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Original article

Reference Interval for Uric Acid Among Libyan Healthy Individuals

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Abstract
The current study seeks to evaluate the possible risks that are related to the use of reference intervals (RIs) obtained from other populations in clinical chemistry analysis, with special reference to the Libyan population. Concerns stem from the significant disparities between the Libyan population and the reference populations, mainly concerning the lifestyle and genetic heritage. These inherent differences leave doubt on the generalizability of the above existing RIs to the Libyan population. The objective of this study is to establish the RIs of serum uric acid (UA) in the Libyan population; there has been no such study done before in Libya. In the present study, blood samples were freshly collected by venipuncture from 139 healthy females after explaining the nature of the study to them; the blood samples were collected aseptically into untreated tubes. After the centrifugation process for 15 minutes, the obtained serum specimens were analyzed through the spectrophotometric assay to determine the UA concentrations. The non-parametric percentile method was applied to establish the RIs of uric acid. The results showed that the number of values and the order of the value were 134.55 and the corresponding concentration is 7.4 mg/dl, and then the reference period is 2.7-7.4 mg/dl. The reference range of UA concentration as identified by this study is between 2.7 mg/dl and 7.4 mg/dl. These observed differences in RIs have implications for advocating the need to develop the RIs for the populations of different geographical locations. The RIs generated in this context are more relevant and fitting in the case of the Libyan population as compared to those mentioned in the manufacturing kit or those that can be sourced from other populations.
Keywords. Uric acid, Reference Intervals, Dichlorohydroxybenzene sulfonate, National Health and Nutrition Examination Survey.

Introduction

Humans utilize various strategies to dispose of nitrogenous waste, a key function in their body's chemistry. Uric acid stands out as a significant excretory product for most organisms, while assessing blood levels of creatinine, urea, and ammonia remains a common practice, uric acid, along with urea, also plays a crucial role in eliminating nitrogenous waste in specific animals [1]. UA, a heterocyclic compound with the chemical formula C5H4N4O3 (systematic name: 7,9-dihydro-3H-purine-2,6,8-trione), exhibits a widespread distribution across diverse organisms [2]. In mammalian systems, UA is the end product of purine metabolism; it has been reported in blood serum and urine. Purine metabolism in primates' final pathway takes place, producing UA from the breakdown of nucleic acids and proteins, then UA is eliminated from the body through the urinary system [3]. Similarly, healthy humans have the highest serum UA levels among all mammals, which also testifies to a unique feature in human purine metabolism as compared to other mammals [4].

Hypoxanthine and xanthine oxidase are involved in the metabolism of UA, which is homeostatically important in human beings. But there are numerous factors capable of disturbing this stability, and as a result, UA levels may exceed or fall short of the desired range [1]. Such factors include the ones derived from extra-intestinal origin, namely diet, and those arising from cellular metabolism. Interestingly, UA excretion has a broad significance that directly contributes to the preservation of this balance [5]. UA has a strong antioxidant activity as a result of urate oxidase loss in the course of evolution, higher concentration is observed in humans and some animals, as well as its possible longer life span [6]. It is involved in reducing peroxynitrite and free radicals and has an effect against oxidative stress and DNA damage, and this is about the vulnerability of neural cells to oxidative stress [7].

However, UA is known to have a two-sided effect; it acts as a natural antioxidant, but it can also have a pro-oxidant effect under some circumstances. In the lipid-rich environment, such as plasma, UA can produce reactive oxygen species and therefore contribute to the situation of oxidative stress [8]. Serum UA levels thus represent an ingeometrical balance between dietary purine intake, xanthine oxidase activity, and renal UA excretion [1]. The healthy normal range for UA concentration falls within 3.5-7.2 mg/dl for males and between 2.6-6.0 mg/dl for females. Disturbances in this equilibrium can cause an elevated serum urate concentration (hyperuricemia) or a low serum urate concentration (hypouricemia). Hyperuricemia is a condition characterized by a serum UA concentration that is above the reference concentration of 7mg/dl, and for women, below 7 mg/dl. On the other hand, hypouricemia refers to a serum UA concentration level, which is below or equal to 2 [7]. Hyperuricaemia is associated with multiple pathological conditions with cardiovascular diseases being the most pronounced. Some effects include gout, which is a type of arthritis whereby patients develop urate crystals in their joints and also eclampsia that can be fatal for pregnant women. Furthermore, studies indicate that UA is related to cardiovascular diseases with elements as acute

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coronary syndromes, strokes, heart failure, and atrial fibrillation. These have brought out the fact of the contribution of UA in cardiovascular problems and mortality [9].
Subsequently, proper assessment of biochemical tests, including UA requires reference to valid reference intervals (RIs) originating from a population about the test [10]. These RIs are calculated as the middle 95% of values, which means ± 2 standard deviations from the mean value. RIs are identified through an intense evaluation of the specimens originating from a selected reference population with specific standards [11,12]. It is not easy to get the RIs for a general population, and thus there is a need to select a good reference group [13]
A substantial variation in RIs for biochemical tests utilized by Libyan clinical laboratories was established. Such discrepancies stem from the use of RIs that have been developed in other populations and may not be very relevant to the Libyan population as a result of such factors as ethnic background, lifestyle, and diet. The observed inconsistency may result in misinterpretation of test outcomes, possibly including erroneous diagnosis. This study aims to define Libyan-specific reference intervals (RIs) for serum UA concentrations. The primary goal is to improve diagnostic accuracy by establishing population-specific RIs for UA. Recognition by the Libyan Ministry of Health would facilitate the implementation of these RIs into clinical laboratory reporting systems, thereby standardizing and enhancing diagnostic precision. To achieve this objective, the study enrolled a group of unrelated healthy Libyan individuals. Peripheral blood samples were collected, and serum UA levels were measured. The data will be subjected to statistical analysis to generate RIs for UA that are specific to the Libyan population.

Methods

Subjects and specimens' collection

This study was conducted at El- Estishari Medical Laboratory in Tripoli, Libya, from June to September 2021, and recruited a total of 139 healthy individuals ranging in age from 14 to 52 years.
The inclusion criteria established were normal blood pressure and no history of smoking, alcohol, or drug abuse. Following a 12-hour overnight fast, venous blood samples were carefully obtained via venipuncture from the right arm of each participant. The collected blood was transferred into untreated (red-topped) tubes (Labchem Sdn Bhd, Damansara Kim, Petaling Jaya, Selangor, Malaysia) and filled to at least two-thirds of their capacity. After a 30-minute resting period to facilitate clot formation, the samples underwent centrifugation for 15 minutes at 1500 rpm. Subsequently, the isolated serum was transferred to clean specimen tubes for immediate analysis of uric acid (UA) content.

Ethics Approval

After securing approval from the El Estishari Medical Laboratory's Biomedical Ethics Committee, informed consent was procured from all participants before blood sample collection.

Instrumentation and analysis

The serum uric acid level of the subjects was estimated using the spectrophotometry method. Based on this principle, the method adapts the enzymatic reactions to create a uric acid product that can be assessed by its ability to absorb light at a certain wavelength. This means that the amount of light absorbed (absorbance) is proportional to the initial concentration of uric acid. Enzymes used in the determination method include Uricase: This enzyme works by hydrolyzing uric acid into allantoin and hydrogen peroxide. Peroxidase: This enzyme catalyzes the oxidation of a colourless indicator using hydrogen peroxide. Other components used in the determination include Potassium hexacyanoferrate(II): This compound is in the assay to act as an expectation so that variations in the amount turning violet can be measured. The method also utilizes buffers such as Tris buffer (pH 8): This buffer ensures that enzymatic reactions proceed at this pH,
Dichlorohydroxybenzene sulfonate (DCHS): This compound could be an example of the substrate that peroxidase works on to produce a colored compound when in the presence of hydrogen peroxide and Aminoantipyrine: This is a colourless indicator that when it reacts with hydrogen peroxide in the presence of peroxidase and DCHS it forms colored product measured at 505nm.

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 MedCalc Software (MedCalc Software, Mariakerke, Belgium).

Results and Discussion

The present study investigated UA concentration in healthy females. A sample of 139 females ranging in age from 14 to 52 years old. Descriptive statistics for age (mean, standard deviation) and uric acid concentration (minimum, maximum) are presented in Table 1. The table shows that the highest age in the dataset is 52 years old. And the lowest age was 14 years old in this data set, and the average age of participants was 32.6 years old.

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Table 1. Descriptive Statistics of Age

StDev	Mean	Max	Min
9.624	32.683	52	14
StDev	Mean	Max	Min

Descriptive Statistics of Uric Acid 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 uric acid. 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 uric acid concentration data. This table shows that the highest uric acid concentration is value of 6,900 mg/dl and the lowest uric acid concentration is 2.300 mg/dl. In accordance with the present study, Sairama et al. found that uric acid concentration was about 2.6–6.3 mg/dl in women aged less than 40 years old [14]. Another Previous research on serum uric acid levels in Japanese populations aligns with the present study, Tani et al. observed a significant difference in uric acid levels between genders, with females exhibiting a mean value of approximately 4.5 mg/dl (standard deviation ±1.0 mg/dl) and males at 6.2 mg/dl (±1.2 mg/dl) [15]. These findings are further corroborated by another study, which reported wider reference ranges for uric acid: 2.6-8.2 mg/dl overall, with gender-specific ranges of 2.5-6.9 mg/dl for females [16]. Similar sex-based disparities were documented by Gardner and Scott in their 1980 study [17].

Table 2: Descriptive Statistics of Uric acid Concentration Data

CV%	StDev	Mean	Max	Min
28.59	1.338	4.678	6,900	2.300

Outliers in uric acid 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 1.000, which is greater than the significance condition, and then it was concluded that there is no outlier, and all the values of uric acid concentration were used to calculate the reference period, and the following figure is attached in it for further clarification.

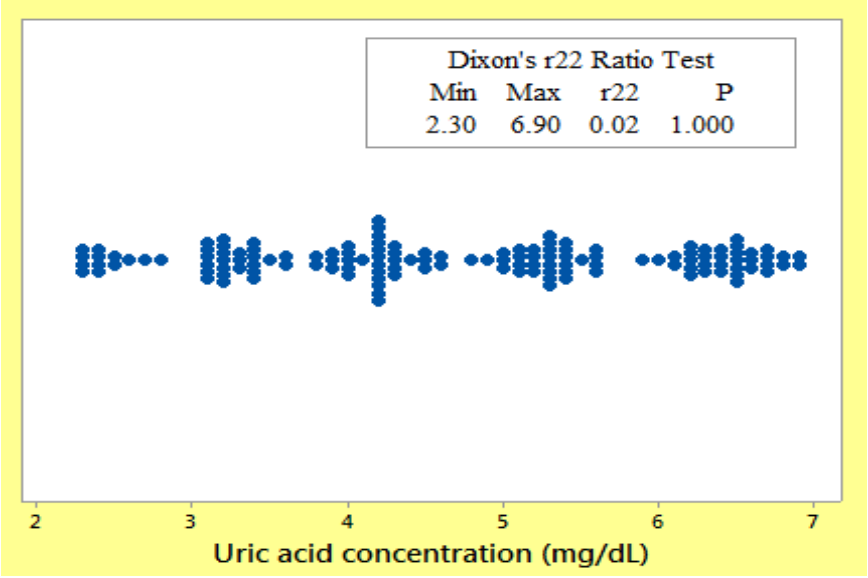

Figure 1. Outliers test results of serum uric acid.

Normality test

The Kolmogorov-Smirnov test was employed to assess the normality of the data. The test results indicated a statistically significant departure from normality (p-value = 0.01 < α = 0.05), suggesting that the data is not normally distributed, and Figure 2 shows that the data is not wrapped around the straight line, which shows that it does not follow the normal distribution.

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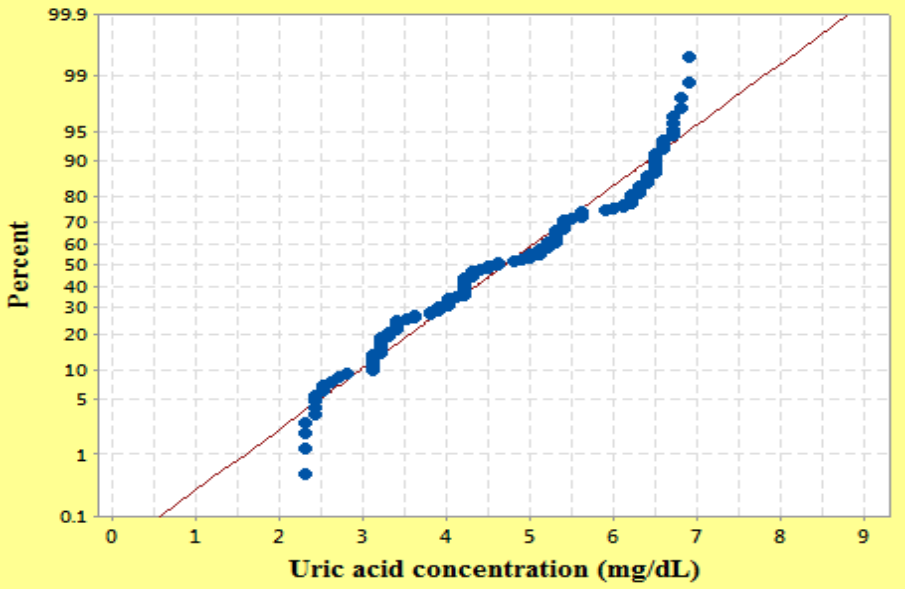

Figure 2. The probability plot of uric acid concentration.

Establishing the reference intervals for uric acid

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 uric acid 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 was 3.45 and the corresponding concentration is 2.7 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 was 134.55 and the corresponding concentration is 7.4 mg/dl, and then the reference period is 2.7-7.4 mg/dl.

Therefore, based on the above outcomes of the present study, it stresses the significance of RIs in using laboratory results in a clinical context and other investigations. Since there is limited data on Libyan RIs, healthcare practitioners use data obtained from Western countries, which might be slightly different due to variations in the physiological baseline. Calculations have revealed differences in the values of the physiological parameters between the populations. Hence, it is necessary to create local reference intervals in Libya, especially for uric acid, as it will enhance healthcare services and the research mission of the country. Consequently, this research is intended to fill this void by providing a preliminary set of uric acid RIs from a Libyan population-based study.

The RI values of serum UA for females (2.7-7.4 mg/dl) in this study are quite similar to the study conducted by Das *et al.* (2.5-6.9 mg/dl for females). They also reported a wide range of 2.6 mg/dl- 8.2 mg/dl RI of uric acid in normal adults of both genders [16]. There is limited literature data on RIs for serum UA, and what is known shows significant geographical variation. For example, RIs in the study on a healthy elderly Chinese population were observed to be 1.9-7.98 mg/dl for the healthy women. This would imply that there might be a lower basal incidence in this precise group compared to other groups [21]. On the other hand, the mean serum UA concentrations seem to be higher in Western countries than in other regions of the world. A cross-sectional survey conducted among Italian youths found that only 17.6% of healthy controls had UA levels below 6 mg/dl [22]. The findings of the National Health and Nutrition Examination Survey (NHANES) in the United States pointed out a national mean of about 4.89 mg/dl for women [23]. There is scarce information on Romania RIs for UA. A particular study screening more than 1,900 healthy individuals found that the mean serum UA level was 4.93 mg/dl (standard deviation 1.42 mg/dl). With (2.40- 5.70 mg/dl for women) for their analysis. This raises questions about potential discrepancies between population-derived RIs and laboratory-defined reference ranges [24].

Conclusion

Results of the present study revealed that serum UA levels did not follow a symmetric normal distribution. This is why a non-parametric method was applied to determine the reference range. The reference range of the uric acid concentration in this study, therefore, falls between 2.7 mg/dl and 7.4 mg/dl. The observed variations in RIs across the different regions show why there is a need to develop population-specific RIs. The RIs identified in this study are more relevant and appropriate to the current Libyan population than the RIs either obtained directly from the manufacturer's kit or derived from external sources. These findings may be useful for the Libyan Ministry of Health to support the Libyan population-specific RIs to standardize laboratory diagnostics. More studies should be conducted to establish the possible correlation between age and gender in determining UA RIs. Further, the other predeterminant biochemical markers RIs need to be set for the Libyan populations in urine, blood, and other fluids.

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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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الملخص

تسعى الدراسة الحالية إلى تقييم المخاطر المحتملة المتعلقة باستخدام الفواصل المرجعية المأخوذة من مجموعات سكانية أخرى في تحليل الكيمياء السريرية، مع التركيز بشكل خاص على السكان الليبيين. تنبع المخاوف من التباينات الكبيرة بين السكان الليبيين والمجموعات السكانية المرجعية، والتي تتعلق بشكل رئيسي- بنمط الحياة والوراثة الوراثية. هذه الاختلافات الجوهرية تترك مجالًا للشك في إمكانية تعميم الفواصل المرجعية المذكورة أعلاه على السكان الليبيين. تهدف هذه الدراسة إلى تحديد الفواصل المرجعية لحمض اليوريك في المصل في السكان الليبيين؛ حيث لم تُجرَ أي دراسة من هذا القبيل في ليبيا من قبل. في هذه الدراسة، جُمعت عينات دم حديثة عن طريق بزل الوريد من 139 أنثى سليمة بعد شرح طبيعة الدراسة لهن؛ جُمعت عينات الدم بطريقة معقمة في أنابيب غير معالجة. بعد عملية الطرد المركزي لمدة 15 دقيقة، حُللت عينات المصل التي تم الحصول عليها باستخدام التحليل الطيفي الضوئي لتحديد تركيزات حمض اليوريك. طُبقت طريقة النسبة المئوية غير المعلمية لتحديد الفواصل المرجعية لحمض اليوريك. أظهرت النتائج أن عدد القيم وترتيبها كان 134.55، وأن التركيز المقابل هو 7.4 ملغ/ديسيلتر، وأن الفترة المرجعية تتراوح بين 2.7 و7.4 ملغ/ديسيلتر. يتراوح النطاق المرجعي لتركيز حمض اليوريك، كما حددته هذه الدراسة، بين 2.7 و7.4 ملغ/ديسيلتر. لهذه الاختلافات الملحوظة في مؤشرات الاستجابة المرجعية آثارٌ على الدعوة إلى تطوير مؤشرات استجابة مرجعية لسكان مختلف المناطق الجغرافية. وتُعدّ مؤشرات الاستجابة المرجعية المُولّدة في هذا السياق أكثر ملاءمةً وأهميةً لحالة السكان الليبيين مقارنةً بتلك المذكورة في مجموعة التصنيع أو تلك التي يمكن الحصول عليها من سكان آخرين.

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