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Original article

Reference Interval for Calcium among Libyan Healthy Individuals

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ABSTRACT

The goal of this study was to determine the reference intervals (RIs) of calcium levels (Ca) in the blood of Libyan individuals. Blood samples were obtained from 120 healthy males and females through aseptic venipuncture. Informed consent was obtained prior to sample collection. Serum was extracted from the centrifuged samples and analyzed spectrophotometrically to determine calcium concentrations. The non-parametric percentile method was applied to establish the RIs of calcium, results showed the reference period is 7.8- 10.1 mg/dl. The study establishes RIs for serum calcium concentration between 7.8 mg/dl and 10.1 mg/dl in the Libyan population. These findings underscore the importance of developing geographically specific RIs, as RIs derived from manufacturer's kits or other populations may not accurately reflect the unique characteristics of the Libyan population. The RIs identified in this study are more relevant and appropriate for the Libyan context.

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INTRODUCTION

Calcium is an important element that is involved in the process of formation of the mineral phase of the bone [1]. Over 99% of the body calcium is combined with phosphate to form crystals of hydroxyapatite in the bones. Calcium in this form offers the mechanical framework that supports skeletal integrity and storage for calcium that can be mobilized back into serum [2]. Calcium, a critical mineral for various bodily functions, circulates in the blood in three forms: protein-bound, ionized, and complexed [3]. Of these, only ionized calcium is biologically active and can be utilized by tissues. The remaining two forms, protein-bound and complexed calcium, serve primarily as storage and transport mechanisms [4]. Maintaining appropriate serum calcium levels is crucial for overall health. Excessive calcium in the blood (hypercalcemia) or insufficient calcium (hypocalcemia) can lead to serious health consequences [5,6]. The endocrine system maintains calcium balance through the interplay of parathyroid hormone (PTH), vitamin D3, and calcitonin [7]. PTH rises when calcium levels drop, stimulating calcium reabsorption in the kidneys and bones and increasing vitamin D3 production to elevate calcium levels [8,9].

Calcitonin is a hormone secreted by the thyroid gland in response to elevated blood calcium levels [10]. It primarily functions to decrease calcium absorption from the intestines, reduce bone resorption by osteoclasts, and increase calcium excretion in the urine [11]. These actions collectively lower serum calcium levels [12]. Calcium is a vital mineral essential for various physiological processes, including bone formation, muscle contraction, and neurotransmission. Approximately 99% of the body's calcium is stored in bones, providing structural support [13]. Muscle contraction is a complex process involving the interaction of calcium with proteins in muscle cells [15]. Calcium influx triggers the release of calcium from intracellular stores, leading to the activation of contractile proteins [2, 15]. Calcitonin plays a crucial role in maintaining calcium homeostasis by opposing the effects of parathyroid hormone, which raises blood

calcium levels [16]. By regulating calcium absorption, bone resorption, and excretion, calcitonin helps prevent hypercalcemia and supports overall calcium balance in the body [17-19].

Both hypercalcemia and hypocalcemia can be life-threatening conditions. Hypercalcemia is diagnosed when serum calcium levels exceed 10.4 mg/dL, but mild cases may not cause symptoms. However, levels above 11.5 mg/dL can lead to a range of symptoms such as nausea, vomiting, altered mental status, headache, dizziness, confusion, abdominal or flank pain, constipation, depression, weakness, muscle or joint pain, excessive urination, thirst, and nighttime urination [20,21]. Hypercalcemiacan lead to severe complications such as coma. Common clinical manifestations include hypertension, bradycardia, hyperreflexia, and tongue fasciculation [22]. Hypercalcemia can be caused by primary hyperparathyroidism, secondary hyperparathyroidism, or other etiologies. PTH-mediated hypercalcemia results from excessive PTH secretion, leading to increased intestinal calcium absorption. Non-PTH-related hypercalcemia can be attributed to malignancies, granulomatous diseases, certain drugs, endocrinopathies, and genetic disorders [2, 22,23]. Acute hypocalcemia, characterized by a serum calcium level below 8 mg/dL, can lead to severe symptoms such as neuromuscular excitability (e.g., tetany, seizures), cardiac dysrhythmias, and respiratory distress [24]. Long-term complications include skin and dental abnormalities. Physical examination often reveals signs of neural hyper-excitability and psychological changes [25]. Chvostek and Trousseau signs are diagnostic for hypocalcemia. Sepsis and certain medications can contribute to hypocalcemia. A thorough medication history is essential for identifying potential causes [26].

Therefore, accurate interpretation of biochemical tests such as Ca involves reference to appropriate RIs derived from a population relevant to the test [27]. These RIs are estimated to be the middle 95%; that is, at ± 2 standard deviations from the mean value. RIs are determined by a rigorous examination of the reference specimens derived from a particular reference population with particular norms [28, 29]. This makes it difficult to get the RIs for a general population and therefore there is need to select a good reference group [30].

Significant disparities in reference intervals (RIs) for biochemical tests, including serum calcium, have been observed among Libyan clinical laboratories. These discrepancies are primarily attributed to the use of RIs derived from other populations, which may not accurately reflect the unique characteristics of the Libyan population. To address this issue and enhance diagnostic accuracy, this study aims to establish Libyan-specific RIs for serum calcium levels. By developing population-specific RIs, we seek to improve the diagnostic confidence of calcium determinations and ensure consistent interpretation of test results across the country. The endorsement of these RIs by the Libyan Ministry of Health would facilitate their integration into the current clinical laboratory reporting model, leading to improved diagnostic accuracy and consistency nationwide.

METHODS

Subjects and specimen's collection

This study was carried out in El- Estishari Medical Laboratory in Tripoli, Libya from June to September 2021 and involved a total of 120 apparently healthy subjects aged between 10 – 50years. The criteria were set as follows: the patients did not have a history of smoking or alcohol and/or drug abuse, and their blood pressure was normal. In order to measure FPG levels, blood collection was done after a 12-hour overnight fasting period, using venipuncture of the right arm of the subjects. The collected blood was transferred to the untreated tubes with red tops (Labchem Sdn Bhd, Damansara Kim, Petaling Jaya, Selangor, Malaysia) and the tubes were filled with blood until at least two thirds full. Following blood coagulation for a period of 30 minutes, the samples were centrifuged with a speed of 1500 rpm for 15 minutes. Following that, the isolated serum was placed in sterile sample tubes for the immediate determination of Ca content.

Ethics Approval

Informed consent was sought from all patients in compliance to the Ethical Standards of the El Estishari Medical Laboratory Biomedical Ethics Committee.

Instrumentation and analysis

Serum Ca level of the subjects was estimated using spectrophotometry method. Based on this principle, calcium in blood is determined by using the arsenazo III method, which forms a blue-purple complex with calcium, Clotted blood samples were centrifuged at 1500 g for 15 minutes to separate the serum. This method involves the formation of a colored complex between Arsenazo III, a triphenylmethane dye, and calcium ions. The absorbance of the colored complex is using a spectrophotometer at a 520 wavelength. The absorbance is directly proportional to the concentration of calcium ions in the sample.

Statistical analysis

Statistical analyses were performed using a combination of software programs. These included Minitab 17 (Minitab Inc., State College, PA, USA), Microsoft Excel 2013 (Microsoft Corp., Seattle, WA, USA), and MedCal Software (MedCalc Software, Mariakerke, Belgium).

RESULTS AND DISCUSSION

The present study investigated Ca concentration in healthy individuals employed a sample of 120 males and females ranging in age from 10 to 50 years old descriptive statistics for age (mean, standard deviation) and Ca concentration (minimum, maximum) are presented in Table (1). The table shows that the highest age in the dataset, which was 50 years old. And the lowest age, was 10 years old in this data set, and the average age of participants was 33.2 years old.

Table 1. Descriptive Statistics of Age

StDev	**Mean**	**Max**	**Min**
9.624	33.2	50	10

** CV% is coefficient of variation*

Descriptive Statistics of Calcium Concentration

Table 2 shows the lowest value, the highest value, the average age, the standard deviation and the coefficient of variation for the concentration of Ca. The table shows the rather high value of the standard deviation and the coefficient of variation (CV%), which indicates the extent of dispersion of the Ca concentration data. This table shows the highest Ca concentration is value of 10.8 mg/dl and the lowest Ca concentration, is 7.5 mg/dl.

In accordance with the present study Kratz & Lewandrowski., [31] found that Ca concentration was about 8.8-10.4 mg\dl in in healthy individuals. Another Previous research on serum Ca levels in aligns with the present study; also, Evan et al., [32] evaluated serum Ca in normal population and indicated the normal level of about 9.08 mg\dl.

Table 2. Descriptive Statistics of Calcium Concentration

CV%	**StDev**	**Mean**	**Max**	**Min**
6.7	0.6	8.94	10.8	7.5

** CV% is coefficient of variation*

Outliers in Calcium Ion Concentration Data

Since the outliers affect the final result when calculating the reference period, a test was conducted to determine the outliers known as Dixon's r22 ratio at the significance condition $\alpha = 0.05$, and the P value of the test was 0.117, which is greater than the significance condition, and then it was concluded that there is no outliers, and all calcium ion concentration values were used to calculate the reference period, and the following figure is attached in it for further clarification (Figure 1).

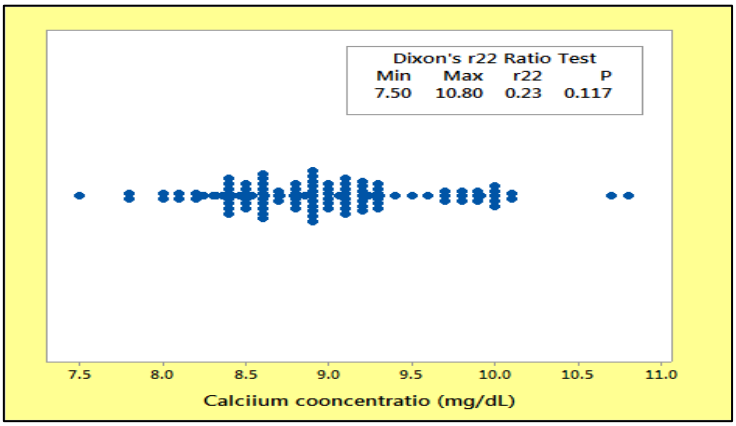

Figure 1. Outliers in calcium ion concentration

Normality test

Because the method of determining the reference period varies according to the nature of the data, the Kolmogorov-Smirnov test was conducted to determine whether the data follows the normal distribution or not, and it was found that it does not follow the normal distribution because the P value of 0.028 was less than the significance condition $\alpha = 0.05$, and the graph shows that the data is not wrapped around the straight line, which shows that it does not follow the normal distribution (Figure 2).

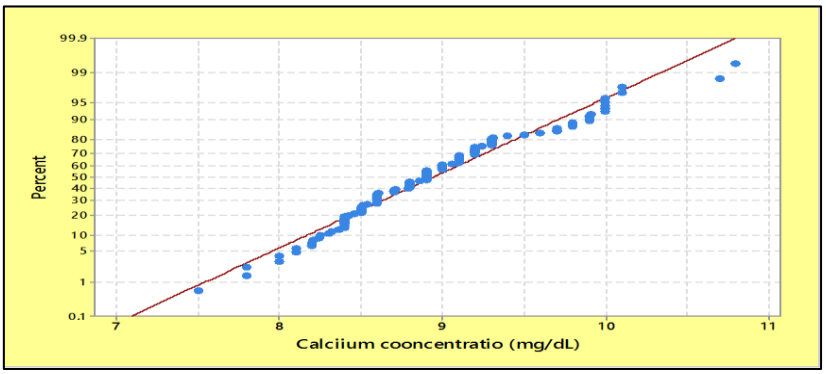

Figure 2. The probability plot of calcium concentration

Establishing the reference period for calcium ion concentration

Because the data do not follow the normal distribution, the non-parametric method (nonparametric) was applied to calculate the reference period; where the values of the concentration of calcium ion were arranged ascendingly, and according to the order in the values representing the minimum of the reference period through the relationship 0.025 X (n + 1) where the number of values and the order of the value is 3 and the corresponding concentration is 7.8 mg/dL, and the order in the values representing the upper limit of the reference period only through the relationship 0.975 X (n + 1) where the number of values and the order of the value is 118 and the corresponding concentration is 10.1 mg/dL, and then the reference period is 7.8 – 10.1 mg / dL.

Given the findings of this study, the importance of establishing RIs for laboratory tests, including calcium, within a clinical context and for research purposes is underscored. As there is a dearth of Libyan-specific RIs, healthcare professionals often rely on data derived from Western populations, which may exhibit slight variations due to differences in physiological baselines. This study highlights the need for developing local RIs in Libya, particularly for calcium, to enhance the accuracy of healthcare services and research endeavors. By providing a preliminary set of calcium RIs from a Libyan population-based study, this research aims to address this critical gap in the current knowledge base.

The RI for serum calcium levels in healthy individuals vary based on age, sex, and specific population studies. Understanding these intervals is crucial for accurate clinical assessments. The RI values of serum calcium in the present study is (7.8-10.1) mg\dl, this aligns with a recent comprehensive analysis of 502,524 adults from the UK Biobank revealed that the reference interval for serum calcium is (8.76 to 10.24 mg/dl) [33], existing reference intervals for calcium vary depending on age and sex, with older women exhibiting higher upper limits compared to younger women and men. The UK Pathology Harmony initiative employed a combined pragmatic and scientific approach to address these inconsistencies, resulting in recommended reference intervals of 2.2–2.6 mmol/L. Another previous study of Mundy [34] confirmed that Normal RI of calcium levels in healthy individuals typically range from 8.5 to 10.5 mg/dL, reflecting the intricate balance of calcium homeostasis involving the gut, kidney, and bone. Several studies stated that normal RI calcium in healthy individuals typically range from 8.5 to 10.2 mg/dl. This range is crucial for various physiological functions, including bone health and muscle contraction. The regulation of calcium levels is a complex interplay involving dietary intake, hormonal control, and organ function.

CONCLUSION

The present study demonstrates that serum calcium levels in the Libyan population exhibit a non-normal distribution, necessitating a non-parametric approach to determine. The established RIs for calcium concentration, ranging from 7.8 mg/dl to 10.1 mg/dl, are more accurately representative of the Libyan population than those derived from manufacturer's kits or external sources. These findings highlight the importance of developing population-specific RIs to ensure accurate laboratory diagnostics. The Libyan Ministry of Health should consider adopting these RIs as a standard for laboratory testing. Future studies should investigate the potential influence of age and gender on calcium RIs and

establish RIs for other biochemical markers in urine, blood, and other body fluids to further enhance the accuracy of laboratory diagnostics in Libya.

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Conflicts of Interest

This study was conducted without sponsorship from any public, commercial or non-profit granting agency.

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الفترة المرجعيه للكالسيوم لدى الأفراد الليبيين الأصحاء

فاطمة المجدوب

كلية العلوم والتقنيات الطبية، طرابلس، ليبيا

المستخلص

تهدف هذه الدراســـة إلى تحديد الفئات المرجعية لمســـتويات الكالســـيوم في الدم لدى الأفراد الليبيين، حيث لم يتم إجراء دراسات سابقة حول هذا الموضوع في ليبيا. تم الحصول على عينات دم من 120 فردًا ليبيًا، تم استخلاص المصل وتحليله طيفياً لتحديد تركيز الكالسيوم. تم تطبيق طريقة النسبة المئوية غير البارامترية لتحديد الفئات المرجعية للكالسيوم، وكانت النتائج بين 7.8-10.1 ملغ/ديسيلتر. توضح الدراسة الفئات المرجعية لتركيز الكالسيوم في المصل بين 7.8 ملغ/ديسيلتر و 10.1 ملغ/ديســيلتر لدى الســكان الليبيين. تشــدد هذه النتائج على أهمية تطوير فئات مرجعية محددة جغرافياً، حيث قد لا تعكس الفئات المرجعية المستمدة من مجموعات البيانات الأخرى الخصـائص الفريدة للسكان الليبيين. وبالتالي، فإن الفئات المرجعية المحددة في هذه الدراسة أكثر ملاءمة للسياق الليبي.

الكلمات المفتاحية. الكالسيوم، الفترة المرجعية، الأفراد الأصحاء، ليبيا.