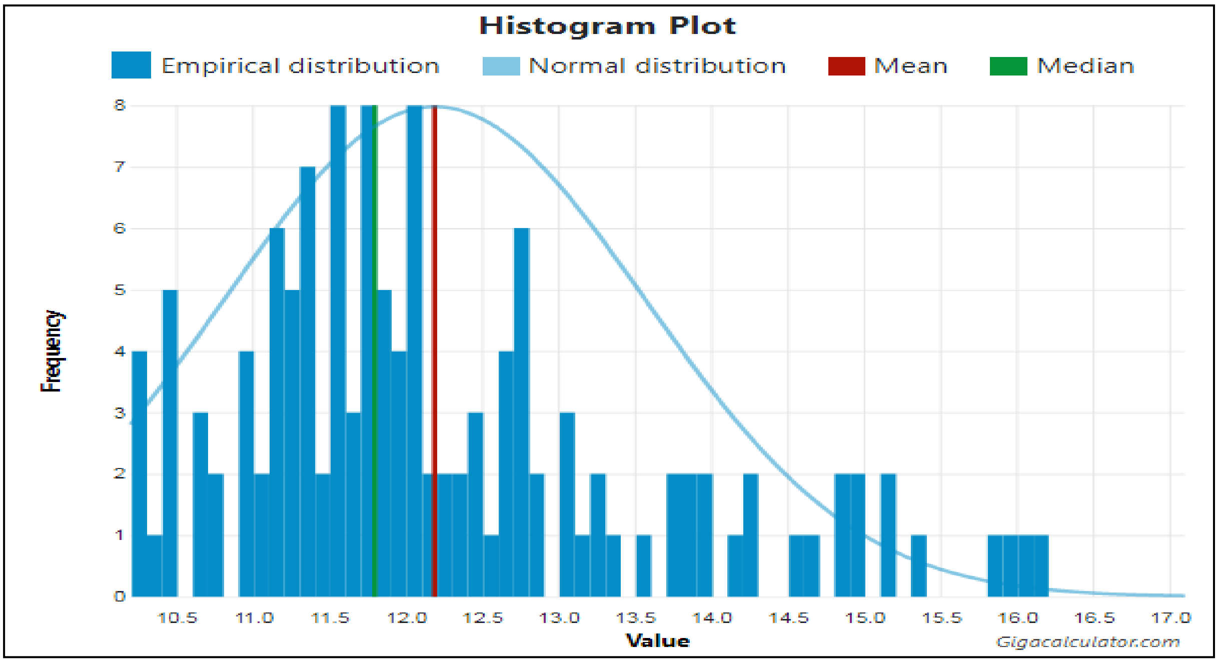

Figure 3 Distribution pattern of hemoglobin concentration demonstrating skewness of the dataset.

Comparative analysis using the Mann–Whitney U test revealed a statistically significant difference in hemoglobin concentration between males and females ($Z = 6.72917$, $p < 0.00001$). Male participants demonstrated significantly higher hemoglobin concentrations compared with female participants.

Because the dataset did not follow a normal distribution, nonparametric methods were used to establish hemoglobin reference intervals. The lower reference limit was determined using the 2.5th percentile, while the upper reference limit was determined using the 97.5th percentile of the ordered dataset. Accordingly, the reference interval for hemoglobin concentration in males was 11.1–16.0 g/dL, whereas the corresponding reference interval for females was 10.2–13.5 g/dL.

Discussion

The present study was conducted to establish reference intervals for hemoglobin concentration among healthy adult males and females in Derna, Libya. The findings demonstrated clear gender-related differences in hemoglobin levels, with males exhibiting higher hemoglobin concentrations than females. The established reference intervals were 11.1–16.0 g/dL for males and 10.2–13.5 g/dL for females. These findings are consistent with the well-documented physiological influence of sex on erythropoiesis and hemoglobin production, where males typically exhibit higher hemoglobin levels because of androgenic stimulation and differences in iron metabolism and menstrual blood loss in females.

The observed gender-based variation in hemoglobin concentration is consistent with findings reported in several regional and international studies. (Radwan *et al.,* 2022), in an Egyptian population, reported significantly higher hemoglobin concentrations in males compared with females and demonstrated noticeable differences between locally derived hematological values and internationally adopted reference ranges. Similarly, (Awad *et al.,* 2019) reported lower hemoglobin concentrations among Sudanese females compared with males, further supporting the influence of demographic and physiological factors on hematological parameters. The similarity between the findings of the present study and those conducted in neighboring African and Middle Eastern populations may reflect shared environmental, nutritional, ethnic, and socioeconomic characteristics.

The current findings also support previous evidence indicating that universal hematological reference intervals may not accurately represent all populations. Several studies conducted in Turkey, Pakistan, Brazil, and Korea have demonstrated substantial variations in hematological parameters according to ethnicity, sex, age, geographic location, and environmental exposure (Tekkeşin *et al.,* 2014; Mazhar *et al.,* 2017; Nah *et al.,* 2017; de Sá *et al.,* 2023). These observations reinforce the growing international consensus that population-specific reference intervals are essential for improving laboratory interpretation and clinical decision-making.

In the present study, hemoglobin values did not follow a normal distribution according to the Anderson–Darling normality test. Consequently, nonparametric statistical methods were used to establish reference intervals. This approach is consistent with internationally accepted recommendations for the determination of laboratory reference intervals, particularly when data demonstrate skewed distributions. The statistically significant difference identified between males and females using the Mann–Whitney U test further validates the necessity of sex-specific hemoglobin reference intervals for the studied population.

Compared with internationally accepted hemoglobin reference ranges, the values obtained in the present study appear relatively lower, particularly among females. Similar findings have been reported in studies from Sudan, Egypt, and Pakistan, where locally derived hemoglobin values differed from standard Caucasian-based reference intervals (Awad *et al.,* 2019; Radwan *et al.,* 2022; Mazhar *et al.,* 2017). Such differences may be attributed to variations in dietary

habits, socioeconomic status, genetic background, environmental conditions, altitude, and prevalence of nutritional deficiencies. These factors collectively influence erythropoietic activity and hematological profiles within different populations.
The importance of establishing localized reference intervals has been increasingly emphasized in recent years. Although international organizations such as the World Health Organization (WHO) provide standardized hemoglobin thresholds for the diagnosis of anemia, several researchers have questioned the universal applicability of these cut-off values across diverse populations (Gonzales *et al.,* 2018; Sarna *et al.,* 2020; Kanu *et al.,* 2023). In Libya, clinical laboratories continue to rely predominantly on hematological reference values derived from non-Libyan populations despite the absence of nationally established standards. The present study therefore represents an important preliminary contribution toward the development of locally relevant hematological reference intervals for the Libyan population.
The findings of this study may have important clinical implications. The establishment of locally derived hemoglobin reference intervals may improve the interpretation of laboratory investigations, enhance diagnostic accuracy for anemia and other hematological conditions, and reduce the risk of misclassification resulting from inappropriate use of foreign reference standards. Furthermore, these data may provide a valuable foundation for future large-scale epidemiological and hematological studies across different regions of Libya.
Despite the significance of the present findings, certain limitations should be acknowledged. The study was conducted within a single city and involved a relatively limited sample size, which may restrict the generalizability of the results to the broader Libyan population. In addition, other potentially influential variables, including nutritional status, iron profile, socioeconomic conditions, and genetic background, were not extensively evaluated. Therefore, larger multicenter studies involving different Libyan regions and broader demographic representation are recommended to establish comprehensive national hematological reference intervals.
Overall, the present study contributes to the growing body of evidence supporting the necessity of establishing population-specific hematological reference intervals. The results highlight the importance of considering regional demographic and environmental characteristics in clinical laboratory interpretation and support the adoption of locally derived hemoglobin reference values for the Libyan population.

Conclusion

The present study established population-specific reference intervals for hemoglobin concentration among healthy adults in Derna, Libya, demonstrating reference ranges of 11.1–16.0 g/dL for males and 10.2–13.5 g/dL for females, with statistically significant sex-related differences in hemoglobin levels. The findings further revealed that hemoglobin values in the studied population did not follow a normal distribution, supporting the use of nonparametric statistical methods for reference interval estimation. These results emphasize the importance of establishing locally derived hematological reference intervals rather than relying exclusively on internationally adopted standards that may not accurately reflect the genetic, environmental, nutritional, and demographic characteristics of the Libyan population. The implementation of population-specific reference values may improve laboratory interpretation, enhance diagnostic accuracy, and contribute to better clinical management of hematological disorders. Nevertheless, the relatively limited sample size and restriction of the study to a single geographic region highlight the need for larger multicenter investigations involving broader demographic representation across different Libyan regions. Future studies should additionally evaluate the influence of nutritional status, environmental exposure, lifestyle, and genetic background on hematological parameters while applying standardized laboratory methodologies and robust statistical approaches. The establishment of comprehensive national hematological and biochemical reference databases for the Libyan population may substantially improve clinical decision-making and healthcare quality within the national medical system.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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