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Original Research Article

Establishment of gender specific reference intervals for hepatic enzymes: A pilot study

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ABSTRACT

Background: Reference interval is the common tools used for interpreting various biochemical parameters. The age, gender, diet, ethnicity, socioeconomic conditions affect the physiology of population and thereby affecting the reference intervals. Thus, it is needed to set up our own reference intervals which are specific to particular gender, region and population.

Aim and Objectives: To establish the gender specific reference interval for hepatic enzymes at BIMS Hospital, Belagavi.

Materials and Methods: This was a hospital based cross-sectional study involving 450 subjects. The selected subjects were from the general population, health professionals, medical students and those attending the Out-Patient Department (OPD) for general health check ups. The serum Alanine transaminase (ALT), serum Aspartate transaminase (AST), serum Alkaline phosphatase (ALP) were estimated based on the recommendations of IFCC without pyridoxal phosphate and serum Gamma glutamyl transferase (GGT) by enzymatic colorimetric method. The 97.5^{th} percentile and 2.5^{th} percentile was used to obtain the reference intervals.

Results: The serum AST and serum GGT showed the statistically significant difference between the male and female values. Thus separate reference should be considered for both males and females. There was no statistically significant difference in serum ALT and serum ALP levels. Thus generalized reference intervals were established.

Conclusion: The study revealed the difference in reference intervals of serum AST and serum GGT between males and females.

1. Introduction

Reference intervals are an important tools in clinical practice for making a decision, for screening and diagnosis of diseases, for monitoring of disease progression and for studying efficacy of treatment. One recquires an accurate reference intervals from the appropriate population to interpret the laboratory tests precisely.[1] Thus, the reference intervals should be obtained from a particular population who satisfy the well-defined criteria. The quality and interpretation of the laboratory reports often depends on the quality of the reference intervals because several factors like age, gender, diet, ethnicity, geographical area, socioeconomic conditions etc., influence the physiology of population. Therefore, it is compulsory to derive once own reference intervals which are applicable to specific population rather than using the reference intervals given by reagent kit manufacturers or by other population based studies.

Liver enzymes like AST(aspartate transaminase), ALT (alanine transaminase), ALP (alkanine phosphatase) and GGT(gamma glutamyl transferase) are important liver function tests and used routinely in clinical practice.[2] The enzymes like AST and ALT are markers of hepatic diseases and hepatocyte injury. The ALP levels are elevated in obstructive lesions of the liver and biliary tract.[3] Serum GGT is marker for alcoholic hepatitis and biliary

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obstruction.[3]

Indian studies on establishment of reference intervals in Indian populations are very few in number. And hence the present study is designed to establish the gender specific reference intervals of hepatic enzymes in BIMS hospital, Belagavi.

2. Materials and Methods

This was a hospital based cross-sectional study done at BIMS Hospital, Belagavi for a period of 2 years (Aug 2017-Aug 2019) involving 450 healthy subjects according to the guidelines given by Clinical and Laboratory Standards Institute.[4] Ethical clearance was taken from the institutional ethical committee before starting the study and informed consent was signed from all the subjects involved in the study. The study subjects were randomly selected based on the history and clinical examinations from the general population, health professionals, medical students and those attending the out patient department(OPD) for general health check ups.

2.1. Inclusion criteria

Subjects aged between 20-60 years, who were born and brought up in Belagavi district and who were willing to participate in the study.

2.2. Exclusion criteria

Subjects aged below 20 years and above 60 years, those who have history of liver diseases, renal diseases, diabetes mellitus, Hypertension, cardiovascular diseases, alcoholics, smokers, pregnancy, intake of oral contraceptive pills or any medications, anemia, chronic diseases, malabsorption syndrome, obesity etc were excluded from the study.

Blood samples were collected under strict aseptic condition after giving sufficient (15mins) time for physical rest[5] and in sitting posture. The 2ml of blood sample was collected in a plain red topped vacutainers containing clot activator and were kept undisturbed for 30minutes following which they were centrifuged at 3000rpm for 5minutes. The serum was separated and analyzed using transasia XL 640 autoanalyzer.

2.3. Method of analysis

All the samples obtained were analysed in duplicates. The analytical procedures were standardised and reagent was calibrated to instrument before sample analysis.[6,7] The instrument was checked for precision on several occasions. The serum Alanine transaminase (ALT), serum Aspartate transaminase (AST), serum Alkaline phosphatase (ALP) were estimated based on the recommendations of International Federations of Clinical Chemistry (IFCC) kinetic method without pyridoxal phosphate[8–10] and serum Gamma glutamyl transferase (GGT) by enzymatic olorimetric method.[11]

Statistical analysis was done using SPSS software 25 and normality of the data was determined by using Kolmogorov-Smirnov test. The descriptive statistics(mean +/- standard deviation) was used for summarizing the normally distributed data and median was taken for skewed data. 2.5^{th} and 97.5^{th} percentile were used to obtain the reference intervals. A p-Value <0.05 was considered as statistically significant.

3. Results

Out of 450 study subjects, 46%(207) were females and 54% (243) were males. The gender specific reference intervals for hepatic enzymes are given in Table 1.

The reference intervals for serum AST was 10.6-36.3 IU/L in females and 10-44 IU/L in males. Serum GGT levels was 6.1-42 IU/L in females and 10- 57 IU/L in males respectively. This shows a statistically significant difference in the reference intervals between males and females for serum AST and serum GGT levels. But the same was not seen in ALT and ALP. The reference intervals for ALT is 4.5-46 IU/l and ALP reference intervals were 47-137.8 IU/L respectively. There was no significant difference in the reference intervals between the males and females for both ALT and ALP. Hence, a single reference interval considered.

4. Discussion

This preliminary study gives the gender specific reference intervals for hepatic enzymes in north Karnataka population specifically in Belagavi district healthy population in the age group 20-60 years. The study included 450 healthy subjects having 207 females and 243 males. The present study showed the gender difference for AST and GGT. The reference interval values were found to be lower in female population than in male population. The lower AST levels in female are probably due to lower muscle mass in female compared to male. Thus the values are always higher in males, in muscular disorders than in females. Kenya, Kampala, In US population and Tanzania studies similar observation were found.[12–14]

The serum GGT reference intervals was also lower in females when compared to males. This is due to the secretion of GGT from the prostate gland in male population. Thus, serum GGT shows higher values in males when compared to females. This observation, goes in par with Saathoff et al.[15] prostatic adenocarcinoma and hepatobiliary diseases causes the increased serum GGT levels in males than in females. Also, the reference values obtained in the present study are higher when compared with the Kit values. The reason may be difference in ethnicity and geographical variation in the study population because the kit reference intervals were obtained for the

Table 1: Gender specific reference intervals for hepatic enzymes in study polpulation

Parameters (IU/L)	Gender (n)	Reference intervals (median)	p -value
Aspartate transaminase (AST)	M(97)	10-44 (23)	0.01
	F(94)	10.6- 36.3 (20)	
Gamma glutamyl transferase (GGT)	M(95)	10-57 (25)	0.003
	F(88)	6.1-42 (19)	
Alanine transaminase (ALT)	M(92)	5.2-48 (19)	0.07
	F(94)	4-44.6 (15.6)	
	M+F(104)	4.5-46 (16)	
Alkaline phosphatase (ALP)	M(104)	53-138.8(78)	0.264
	F(96)	37-137.3(76.9)	
	M+F(200)	47-137.8 (80)	

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

study done in Ugandan population. [10,16]

However, the serum ALP and ALT levels did not show any gender differnces and hence a single reference interval value can be used for both male and female in present study population. The finding in present study were in contrast with the finding of other studies. [17–19].

5. Limitation of the Study

1. Small sample size.
2. Male : Female ratio less.
3. Cannot generalize for whole population.
4. Hence, a study is needed in a larger population for establishment of accurate reference intervals to apply for general population.

6. Conclusion

1. The study showed the gender differences in the reference intervals of aspartate transaminase and Gamma Glutamyl transferase. This finding was different from the other studies.
2. The reference interval levels obtained in the present study was higher when compared with the values of studies used to frame kit values.
3. Therefore, a larger study is needed in a specific population for establishment of reference intervals in that particular population.

7. Source of Funding

None.

8. Conflict of Interest

None.

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