Original Article

Establishing Reference Value of Biochemical Parameters - A Must Before Ensuring Quality in Biochemistry Diagnostic Lab

Gitanjali* and Panag KMDS

Dept of Biochemistry GGS Medical College, Faridkot, Punjab, India

Keywords: *Reference values, Biochemistry, General population*

ABSTRACT

Background: Quality assurance in a laboratory comprises of a set of factors which besides numerous preanalytical, analytical and post analytical variables also include effect of age and sex on various parameters. Present study was done to know the impact of these factors on various biochemical variables and reference values of the various biochemical parameters were set according to age and sex.

Methodology: The present study was conducted on 200 apparently healthy individuals from Malwa belt of Punjab. The participants were divided according to age and sex. All the parameters were measured by standard lab protocols/ methods.

Result: In addition to various preanalytical, analytical & post analytical variables, some of the parameters showed significant variations according to age and sex. Serum creatinine,uric acid, AST,ALP and Calcium showed significant difference in males as compared to females($p<0.05$). Parameters like Urea, Creatinine, uric acid ,total protein and cholesterol showed significant difference in individuals who were more than 50 years when compared with individuals less than 50 years ($p<0.05$).

Conclusion: These reference values will be used to compare the results of various biochemical parameters of patients coming to our tertiary care hospital. Also the setting of reference value should be done as a protocol before assuring quality control in the lab. But there is need for more elaborative studies with more number of participitants and it should be comparable with the results from multiple laboratories that are using same methodology and instruments.

***Corresponding author:**
Dr Gitanjali, Associate Professor, Dept of Biochemistry GGS Medical College, Faridkot, Punjab, India
Email: gitanjaligoyal@yahoo.co.in

Introduction

Health of an individual is conceptually different in different countries, in the same country at different times and in same individuals at different ages. It is thus a relative and not an absolute state. This means that the condition of individuals must be related to or compared with reference data. On comparing the individuals data collected during the medical interview, clinical examination, and supplementary investigations with the reference data, the condition of individuals can be interpreted. A patient's laboratory result simply is not medically useful if appropriate data for comparison are lacking. It is thus the central role of the laboratory scientist to aid the clinician in interpreting observed values by providing relevant reference values and presenting them in a convenient and practical form.[1]

A good quality assurance in a tertiary care diagnostic laboratory requires establishing physiological ranges of various biochemical parameters which may vary according to age, gender and geographic distribution.

The concept of reference values was introduced in 1969.[2] Reference values are result of tests obtained from sample(s) of an individual or individuals of a defined description. [3]Determining reference values of healthy subjects of a geographic location in a hospital laboratory assumes significance as results can vary in different setting and it would serve as reference for less heterogeneous population and subjects attending the hospital. [4]

Reference ranges for biochemical parameters are nonexistent in this population where, like in many other countries of the third world it is common practice to use reference ranges established for western population for interpretation of lab results.[5]

As recommended by the IFCC at least 120 reference subjects are required for the establishment of reference values.[4]

The need for establishment of baseline reference with which to monitor the complete quality protocol as well as to monitor pathological changes in the patients coming for investigations is there.

The present study was aimed to establish reference values of various biochemical parameters in healthy individuals according to sex and age.

Materials and Methods

After appropriate informed and written consent, detailed history regarding socioeconomic status and medical history to rule out common illnesses, 200 healthy individuals were enrolled for the study. The persons with abnormal blood cell counts, deranged blood sugar level, abnormal triglyceride levels were excluded from the study. About 10 ml of blood was collected after overnight fasting. Serum was separated after centrifugation at 3000 rpm for 10 min.

Routine investigations were done on fully autoanalyzer Beckman Coulter AU 480. Blood glucose was based on hexokinase enzymatic method,[6] Urea/BUN was measured by urease reaction coupled to decrease in NADH by l-glutamate dehydrogenase,[7] Creatinine measurement was based on Jaffe reaction,[8] uric acid was measured by reaction of uricase and peroxidase reaction. [9] Cholesterol measurement was based on cholesterol esterase and peroxidase,[10] Triglyceride was based on GK GPO Peroxide,[11] HDL was measured by release of HDL and reaction with CHE and peroxidase,[12]TBI, DBI were measured by Vanden berg reaction and Diazo method.[13] ALP was measured by rate of conversion of p-nitro-phenylphosphate to p-nitrophenol.[14] ALT and AST were based on coupling of transamination reaction with consumption of NADH for LDH reaction,[15]TP was estimated by reaction with cupric ion in alkaline medium,[16] Albumin estimation was based on its reaction with bromocresol green.[17]

Statistical Analysis: Stastical analysis was done using SPSS version 20.All the values were given as Mean + SD .p Value was calculated using Karl Pearson formula.

Quality Control

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. The reference ranges were considered only after verification of control ranges. Westgard Rules were followed to ensure quality.

Results

The subjects were divided according to sex and age. The parameters were compared among males and females (Table 1).

The values were comparable to those as given in the protocols but the values of some parameters which show significance difference according to gender and age as highlighted by asterisk in tables ($p<0.05$). Parameters like Urea, Creatinine, uric acid ,total protein and cholesterol showed significant difference in individuals who were more than 50 years when compared with individuals less than 50 years. Similar findings were found by other authors also.[4]

Table 1: showing reference range of various biochemical parameters in males and females

Parameter	Mean (SD) value in Males	Mean (SD) value in Females	Reference Range as per kit insert
Glucose (mg%)	87.3 (10.9)	86.4 (9.6)	70-105
Urea/BUN (mg%)	18.1 (5.1)	17.4 (4.1)	7-25
*Creatinine (mg%)	0.90 (0.25)	0.80 (0.25)	M = 0.7-1.3 F = 0.6-1.2
*Uric acid (mg%)	5.38 (1.0)	4.90 (0.85)	M = 4.4-7.6 F = 2.3-6.6
TBI (mg%)	0.88 (0.4)	0.80 (0.3)	0.3-1
DBI (mg%)	0.3 (0.14)	0.2 (0.09)	0-0.2
*AST (U/L)	14.9 (5.3)	13.8 (4.1)	13-39
ALT (U/L)	20.65 (5.8)	19.2 (6.0)	7-52
*ALP (U/L)	69.6 (12.4)	67 (11.8)	34-104
TP (g%)	7.33 (1.4)	7.17 (1.1)	6.4-8.9
ALB (g%)	4.67 (0.71)	4.01(0.5)	3.7-5.3 4.2-5.5
Chol (mg%)	193.5(38)	189.8(36.6)	136-290
TG (mg%)	113.4(30.9)	109.9(28.8)	48-352
HDL (mg%)	47.1(8.9)	49.0(8.4)	30-67
*Ca (mg%)	9.38(1.5)	9.03(1.3)	8.6-10.3

Serum creatinine,uric acid, AST,ALP and Calcium showed significant difference in males as compared to females($p<0.05$)

The subjects were divided in two groups according to age.Group I less than 50 years and group II with age group more than 50 years.

Table 2: showing reference range of various biochemical parameters according to age.

Parameter	Mean(S.D) Age <50 years	Mean(S.D) Age >50 years
Glucose (mg%)	87(11.3)	87.6(10.5)
*Urea/BUN (mg%)	16(10.0)	19(12.1)
*Creatinine (mg%)	0.7(0.2)	0.81(0.3)
*Uric acid (mg%)	4.50(1.1)	4.8(0.9)
TBI (mg%)	0.80(0.2)	0.81(0.29)
DBI (mg%)	0.3(0.14)	0.3(0.1)
AST (U/L)	13.98(5.92)	14.67(5.37)
ALT (U/L)	21.2(6.1)	20.9(5.6)
ALP (U/L)	65(22.3)	65(23.2)
*TP (g%)	7.5(2.0)	6.38(2.14)
ALB (g%)	4.11(0.6)	4.04(0.4)
*Chol (mg%)	184(24.3)	198(24.7)
TG (mg%)	102.3(35.3)	123(30.3)
HDL (mg%)	47.8(7.30)	46.9(7.10)
Ca (mg%)	9.60(0.7)	9.11(0.6)

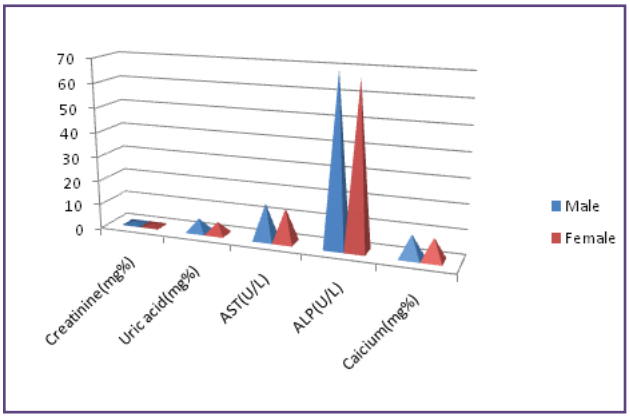

Fig. 1: showing values of parameters having significant difference according to sex.

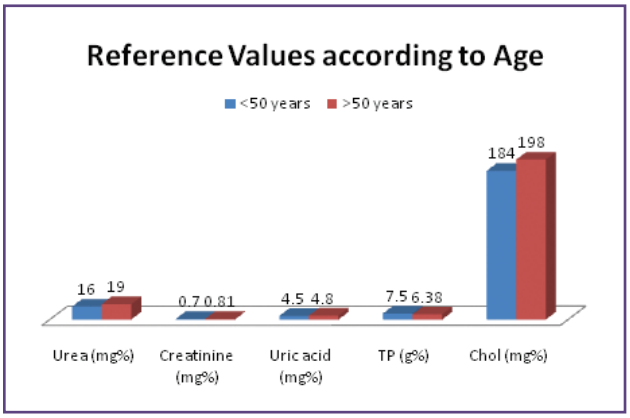

Fig. 2: showing reference value of some Biochemical parameters according to age

Discussion

A reference range of a clinical chemistry parameter is a set of values used in the interpretation of a clinical chemistry report. There are two types of reference ranges categorized as subject based and group based. When doing a follow up on patients, a clinician often use a subject-based reference range to determine the progress made in the management of a pathological disorder. To establish whether a patient has a certain pathological disorder however, group-base reference range is used in the interpretation of laboratory report.[18] In clinical management of patients, physicians rely on blood chemistry analytes for accurate diagnosis, proper treatment and follow-up of patients. Correct interpretation of the results from these analytes presupposes that the clinician and the laboratory medicine physician have good reference information. Published reference ranges in literature do not sometimes represent adequately the specific population from which the patient comes from based on age, sex, genetics,diet, and altitude. In addition, reference ranges produced by reagent manufacturers are determined from analysis of blood samples of a few health workers who do not represent the general population. Reference information is often the weakest data provided by clinical laboratories even though such data is very useful for the correct and proper interpretation of laboratory results. It is therefore recommended that each clinical chemistry laboratory establish its own reference range for biochemical parameters.[19]

Our findings are in accordance with other authors which states that differences in the reference limits could be due to differences in the geographical location ,methods and equipments used, sample size, posture, race, regional differences in the dietary intakes of foods rich in these analytes, and genetics. [20-22]Increase in creatinine with the advancement of age for both sexes could be due to muscle degradation with age. Higher creatinine levels in males than in females may be due to the greater muscle mass in males than females. [23] The higher UA in males relative to females could be explained by the higher clearance rate in females than in males. [24] Increase of ALT with advancing age in both males and females suggests that ALT levels are age dependent Ideally, specimens for the production of reference values for clinical use should be collected under conditions as similar as possible to those prevailing in clinical practice. As several factors cause increased variability of analytes, it is necessary to standardize the pre-analytical procedures. Important among biological factors are those that modify the metabolism of lipids, amino acids, and carbohydrates. Meals,prolonged fasting, pharmacologically active substances, hormonal supplementation therapy,stress, and physical exercise may have an effect on the metabolic state of the reference individual.Hemodynamic factors like change in posture,[25] recent exercise, or tourniquet pressure can bring about an increase in the concentrations of proteins, calcium, fatty acids, and bilirubin. Intake of ethanol,anticonvulsant drugs may induce synthesis of liver enzymes (eg. gammaglutamyltransferase) and this may increase the clearance of many substances, thus affecting their concentration in serum.

Limitation of the Study: 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 .

Conclusion

These reference values will be used to compare the results of various biochemical parameters of patients coming to our tertiary care hospital. Also the setting of reference value should be done as a protocol before assuring quality control in the lab. But there is need for more elaborative studies with more number of participitants and it should be comparable with the results from multiple laboratories that are using same methodology and instruments.

References

1. Solbergm H. International Federation of Clinical Chemistry, Expert Panel on Theory of Reference Values: Approved Recommendation on the theory of reference values. Part 1. The Concept of Reference Values. J. Clin. Chem. Clin.Biochem. 1987;25: 337-342.
2. Grasbeck R, Saris NE. Establishment and use of normal values. Scand J Lab Invest 1969; 26:62-3
3. Soldberg HE. Establishment and use of reference values. In: Teitz text book of clinical chemistry, 2nd Ed. Burtis CA and Ashwood ER eds. Saundres, Philadelphia 1996; 356-86.
4. SujataWangkheimayum, Determination of reference values of some routine clinical biochemical parameters of apparently healthy North Indian subjects. J of Biochemistry Research 2013; 1(1):1-6.
5. G.A. Alemnji, J.Mbuagbaw, G.Teto, S. Nkengafac, E.Folefac, N.Atems, B.K wingwah and T.Asonganyi. Reference ranges for serum biochemical parameters among Healthy Cameroonians to support HIV Vaccine and Related Clinuicxal Trials.The oprn Clinicaql Chemistry2010;3:66-71.
6. Stein M W,Clinical methods of enzymatic analysis,Academic Press,1965;117.
7. Talke H, Schubert GE. Enzymatic urea determination in the blood and serum in the warburg optical test. Klivische Wochenschrift1965;43:174.
8. Fabiny DI, Ertingshausen G. Automated reaction rate method for determination of serum creatinine.Clin Chem.1971;17:696.
9. Fossati P, Prencipe L, Berti . Enzymic Creatinine Assay: A New Colorimetric Method Based on Hydrogen Peroxide Measurement G.Clin Cherm,1980;26:227.
10. Allain CC, Poon L S, Chan C S G,Richmond W Fu P C. Enzymatic determination of total serum cholesterol .Clin chem. 1974;12:403.
11. Trinder P,Ann Determination of Glucose in Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor. Clin Biochem 1969;6:24.
12. Vnaden Berg A Mueller P, Biochem Z, Uber eine direct and eine indirect Diazo reaction of Bilirubin1916;77:90
13. Bowers GN, Mc Comb RB, Measurement of total alkaline phosphatase activity in human serum.Clin Chem 1975;21:1988-1995.
14. IFCC Methods for the Measurement of Catalytic Concentration of Enzymes Clin Chem.1977; 23:887.
15. Wroblewski F, La Due JS. Serum glutamic pyruvic transaminase in cardiac and hepatic disease.Pros Soc Exp Biol Med 1956;91:569.
16. Weichselaaum TE, Amer An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. J Clin Path1946 ;16:40.
17. Rodkey FL Direct Spectrophotometric Determination of Albumin in Human Serum Clin chem. 1965;2: 478.
18. IFCC. Approved recommendation on the theory of reference values. Journal of Clinical Chemistry and Clinical Biochemistry 1981; 25(5): 337-342.
19. Ferre Masterrer, M, Fuentes, A.X. Puchal, An indirect reference limit estimated from patients results by three mathematical procedures. Clinica Chemica Acta 1999; 279(1-2): 97-105.
20. Rosen, FS., Cooper, M.D. and Wedgwood,R.J.The primary immunodeficiencies. New England Journal of Medicine 1984; 311: 235-242.
21. Erasmus RT., Ray U., Nathaniel K. and Dowes G. Reference ranges for serum creatinine and urea in elderly coastal Melanesians. Papua New Guinea Medical Journal 1997; 40(2): 89-91.
22. Reidenberg MM., Gu, Z-P., Lorenzo, B., Coutinho, E., Athayde, C., Frick, J., Alvarez, F., Brache, V.and Emuveyan, E.E. Differences in serum potassium concentrations in normal men in different geographic locations. Clinical Chemistry 1993; 39(1): 72-75.
23. Jagarinec, N., Fleger, M., Surina, B., Vrhovsks, H.,Hebrung, N. and Predenkerokuvic, V. Pedriatic Reference Interval for 34 biochemical analytes in urban school children and aldolescent. Clinical Chemistry Laboratory Medicine 1998; 36(5): 327-337
24. Jannie W, Treuting JJ, Donald, C.C. Metabolic intermediates and inorganic ions. In:Clinincal diagnosis and management; 16th edition; Philadelphia; London and Toronto; WB Saunders company. 1979; pp 259-304.
25. Felding, P., Tryding, N., Hyltoft, P. et al. Effects of posture on concentrations of blood constituents in healthy adults: Practical application of blood specimen collection procedures recommended by the Scandinavian Committee on Reference Values. Scand. J. Clin. Lab. Invest.1980: 40; 615-621.