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Original Research Article

An Attempt to Revise and Update the Reference Interval of Lipase in the Population Attending a Teaching Hospital in Eastern India

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Abstract

Background: As per the proposal of ISO: 15189: NABL -112& CLIA guidelines, a laboratory should ideally establish its own reference intervals for all the parameters specific to the population it serves. Accordingly, a project was taken up to revise and update the reference interval of lipase in the population attending the departmental clinical lab.

Material & Methods: This observational, cross sectional database study was carried out at Department of Biochemistry in a teaching hospital. 126 individuals were included in the study provided they satisfied the inclusion and exclusion criteria.

Results: On statistical analysis of the compiled data, it was found that the values of lipase ranged from 47.96 U/l – 51.79 U/l(95% confidence interval) with a mean was of 49. 87 U/L. Skewness was found to be 0.990 and kurtosis 1.572 which assured the normal distribution of healthy study population. No significant age and sex differences were found among males and females. Significance was considered at P value $\leq$0.05.

Conclusion: We found that the reference interval of the reagent manufacturers were set at a bit higher level for the population and it needs to be updated. This study also established reference interval of lipase did not show significant age and sex differences, suggesting that males and females share the same reference interval.

Keywords: Pancreatitis, amylase, lipase, Biological reference interval.

Introduction

The reference interval is an interval that, when applied to the population serviced by the laboratory, correctly includes most of the subjects with characteristics similar to the reference group to be identified as "healthy' and excludes the others. For most analytes, the lower and upper reference limits are assumed to demarcate the estimated 2.5th and 97.5th percentiles of the underlying distribution of values, respectively [1].

Usually, 95% of the observations of healthy individuals fall within these two thresholds. Accurate and reliable laboratory results and applicable reference intervals are essential for health assessment, disease diagnosis, treatment monitoring, and prognosis judgment [2]. Therefore, establishing an appropriate and reliable reference interval is essential for clinical laboratories.

At present, most laboratories' reference intervals are from industry manuals, textbooks, manufacturers or reagent instructions and these often have some limitations which require modifications. According to the Clinical Laboratory Standard Institute (CLSI) guidelines, established reference intervals should reflect the subgroup distinction, such as age, sex, etc. For particular groups, such as children and adolescents, older people, subgroup differentiation is essential.

The concentration of amylase and lipase are indicators to assess pancreatic function. The α-amylase being a nonspecific enzyme derived from the pancreas, salivary glands, along with other tissues, can catalyze the hydrolysis of glycosidic bonds in starch& glycogen molecules. Lipase offers a larger diagnostic window than amylase since it is elevated for a longer time and more specific for pancreatitis thus allowing it to be a useful diagnostic biomarker in early and late stages of acute pancreatitis. Several recent evidence-based guidelines recommend the use of lipase over amylase [3].

The reference interval of lipase used in the clinical laboratory in the Department of Biochemistry, CMSDH is <60 U/L. But in the last few years, we are frequently observed elevated values in healthy

individuals with no underlying pathology. So there was felt need to verify or revise the reference interval of lipase in this population, if required ,as per the proposal of ISO: 15189: NABL -112& CLIA guidelines [4].

Aims and Objectives

The aim of the study is to verify the reference interval of lipase in the population attending the hospital andto update the reference interval of lipase if required**.**

Materials & Methods

The study took place at Department of Biochemistry of College of Medicine & Sagore Dutta Hospital-a tertiary care teaching hospital from April 2021 to March 2022 after approval from the Ethics committee. It's an observational, cross sectional database study.

All the individuals attending the outdoor patient's department who were found to be healthy after thorough clinical examination (including anthropometry) and laboratory investigations were included in the study. Persons who matched the inclusion criteria within the stipulated time duration (12 months) were included in the study At the end of 12 months, the number of patients was found to be 126 .Fasting blood samples (5 ml) were collected in a clotted vial and assayed for amylase, lipase, liver function tests, lipid profile, thyroid profile, c-reactive protein, electrolytes, random blood sugar, and lactate dehydrogenase. A questionnaire for selection of individuals was set as per ICMR guidelines. Informed consent was obtained from each study subjects.

Inclusion Criteria

All healthy individuals, both males& females who satisfied the inclusion criteria as per ICMR guideline and aged between 18-70 years attending the OPD of College of Medicine & Sagore Dutta Hospital.

Exclusion Criteria

Individuals with a history of habitual alcohol intake, smokers, those with any other addiction/drug abuse like cannabis, opioids, sedative etc, or taking any medications affecting liver, pancreas like statin, NSAIDs, methotrexate, barbiturate, OCP were excluded from the study.

All patients with documentary evidence of any liver, renal, thyroid disorders, cardiovascular disorders, COPD, pancreatic pathology, gall bladder pathology and any acute/chronic inflammatory or febrile condition were excluded.

Standard Approach

The standard approach to set up a reference interval uses 120 healthy individuals from a reference sample group. The values obtained from the sample group are then analyzed statistically to determine the 2.5th and 97.5th percentiles that form the 95% of the values.

In any analysis of reference interval (RI) data, a visual plot of all the data, such as histogram, a stem-and-leaf plot and/or a box plot should be done first with the consideration that skewed values may be caused by diseased individuals in the sample. If three or more of the values lie outside the interval, then one cannot use the manufacturer's RI; instead, the lab should set up their own RI [5] [6].

A pilot study was performed over 2 months among all the healthy individuals befitting the inclusion & exclusion criteria & all the routine parameters found to be within normal limit. At 95% CI, the value of lipase level found within 44.65-55.86 U/L, with a mean of 50.25 U/L, skewness was 0.434 which was acceptable for a normal distribution. [7]. After the successful pilot study we formulated the appropriate hypothesis and performed the proper study for an one year duration.

Data Analysis

All the data obtained were analyzed by standard statistical software. Results were arranged in tabular & graphical forms.

Results

The lab is under well maintained internal quality assurance program as well as external quality control assurance scheme on monthly basis. All Z scores in the EQAS reports were within +/- 2 which is well accepted (vide table-1). That assures about accurate and precise testing of patients' sample by the lab.

Table 1: Z score and Bias Calculation of Lipase from EQAS sample for last 1 year

Sample No	True value(U/L)	Population means(U/L)	Abs bias (U/L)	Absolute bias%	Z score
1	47.2	46	1.2	2.6	0.39
2	91.3	119	27.7	23.2	-1.50
3	91.5	92.2	0.7	0.7	-0.07
4	61.7	65.9	4.2	6.37	-0.78
5	52	46.1	5.9	12.8	1.82
6	80.1	92.5	12.4	13.4	-1.29
7	103.1	119	15.9	13.4	-0.86

8	46.2	46.1	0.1	0.2	0.03
9	80.3	91.4	11.1	13.8	-1.01
10	107.1	117	9.9	8.48	-0.49
11	65.5	65.3	0.2	0.3	0.03
12	61	66	5	7.57	-0.98

There was no significant difference in the mean values according to age or sex difference in lipase concentration in the study population. (Vide table-2).

Table 2: One way ANOVA according to age and sex distribution in lipase data set

		Sum of Squares	df	Mean Square	F	Sig.
lipase	Between Groups	224.764	1	224.764	1.920	0.168
	Within Groups	14516.289	124	117.067		
	Total	14741.054	125			
age	Between Groups	53.647	1	53.647	0.231	0.632
	Within Groups	28798.488	124	232.246		
	Total	28852.135	125			

** Difference is significant at P </= 0.05.

Outliers (those values outside the range) found to be 10; 5 in the lower side and 5 in the higher side.

Table 3: Exclusion of outliers' lipase data set in the given population

			Case Number	Value
Lipase	Highest	1	113	93.90
		2	11	80.90
		3	96	77.00
		4	103	75.20
		5	104	73.20
	Lowest	1	99	29.40
		2	108	29.50
		3	13	32.00
		4	26	34.00
		5	123	37.00[a]

All the subjects under the study having lipase value within 47.96 U/l – 51.79 U/l considering 95% confidence interval. Mean was determined to be 49. 87 U/L. Skewness is found to be 0.990 and kurtosis 1.572. (vide table 4)

Table 4: Descriptive Statistics of lipase data set in the given population (with bootstrap adjustment)

Lipase			Statistic	Std. Error	Bootstrap			
					Bias	Std. Error	95% Confidence Interval	
							Lower	Upper
	Mean		49.8754	0.96744	-0.0200	0.9641	48.0235	51.8045
	95% Confidence Interval for Mean	Lower Bound	47.9607					
		Upper Bound	51.7901					
	Median		48.0000		0.1171	1.2212	45.4000	50.1500
	Variance		117.928		0.021	20.068	81.417	161.643
	Std. Deviation		10.85949		-0.03796	0.91939	9.02315	12.71388
	Minimum		29.40					
	Maximum		93.90					
	Range		64.50					
	Interquartile Range		16.20		-0.79	1.42	12.28	18.00
	Skewness		0.990	0.216	-0.061	.298	.405	1.494
	Kurtosis		1.572	0.428	-0.279	1.144	-.455	3.635

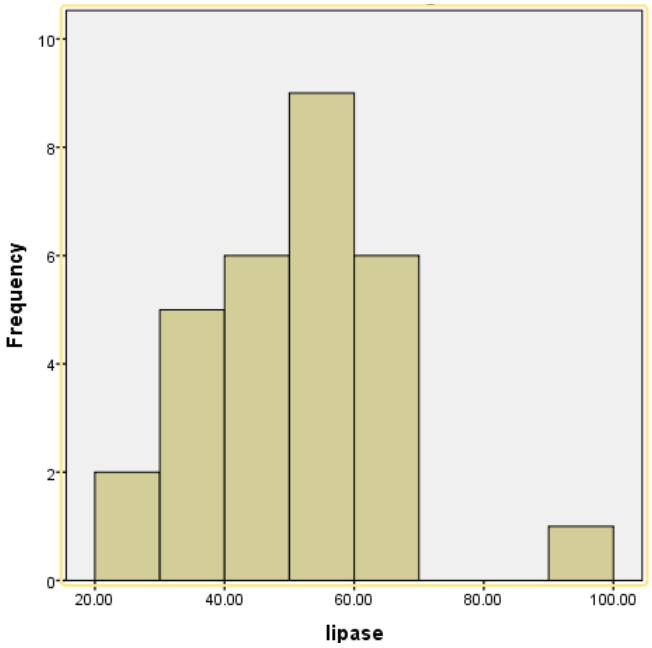
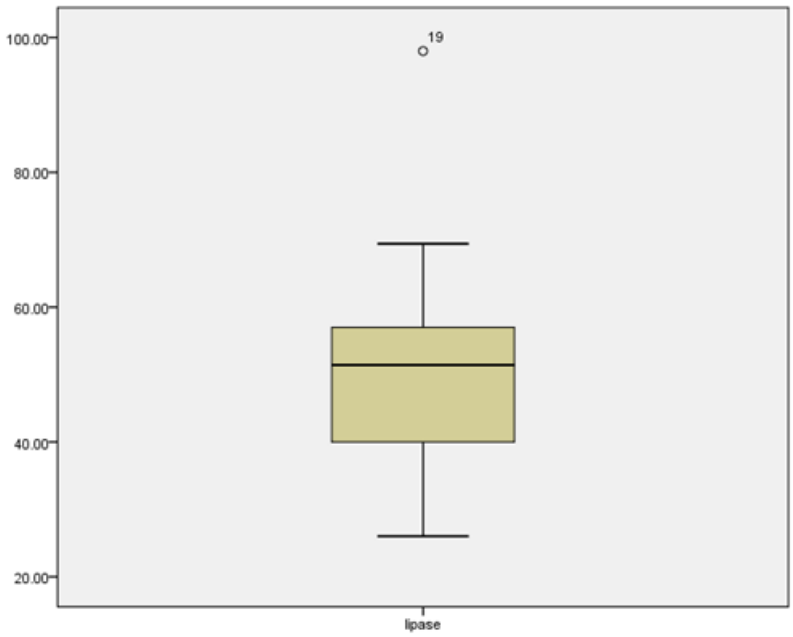


Figure: 1: Histogram showing frequency distribution of lipase



Median: 48, Upper extreme- 93.4, Lower extreme- 29.4, Upper quarantile- 64.2, Lower quarantile- 31.8.
Figure 2: Box and whisker plot displaying variation in Lipase data set among healthy individual.

Discussion

The establishment of reference intervals for any laboratory parameter is one of the trickiest tasks for laboratories because it is difficult to directly collect blood samples from healthy population. Here we've established serum lipase reference interval from 126 healthy populations attending the OPD of this hospital. This is a larger reference set than used in other studies of this type.

The standard diagnostic criteria of acute pancreatitis include clinical features with elevation of amylase &lipase within48 hours above threefold the normal range, assisted by radiological findings when necessary. During acute pancreatitis, serum lipase increases within four to eight hours, peaks at 24 hours, and remain elevated for one to two weeks. It is excreted by the kidneys. Thus, impaired renal function leads to an increased level of lipase. In routine practice, lipase levels can be useful for physicians in the detection of this disorder. So, a normal lipase level is relevant to rule out diagnosis of acute pancreatitis.[8] The results of this study confirmed that there was no age or sex difference in lipase concentration, which was consistent with the results of other studies

(vide table-2). The reference interval of serum lipase concentrations in adults established by the Nordic Reference Interval.

Project also did not show significant age and sex differences, suggesting that males and females share the same reference interval.[6]

The best approach to determine the reference range is by a multicentre study [9-11] by using samples from different centres. The samples should be a varied one, consisting of wide distribution of age, gender and race. However, this approach requires separate studies and therefore involves a huge amount of time, manpower and money. Thereby, it is often not feasible; so, most laboratories do not derive their own RIs and rely on manufacturer's values. In order to use manufacturer published RIs, one must verify that these values are applicable to the specific service population of the laboratory. This is often termed transference of reference values.

In this study, we estimated the serum lipase levels in different age and sex matched groups ranging from18-70 years in healthy population to verify whether the biological reference range established by the reagent manufacturers can be projected on the general population. Outliers found to be 10 in the lipase data set; 5 in the lower side and 5 in the higher side which fulfills the criteria of establishing the lab's own biological reference range for lipase as per standard approach. [5,6].

Next by descriptive statistics we found that all the subjects under the study had lipase values within 47.96 U/l – 51.79 U/l considering 95% confidence interval. Mean was determined at 49. 87 U/L. Skewness was found to be 0.990 and kurtosis 1.572 (vide table-4) which assured the normal distribution of healthy study population[7].

The range set by the reagent manufacturers was found to be a bit higher (≤ 60 U/L).[12] So we felt the reference interval for lipase in our lab needs to be updated for the population served by this lab. The new Reference Interval (RI) updated by the lab (≤50U/L) can be applied to the population it serves. Both the figures including Histogram and Box and whisker plot are in agreement with the normal distribution of the study population thereby adding validity to the present study (vide Figure1&2). It may be noted here that the new reference interval set up is less than the one suggested by the manufacturers, most likely due to the phenomenon of biological variation.

Limitations

It is suggested to perform the study with a multicentric approach in a larger population to impart it better credibility.

Conclusion

The authors found that the RI of the reagent manufacturers were set at a bit higher level for the population, and it required to be updated. The updated RI now stands at </=50 U/L considering 95% CI for the population it serves. This study also established that RI of Lipase did not show significant age and sex differences, suggesting that males and females share the same reference interval in this population.

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