■ *Short Communication*

ESTABLISHMENT OF REFERENCE INTERVALS FOR BLOOD UREA LEVELS IN YOUNG PROSPECTIVE MEDICAL STUDENTS UNDERGOING HEALTH CHECKUP AT BPKIHS, DHARAN, NEPAL

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Abstract

Background: Reference values or reference intervals are set of values of a certain type of quantity obtainable from a single individual or a group of individuals corresponding specific description. **Objective:** This study highlights the approach for determining the reference intervals for blood urea in a healthy population and establishes its upper and lower reference limits. **Subjects and methods:** A descriptive study was carried out in the Department of Biochemistry B. P. Koirala Institute of Health Sciences from June 2009 to August 2009. International federation of Clinical Chemistry and Laboratory Medicine (IFCC) priori sampling technique was used. Blood urea was estimated by diacetyl monoxime (DAM) and Glutamate Dehydrogenase (GLDH) kinetic methods. Reference intervals were defined as mean ± 1.96 SD. Mean and standard deviation for blood urea values were expressed as descriptive statistics. **Results:** The study included 60 individuals (36) 63% males and (24) 37 % females . Blood urea values by GLDH kinetic and DAM methods were 22.07 ± 5.6 mg/dl and 27.1 ±8.79 mg/dl respectively. Reference values of blood urea by GLDH kinetic and DAM methods were 16-28 mg/dl and 18-36 mg/dl respectively. **Conclusions:** This study highlights the establishment of reference intervals of blood urea levels from a healthy population. The reference intervals would enable the laboratory personnel and the clinicians to interpret the medical data.

Keywords: blood urea, reference intervals, reference values.

Introduction

Reference values are a set of values of a certain type of quantity obtainable from a single individual or a group of individuals corresponding to a specific description. Homogenous reference groups for variables such as age, sex, diet and ethnic background, make the relative reference values more effective for a population. Therefore, each analyte may require several reference intervals, even for persons in good health.[1] The term "normal values" is less frequently used and is considered obsolete for referring medical data used for the purpose of comparison.[2]

The establishment of suitable reference population is essential but difficult and there are still cases in which unrepresentative reference populations - medical students, hospital employees, blood donors or other volunteers, variably classified as healthy have been used.[3] Accurate definition of reference intervals is particularly important in laboratories, such as diagnostic centres, expecting to encounter a sizeable number of healthy individuals among the presenting patient population or a small deviation from normality of test results.[2,3]

On comparing the individuals data collected during the medical interview, clinical examination and supplementary investigation with the reference data,

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the conditions of individual can be interpreted. A patient's laboratory result simply is not medically useful, if appropriate reference values for comparison are not present. It is thus, the central role of the laboratory personnel to aid the clinician in interpreting the observed values by providing relevant reference values and present them in a convenient and practical form.[4]

So far, there are limited sources of established population based reference values of blood urea and other biochemical parameters in Nepalese population. The upper and lower reference limit of measurement varies depending on the source of information as well as the methodology followed. It is important to realize that there is a requirement for restructuring the reference intervals of blood urea for the Nepalese population by different methods of urea estimation. The primary objective of this study is to assist in interpreting medical data through evaluation of reference intervals for an analyte such as urea.

Subjects and methods

A descriptive pilot study was conducted in the Department of Biochemistry, B. P. Koirala Institute of Health Sciences, from June 2009 to August 2009. The sampling technique used was priori direct sampling as recommended by International federation of Clinical Chemistry and Laboratory Medicine (IFCC). Seventy-five individuals were selected from the population attending health check up during admission in the medical college. Blood specimen was drawn from the individuals in plain vials and serum was separated by centrifugation at 3000xg for 10 minutes. All samples, those were lipemic, haemolysed or icteric were excluded. The sample size was 60 after exclusion. Blood urea was measured by diacetyl monoxime (DAM) method and Glutamate Dehydrogenase (GLDH) kinetic Urease UV method. Non-enzymatic DAM method for blood urea was performed by colorimetric, the WHO manual for clinical chemistry recommended method.[4,5] The intra assay coefficients of variations (CVS) of both methods* were 7% and 5% respectively.
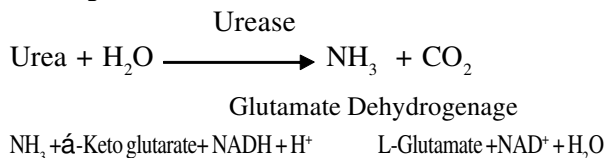
Principle

Urea reacts directly with diacetyl monoxime under strong acidic conditions to give a yellow condensation product. The reaction is intensified by the presence of ferric ions and thiosemicarbazide. The intense red colour formed is measured at 540nm or using yellow green filter.

GLDH kinetic Urease UV method[6] was performed by Vitalab Selectra E, Autoanalyzer.

Principle:

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} NH_3 + CO_2$$

$$NH_3 + \acute{a}\text{-Keto glutarate} + NADH + H^+ \xrightarrow{\text{Glutamate Dehydrogenage}} \text{L-Glutamate} + NAD^+ + H_2O$$

The rate of decrease in absorbance at 340nm due to the oxidation of NADH to NAD^+ was proportional to urea concentration in the sample.

Data were expressed as mean and standard deviation. The reference interval for the blood urea was calculated by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) referred method.[5] Upper and lower reference limits were defined as mean ± 1.96 Standard Deviation.

Results

Sixty subject of age range 18-25 year old were enrolled in the study. The average age a female here 21.5and 19 respective. The sex wise distribution of subjects is depicted on table 1, among which 63% were male (n=38) and 37% were female (n=22). Blood urea levels in male and female by the two methods GLDH and DAM are shown in Table: 1. The blood urea values by GLDH and DAM methods were 22.07 ± 5.6 mg/dl and 27.1 ± 8.79 mg/dl respectively. In GLDH method, blood urea levels in male and female were 22.00 ± 5.78 mg/dl and 22.18 ± 5.33 mg/dl respectively. In DAM method, blood urea in male and female were 25.5 ± 9.13 mg/dl and 29.867 ± 7.48 mg/dl respectively. The difference in both the methods was not statistically significant. Table: 2 shows the reference values of blood urea by the two different methods. The reference range of blood urea was 16-28 mg/dl by GLDH method and 18-36 mg/dl by DAM method.

Table 1: Blood urea levels by GLDH and DAM methods

Gender	GLDH method (mg/dl)	DAM method (mg/dl)
Male(n=38)	22.00 ± 5.78	25.5 ± 9.13
Female(n=22)	22.18 ± 5.33	29.867 ± 7.48
Total	22.07 ± 5.6	27.1 ± 8.79

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Table 2: Reference intervals for urea by GLDH and DAM methods

Methods	Reference Intervals (mg/dl)	Minimum (mg/dl)	Maximum (mg/dl)
GLDH	16-28	14	30
DAM	18-36	15	41

Discussions

The medical interpretation of clinical laboratory data is a comparative decision making process in which a laboratory test result for an individual is compared with a reference interval derived from a reference population. Therefore, reliable reference values are required for all tests in the clinical laboratory and must be provided by clinical laboratories and diagnostic test manufacturers.[2] The reference interval or the reference values for a specified test for an analyte of a population is rarely found in Nepal and very few databases are found in South-Asia. It is a compulsion for us to interpret the results in terms of manufacturer reference interval, or consult a textbook for the reference value, which is generally based on the studies conducted on Western population. So, the reference intervals in our part of the globe is poorly determined.[7, 8]

The establishment of reference ranges possess many problems, particularly in the choice of subjects and it is often difficult to examine large number of people and to standardize the conditions under which specimen has been collected.[3] This study was performed with the priori sampling technique which is a direct method. Individuals were selected for specimen collection if they fulfilled the inclusion criteria. The priori sampling technique is best suited for a small sample size. In this study, blood urea levels were not significantly different in male and female by both the methods, which might be due to similar age group, dietary habits, geographic locations, ethnic backgrounds, etc. Both of the methods showed quite similar values for the blood urea levels, which adds to the accuracy and precision of these tests. Moreover, there was no statistically significant difference in the blood urea values by these two methods.

Also, reference value obtained by the GLDH method is lower than that of DAM method, which creates a dilemma towards which method to choose. This might be due to the intra- personnel variability while performing the DAM method, which is a manual

one. GLDH method can be called more accurate because it is based on the autoanalyzer which reduces personnel work load and also gives reliable result. Regarding previous studies of reference intervals of blood urea, Gallo et al in (1985)[1] found reference range for urea 17- 41 mg/ dl, Grossi et al (2005)[3] found 13-50 mg/dl in males and 19-38 mg/dl in females. Ashavaid et al, (2005)[8] found reference intervals of blood urea 29-31 mg/dl in males, 28-30 mg/dl in females.

Limitations

This was a pilot study of with short time period a small sample size less than 120. The IFCC recommends more than 120 samples to be included for calculation of a reference interval of an analyte in a population.[6] More parameters could be estimated rather than only urea for the same populations wo need to mans.

Conclusion

Establishment of reference intervals for urea and other biochemical parameters is a dire need for our part of the sub-continent. Large cohort studies have to be performed with appropriate sampling techniques for the reference intervals of all biochemical parameters. This study highlights the possibility of formulation of internal reference values of blood urea in the healthy subjects on our population. More study is needed in this part to fulfil the gaps between clinician's response, laboratory personnel's guidance and the patient satisfaction through proper use of medical data while interpreting the laboratory results.

Acknowledgement

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