



Estimation of Reference Range of Serum Protein Fractions in an Urban South Indian Population

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ABSTRACT

Reference interval in a laboratory when applied to the population serviced by the laboratory includes most of the subjects with characteristics similar to the reference group to be identified as 'healthy' and excludes the others. The reference range and reference limits for various Biochemistry parameters has not been established in our Indian population. As on date, most of our laboratories are using the text book values, literature referred values or manufacturer's reference value in the kit inserts. In India, like many other biochemical parameters the interpretation of serum protein fractions obtained by the electrophoresis and the total protein by Biuret method is usually dependent on the reference range which is mainly obtained from Western population. The aim of the present study is to determine the reference range of serum proteins fractions and total protein of a sample population attending a tertiary care center in Bangalore, Karnataka the by semi automated cellulose acetate electrophoresis method and Biuret method and to compare the newly obtained reference range of serum proteins with the currently used manufacturers reference range in the laboratory which is based on western population. The samples obtained from individuals coming for executive health check up at this tertiary care center were used for this study. Serum Total protein and other protein fractions were measured in the samples and reference range was determined. We found noticeable deviation in the new reference ranges of some parameters such as total protein, albumin, γ globulin from the manufacturers values. We conclude that the reference ranges observed in a small population in a city could be significantly different from the literature referred values and generating any such data for any biochemical parameters in more partitioned groups and larger sample size will be of great significance to the clinicians using such data during clinical intervention.

Keywords: Reference Range; Reference interval; Serum proteins; Serum protein electrophoresis.

INTRODUCTION

Health is relative, and not an absolute status as it varies from country to country, and in the same country from one region to another region, and in

the same region from person to person, and in the same person, in different ages. The condition of an individual can be interpreted with clinical examination and necessary laboratory investiga-

tions with reference data. A patient's laboratory test is of no use if appropriate data for comparison is missing. The main role of the laboratory to aid the clinician to interpret the results by providing reliable reference value.⁽¹⁾

The Reference value is the most commonly used decision-making tool in medical field.⁽²⁾ It is important to the determine whether or not an individual is healthy. The reference interval (RI) theory was first put forth by Schneider in his 1960s paper entitled 'Some thoughts on normal, or standard, values in clinical medicine'.⁽³⁾ It determines the statistical probability of having a specific disease or not when values fall within or outside the RI. Reference interval in a laboratory when applied to the population serviced by the laboratory includes most of the subjects with characteristics similar to the reference group to be identified as 'healthy' and excludes the others.⁽⁴⁾

The only way for understanding the health status of an individual is with the aid of reliable validated laboratory data. Clinicians use the data collected by the laboratory to interpret relative health status of an individual and then use their skill, knowledge and decide regarding further action towards the patient care. A major need for clinical chemistry personnel in particular, is to provide the clinicians updated & appropriate reference Values.

There is also a need for establishing in house reference ranges considering most variable biological factors such as age, gender, nutritional status and other physiological factors⁽⁵⁾ we have not yet established reference ranges for various analytes in clinical biochemistry in our Indian population. As on date, most of our laboratories are using the text book values, articles or Kit insert literature from the manufacturers.

The aim of the current study was to develop the reference range from healthy individuals of a population for which it can be used as reference. The concept of reference values of serum proteins is of great significance in diagnostics. There is also a need for laboratory-developed reference ranges of serum proteins as the required data is

not available in this part of our country. Aim of this study is to determine the reference range of various serum proteins in reference and to compare the obtained results with the reference range of serum protein currently used in the laboratory.

MATERIALS AND METHODS

The study was conducted prospectively. The subjects were chosen from population visiting the Health Plan Clinic, which is a preventive medical center, a part of a tertiary hospital in Bangalore.. 237 individuals belonging to the age group between 20 to 60 years were selected for the study after excluding the subjects as per the criteria⁽⁶⁾ Among 237 individuals 111 were women and 126 were men.

Subjects with the following history are excluded from the study.

Acute inflammatory diseases, Diabetes Mellitus, Tuberculosis Malignancy, Dyslipidemia Liver dysfunction, Contact with jaundiced patients, Cardiovascular abnormalities, Renal abnormalities, Medication, Excessive body weight, Smoking, Alcohol abuse, pregnancy

METHOD OF ANALYSIS

The blood specimens were drawn from the individuals in the morning between 8:30 AM and 9:30 AM. Vacutainers specific for serum were used for the collection of venous blood sample. Blood was collected using recommended procedures for collection of diagnostic blood specimens by venipuncture^[7,8] after an overnight fast. All serum samples were analyzed for serum total protein Modified Biuret method^(9,10,11) using Dade Behring Dimension RxL auto analyzer in the Biochemistry Laboratory, and also analyzed for serum protein fractions by semiautomated cellulose acetate electrophoretic system in the Biochemistry Laboratory.⁽¹²⁾ All the analytical procedures were calibrated to the instrument before sample analysis was done^(13,14)

Statistical Method

Statistical Methods: Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. The following assumptions on data is made, Assumptions: 1. Dependent variables should be normally distributed, 2. Samples drawn from the population should be random, Cases of the samples should be independent Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups Inter group analysis) on metric parameters. LevenIs test for homogeneity of variance has been performed to assess the homogeneity of variance.

Significant figures

- + Suggestive significance (P value: 0.05<P<0.10)
- * Moderately significant (P value:0.01<P ≤ 0.05)
- ** Strongly significant (P value: P≤0.01)

Statistical Software

The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, Med Calc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

RESULTS

Table 1: Age distribution according to gender

Age in years	Male		Female	
	No	%	No	%
20-40 years	56	44.4	49	44.1
41-60 years	70	55.6	62	55.9
Total	126	100.0	111	100.0

Figure1: Distribution of males

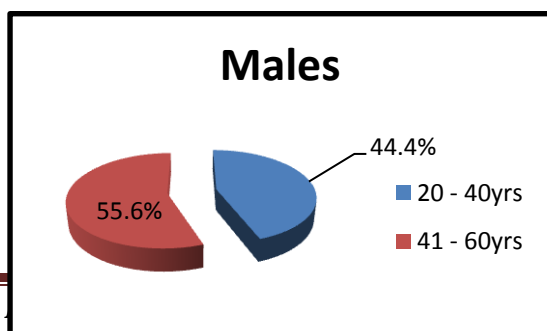
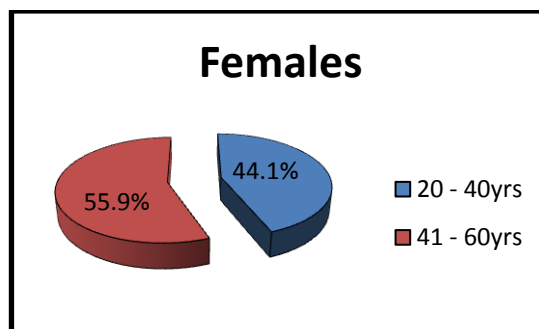


Figure 2: Distribution of females



Out of 237 subjects 126 were males and 111 were females. In males 56 were in 20-40 year group and 70 were in 40-60 years group. In females 49 were in 20-40 year group and 62 were in 40-60 years group.

Table 2: Mean and SD of study variables in two age groups studied for female

Variables	20-40 yrs	41-60 yrs	P value
ALBUMIN	3.85±0.34	3.86±0.4	0.891
α1 GLOBULIN	0.21±0.05	0.21±0.05	0.666
α2 GLOBULIN	0.81±0.13	0.81±0.14	0.855
β GLOBULIN	0.91±0.12	0.88±0.14	0.214
γ GLOBULIN	1.23±0.25	1.22±0.27	0.797
A/G ratio	1.24±0.19	1.26±0.22	0.483
TOTAL PROTEIN	7.01±0.55	6.98±0.64	0.755

Results are presented in Mean ± SD, P value obtained by student t test.

There is no significant difference between the mean values of various protein fractions in between the two age groups in females

Table 3: Mean and SD of study variables in two age groups studied for male

Variables	20-40 yrs	41-60 yrs	P value
ALBUMIN	4.02±0.46	3.93±0.47	0.306
α1 GLOBULIN	0.20±0.06	0.21±0.06	0.833
α2 GLOBULIN	0.78±0.16	0.78±0.15	0.870
β GLOBULIN	0.89±0.14	0.97±0.23	0.642
γ GLOBULIN	1.28±0.24	1.20±0.24	0.052+
A/G ratio	1.30±0.21	1.32±0.21	0.561

TOTAL PROTEIN	7.16±0.64	6.95±0.56	0.050*
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Results are presented in Mean ± SD, P value obtained by student t test

There is no significant difference between the mean values of following protein fractions in between the two age groups in males.

Albumin, α1 GLOBULIN, α2 GLOBULIN, β GLOBULIN, A/G ratio.

Suggestive significant differences in the mean values of γ GLOBULIN

Moderately significant difference between the mean values of TOTAL PROTEIN

Table 4: Reference interval study variables in two age groups studied for female

Variables	20-40 yrs	41-60 yrs	All subjects
ALBUMIN	3.19-4.52	3.07-4.65	3.12-4.59
α1 GLOBULIN	0.11-0.32	0.11-0.30	0.11-0.31
α2 GLOBULIN	0.55-1.07	0.55-1.08	0.55-1.07
β GLOBULIN	0.68-1.14	0.61-1.14	0.64-1.14
γ GLOBULIN	0.74-1.72	0.69-1.74	0.71-1.73
A/G ratio	0.86-1.61	0.83-1.69	0.84-1.66
TOTAL PROTEIN	5.93-8.10	5.72-8.23	5.81-8.17

95% CI reference Interval

ALBUMIN: reference range is slightly narrower in the age group of 20-40 years with increase in the lower limit and decrease in the upper limit.

α1 GLOBULIN, α2 GLOBULIN: No major difference in the reference range between the groups.

β GLOBULIN: reference range is narrowed in 20-40 years group with increase in the lower limit.

γ GLOBULIN: reference range is narrowed in 20-40 years group with increase in the lower limit.

TOTAL PROTEIN: reference range is narrowed in 20-40 years group with increase in the lower limit and decrease in the upper limit A/G ratio: reference range is narrowed in 20-40 years group with increase in the lower Limit and decrease in the upper limit

Table 5: Reference interval study variables in two age groups studied for Male

Variables	20-40 yrs	41-60 yrs	All subjects
ALBUMIN	3.13-4.91	3.02-4.85	3.06-4.88
α1 GLOBULIN	0.09-0.32	0.10-0.32	0.09-0.32
α2 GLOBULIN	0.46-1.10	0.48-1.08	0.47-1.09
β GLOBULIN	0.61-1.18	0.48-1.37	0.49-1.30
γ GLOBULIN	0.80-1.76	0.73-1.66	0.76-1.71
A/G ratio	0.88-1.72	0.92-1.73	0.90-1.72
TOTAL PROTEIN	5.90-8.43	5.83-8.07	5.85-8.24

95% CI reference Interval

ALBUMIN: reference range is slightly narrower in the age group 20-40 years with increase in the lower Limit and there is also an increase in upper limit.

α1 GLOBULIN, α2 GLOBULIN: No major difference in the reference range between the groups

β GLOBULIN: reference range is narrowed in 20-40 years group with increase in the lower limit and decrease in lower limit.

γ GLOBULIN: reference range is narrowed in 40-60 years group with decrease in upper and lower limit.

TOTAL PROTEIN: reference range is wider in 20-40 years group with increase in the lower limit and increase in the upper limit.

A/G ratio: reference range is wider in 20-40 years group with decrease in the lower limit.

Table 6: Reference ranges for the following parameter in different age group of both sexes:

Age gps in yrs.	Sex	TP g/dl	ALB g/dl	α1 GLB g/dl	α 2 GLB g/dl	β GLB g/dl	γ GLB g/dl
20 - 40	M	5.9 - 8.4	3.1 - 4.9	0.1 - 0.3	0.4 - 1.1	0.6 - 1.2	0.8 - 1.8
	F	5.9 - 8.1	3.1 - 4.5	0.1 - 0.3	0.5 - 1.1	0.7 - 1.1	0.7 - 1.7
41 - 60	M	5.8 -	3.0 - 4.8	0.1 - 0.3	0.5 - 1.1	0.5 - 1.4	0.7 - 1.7

		8.1					
	F	5.7 - 8.2	3.1 - 4.6	0.1 - 0.3	0.5 - 1.1	0.6 - 1.1	0.7 - 1.7

41 - 60	M	5.8 - 8.1	3.0 - 4.8	0.1 - 0.3	0.5 - 1.1	0.5 - 1.4	0.7 - 1.7
Ref. range used in lab	Over all	6.4 - 8.2	3.2 - 5.6	0.1 - 0.4	0.4 - 1.2	0.5 - 1.3	0.5 - 1.6

Total Protein

Age group 20-40 years: reference range is narrower in females with decrease in the upper limit compared to males.

Age group 40--60 years: There is no major difference in reference range between males and females.

Albumin:

Age group 20-40 years: the reference range in females shows a narrower decrease in the upper limit compared to males.

Age group 40--60 years: the reference range in females is narrower with a decrease in the upper limit compared to males.

α1 GLOBULIN, α2 GLOBULIN

Age group 20-40 years: There is no major difference in the reference range between males and females.

Age group 40--60 years: There is no major difference in the reference range between males and females.

β GLOBULIN :

age group 20-40 years: reference range slightly narrower in females with decrease in the upper limit and increase in the lower limit compared to males.

age group 40--60 years: reference range narrower in females with decrease in the upper limit and increase in the lower limit compared to males

γ GLOBULIN

age group 20-40 years: No major difference in the reference range between males and females

age group 40--60 years: No difference in the reference range between males and females

Table 7: Comparison of reference ranges for men obtained in our study with the reference range used at our laboratory.

Age gps in yrs.	Sex	TP g/dl	ALB g/dl	α1 GLB g/dl	α 2 GLB g/dl	β GLB g/dl	γ GLB g/dl
20 - 40	M	5.9 - 8.4	3.1 - 4.9	0.1 - 0.3	0.4 - 1.1	0.6 - 1.2	0.8 - 1.8

Total Protein

age group 20-40 years: reference range wider with decrease in the lower Limit and a increase in upper limit compared to reference range used in lab

age group 40--60 years:- reference range wider with decrease in the lower Limit compared to reference range used in lab

Albumin

age group 20-40 years: reference range narrower with decrease in the upper Limit compared to reference range used in lab

age group 40--60 years: reference range narrower with decrease in the upper Limit compared to reference range used in lab

α1 GLOBULIN, α2 GLOBULIN

age group 20-40 years: No major difference reference range compared to ref range used in lab

age group 40--60 years: No major difference reference range compared to ref range used in lab

β GLOBULIN

age group 20-40 years: reference range narrower with decrease in the upper Limit compared to reference range used in lab

age group 40--60 years: No major difference reference range compared to reference range used in lab

γ GLOBULIN

age group 20-40 years: reference range slightly narrower with increase in the lower limit compared to reference range used in lab

age group 40--60 years: reference range slightly narrower with increase in the lower limit compared to reference range used in lab

Table 8: Comparison of reference ranges for Women obtained in our study with the reference range used at our laboratory.

Age gps in yrs.	Sex	TP g/dl	ALB g/dl	α1 GLB g/dl	α 2 GLB g/dl	β GLB g/dl	γ GLB g/dl
20 - 40	F	5.9 - 8.1	3.1 - 4.5	0.1 - 0.3	0.5 - 1.1	0.7 - 1.1	0.7 - 1.7

41 – 60	F	5.7 – 8.2	3.1 – 4.6	0.1 – 0.3	0.5 – 1.1	0.6 – 1.1	0.7 – 1.7
Ref. range used in lab	Over all	6.4 – 8.2	3.2 – 5.6	0.1 – 0.4	0.4 – 1.2	0.5 – 1.3	0.5 – 1.6

Total Protein

age group 20-40 years: reference range wider with decrease in the lower Limit and increase in upper limit compared to ref range used in lab

age group 40--60 years: reference range wider with decrease in the lower Limit compared to ref range used in lab

Albumin

age group 20-40 years: reference range narrower with decrease in the upper Limit compared to reference range used in lab

age group 40--60 years: reference range narrower with decrease in the upper Limit compared to reference range used in lab

 α 1 GLOBULIN, α 2 GLOBULIN

age group 20-40 years: No major difference reference range compared to reference range used in lab

age group 40--60 years: No major difference reference range compared to reference range used in lab

 β GLBULIN

age group 20-40 years: reference range narrower with decrease in the upper limit and increase in the lower limit compared to reference range used in lab

age group 40--60 years: reference range narrower with decrease in the upper limit and increase in the lower limit compared to reference range used in lab

 γ GLOBULIN

age group 20-40 years: reference range slightly narrower with increase in the lower Limit compared to reference range used in lab

age group 40--60 years: reference range slightly narrower with increase in the lower Limit compared to reference range used in lab

DISCUSSION

Reference intervals are based on the testing of reference population⁽⁶⁾ The reference population

may represent healthy subjects, non-healthy subjects without a disease, which can affect the reference interval. Clinical chemists and governmental organizations have adopted recommendations on reference values as they are part of quality standards.

Grasbeck and Alstrom⁽¹⁵⁾ and Harris and Boyd⁽¹⁶⁾ published basic method of determination of reference ranges To establish the reference values the IFCC has given guidelines by articles on the theory of Reference Values⁽¹⁷⁾ Few other studies published articles on determination of reference ranges either general or pertaining to age sex and local population^(18,19). Since collecting data from large reference population is not possible for many laboratories they use literature data and manufacturers value in the kit insert.

The present study which aimed to evaluate the reference range for biochemical parameters such as total protein by biuret method, albumin, α 1 globulin, α 2 globulin, β globulin, γ globulin by cellulose acetate electrophoresis showed varying results when compared to the values that are currently being used in our hospital laboratory, which are taken from western literatures.

The reference intervals α 1 globulin, α 2 globulin obtained in present study is similar to manufacturers reference interval followed by the laboratory where as it is wider for total protein and narrower for albumin, β globulin, γ globulin in both males and females. We have observed that there is difference in the observed reference range of total protein, β globulin, γ globulin between males and females.

These observed differences could be generally due to differences in methodology, race of the population and environmental and as physiological factors such as nutrition, dietary habits of the Indian population. Also uniform dietary pattern was not followed by all the subjects included in this study, could have caused the shift in reference ranges. The possibility of repeated infection in a developing country also could be contributing factor.

The detailed analysis of our results for each of the parameters though restricted to small sample size, we believe that the observed changes are of significance especially with reference to the total protein and albumin β globulin, γ globulin.

Though our attempts were to partition the population, which is a mixture of various subsections such as vegetarians/non vegetarians etc and also based on the nutritional status and the type of food they consume, we were unable to do so because of the small sample size. So, our study needs to be revalidated with large sample study.

We wish to conclude as detailed in the discussion, reference ranges observed in a small population in a city could be significantly different from the literature referred values. Hence generating any such data for any biochemical parameters in more partitioned groups and larger sample size will be of great significance to the clinicians using such data during clinical intervention.

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