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Original Research Article

Evaluating effectiveness of ultracentrifugation for removal of interference caused by lipemia in estimation of amylase, urea, creatinine, glucose and uric acid

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ABSTRACT

Introduction: Lipemic samples are affecting sample integrity and may raise questions on accuracy of test results. To overcome lipemic interference ultracentrifugation, high speed centrifugation, lipid clearing agent, dilution with normal saline or sample blanking can be used. Out of these, ultracentrifugation is likely to cause least interference as there is minimal exposure to additional chemicals.
Aims and Objectives: The study was conducted to compare the results obtained for some commonly asked biochemical parameters, before and after ultracentrifugation and to assess the need of removal of lipemia using ultracentrifugation as a tool for these parameters.
Materials and Methods: From 50 lipemic samples, 2 aliquots were prepared each having 500 microliter of serum. Out of these 2, one was used to perform amylase, urea, creatinine, glucose and uric acid. From Second aliquot, same parameters were performed after ultracentrifugation and comparison of results obtained, was done by Student's t-test. Moreover, ratio of % lipemic bias to CLIA criteria, biological variation and % coefficient of variance of laboratory were also calculated to see whether the difference is clinically significant too.
Results: Statistically significant difference between lipemic and ultracentrifuge samples was observed in amylase, urea, creatinine, uric acid and glucose while only glucose and uric acid showed clinically significant difference.
Conclusion: Lipemia causes statistically significant interference in estimation of amylase, urea, creatinine, glucose and uric acid and ultracentrifugation is very effective process in removal interference caused by lipemia. When this statistically significant interference was studied in the light of clinical need, only glucose and uric acid showed clinically significant interference. Laboratories not equipped with ultracentrifuge, can use kinetic method, two point kinetic method or at least two reagents method as alternative.

1. Introduction

Healthcare system is very complex and it involves the multiple service providers across the various disciplines of medicine. To achieve favorable and desired health outcome of patients, timely and correct clinical decision has to be taken by the treating physician, which in turn depends on test reports generated by a laboratory which is used by clinicians either, to confirm or exclude diagnosis, to select, optimize and monitor treatment or to provide prognosis.[1] So, it is vital to reduce errors in testing, in every step including preanalytical, analytical and post analytical phase. Traditionally, laboratory errors were identified as analytical problem but nowadays due to advancement in medical technology and automation in clinical laboratories, errors in analytical and post analytical process has reduced to great extent but still, pre-analytical errors represent more than half of the total errors which occur in the clinical laboratories.[2–7] Out of various factors which affect pre analytical phase, lipemia, hemolysis and icterus are

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endogenous factors which cause interference and adversely affect sample integrity and test results. Though not encountered frequently, lipemic samples poses problems to the clinical laboratories, particularly in establishing good clinical practice.[6,8–10]

Lipemia seen in post prandial sample or in samples of patient receiving total parenteral nutrition (TPN) can be avoided by repeat sample at other time but, repeat sampling may not always get clear serum specially, when lipemia is caused by chronic diseases like diabetes mellitus (DM) particularly type 1, insulin resistance, metabolic syndrome, pancreatitis, hypothyroidism and chronic renal failure.[8,9,11] So, to minimize the interference, laboratories have to develop standard procedures of removal of lipemia from the same sample rather asking repeat sample at other time, which is also more cost effective and practical approach. Lipemia causes analytical interference in estimation of various biochemical parameters by various mechanisms not necessarily, all affecting measurement of all analytes. Light scattering and light absorption by lipid compounds mechanism particularly affect photometric assay while volume displacement mechanism affect electrolyte analysis by indirect ion selective electrode (ISE) method.[7,8,10,12]Figure 1. Other mechanisms of interference include, partition between polar and non-polar part and binding of lipophilic substance to lipoproteins affect immunoassay. For clearing of lipemia, various methods can be used like dilution with normal saline, lipid clearing agent, high speed centrifugation and ultracentrifugation. Sample blanking can also be used as an alternative method to minimize the interference.[8,9] As there is no addition of chemicals as in lipid clearing agents, ultracentrifugation, physical removal of lipemia is likely to cause least interference and hence in this study ultracentrifugation was used as a tool to remove lipemia and an attempt was made to assess the need of removal lipemia using ultracentrifugation in some biochemical parameters.

2. Aims and Objectives

The study was conducted to compare the results obtained for some of commonly asked biochemical parameters, before and after ultracentrifugation and to assess the need of removal of lipemia using ultracentrifugation as a tool for these parameters.

3. Materials and Methods

This study was conducted in the clinical biochemistry service laboratory of tertiary care center and teaching hospital in Gujarat from September 2011 to May 2013 after obtaining approval from Institutional Human Research Ethics Committee (HREC). The laboratory, on an average, receives 200 to 300 samples daily for routine analysis. Out of these samples, total of 50 visibly turbid samples even after routine centrifugation were selected. Samples were included in the study in a manner that it does not reveal the identity of patient. Those samples which simultaneously presented with hemolysis or icterus were excluded. Two aliquots, each containing five hundred microliter of sera were prepared from lipemic samples out of which one was used to perform amylase, urea, creatinine, glucose and uric acid. Analysis was done using fully automated biochemistry analyzer Erba XL-640 by Transasia. Table 1 Second was used to carry out ultracentrifugation at 30000 rpm for 10 minutes at 37000 g at + 4^0 C in a SORVALL Discovery M120 Ultracentrifuge and same parameters were performed from the infranatant, carefully collected in separate aliquot. In case of inadequate serum, two subsequent samples having inadequate sera were mixed. Whenever required, sera were stored at -40^0 C not exceeding two weeks. Analysis and comparison was done by paired Student's t-test in Microsoft Office 2007. Further, ratio of lipemic bias to criteria of clinical laboratory improvement act (CLIA), biological variation and % coefficient of variance of laboratory were used to find whether statistically difference is clinically significant too.

4. Results and Discussion

Average results of various analytes, with and without exposure to ultracentrifugation were calculated and comparison of results was done using Student's t-test. Table 2 shows statistically significant difference for amylase, urea, creatinine, glucose and uric acid between pre and post ultracentrifuge samples. ($p - Value < 0.05$).

Later to find whether statistically significant difference is clinically significant too, ratio of lipemic bias to criteria of clinical laboratory improvement act (CLIA),[13] biological variation and % coefficient of variance of laboratory were studied.

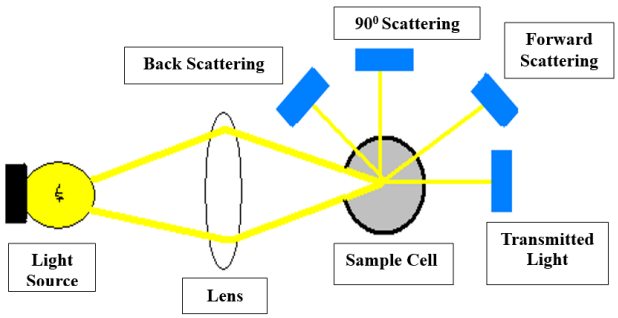

Fig. 1: Light scattering mechanism of lipemic interference

Percentage of lipemic bias was calculated using the formula, % lipemic bias = [{average of lipemic samples} / {average of ultracentrifuge samples}] x 100. To calculate % biological variation, biological variation values were obtained from randox internal quality assessment scheme.

Table 1: An alytical methods used for various parameters done in fully auto-analyzer Erba XL-640

Parameter	Principle	Method
Amylase	pNP Maltotriose substrates 37°C	Kinetic
Urea	Urease GLDH	Two point
Creatinine	Jaffe two point	Two point
Glucose	GOD-POD end point	End point
Uric Acid	Uricase peroxidase	End point

Table 2: Mean values of results in post and pre ultracentrifuge samples and p - value

Parameter	Examination		p – Value
	Post-ultracentrifuge	Pre-ultracentrifuge	
AMY	90.92	84.74	0.0027
URE	30.98	32.45	0.0175
CR	1.35	1.23	0.0000
GLC	144.46	181.46	0.0000
UA	4.29	13.24	0.000

Table 3 shows ratio of % bias to % biological variation of various examinations in ascending order. Lipemic interferences for amylase, urea and creatinine are not large enough to warrant clearing of lipemia because intra-individual variation is larger than the bias introduced by lipemia. While for glucose and uric acid the bias is significant enough as compared to biological variability. Hence, removal of lipemia has to be carried out for accurate and unbiased estimation of glucose and uric acid.

Similarly, % lipemic bias was compared with % CLIA acceptability criteria as shown in Table 4 and with twice the % coefficient of variance as shown in Table 5.

These ratios help considering these statistically significant biases in the light of biological variability of the analytes, laboratory precision and clinical need for accurate results. For example, in estimation of serum amylase, with wide biological variation and low clinical need for accurate and precise results, small bias introduced by the lipemia may not be clinically important and may not alter the clinical decision making or overall health outcome of patient. Thus, rather relying purely on statistics, lipemic interference must be evaluated in light of clinician's expectation for accurate result. This comparison, also made it clear that step of removal of lipemia is necessary for accurate results in estimation of glucose and uric acid.

As shown in Table 6 kinetic and two-point methods are less likely to be interfered by lipemia compared to end point methods. In present day automated analyzers, the sequence of reagent and sample mixing is reagent 1then sample and lastly reagent 2 is added. For end-point method reading is taken after addition of reagent but before adding sample so light absorption and light scatter caused by lipemic sera is taken in to account in the final reading which is the major cause of interference by lipemia. While in two point and kinetic method reading is taken after addition of sample so in final reading the interference caused by lipemia is minimized to great extent. Thus to minimize the lipemic interference it is advisable to use kinetic method, two point method or at least two reagents method if possible.

5. Limitations of the study

Ultracentrifugation is very effective method to remove lipemic interference but it not always possible for all laboratories to be equipped with the ultracentrifuge. Such laboratories can use high speed centrifugation, lipid clearing agents, dilution with normal saline, precipitation by polyethylene glycol, extraction of lipid with organic solvents to remove lipemia if required. Present study does not compare the effectiveness of removal of lipemia for these different methods. Some of the analytes like sodium, potassium and chloride were not included in the study due to limited availability of left-over lipemic serum and requirement of at least additional two hundred microliter of sample if they are to be included in the study. The study included samples with gross turbidity, so the effect of minor lipemia cannot be predicted from the study. Moreover, the study depended on visual index of lipemia, instead of measuring lipemic index in automated analyzer.

In present study two consecutive samples were pooled to have enough amounts of sera when ever insufficient quantity of sera was obtained in the laboratory. Disease profile of those two samples may not be same. Present study does not considered the effect of pooling of two different samples from different patient with different disease profile as lipoprotein composition every individual is not same and various disease also alter the lipoprotein composition of the patient.

6. Conclusion

Lipemia causes statistically significant interference in estimation of amylase, urea, creatinine, glucose and uric

Table 3: % bias in lipemic sample and ratio of % lipemic bias to % biological variation

Parameter	% Bias in Lipemic results	% Biological Variation (Source: RIQAS)	[%Bias]/[%Biological Variation]
AMY	7	15	0.5
URE	5	16	0.3
CR	9	8	1.1
GLC	26	7	3.8
UA	209	12	16.8

Table 4: % bias in lipemic sample and ratio of % lipemic bias to % CLIA acceptability criteria

Parameter	% Bias in Lipemic results	% CLIA Acceptability Criteria	[%Bias]/[%CLIA Acceptability Criteria]
AMY	7	30	0.2
URE	5	9	0.5
CR	9	22	0.4
GLC	26	10	2.6
UA	209	17	12.3

Table 5: % bias in lipemic sample, % coefficient of variance of laboratory and ratio of % lipemic bias to twice the % coefficient of variance of laboratory

Parameter	Laboratory %CV	2*CV% (95% probability)	% Bias in Lipemic results	[% Bias]/[2*CV%]
AMY	6.4	12.8	7	0.5
URE	6.1	12.2	5	0.4
CR	5.9	11.8	9	0.8
GLC	4.6	9.2	26	2.8
UA	6.7	13.4	209	15.6

Table 6: Method kinetics and various ratios

Parameter	Method Kinetic	%Bias]/[2*CV%]	[%Bias]/[%CLIA Acceptability]	[%Bias]/[%Biological Variation]
AMY	Kinetic	0.5	0.2	0.5
URE	Two point	0.4	0.5	0.3
CR	Two point	0.8	0.4	1.1
GLC	End point	2.8	2.6	3.8
UA	End point	15.6	12.3	16.8

acid and the ultracentrifugation is very effective process in removal interference caused by lipemia. When this statistically significant interference was studied in the light of clinical need, only glucose and uric acid showed clinically significant interference. For laboratory not equipped with ultracentrifuge, lipemic interference can be minimized by using kinetic and two point kinetic methods or at least two reagents method.

7. Source of funding

None

8. Conflict of interest

None

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