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Research Article

Effect of lipemia interferences in routine clinical Biochemical Tests (Intralaboratory Study)

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Abstract

Introduction: Lipemic specimens are common and frequent, but yet unresolved problem in clinical chemistry, and may produce significant interferences in the analytical results of different biochemical parameters. The aim of this study was to examine the effect of lipid removal using ultracentrifugation and LipoCleare treatment of lipemic samples, on some routine biochemistry parameters. Material and Methods: The samples obtained daily in the laboratory, the ones which were visibly muddy were selected and underwent to a process of LipoClean treatment and ultracentrifugation, being determined a variety of biochemical tests before and after investigationn. A total of 150 samples were studied. Results: Using the ultracentrifugation we found greatest differences in the concentration of alanin amino peptidase (10.6%) and the smallest one in the concentration of glucose (0.61%). Clinical interferences were found for urea, creatinine and calcium(those generally lead to significant errors in the interpretation of laboratory results).Results showed no significant differences in analyte values before and after LipoClean treatment, except for the total proteins, cholesterol and triglycerides. Conclusion: The individual laboratories most is quantify interference from lipemia for their specific methods and instruments, as the interference could be analyzed and/ or reagent-specific. Only if there is significant interference should the use of lipid clearing agents be considered. LipoClear does reduce lipemia but most methodologies are often sufficiently robust to avoid interference from lipemia.The percentage change in the concentration of different analytes before and after ultracentrifugation in hyperlipidemic sera never exceeded the total allowable error, significant differences in all parameters were found except for total bilirubin, glucose, and AST. This is the importance of proper treatment of lipemic samples and the significance of interferences in preanalytical phase and in the whole process of biochemical analysis of serum samples.

Keywords:Lipemia, Ultracentrifugation, LipoClear, Biochemical Tests, Interferences

INTRODUCTION

The data from the most representative studies show that preanalytical errors represent more than half of the total errors which occur in the clinical laboratory (Glick and Ryder, 1987) and within this type of errors, for its importance, it is remarkable for the quality of the samples to analyze (Brady and O'Leary, 1994; O'Leary et al., 1992). Analytical interference is a deviation from the true value of the analyte caused by presence of some endogenous or exogenous substance.In the clinical laboratory setting, interferences can be a significant source of laboratory errors with potential to cause serious harm for the patient (Glick and Ryder, 1987).

Lipemic sera are often found in the practice of clinical laboratories and can cause significant interferences in the analytical results of different biochemical parameters(Kroll and Elin, 1994).The establishment of the concentration of lipids compounds which produce signifcant interference depends on the analyzer, reagents, analytical method and the concentration of interfering constituents that are measuring (Glick et al., 1986). The overall frequency of lipemic

samples ranges from 0.5-2.5%, depending on the type of hospital and proportion of in-patient and out-patient samples (Glick and Ryder, 1987; Glick et al., 1986). However, in the outpatient unit, lipemia was the leading cause of unsuitable samples with the frequency almost 4-fold higher than in hospital patients (Simundic and Topic, 2008). Most errors occur in the pre-analytical phase of clinical laboratory testing (Lippi, 2009; Plebani et al., 2006). Successful monitoring and management of pre-analytical sources of interferences is therefore crucial to the quality of laboratory diagnostic process and to the quality of patient care. Results from lipemic samples may be inaccurate and can lead to medical errors, and as such represent a considerable hazard to patient health. Modern clinical chemistry analyzers are equipped with automated systems for detection of lipemic, icteric, and hemolyzed samples. With continuous technological development and laboratory automation, a significant reduction in laboratory errors can be expected (Simundic and Topic, 2008). However, in laboratories that do not use automated systems for detection and management of pre-analytical interferences, unsuitable samples are detected by means of visual inspection by individual laboratory personnel. Visual inspection is not only time consuming, but also highly subjective, non-standardized and may be a potential source of error (Simundic et al., 2009). Lipemia is reported to interfere in many routine assays. Many reagent suppliers provide information on the effect of lipemia in their assays, but this is often vague, not quantified and may not be instrument-specific. Lipemia interference is also due to increased light scatter and the absorption of the light by the lipids (mainly chylomicrons and very low density lipoproteins) in the spectrophotometric methods. This phenomenon causes a decrease in the intensity of light reaching the solution, which will be absorbed, so the turbidity most likely affects the photometric methods than the non-photometric methods (Dimeski, 2008).Both chylomicrons and VLDL particles producethis phenomenon, but in both cases the particles are very heterogeneous and there is an enormous variation in their size and triglyceride content, so the direct measurement of triglyceride content does not show good correlation with the phenomenon of light scattering. Moreover, the turbidity of the samples was very weakly correlated with the concentration of triglycerides present in the sample (Simundic et al., 2009; Twomey et al., 2003).The interference from lipemia can be minimized in a number of ways, including the use of a sample blank reading, kinetic analysis, changing the wavelength at which the reaction is read to one at which there is minimal absorbance from the interfering (Glick et al., 1986; Kroll and Elin, 1994) and the use of commercial preparations that clear the lipid content from serum. In the laboratory setting, staff use different methods such as visual inspection, lipemia index, serum indices and triglyceride concentration to determine the degree of turbidity from lipemia. These assessments, however, may be inaccurate as the degree of interference from lipemia is method and instrument-dependent. Lipemia may interfere with tests which use transmission of light as part of their measurement system. The interference caused by lipemia is due mainly to three distinct mechanisms: light scattering, increasing non-aqueous phase and effects of partition between polar and non polar phases (Simundic and Topic, 2008).

The aim of this study, sera with a high content of triglycerides (visibly turbid), but in different concentrations were used and these samples were subjected to an ultracentrifugation process to clarify the samples. Ultracentrifugation separates lipid complexes, preferentially larger, less dense (chylomicron) and VLDL particles, being both located on the top and we have determined the concentration of the different routine biochemistry parameters in the samples before ultracentrifugation and after ultracentrifugation. Also same sample evaluate the effects of lipemia and LipoClear (a non-toxic polymer for serum lipid clearance) on 10 tests commonly analyzed on the Dimension RL MAX analyzer (Simens, Germany), prior to the introduction of LipoClear into our routine laboratory repertoire.

MATERIAL AND METHODS

Sample

This preliminary study was performed in the biochemistry laboratory of the Clinical centre in Tetovo (Macedonia), from January to June of 2013. This is a tertiary hospital, that receive on routine analysis about 300-350 samples daily of which 5-7 samples are visibly turbid (1.6-1.7%). Among all of these routine samples, we selected the samples which were visibly turbid and after their centrifugation (3.000 x g for 15 minutes) aliquots were obtained, one of which was used to determine appropriate parameters, another was further subjected to the ultracentrifugation process, and third was treatend with LipoClear(phiTec International, UK). The process by which samples were subjected involved ultracentrifugation at 40.000 x g and $^{+}$4 °C, without adjustment of density T-1080 Ultracentrifuge (Kontron AG, Switzerland).

A total of 10 analytes were measured in up to 150 serum samples with varying degrees of lipemia (mean serum triglyceride 6.89 (range 1.35-24.4 mmol/L) using methods recommended for use by the instrument manufacturer (Table 1). Each analyte was determined before and after ultracentrifugation and treatment with LipoCleare.

METHODS

The analytical parameters determined were the following: cholesterol, triglycerides, aspartate amino transferase (AST), alanine aminotransferase (ALT), total bilirubin, total calcium, creatinine, glucose, urea and total protein in a Dimension RL MAX biochemical analyzer (Simens, Germany). Analytical methods employed for each of the biochemical parameters that have been determined are presented in table 1. The maximum number of samples analyzed was 120 for glucose,urea, creatinine and total proteins.

Statistical analysis

Normality was tested for each variable. Variables that were distributed normally were presented with arithmetic mean and standard deviation. To compare the average values of the various parameters measured before and after being subjected to ultracentrifugation parametric and LipoClear treatment, Student t test for paired data (for normally distributed data) and nonparametric Wilcoxon test (for triglycerides) were used. Percentage of change was calculated for each analyte before and after ultracentrifugation and Lipocler treatment compared to desirable inaccuracy according to data published in the literature (Anderson et al., 2003).

RESULTS

A total of 10 analytes were measured in up to 150 serum samples with varying degrees of lipemia (mean serum triglyceride 6.89 (range 1.35-24.4 mmol/L) using methods recommended for use by the instrument manufacturer (Table 1).

Table 1. Analytical methods used in the Dimension analyzer (Siemens, Germany) and analyte range

Analyte	Reagent supplier	Method	Analyte range
Urea,mmol/L	Siemens	Kinetic, urease	0 – 53.5
Creatinine, µmol/L	Siemens	Kinetic, Jaffe	0 - 1768
AST,U/L	Siemens	IFCC	0 - 1000
ALT,U/L	Siemens	IFCC	0 - 1000
Trigliceride, mmol/L	Siemens	Enzymic end point	0.17 – 11.3
Cholesterol, mmol/L	Siemens	Cholesterol oxidase	1.3 – 15.5
Glucose,mmol/L	Siemens	Hexocinase	0 - 27.8
Bilirubin, µmol/L	Siemens	Diaso	2 - 428
Calcium, mmol/L	Siemens	o-cresolftralein	1.2 – 2.9
Total protein, g/L	Siemens	Biuret	59-138

With the exception of alanine transaminase (ALT) and AST , significant differences in the other analyte values before and after treatment with LipoClear were seen using standard statistical techniques (Table 2). When analytical CV was taken into account total protein, cholesterol and triglyceride showed significant analytical change (Table 2).

Table 2. Mean and median analyte results before and after LipoClear treatment

Analyte	Number tested	Value before lipid extraction	Value after lipid extraction
Urea	120	6.25+/4.0	6.51+/-4.75
		(4.3-8.7)	(4.8-9.5)
	120	128.3+/-139.0	135.4+/-142.0
Creatinine		(89-167)	(93.0-175.0)
	150	32.4+/-8.0	33.0+/-9.5
AST		(24.0-40.0)	(23.5-42.5)
	150	33.8+/-10.0	34.2+/-12.0
ALT		23.8-43.8)	(22.5-46.8)
	150	7.75+/-2.3	3.12+/-1.95***
Trigliceride		(5.3-16.5)	(1.22-5.02)
	150	6.85+/-2.9	3.82+/-1.6***
Cholesterol		(3.5-9.8)	(2.2-5.4)
	120	7.25+/-1.6	7.55+/-2.2
Glucose		(5.65-8.85)	(5.45-9.85)

Continuation of Table 2

	150	27.3+/-6.0	25.9+/-9.5
Bilirubin		(21.3-33.3)	(16.4-35.4)
	150	2.25+/-0.15	2.20+/-0.16
Calcium		(2.1-2.40)	(2.12-2.3)
	120	69+/-19.0	59+/-7.1*
Total protein		(50.0-88.0)	(48.9-63.1)

*p<0.05;***p<0.001;

The results of the various serum parameters measured by spectrophotometric methods before and after samples were subjected to ultracentrifugation are presented in Table 3.

Table 3. The concentration of the different parameters before and after subjected to ultracentrifugation

Analyte	Number tested	Before, native sample	Ultracentrifuged sample	Difference %
Urea	120	6.25+/-5.38	6.40+/-5.6	2.42*
Creatinine	120	128.3+/-113.6	131.4+/-118.1	3.95*
AST	150	32.4+/-37.2	31.8+/-38.0	1.85 n.s.
ALT	150	33.8+/-45.4	30.2+/-46.1	10.6*
Trigliceride	150	7.75+/-0.9	5.92+/-0.6	23.6**
Cholesterol	150	6.85+/-1.91	6.35+/-5.95	7.29**
Glucose	120	7.25+/-3.21	7.15+/-4.2	0.61 n.s.
Bilirubin	150	27.3+/-24.3	26.9+/-28.1	1.46 n.s.
Calcium	150	2.25+/-0.45	2.35+/-0.24	4.45*
Total protein	120	68.0+/-6.4	70.3+/-7.3	1.88 n.s

*p<0.05;**p<0.01;

In this table the mean concentration before and after ultracentrifugation and the number of specimens analyzed is expressed as well as the median, the standard deviation for each parameter and the percentage change in the mean values. Aside from the expected changes in the concentration of triglycerides (23.6%) and cholesterol (7.29 %), the greatest difference in the parameters analyzed was found for ALT (10.6%). Minor changes were found in the concentration of calcium (4.45%) creatinine (3.95%), urea (2.42%), total protein (1.88%), total bilirubin (1.46%), and AST (1.85%). Glucose with less than 0.61% difference was the least affected parameter.

When the values of various parameters measured before and after ultracentrifugation were compared, significant differences were observed in all cases

except for total bilirubin ,glucose,total proteins and AST. In the rest of the parameters we obtained significant differences with a significance level of $p < 0.01$ except for creatin in which had a significance level of $p < 0.05$.

DISCUSSION

Methods for removal lipids of the samples include ultracentrifugation (the gold standard), high speed centrifugation and lipid clearing agents, mainly Lipoclear(Anderson et al., 2003; Dimeski and Jones, 2011; Simundic and Topic, 2008). In this study when the values of various parameters measured before and after ultracentrifugation were compared, significant differences were observed in all cases except for total bilirubin, glucose,total proteins and AST. In the rest of the parameters we obtained significant differences with a significance level of $p < 0.01$ except for creatinin which had a significance level of $p < 0.05$. Studies by Brady and O'Leary and Jabbar et al. show that the majority of analytes are affected by hyperlipidemia (Brady and O'Leary, 1994; Jabbar et al., 2006). Steen et al. (2006) conducted a multicenter analysis of sixteen (16) Dutch clinical laboratories, evaluating the interference caused by hemolysis, hyperbiliru binemia and lipemia in the determination of thirty two (32) different analytes(Steen et al., 2006). On the basis of biological variation these authors suggested a cut-off value above which, clinically significant interference exists. They found clinically significant interference from lipemia in twelve (12) of the thirty two (32) analytes studied. According to Glick the differences between the samples with or without interference must be below 3%, when assessing the result of a sample for a short term follow up, and to monitoring a patient for a long-time(Glick and Ryder, 1987). This author can accept a maximum range equal to the objective of inaccuracy of the method, except for ALT, CK and GGT in which the change should not exceed 10% (Glick et al.,1986). Vermeer et al(2007). compared reducing lipemia by high speed centrifugation or treating sera with Lipoclear and irrespective of the methodology used found excellent recovery in most of cases, but using high speed centrifugation the recovery was unacceptable for total bilirubin and CRP and using Lipoclear the recovery was inacceptable for GGT, HDL cholesterol, cholesterol and CRP(Vermeer et al., 2007). In our

study under consideration the differences between the two measurements, native and ultracentrifugated samples did not exceed 10% and the total maximum error allowed has not been exceeded in any of the techniques. On the other hand, Anderson et al. using a clarifying agent Lipoclear, found no critical differences in the concentration of the analytes studied before and after treatment with Lipoclear, except in the concentration of total protein, phosphorus and an expected fall for cholesterol and triglycerides(Anderson et al., 2003). Most methodologies used on the Dimension analyzer appeared to be subject to statistically significant interference from lipemia when evaluated by standard statistical methods, but these do not consider the analytical imprecision of assays. When the analytical CV was taken into account, most of the differences failed to achieve critical significance. In these studies only total proteins, cholesterol and triglyceride values remained critically different after the addition of LipoClear.These findings support a previous study of LipoClear effects and were expected as LipoClear, a nonionic polymer, precipitates lipoproteins and phospholipids. Lipemia did not critically affect measurement of other analytes, probably because the Dimension analyzer performs an initial blank reading at the start of the reaction, supporting previous reports recommending the use of serum blanks in minimizing lipemic interference.

CONCLUSION

Although, the percentage change in the concentration of different analytes before and after ultracentrifugation in hyperlipidemic sera exceeded the total allowable error, significant differences in all parameters were found except for total bilirubin, glucose, and AST and a variation that exceeds the allowed desirable inaccuracy. It is the importance of proper treatment of lipemic samples and the significance of interferences in preanalytical phase and in the whole process of biochemical analysis of serum samples. LipoClear does reduce lipemia but most methodologies are often sufficiently robust to avoid interference from lipemia. The individual laboratories should quantify interference from lipemia for their specific methods and instruments, as the interference could be analyzerand/ or reagent-specific. Only if there is significant interference should the use of lipid clearing agents be considered.

Potential conflict of interest

None declared.

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