Research Article

Lipemic index – a tool to measure lipemia

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Key words: lipemia, interference in analysis, lipemic index.

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Abstract

Introduction: Lipemia is represented as turbidity in the serum or plasma which becomes evident before the analytical process. It is mainly caused by large particles of lipoproteins such as chylomicrons or VLDL, the main lipid component of which is triglyceride. Lipemic interference is commonly found in routine clinical chemistry tests. It can, not only influence measurements of analytes, but can also cause false increase or decrease intheir levels. The aim was to use Lipemic index (LI) as an automated determinant of lipemia in venous blood specimens sent to our clinical chemistry laboratory and measure the extent of turbidity. **Methods:** The study was conducted in Clinical Biochemistry laboratory in the month of January 2016.Total of 809 samples were collected and lipemic index (LI) was estimated in autoanalyzer, transasia XL-640.LI values were categorized from L^- to L^{++++}.Percentage of sample in each category was calculated. **Results:** Most of our patients (68.23%) had LI <10,that is L^-.A considerable group (28.3%) were in L^+ range. Highest degree of lipemia was observed in 0.98% patients. Females had less turbid samples as compared to men. A greater proportion of women (58.7%) had LI <10 as compared to men (41.3%).From L^+ to L^{++++} ,men had higher LI as compared to women. **Conclusion:** Lipemic index estimation ensures that the sample is fit for analysis. The use of automated LI estimation overcomes the limitations associated with visual estimation by providing a more objective and accurate estimate of lipemia.

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Introduction

Interference is defined as “the effect of an endogenous substance present in the sample that alters the appropriate value of the result” by Kroll and Elin [1]. Analytical interference is a variation from the true value of the analyte caused by presence of some endogenous or exogenous material in the blood [1]. In the clinical laboratory setting, interferences can be a significant source of laboratory errors with potential to cause serious harm for the patient [2]. Lipemia is one among them. Lipemia interferes by scattering the light and disturbing the transmission of light through the reaction mixture. In lipemia there are a number of lipid components that causes scattering of light to produce a milky appearance or turbidity. The degree of light scattering depends on the number, size and refractive index of the suspended lipid particles. As patient serum samples are a mixture of various particle sizes, the sample appears white because the light is scattered at all angles.

Larger lipid entities such as chylomicrons and VLDL cause light to be scattered to the greatest degree. Chylomicrons constitute a diverse group of particles with varying sizes and vary from individual to individual. VLDL particles are a heterogeneous mixture of sizes and lipid content and the number of VLDL particles can be increased in various disease states. The interference can be either positive or negative depending on the blanking procedure of the assay.

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Lipemiaartificially increases values of the following analytes: glucose, phosphorus, bilirubin, uric acid, total protein,Hb_{A1c},fructosamine, triglyceride, D-dimer and decreases values of the following analytes : sodium, HDL cholesterol, ceruloplasmin, prealbumin, transferrin [1,3]. At high turbidity, no measurement may be possible due to the limits of the linearity of the spectrophotometer. Lipemia also interferes with the assays with volume displacement and optical clot detection methods. Such interference poses serious problem in the analytical process. Detection of spectral interferences is either by visual assessment or by an automated measurement of serum index which can be performed on most biochemical analyzers [4,5]. Since visual assessment is not accurate, it is preferable to assess with autoanalyzer.

Objective of the study is to assess the degree of turbidity by measuring lipemic index of the received samples in the clinical biochemistry laboratory for routine clinical investigations and to compare LI among both the genders.

Methodology

This study was conducted in the Department of Biochemistry, Karwar Institute of Medical Sciences. A total of 809 patient samples were collected in the month of January 2016, out of which 386 males and 423 female patients. Institutional ethics committee permission was sought to conduct the study. Blood samples were collected in the clinical laboratory in EDTA bottles, vacutainers or in syringes. Frequently samples were giving erroneous results due to lipemia. We used to assess the extent of lipemia by visual assessment which was not accurate. Visual detection is dependent on subjective assessment and unreliable as it may over- or under-estimate the exact amount of turbidity in the specimen. An automated serum index detection by photometric method has been implemented. We used Transasia XL -640, automated clinical chemistry analyzer in our laboratory that measures the degree of turbidity.

Principle of assay: The assay is based on calculations of absorbance estimation, of diluted samples at different bichromatic wavelength pairs to provide a semi-quantitative assessment of levels of turbidity in serum and plasma samples. The XL-640 analyzer takes an aliquot of the patient specimen and dilutes it with saline (0.9% sodium chloride to measure the absorbance for lipemia 660 nm (primary wavelength) and 700 nm (secondary wavelength). From these absorbance values the instrument calculates the serum index value for lipemia.

Statistical analysis was done by descriptive statistics.

Results

Lipemia was graded as L^- to L^{++++}. Samples were categorized in to different grades based on their lipemic indices and represented as percentage (Table 1).A comparative study of lipemic indices was done among males and females and percentage was expressed (Table 2). Percentage distribution of LI is represented in figure 1.
Majority of our patients (68.23%) had LI <10,that is L^-.A considerable group (28.3%) were in L^+ range. Highest degree of lipemia was observed in 0.98% patients. Compared to men, females had less turbid samples. A greater proportion of women (58.7%) had LI <10 as compared to men (41.3%). From L^+ to L^{++++}, men had higher LI as compared to women (Table 2).

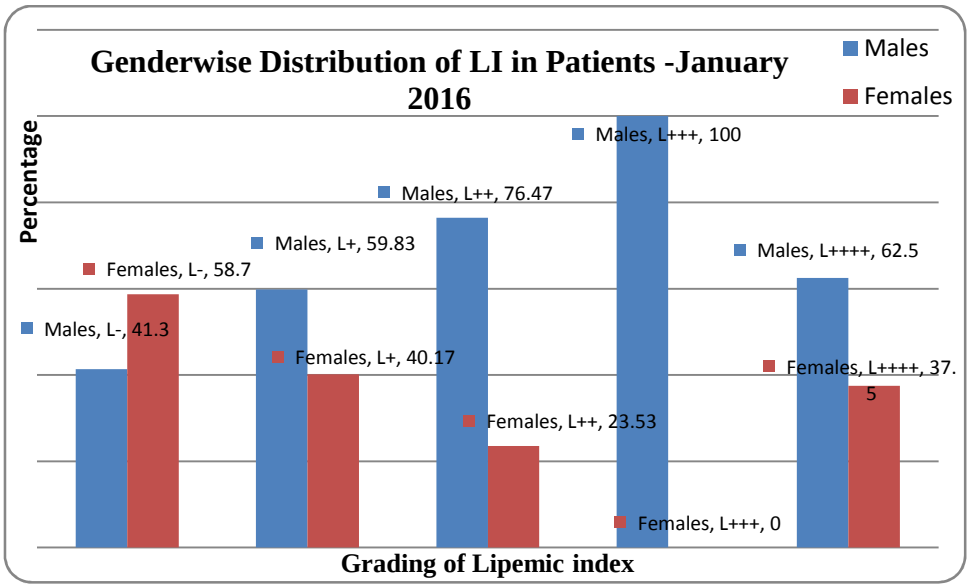
Table 1: Lipemic index in patient samples in the month of January 2016.

Lipemic index grading	Range	No. of samples	Percentage (%)
L^-	<10	552	68.23
L^+	10-20	229	28.31
L^{++}	20-30	17	2.10
L^{+++}	30-40	3	0.37
L^{++++}	>40	8	0.99
	Total	809	

Table 2: Gender wise distribution of lipemic index in January 2016

Lipemic index grading	Total samples	Gender wise distribution	
		Males	Females
L^{-}	552	228	324
L^{+}	229	137	92
L^{++}	17	13	4
L^{+++}	3	3	0
L^{++++}	8	5	3
	Total = 809		

Figure- 1: Percentage distribution of LI



Discussion

In our study majority of our patients (68.23%) had LI <10,that is L-.A significant portion of patients (28.3%) were in L^{+} range. Highest degree of lipemia ((L^{++++}) was observed in 0.98% patients.

Compared to men, females had less turbid samples. A greater proportion of women (58.7%) had LI <10 as compared to men (41.3%). From L^{+} to L^{++++}, men had higher LI as compared to women. The high lipemic index in men might be justifiable by the fact that blood samples were more turbid in males due to higher triglyceride levels [6]. Lipemia becomes visible if the concentration of triglycerides in patient sample is above 3.4 mmol/L [7]. In the full blood samples, visual detection is difficult to perform and can be observed at much higher concentration of triglycerides (over 11.3 mmol/L) [7].

Because of that, lipemia of the full blood sample often remains undetected. This was demonstrated by Salvagno*et al.* in a research to determine frequency of lipemia in full blood arterial samples received to laboratory for blood gas analysis [8]. So analysis of lipemic status with the help of automated analyzer is proved to be more accurate and useful.

Advantages of automatic detection are low cost, high speed, increased reproducibility and shortening of turn-around-time. However, there are some disadvantages as well. False positive results can occur in the presence of sample turbidity that might not be because of lipids, but due to the presence of other components. There are several articles describing falsely elevated LI with low lipid values due to the presence of paraproteins in the sample [9,10].

Lipoprotein particles in the sample absorb light. The concentration of absorbed light is inversely proportional to the wavelength and decreases from 300 to 700 nm, without any specific absorption peaks in between [11].

Therefore, methods that use lower wavelengths are more affected by lipemia, because the absorbance is the highest in that part of the spectra. Many clinical chemistry methods like ALT , AST and glucose use the reaction, NAD(P)+ ↔ NAD(P)H + H+ as an indicator reaction for determining concentration of the analyte. As the change of absorbance is measured at 340 nm, most of these methods are strongly affected by lipemia.

The problem of interference of lipemia can be overcome by several ways. Ultracentrifugation is one among them which removes lipemia and allows measurement of various analytes [12,13]. But limitation of the method is non-availability of the instrument due to high cost. Extraction or dilution of the sample is the other methods to reduce turbidity.

Lipids can be extracted using polar solvents. Some laboratories still use manual protocols with polyethylene glycol or cyclodextrin [14], while this principle is now utilized in commercially available kits. But this method cannot be followed as all the parameters cannot be recovered fully after treatment with such solvents.

Sample dilution may be the simplest method to remove interference with lipemia.

Conclusion

Lipemic index estimation is the systematic way of ensuring that the sample is fit for analysis. The use of automated LI estimation overcomes the inherent limitations of classical visual estimation by providing a more objective and accurate estimate of lipemia. It guides the measures to be taken to reduce the turbidity of the sample so as to ensure error free measurement of the analyte.

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