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ESTIMATION OF REFERENCE VALUES IN LIVER FUNCTION TEST IN HEALTH PLAN INDIVIDUALS OF AN URBAN SOUTH INDIAN POPULATION

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ABSTRACT

Reference intervals in clinical chemistry are commonly based on results of measurements in reference western population or are taken from the western literature. Reference Values are thought to aid physicians to interpret results of measurements and should be representative of a defined group of individuals. This group should be as similar as possible to the patients under investigation. The reference population in this study has been recruited from the individuals attending the Health Plan Clinic who fulfill the defined inclusion and exclusion criteria as well as defined partition criteria. The samples were sorted based on the decision by the physician. The emerging group of individuals was considered as a reference population for the hospital patients and the results of measurements in this study was evaluated statistically to stress on the urgent need to establish the in-house reference values. The reference limits are defined as the central 95 percentile of the population after eliminating the outliers. The lower reference limit is the 2.5 percentile while the upper reference limit constituted the 97.5 percentile for the population.

KEY WORDS

Reference Intervals, Age, Gender, Reference Population.

ABBREVIATIONS

AST, Aspartate Aminotransferase; ALT, Alanine Aminotranferase; ALP, Alkaline Phosphatase; γ-GT, Gamma - Glutamyl Transferase.

INTRODUCTION

A major need for laboratory medicine and clinical chemistry personnel in particular, is to provide the clinicians updated & appropriate information in Reference Values, previously known as normal values. Introduction of the concept of Reference Values and Reference population simplifies the task for laboratories; as long as they define the reference population, the outcome can always be recognized as Reference Values and Reference Intervals. Selecting Reference individuals is an essential but difficult step in the production of Reference Values throughout the world (1) (Fig 1).

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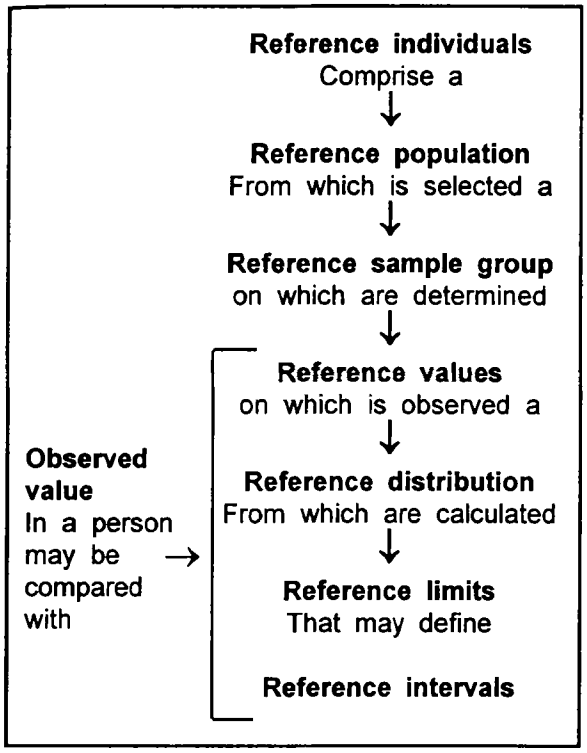
There have been many attempts to create reference Intervals without going through the laborious methods originally described by Grasbeck and Alstrom (2) or in the IFCC recommendation (3). Harris and Boyd (4) have comprehensively discussed the theory of Reference Values from a bio-statistical viewpoint. In recent years' Nano *et al.* (5), have shown considerable usefulness of defining Reference Intervals for a few quantities from carefully selected hospitalized patients.

So far there is, no established large population based study, on reference limits in Indian population. The reference limits in use are either borrowed from the textbooks and articles or insert literature from the kit manufacturers. The upper and lower limit of measurements varies dependent on source of information as well as the methodology followed. There is a need to realize, whether, there is a requirement for restructuring the reference interval for an Indian population. The aim of the current study was conducted based on

Fig. 1. Standard terminology for the description and discussion of reference values



Redrawn from How to define and determine reference intervals in the clinical laboratory; approved guidelines C28-A, PA: NCCLS, 1995.

these needs so that parameters evaluated in the healthy defined group of individuals would serve as the Reference Values for the Reference population.

MATERIALS AND METHODS

This retrospective study was conducted in the Department of Clinical Biochemistry at St. John's Medical College Hospital for a period of 4 years (1999 to 2002). The sampling strategy used was the posteriori sampling. In the posteriori sampling, a direct method which uses a database containing both analysis results and information on a large number of individuals. Values of individuals fulfilling defined inclusion criteria are selected. The individuals were selected from the population attending the Health Plan Clinic, which is a preventive medical center, a part of St. John's Medical College Hospital. In the Health Plan clinic, all individuals are evaluated by a physician and for most of the biochemical parameters. Individuals opting for Executive Health Check up are evaluated in detail by a physician and a consultant as per the individual preferences. The biochemical evaluation is also done to assess liver and renal function, lipid profile and glucose levels. Amongst 2500 individuals attending the clinic, 1500 individuals had opted for executive health check up. Remaining 1000 individuals were eliminated because they opted for health check up, which was not accompanied by the above-mentioned evaluation with the physician. Out of the 1500 selected subjects, 664 individuals belonging to the age group between 20 to 70 years were selected for the study after excluding the subjects as per the criteria (6) given in the table no.1. Among the 664 individuals, 464 individuals were men and the remaining 200 individuals were women.

The blood specimens were drawn from the individuals in the morning between 8:30 AM and 9:30 AM. Vacutainers (Becton Dickinson) with clot activator specific for serum were used for the collection of venous blood sample. All the samples were drawn after an overnight fast. This is the regular protocol followed in the hospital for the individuals attending the Health Plan Clinic.

In the study group, defined by gender and age, the first step was to eliminate all results, which were marked lipemic, hemolytic or icteric. Then the median and central 98 percentiles were calculated and results outside these limits eliminated before the final median and central 95 percentile was calculated. The 97.5 percentile and 2.5 percentile formed the upper and lower reference limits of the population. A summary of the results is given in Table 2 and 3 together with the reference intervals in use at the laboratory.

Measurement Procedures

To ensure the reproducibility and repeatability of the test results, the laboratory participated in established external quality assessment programs (BIORAD) and had a comprehensive internal quality control program and results were accordingly released. The Quality control check was done every day and SD, percentage of coefficient of variation (CV) was calculated (Table 4). Liver Function Parameters in serum were analysed using Dade Dimensions (Dade RxL). Statistical analysis was done using SPSS package. The median and central 98 percentiles were calculated and results outside these limits eliminated before the final median and central 95 percentile were calculated.

Liver function parameters were estimated using IFCC approved method. Total Protein and Serum Albumin were estimated using Biuret (7, 8) and Bromocresol Green method (9, 10). Albumin to

Globulin ratio is calculated. Total Bilirubin and Direct Bilirubin were estimated based on the modification of the Doumas reference method (11). Transaminases (AST & ALT) methods are an adaptation of the recommended procedure of the IFCC as described by Bergmeyer (12, 13). Alkaline phosphatase method is based on a procedure by Bowers and McComb (14). γ-GT is an adaptation of the methodology recommended by the IFCC (15).

RESULTS

The results obtained for the analytes used to measure the liver function is tabulated in the Table 2 and 3. The reference interval, which has been utilized by the lab, is tabulated alongwith the results so as to enable us to compare the results obtained from the study. Table 4 gives the overview of the reference material used for the evaluation of the assay alongwith the day-to-day coefficient of variation.

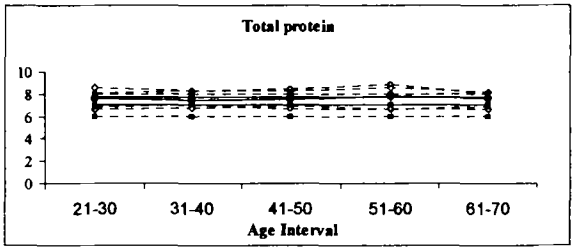
Table 1. Exclusion criteria for defining reference individuals

History of	Risk factors
Acute inflammatory condition	Excessive body weight
Diabetes Mellitus	Smoking
Tuberculosis	Alcohol abuse
Malignancy	Physiological state e.g. pregnancy
Dyslipidemias	strenuous exercise
Liver dysfunction	
Contact with jaundiced patients	
Cardiovascular abnormalities	
Renal abnormalities	
Medication	
Exposure to Carbon tetrachloride	
Beryllium or Vinyl Chloride	

From National Committee for Clinical Laboratory Standards: How to define, determine and utilize reference intervals in the Clinical laboratory: Proposed guidelines, ed. 2, NCCLS Document C28-A2, Villanova, PA, 2000, NCCLS.

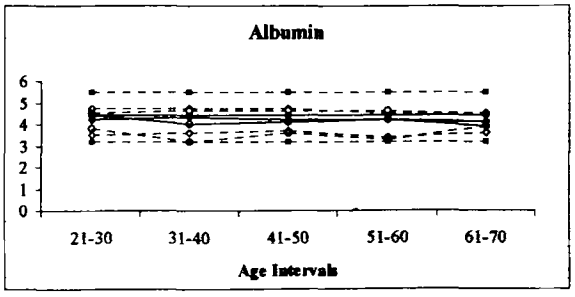
The total protein concentration was observed to be higher in the men in comparison to women except in the age group of 51 to 60 years wherein the median was higher by 0.1 g/dL and the upper limit was higher by 0.2 g/dL. Serum Albumin concentration was found to be slightly elevated in men when compared to women except in the age group of 21 to 30 years, the median was higher by 0.2 g/dL though the range was found to be similar. These observations were found to be less than the upper limit of the reference interval used in the lab (Fig. 2, 3).

Fig. 2. Graphical representation of measurement of total protein in men and women and changes occurring with age



Legend : Open diamonds and open circles refer to men and women respectively. Filled squares represent the reference ranges used in the laboratory. Dotted lines represent the 2.5 and 97.5 percentiles. Unbroken lines represent the median.

Fig. 3. Graphical representation of measurement of albumin in men and women and changes occurring with age



Legend : Open diamonds and open circles refer to men and women respectively. Filled squares represent the reference ranges used in the laboratory. Dotted lines represent the 2.5 and 97.5 percentiles. Unbroken lines represent the median.

Table 2. Comparison of reference ranges for men obtained in our study with the reference range used at our lab, St. John's Medical College Hospital, Bangalore

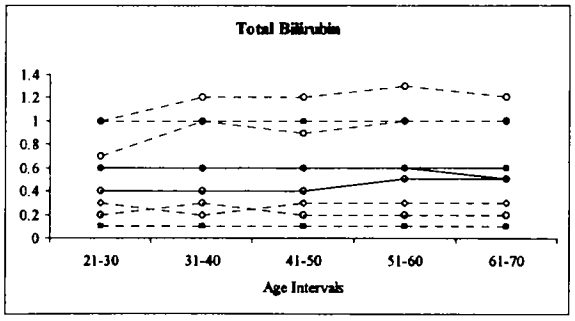
Age Interval (years), Median / Reference Interval	Reference range used in the lab	21-30	31-40	41-50	51-60	61-70
No. of Individuals (n)		69	79	125	119	72
Total protein, g/dL	6.0-8.0	7.7 / 6.6-8.6	7.7 / 6.7-8.3	7.7 / 6.9-8.3	7.7 / 6.6-8.6	7.7 / 6.6-8.2
Albumin, g/dL	3.2-5.5	4.2 / 3.5-4.7	4.3 / 3.6-4.7	4.2 / 3.7-4.7	4.2 / 3.4-4.5	4.1 / 3.6-4.5
AG Ratio	1.0-1.5	1.1 / 0.7-1.7	1.2 / 0.7-1.5	1.1 / 0.8-1.7	1.2 / 0.7-1.6	1.1/ 0.7-1.5
Total Bilirubin, mg/dL	0.1-1.0	0.6 / 0.3-1.0	0.6 / 0.2-1.2	0.6 / 0.3-1.2	0.6 / 0.3-1.3	0.5 / 0.3-1.2
Conjugated Bilirubin, mg/dL	0.0-0.4	0.3 / 0.1-0.4	0.2 / 0.1-0.4	0.3 / 0.1-0.4	0.2 / 0.1-0.4	0.2 / 0.1-0.3
AST U/L	Up to 37	24 / 16-39	25 / 14-42	24 / 15-43	25 / 14-44	24 / 11-40
ALT, U/L	Up to 65	38 / 28-67	43 / 27-86	45 / 30-82	44 / 25-86	38 / 26-64
ALP, U/L	50-140	91 / 60-128	88 / 56-148	92 / 59-147	91 / 60-134	91 / 56-130
GGT, U/L	11-50	29 / 17-67	34 / 15-75	41 / 21-78	34 / 19-74	33 / 18-51

The median and the reference range for Total Bilirubin was observed to be higher for men in comparison to women. The concentration of conjugated bilirubin was found to be similar in both sexes (Fig. 4, 5).

The reference range for AST in both sexes was found to be wider than the reference range used in the laboratory. The Median for AST was slightly higher in case of men than the women (Fig. 6).

The reference range for ALT in men was found to be wider in age groups from 20 to 60 years. However, the range was similar to the reference range used in the laboratory in the age group 61 to 70 years. The reference range for ALT in women was similar to the reference range in the laboratory in the age group 20 to 50 years while it was found to be wider in the age group 51 to 60 years. But in 61 to 70 years age group, the reference range was similar to the one utilized in the laboratory (Fig. 7).

Fig. 4. Graphical representation of measurement of total bilirubin in men and women and changes occurring with age

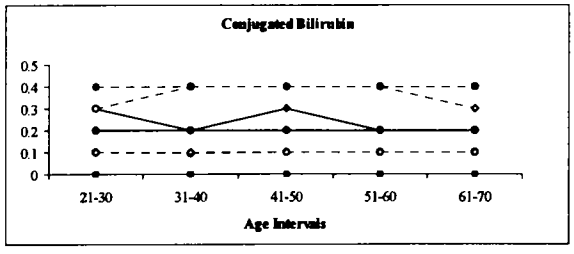


Legend : Open diamonds and open circles refer to men and women respectively. Filled squares represent the reference ranges used in the laboratory. Dotted lines represent the 2.5 and 97.5 percentiles. Unbroken lines represent the median.

Table 3. Comparison of reference ranges for women obtained from our study with the reference range used at our lab, St. John's Medical College Hospital, Bangalore

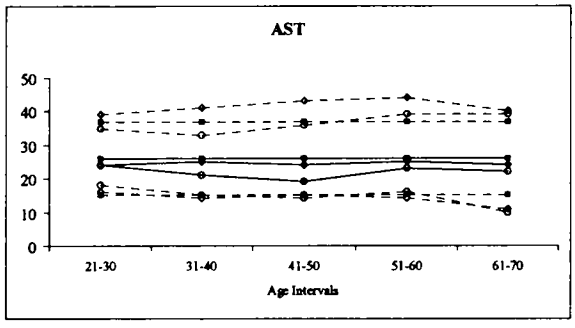
Age Interval (years), Median / Reference Interval	Reference range used in the lab	21-30	31-40	41-50	51-60	61-70
No. of Individuals (n)		38	41	38	47	36
Total protein, g/dL	6.0-8.0	7.6 / 6.8-8.1	7.4 / 7.0-8.3	7.5 / 6.6-8.5	7.8 / 6.6-8.8	7.6 / 6.8-8.0
Albumin, g/dL	3.2-5.5	4.4 / 3.8-4.5	4.0 / 3.2-4.6	4.1 / 3.6-4.6	4.2 / 3.3-4.6	3.9 / 3.5-4.4
AG Ratio	1.0-1.5	1.4 / 1.0-1.6	1.3 / 0.8-1.6	1.2 / 0.9-1.4	1.1 / 0.7-1.5	1.0 / 0.8-1.6
Total Bilirubin, mg/dL	0.1-1.0	0.4 / 0.2-0.7	0.4 / 0.3-1.0	0.4 / 0.2-0.9	0.5 / 0.2-1.0	0.5 / 0.2-1.0
Conjugated Bilirubin, mg/dL	0.0-0.4	0.2 / 0.1-0.3	0.2 / 0.1-0.4	0.2 / 0.1-0.4	0.2 / 0.1-0.4	0.2 / 0.1-0.4
AST U/L	Up to 37	24 / 18-35	21 / 15-33	19 / 14-36	23 / 16-39	22 / 10-39
ALT, U/L	Up to 65	36 / 25-51	36 / 26-54	33 / 26-54	38 / 22-75	38 / 30-63
ALP, U/L	50-140	87 / 67-111	82 / 56-92	95 / 52-126	108 / 55-147	99 / 60-144
GGT, U/L	7-32	21 / 16-39	20 / 13-34	23 / 14-53	31 / 18-55	28 / 18-42

Fig. 5. Graphical representation of measurement of conjugated bilirubin in men and women and changes occurring with age



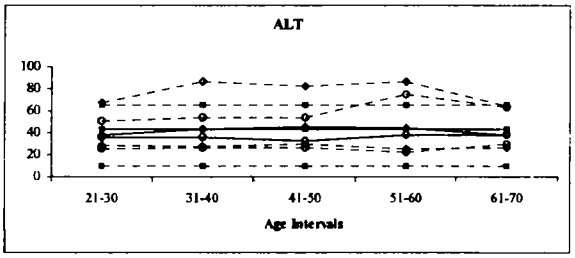
Legend : Open diamonds and open circles refer to men and women respectively. Filled squares represent the reference ranges used in the laboratory. Dotted lines represent the 2.5 and 97.5 percentiles. Unbroken lines represent the median.

Fig. 6. Graphical representation of measurement of AST in men and women and changes occurring with age



Legend : Open diamonds and open circles refer to men and women respectively. Filled squares represent the reference ranges used in the laboratory. Dotted lines represent the 2.5 and 97.5 percentiles. Unbroken lines represent the median.

Fig. 7. Graphical representation of measurement of ALT in men and women and changes occurring with age



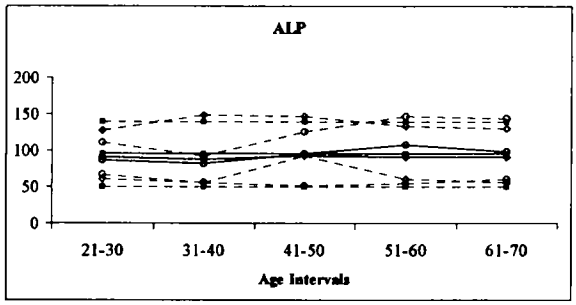
Legend : Open diamonds and open circles refer to men and women respectively. Filled squares represent the reference ranges used in the laboratory. Dotted lines represent the 2.5 and 97.5 percentiles. Unbroken lines represent the median.

Table 4. A comparison of reference material Lyphochek level 2 from BIORAD with the mean obtained in the lab and the everyday percentage of coefficient of variation

Analyte (Units)	Reference Mean	Mean obtained in the lab	Percentage of coefficient of variation
Total protein (g/dL)	4.2	4.00	4.2
Albumin (g/dL)	2.4	2.60	2.9
Total Bilirubin (mg/dL)	5.0	5.34	2.9
Conjugated Bilirubin (mg/dL)	1.66	1.70	8.8
AST (U/L)	200	195.93	7.1
ALT (U/L)	104	101.44	4.2
ALP (U/L)	394	358.19	3.8
γ-GT (U/L)	193	189.12	2.5

The reference range for ALP in men was found to be wider in the age group 31 to 50 years while it was similar in the other age groups when compared to the reference range utilized in the laboratory. In case of women, the reference range for ALP was wider in the age group 51 to 60 years while it was found to be similar in all other age groups (Fig. 8).

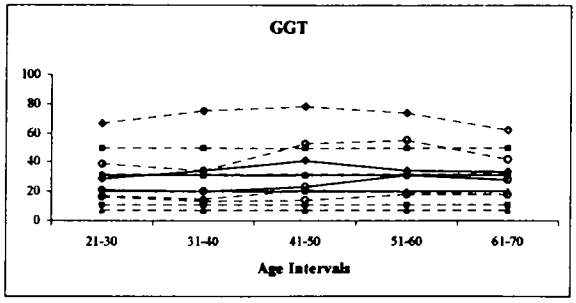
Fig. 8. Graphical representation of measurement of ALP in men and women and changes occurring with age



Legend : Open diamonds and open circles refer to men and women respectively. Filled squares represent the reference ranges used in the laboratory. Dotted lines represent the 2.5 and 97.5 percentiles. Unbroken lines represent the median.

The reference range was wider for γ-GT in men for all age ranges. The elevation was found to be more pronounced in the age groups 31 to 60 years. The γ-GT was found to be slighter wider in case of age groups 20 to 30 years and 61 to 70 years while in the other age groups it was found to have more pronounced elevation (Fig. 9).

Fig. 9. Graphical representation of measurement of GGT in men and women and changes occurring with age



Open diamonds and open circles refer to men and women respectively. Filled squares represent the reference ranges used in the laboratory. Dotted lines represent the 2.5 and 97.5 percentiles. Unbroken lines represent the median.

DISCUSSION

Reference intervals in the clinical chemistry are commonly based on the measurements in reference population which is representative of a defined group of individuals (1) (Fig. 1). The reference population that is thus identified might represent healthy individuals, non-healthy individuals without a disease, which is known to affect a particular property. In many cases military recruits, medical students or other more or less institutionalized, young and 'non-diseased' people have constituted the reference population. In those cases, the reference population is a poor representative of individuals seeking the health care and who might suffer from abnormal conditions.

The IFCC has published articles on the theory of Reference Values, which are the guidelines for the establishment of Reference Values (3). The recommended procedure to identify, collect and measure enough samples from a sufficiently large reference population is not feasible for most laboratories, which thus have to rely on literature data or manufacturers' insert sheets. Besides the fundamental publications by Grasbeck and Alstrom (2) and Harris and Boyd (4), numerous compilations have been published, either general or focussing on selected age groups. BUPA Medical Research in 1994 (16) published a comprehensive study. A detailed study of reference intervals pertaining to Swedish population related to age and sex which included the subjects in the lower age groups was carried out by Anders Kallner et al has been published (17).

In our study, we present an approach based on the assumption that physicians do not continue to request for repeat measurements, when the results have proved normal. The reference population of this approach represents an average outpatient who presents with symptoms that lead the physician in a certain direction that, however, cannot be verified by the initial laboratory investigation. The distribution of values will suffer from possible inconsistencies of the many physicians' opinions and decisions. Therefore the central 95 percentile is assumed to be wider than in a population which has stricter inclusion criteria.

The reference intervals that have been obtained using the present approach are in some cases similar to or identical with published reference intervals. The intervals are often wider than those given in the literature, which may be caused by the non-standard selection of subjects and physicians' inconsistent decisions and the collection of samples. However, the medians seem to tally in comparison with literature data.

Reference ranges for Total Bilirubin is wider because of distribution of values, which does not assume a symmetrical bell shaped curve. Instead it is a mildly skewed towards the right side (18), which is in agreement with the literature.

The wider reference range in case of liver enzymes could be due to non-uniformity in diet pattern of the individuals selected for the study and also could be due to mild amount of infiltration of lipid without any clinical evidence, which needs to be considered. The other shortcoming of the study is non-inclusion of age groups beyond 70 years and below 20 years. Further studies are being carried out to evaluate these age ranges.

Our study is representative of south Indian urban population of Bangalore city because amongst the 664 subjects selected for the study, 40 % of the individuals patients were from Karnataka proper settled in Bangalore. 20% of individuals from Kerala, 10% from Andhra Pradesh while 30% of individuals were from North settled in Bangalore. Since Bangalore is a cosmopolitan city, we were able to acquire data, which could be extrapolated to South Indian Urban population.

CONCLUSION

This pilot study ensures that there is a need for further study to establish the Reference Interval, according to the client population the institution caters to. This issue needs to be addressed on the following areas (a) the strategic statistical planning of the reference samples. (b) The establishment of reference values with appropriate methodology. (c) The determination of reference intervals which will demarcate critical care decision limits and could we extrapolate clinical sensitivity and clinical specificity from such a detailed study.

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