

Establishment of Reference Intervals of Serum Hepatic Enzymes in Tertiary Care Hospital: A Pilot Study

¹P Shruthi Rai, ²Sukanya Shetty, ³Priya Patil, ⁴Roopa Bhandary

ABSTRACT

Introduction: Reference intervals are the most common decision-making tools for the interpretation of various biochemical reports. Age, sex, ethnicity, diet, physical, and socioeconomic conditions affect the physiology of population. Hence, it is necessary to set up the reference values that are applicable to a specific population rather than using the reference values established for other population-based studies or from the literature provided by the reagent kit manufacturers.

Aims and objectives: To establish the reference intervals of serum hepatic enzymes in tertiary care hospital.

Materials and methods: Hospital-based cross-sectional study was done involving 200 subjects. The subjects were randomly selected from the population, which included medical students, health professionals, and those attending different outpatient departments and health camps organized by the hospital for general checkups under health plan scheme. Serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) were estimated by the International Federation of Clinical Chemistry-recommended kinetic method and GGT by enzymatic colorimetric method. To obtain reference interval, 97.5th percentile and 2.5th percentile were used.

Results: The results for serum AST and GGT were found to be statistically significant. Hence separate reference intervals for both male and female were set up. Generalized reference intervals were established for serum ALT and ALP levels.

Conclusion: The present study revealed a significant difference in the reference intervals between males and females.

Keywords: Alanine transaminase, Alkaline phosphatase, Aspartate transaminase, Gamma-glutamyl transferase, Hepatic enzymes, Reference interval.

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^{1,3}Postgraduate Student, ²Professor and Head, ⁴Lecturer

¹⁻⁴Department of Biochemistry, K.S. Hegde Medical Academy Mangaluru, Karnataka, India

Corresponding Author: P Shruthi Rai, Postgraduate Student Department of Biochemistry, K.S. Hegde Medical Academy Mangaluru, Karnataka, India, Phone: +919483922318, e-mail: shrupud26@gmail.com

INTRODUCTION

One of the most important tools in decision-making in clinical practice is clinical laboratory testing, a crucial process for diagnosis and screening of the disease. It also helps in monitoring of the disease progression and also to see the efficacy of the treatment. For accurate and precise interpretation of laboratory tests, one requires a very accurate reference interval from appropriate information.¹ The establishment of reference interval is done by assaying specimens from a particular group of people who satisfy well-defined criteria.

The quality of reference interval plays a major role in the interpretation of results as the quality of result itself. Various factors like age, sex, ethnicity, diet, physical, and socioeconomic conditions influence the physiology of any population. Therefore, it is mandatory to set up reference values which are applicable to that specific population rather than borrowing it from other population-based studies or by the ones provided by the reagent kit manufacturers.

Hepatic enzymes like alanine transaminase (ALT) and aspartate transaminase (AST) are the important markers of the hepatocyte injury and the concentration of these enzymes are elevated in hepatic disorders. Alkaline phosphatase (ALP) is the biomarker for the post hepatic obstructive lesions, and this is related as an increase in the serum ALP level. Although gamma glutamyl transferase (GGT) is considered as one of the biomarkers for alcoholic hepatitis, it is more specific for biliary obstruction.

Very few Indian studies have been done regarding reference intervals for biochemical parameters of liver function in Indian population. The present study was hence designed to study and establish reference interval of hepatic enzymes in Dakshina kannada population.

MATERIALS AND METHODS

This prospective hospital-based cross-sectional study was done at K.S. Hegde Charitable Hospital, Mangaluru, Karnataka, India, for a period of 2 years (2014–2016) involving 200 apparently healthy subjects (Clinical and Laboratory Standards Institute guidelines state that the sample number should preferably be at least 120).²

After obtaining institutional ethical clearance, the subjects were randomly selected based on the clinical

history and clinical examination, which included medical students, health professionals, and those who attended different outpatient departments and health camps organized by the hospital for general checkup, under the health plan scheme.

Subjects in the age group between 20 and 60 years, born and brought up in Dakshina kannada, and who were willing to participate in the study were included in the present study. Subjects below 20 and above 60 years with history of hepatic or renal diseases, diabetes mellitus, hypertension, cardiovascular diseases, intake of oral contraceptive pills, smokers and alcoholics, pregnancy, obesity, malabsorption syndromes, and subjects with nutritional anemia were excluded from the study.

Blood samples were collected randomly, after giving 15 minutes of physical rest,³ in sitting posture. In a plain red-topped vacutainer tubes containing clot activator, 2 mL of blood sample was collected from antecubital vein. The samples were left undisturbed for 30 minutes following which they were centrifuged for 5 minutes at 3000 rpm. Sera was separated and analyzed in Roche Hitachi c311 autoanalyzer.

Method of Analysis

All the samples were performed in duplicate. The precision of the instrument was checked on many occasions. All the analytical procedures are standardized, and the reagent was calibrated to the instrument before the analysis of the sample.^{4,5} Serum ALT, AST, ALP, and GGT were estimated by International Federation of Clinical Chemistry-recommended kinetic method⁶⁻⁸ and serum GGT by enzymatic colorimetric method.⁹

Statistical analysis was done using Statistical Package for the Social Sciences version 16. Normality of the data was determined using Kolmogorov–Smirnov test. Data were summarized using descriptive statistics (mean \pm standard deviation) for normally distributed data and median for skewed data. To obtain reference interval, 2.5th and 97.5th percentile were used. The comparison of reference interval between the genders was made using Mann–Whitney U-test; p-value <0.05 was considered to be statistically significant.

RESULTS

Of the 200 participants, 104 (52%) were males and 96 (48%) were females. The results of the reference interval of hepatic enzymes based on gender obtained are tabulated in Table 1.

The reference intervals of serum AST and GGT were observed to be statistically different in females compared with that of males. Accordingly, the reference interval

Table 1: Reference intervals for serum hepatic enzymes in the study population

Parameters (IU/L)	Gender (n)	Reference intervals (median)	p-value
Alanine transaminase	M (92)	5.3–47 (19)	0.07
	F (94)	4–45.3 (15.5)	
	M + F (186)	4.6–47 (16)	
Aspartate transaminase	M (97)	11–43 (22)	0.01
	F (94)	10.7–37.2 (19)	
Alkaline phosphatase	M (104)	53.6–139.3 (82)	0.264
	F (96)	38.5–138.5 (77.5)	
	M + F (200)	48–138.9 (80)	
Gamma-glutamyl transferase	M (95)	11.4–56.6 (25)	0.003
	F (88)	6.2–43 (19)	

n: number of subjects; p-value: Comparison between male and female subjects; M + F: Male and female combined; M: Male; F: Female

for serum AST was found to be 11 to 43 IU/L in males and 10.7 to 37.2 IU/L in females and for serum GGT it was 11.4 to 56.6 IU/L in males and 6.2 to 43 IU/L in females respectively. There was no significant difference in reference interval for serum ALT and ALP in females compared with males. Therefore single reference interval was considered. The reference interval for ALT is 4.6 to 47 IU/L and for ALP is 48 to 138.9 IU/L.

DISCUSSION

The present preliminary study gives a reference interval of liver function parameters of southern coastal Karnataka apparently healthy population belonging to the age group of 20 to 60 years of both the sex. In the study, 200 subjects were involved, among which 104 were males and 94 females.

The reference values of hepatic enzymes like serum AST and GGT showed gender difference in the present study. Serum AST and GGT values were found to be higher in the male population. Serum AST values in males are higher probably due to higher muscle mass found in males when compared with females. Therefore, in muscular disorders, the values are seen higher in males compared with females. Similar findings are observed in the studies done in Kampala, Kenya, Tanzania, and in US population.¹⁰⁻¹² The reference value of GGT was also found to be higher in males as compared with females. This observation of higher serum GGT in males may be due to the secretion of GGT from the prostate gland in males. The result generated in the present study is in agreement with the results reported by Saathoff et al.¹³ The predominant source for increased GGT activity is hepatobiliary diseases. Prostatic adenocarcinoma is associated with the increased levels of GGT in males.

The reference values for serum ALT and GGT observed in the current study are higher compared with

the kit values.¹⁴ The reference value obtained for serum ALP is lower compared with the kit values¹⁴ of Ugandan population.¹¹ These differences may be due to the ethnic variation in the study population.¹⁵

The serum ALT and ALP reference values did not show gender difference and hence single reference value has been followed for both males and females in the present study population. These findings are in contrast to the findings observed in other studies.¹⁶⁻¹⁸

LIMITATION

As the sample size is small and male to female ratio is less, the result generated in the present study cannot be directly generalized for the whole population. Hence, further study is needed with a larger sample size for the establishment of more accurate reference interval for our general population.

CONCLUSION

The reference intervals observed in this study showed difference in the reference intervals when compared with the ones from other sources. Gender differences in reference intervals of serum AST and GGT were observed. The reference values for the analytes determined in this study varied from the other population, indicating that there is a need for establishment of reference values that are applicable for a specific population, taking into consideration of different age groups and gender.

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