Establishment of Reference Intervals for Biochemical Parameters in Elderly Population in a Coastal Village of South Kerala

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ABSTRACT

BACKGROUND
Reference intervals (RI) are one of the most common decision support tool used for interpretation of laboratory reports. Quality of the RI plays as an important role in interpretation of result and clinical decision making. RI vary with metabolic and physiological changes with advancing age. Reference intervals of Indian elderly population are not available. This study was done to see if there is any difference in the reference intervals of biochemical parameters among elderly compared to the reported value.

METHODS
This community based study was conducted by the Department of Biochemistry in a tertiary care teaching hospital of South Kerala. 134 apparently healthy persons above 60 years of age, using defined criteria, were recruited for the study. All the biochemical parameters of the subjects were analysed on automated analysers using standard IFCC methods using AU680 from Beckman Coulter.

RESULTS
The reference limits are defined as the central 95% of the population comprised between quantiles 2.5 and 97.5, leaving aside 2.5% of the individuals on both sides of the distribution and reference limits were estimated following standard methods of the International Federation of Clinical Chemistry (IFCC). The lower reference limits were estimated as the 2.5th percentile and the upper limits as the 97.5th percentile of the distribution of test results.

CONCLUSIONS
Establishment of age specific reference value should be done as a protocol before assuring quality control in the lab.

KEYWORDS
Reference Intervals, Biochemical Parameters, Elderly Population

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BACKGROUND

Clinical laboratory testing is an integral part of healthcare whether in the diagnosis, treatment or prognosis and plays a pivotal role in the delivery of quality and effective health care. Reference intervals (RI) are one of the most common decision support tool used for interpretation of laboratory reports. Thus, the quality of the RI plays as an important role in interpretation of result and clinical decision making.[1] Reference intervals serve as a means for physicians to compare results of patients to the expected values in their respective clinical settings. Several factors may alter the biochemical parameters in different populations including genetics, dietary patterns, gender, age, level of physical activity, urban or rural location and geographical area. interval can vary based on the instrument or methodology being used. Hence it is essential for individual laboratories to establish their own reference ranges specific for their population to whom they provide service. According to the IFCC it is necessary for every laboratory to have their own set of reference limits.[2-5]

Reference interval of Indian elderly population are not available and the values used are of young healthy adults which are either taken from the textbooks and articles or insert literature from the Kit manufactures based on relatively small sample of healthy young adults RI vary with metabolic and physiological changes with advancing age. This study was done to see if there is any difference in the reference Intervals of Biochemical parameters among elderly compared to the reported value.

We wanted to establish and compare reference interval for routine biochemical parameters among the elderly males and females.

METHODS

This community based study was conducted by the Department of Biochemistry in a tertiary care teaching hospital of South Kerala. The sample was drawn from the rural area around institution. 134 apparently healthy persons above 60 years using defined criteria, were recruited for the study by conducting a medical camp in the selected wards of after obtaining necessary permissions from the concerned authorities. The information and instructions were spread in the selected wards by distributing the notices through ASHA workers of the respective wards. The subjected were recruited to the study after obtaining informed consent. Those suffering from diabetes, hypertension, coronary artery disease and persons on any pharmacologically active agents as in drug treatment for disease or suffering, oral contraceptives, drug abuse, alcohol, tobacco etc., were excluded from the study.

Sample Collection

Blood collection Camp was conducted from 7.30 to 9.30 am. Demographic data, detailed history regarding past and present disease if any, dietary habits, physical activity, smoking or alcoholic habits, anthropometric parameters of each subject were obtained using a performa. Physical examination was done by trained junior doctors. General and system examinations were done and vitals were recorded. Under aseptic conditions 2 ml of venous fasting blood sample was collected from antecubital vein in plain vacuum tube containing clot activator and 1 ml in fluoride vacutainer for fasting blood glucose estimation. All the pre-analytical factors were standardized, and the study parameters were analysed using separated serum.

Method of Analysis

All the biochemical parameters of the subjects were analysed on AU680 from Beckman Coulter using standard IFCC methods using. All pre-analytical, analytical and post analytical precautions were taken into consideration for ensuring proper quality. All Standard Operating Procedures (SOPs) were followed for sample collection, processing, storage and handling. Internal quality control (QC) was done for each parameter by using lyophilized Quality Control levels from BIORAD. Prior to analysis, the instruments were calibrated using calibrators and the controls were run at normal and pathological concentration of the analyte.

Measurement of analytes were done as follows .Blood glucose based on hexokinase enzymatic method.[6] Urea/BUN by urease reaction coupled to decrease in NADH by L-glutamate dehydrogenase.[7] Creatinine by Jaffe reaction[8] uric acid by reaction of uricase and peroxidase reaction.[9] Cholesterol by cholesterol esterase and peroxidise.[10] Triglyceride by GK GPO Peroxide method.[11] HDL was measured by release of HDL and reaction with cholesterol esterase and peroxidase.[12] Total Bilirubin and direct bilirubin by Vanden berg reaction and Diazo method.[13] ALP by rate of conversion of p-nitro-phenylphosphate to p-nitrophenol.[14] ALT and AST by coupling of transamination reaction with lactate dehydrogenase reaction with decrease of NADH.[15] Total Protein by reaction with cupric ion in alkaline medium.[16] Albumin bromocresol green method.[17]

Statistical Analysis

Data analysis was done by using Statistical Software (SPSS-17). Calculation of RIs includes parametric and nonparametric calculation methods, detection of outliers, partitioning, and confidence intervals. The interpercentile is the most commonly used method for establishing reference interval, and recommended by the IFCC. It is defined as an interval bounded by two percentiles of the reference distribution. It is an arbitrary but common convention to define the reference interval as the central 95% interval bounded by the 2.5 and 97.5 percentiles.

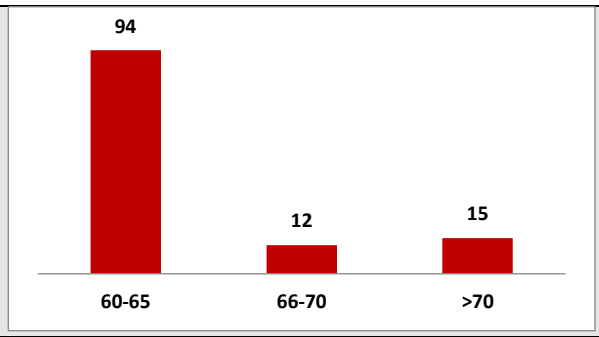
RESULTS

The demographic characteristics of the study subjects are presented in Table 1. 129 (60.56%) were males. The mean age for males was 64.77 (SD-4.998) and females 62(3.691).

Minimum age as 60 and maximum-76 years. Ninety four (77.68%) were below 65 years.

Characteristics	N=121	
Age	Mean (SD)-63.34 (4.210)	
Sex	Male -31	Female 88
Occupation	No job- 50 (41.32.9%)	
Habits	Smoking- 8 (22.58 % of males)	Alcohol -7 (5.8% of males)
Diet	Vegetarian-10 (8%)	Mixed -111 (91.7%)
Family history of NCD	No-64(52.9%)	Diabetes-13 (10.74%), Hypertension-21 (17.35%) CAD-19 (16.4%), Cancer -2 (1.6%), Thyroid diseases-3 (2.45%)
Blood Pressure	Mean (SD)-Systolic BP-130.36(31.705)	Diastolic BP – Mean (SD)- 79.77 (19.267)

Table 1. Sociodemographic Characteristics of Study Subjects



Graph 1. Age Group Distribution

Parameters with Unit N=121	Mean (SD)	Median (Q1,Q3)	2.5th - 97.5th Percentile	Current Reporting Range
Glucose (mg/dl)	97.18 (37.981)	83 (69.50,112)	58.10-207.95	70-110 mg/dl
Urea (mg/dl)	22.75 (6.968)	22 (18,22)	12.05-41.75	20-40 mg/dl
Creatinine (mg/dl)	0.96 (0.214)	1 (0.84,1.0)	0.63-1.96	0.7-1.4 mg/dl
Uric acid (mg/dl)	4.93 (1.165)	2 (4,6)	3.0-7.0	3-7 mg/dl
Total Protein (gm/dl)	7.47 (0.555)	7.42 (7,8)	6.0-8.596	6-8 gm/dl
Albumin(gm/dl)	4.12 (0.262)	4.00 (4,4.21)	3.86-4.997	3.5-5 gm/dl
Total Bilirubin (mg/dl)	0.92 (0.315)	1 (0.77,1)	0.00 -1.96	0.2-0.8 mg/dl
Direct Bilirubin (mg/dl)	.71 (.825)	0.008 (0.0,0.13)	0.06 – 0.298	0.0-0.2 mg/dl
ALT IU/L	28.34 (12.017)	26 (21,30)	17.43 -77.38	15-40 IU/L
AST IU/L	23.22 (13.726)	20 (15,27)	8.05 – 61.85	15-40 IU/L
ALP IU/L	79.08 (25.812)	78 (63,93.5)	20.65 – 132.95	50-150 IU/L
Total Cholesterol (mg/dl)	212.61 (58.696)	213 (171.5,250)	76.16 – 329.96	150-200 mg/dl
Triglycerides (mg/dl)	118.98 (52.309)	102.00 (81,142.5)	54.05 – 251.35	<150 mg/dl

Table 2. Established Reference Intervals of Routine Biochemical Parameters

Parameters with Unit N=121	Mean (SD)	Median (Q1-Q3)	2.5th - 97.5th Percentile	Current Reporting Range
Sodium (mEq/L)	139.72 (5.96)	(137,143) 141	126.05-149.85	135-145 mmol/L
Potassium (mEq/L)	4.01 (0.488)	4 (3,4)	3.00-5.00	3.5-5.5 mmol/L
Calcium (mg/dl)	9.348 (0.482)	9.3 (9,9.59)	8.020-10.495	8-11 mg/dl
Phosphorus (mg/dl)	3.39 (0.541)	3.21 (3,3.98)	2.30-4.30	2.5-4.5 mg/dl

Table 3. Established Reference Intervals for Serum Electrolytes

Kolmogorov-Smirnov was applied for all the parameters to check normality. As many parameters displayed non-Gaussian distributions, non-parametric methods were used. The reference limits are defined as the central 95% of the population comprised between quantiles 2.5 and 97.5, leaving aside 2.5% of the individuals on both sides of the distribution and reference limits were estimated following standard methods of the International Federation of Clinical Chemistry (IFCC).[18] Outliers were identified in the data by calculating the first quartile (Q25), the median (Q.50) and third quartile (Q.75). The interquartile range (IQR) was calculated by subtracting the first quartile from the third quartile (Q.75 - Q.25). Any data observation which lay more than 1.5 × IQR lower than the first quartile or 1.5 × IQR higher than the third quartile was considered an outlier and deleted from data. Data of 8 subjects were thus removed.[19] The lower reference limits was estimated as the 2.5th percentile and the upper limits as the 97.5th percentile of the distribution of test results for the reference population was calculated from 121 samples.

Mann Whitney U test was used to compare the values in males and females. Statistically significant differences are observed between the mean values of Sodium (U=898, p 0.005) and Phosphorus (U=885, p 0.008). No significant difference was observed in different age groups.

DISCUSSION

The establishment of reference interval for individual laboratory is difficult, because of many factors like selection of reference individuals, the control of pre analytical variables etc. Reported values are usually based on the data derived from healthy volunteers and not based on geriatric population. The primary objective of the present study was to analyse the biochemical reference intervals among the elderly persons of the rural population of costal area of South Kerala to see any differences in the biochemical parameters among this defined population compared to the reported values. No significant difference in reference was observed between male and female in parameters except serum sodium and phosphate. Studies show there is gender difference in dietary sensitivity and cellular sodium homeostasis.[20,21] Difference in inorganic phosphate is consistent with previous studies as suggested by effect modification by sex.[22-24] RI for glucose, Total cholesterol and Triglycerides show a significantly high range. RI of Serum sodium is different from the reported value. No significant differences were observed for other parameters .Even though previous study reports an increase in many parameters like S. creatinine, ALT etc., but our study did not show such findings.[25] Differences in the reference limits could be due to differences in the geographical location, methods and equipment used, sample size, posture, race, regional differences in the dietary intakes of foods rich in these analytes, and genetics.[26-28]

Limitations

The limitation of the study is that the participants were considered to be normal which is a broad term. Also, the sample size was small according to age, and sex.

CONCLUSIONS

The reference intervals obtained for blood glucose, total cholesterol and triglycerides from the study showed

deviation from the values provided by the diagnostic kit inserts or literature. No significant gender difference was observed in most of the parameters except for serum sodium and inorganic phosphate. Establishment of age specific reference value should be done as a protocol before assuring quality control in the lab. This will be of great significance to the clinicians in making the decisions.

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