ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 10 | October - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

A NOVEL IN-HOUSE MATRIX-SYNCED METHOD OF OVERCOMING LIPEMIA-INDUCED INTERFERENCE AMONG SELECTED BIOCHEMICAL PARAMETERS, IN A SUPER SPECIALITY HOSPITAL OF KOLKATA

Clinical Biochemistry

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ABSTRACT

Introduction: Interference due to lipemia, accounts for a significant source of analytical errors in clinical laboratory settings, and necessitate removal prior to measuring biochemical parameters. We aimed to investigate whether such interference in serum/plasma samples can be removed using a method that is easier and more practicable than ultracentrifugation, the current reference method. **Methods:** A total of 134 serum samples were screened for two specific parameters, particularly vulnerable to lipemia-related interference; these being ALT (Alanine transaminase) and AST (Aspartate transaminase), from 01/04/2023 to 30/04/2023. In parallel, for overcoming lipemia, separate sera having albumin > 3.5 g/dL were pooled, to constitute our matrix-synced diluent. Lipemia was induced into serum samples where interference was to be studied, using intralipid solution (5 µL/250 µL serum). These samples were then diluted with our matrix-synced diluent, in 1:3 and 1:4 ratio and re-estimated for ALT and AST activities. The values thus obtained were then adjusted for the contribution by the pooled sera and the final values recorded. The data was analyzed by GraphPad Prism (v.8.0), considering a P value of ≤ 0.05 to be statistically significant. **Results:** Out of the 134 samples, 34 and 53 in 1:3 & 1:4 dilutions respectively showed positive and significant correlation (Spearman r=0.9556, P <0.0001; Pearson r=0.9990, P <0.0001), between ALT activity in non-lipemic serum and in lipemia-induced serum diluted with our matrix-synced diluent. A similar correlation (Spearman r=0.9432, P <0.0001; Spearman r=0.9764, P <0.0001), was found between AST activity in non-lipemic serum and in lipemia-induced serum in in 57 and 77 samples with 1:3 & 1:4 dilutions respectively. **Conclusions:** Our matrix-synced diluent thus proved to be a simple and effective strategy in overcoming interference dure to lipemia, w.r.t. ALT and AST activities, which are highly prone to give erroneous values in grossly lipemic samples.

KEYWORDS

Lipemia, Interference, Matrix-sync, Diluent

INTRODUCTION

Analytical interference is classically defined as any deviation from the true value of the analyte, often as a result of hindrance posed by the presence of some endogenous or exogenous substance [1]. In the clinical laboratory setting, such interferences can potentially translate into significant error in reporting of patient results and with serious repercussions with regard to patient welfare [2]. Unlike hemolysis where flagging out is far more common [3], lipemia tends to get overlooked, although the overall percentage of lipemic samples is not negligible; ranging between 0.5-2.5%, depending on the type of clinical setting and proportion of inpatient and outpatient samples [4,5,6]. This lipemia classically is the result of accumulation of lipoprotein particles with the greatest contribution by chylomicrons [7]. The lipemia increases absorbance of light and thereby decreases light transmittance; disrupting spectrophotometric analysis. Apart from short fasting time which is by far the commonest cause, a wide number of conditions such as diabetes mellitus, alcoholism, acute pancreatitis, total parenteral nutrition (TPN), are contributory to lipemia [8]. It is in fact these intractable and unavoidable lipemia that can pose as serious hindrance in routine testing of various biochemical parameters and result interpretation. Intractable lipemia common in critically ill patients receiving TPN (Total Parenteral Nutrition). Serum TG level >1000 mg/dL is known to interfere in reporting of several biochemical parameters. This is a common occurrence when sampling is done too soon after administration of lipid emulsions namely Intralipid solutions, common in neonatal units and intensive care set-ups [9]. It is recommended, draw the sample at least 5-6 hours after administration of Intralipid, to order to avoid lipemia [10]. Despite this precaution, in case of persistent and intractable lipemia, a number of strategies may be employed viz: centrifugation (ultra and high speed), lipid extraction (using polar solvents), sample dilution, and serum blank [8, 11, 12]. Of these, ultracentrifugation is a relatively expensive option and not feasible for many set-ups [13]. Commercially available diluents often prove to be unsatisfactory owning to matrix disparity vis-a-vis patient sera.

We hence developed a matrix synced diluent which could effectively remove lipemia induced turbidity while simultaneously ensuring that even after maximum dilution sample matrix remained unchanged. We zeroed in on serum with albumin > 3.5 g/dL. Such a choice was consciously made keeping in mind the inherent ability of albumin to bind triglycerides as well as free fatty acids efficiently, being their natural carrier while matrix compatibility would ensure freedom from undue bias and far better likelihood of achieving the true value.

MATERIALS AND METHODS:

Study variables:

The study was a hospital-based cross-sectional observational study, undertaken in the Department of Laboratory Medicine of CK-Birla Hospitals, CMRI & BM Birla Heart Research Centre, Kolkata, from 1st April 2023 to 30th April 2023. A total of 134 serum samples routinely sent to hospital laboratory of both institutes, for assay of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were selected, following set inclusion and exclusion criteria. In brief all visibly clear sera were included while all visibly hemolyzed, lipemic and icteric sera were excluded.

Selection of matrix-synced diluent:

For preparing an appropriate diluent capable of overcoming lipemia, while simultaneously possessing the same matrix as the lipemic sample, we selected patient sera with albumin > 3.5 gm/dL. The pooled sera were tested for the same biochemical parameters as that of the

samples under study i.e., ALT and AST.

Induction of lipemia into study samples
Lipemia was induced into serum samples where interference was to be studied, using commercially available, Intralipid solution (5 µL/250 µL serum). These samples were then diluted with our matrix-synced diluent, in 1:3 and 1:4 ratio and re-estimated for ALT and AST activities.

Ethical and safety considerations:
The study was conducted after obtaining clearance from Institutional Ethics Committee. For bio-safety purposes, pooled serum was subjected to full serological testing**,** prior to use. Since entire study was conducted from those samples which were sent to the hospital laboratory for testing of routine biochemical parameters, and no human subjects were recruited separately for the study, no informed consent from patients were taken separately.

Estimation of serum ALT & AST activities:
Serum activities of ALT and AST were estimated on Roche Cobas c 501 automated platform using manufacturer recommended reagents. Serial measurements were performed on neat samples, on pooled sera functioning as matrix synced diluent and, on the samples, spiked with commercially available, Intralipid solution (5 µL/250 µL serum).

Calculation of actual values of ALT & AST activities in lipemia induced samples diluted with matrix-synced diluent:
This involved first noting the activity of the enzyme in the neat (non-lipemic) sample. The activity was designated as **A0.** The enzyme activity in the lipemia-induced serum was designated as **A1** and the volume proportions for **1:3** & **1:4** dilution [serum to diluent] were **0.25 [V1]** and **0.20 [V1]** respectively. Likewise, the enzyme activity in the matrix-synced diluent was designated as **A2** and the volume proportions for **1:3** & **1:4** dilution [serum to diluent] were **0.75 [V2]** and **0.80[V2]** respectively. The final enzyme activity was thus summation of activities contributed by the lipemia-induced serum and that contributed by the matrix-synced diluent, designated as **A3** with the volume proportion being **1** [**0.25 + 0.75 or 0.20 +0.80** for **1:3** and **1:4** dilutions, respectively]. Hence, the calculation of the actual enzyme activity was based on the following formula

A1V1 + A2V2 = A3V3 or **A1 = (A3V3 - A2V2)/V1**

Prototype example (from study sample):
A0: ALT activity in neat sample = **45 U/L, A2:** ALT activity in matrix synced diluent = **22 U/L, A3:** ALT activity in mixture of lipemia-induced serum and matrix synced diluent in **1:3** ratio [serum to diluent] = **27 U/L.**

Applying the above formula: **A1** [actual activity in lipemic sample] = **[27x1 -22x0.75]/0.25 =42 U/L**

Hence the values of ALT activity prior to lipemia induction and post lipemia induction & its subsequent overcoming by our matrix-synced diluent, were quite compatible at **45 U/L** & **42 U/L,** respectively, exhibiting an agreement of 93.33%.

Below is the actual workflow adopted for 1:3 dilution:
Step1. Choose any patient sample with albumin > 3.5 g/dL (preferably ≥ 4 g/dL)

Step2. Run the above sample and note the value of parameter(s) which were showing flag in lipemic sample

Step3. Take 3 parts of this sample and 1 part of undiluted lipemic sample and mix well (Dilution factor 4)

Step4. Run the mixture in analyzer and note the value of parameter(s)

Step5. Multiply the value of parameter(s) obtained in Step 2. by 0.75

Step6. Subtract this value from value of parameter(s) obtained in Step 4.

Step7. Multiply the above value(s) obtained in Step 6. By 4 (to correct for dilution factor)

Step8. Report this value (s) obtained in Step 7, for the lipemic sample

A similar workflow was adopted for 1:4 dilutions, with the necessary numerical changes.

Statistical analyses:
The data generated was analysed by Graph-Pad Prism v.8.4.3, using appropriate statistical tools. The data were expressed as Mean ±S.D. and the normality of the sample distribution was tested by D'Agostino & Pearson omnibus normality test. For analyzing correlation Pearson or Spearman Correlation tests were used, as applicable. In all statistical analysis P value ≤0.05, was considered as statistically significant.

RESULTS:

1. Impact of using matrix-synced diluent on ALT activities of lipemic samples in ratio of 1:3 & 1:4 [sample to diluent]:
Out of the 134 samples collected, 34 showed similar test result for serum ALT activity both in non-lipemic samples and in the lipemia induced samples after treating them with the matrix-synced diluent, in 1:3 ratio [sample to diluent]. Likewise, 53 out of the 134 samples collected, showed result compatibility for serum ALT activity both in non-lipemic sample and in the lipemia induced sample after treating it with the matrix-synced diluent, in 1:4 ratio [sample to diluent]. **Table 1** summarizes the range along with the mean values obtained w.r.t ALT activity.

In addition, a positive and significant correlation [Spearman r=0.9556, P <0.0001; **Fig 1A**] was found to exist between serum ALT activities of the 34 non-lipemic samples and the same samples following lipemia induction and treating the matrix-synced diluent, in 1:3 ratio [sample to diluent]. A similar positive and significant correlation [Pearson r=0.9990, P <0.0001; **Fig 1B**] was found to exist between serum ALT activities of the 53 non-lipemic samples and the same samples following lipemia induction and treating the matrix-synced diluent, in 1:4 ratio [sample to diluent].

2. Impact of using matrix-synced diluent on AST activities of lipemic samples in ratio of 1:3 & 1:4 [sample to diluent]:
Out of the 134 samples collected, 57 showed similar test result for serum AST activity both in non-lipemic samples and in the lipemia induced samples after treating them with the matrix-synced diluent, in 1:3 ratio [sample to diluent]. Likewise, 77 out of the 134 samples collected, showed result compatibility for serum AST activity both in non-lipemic sample and in the lipemia induced sample after treating it with the matrix-synced diluent, in 1:4 ratio [sample to diluent]. **Table 2** summarizes the range along with the mean values obtained w.r.t AST activity.

In addition, a positive and significant correlation [Spearman r=0.9432, P <0.0001; **Fig 2A**] was found to exist between serum AST activities of the 57 non-lipemic samples and the same samples following lipemia induction and treating the matrix-synced diluent, in 1:3 ratio [sample to diluent]. A similar positive and significant correlation [Spearman r=0.9764, P <0.0001; **Fig 2B**] was found to exist between serum AST activities of the 77 non-lipemic samples and the same samples following lipemia induction and treating the matrix-synced diluent, in 1:4 ratio [sample to diluent].

DISCUSSION:

Lipemia is a very commonly encountered and recurrent interference faced in the clinical laboratory. In certain cases, it poses as an intractable condition that cannot be resolved merely by opting for a fasting sample [8]. According to the National Cholesterol Education Program Adult Treatment Panel (NCEP ATP III) guidelines, triglyceride levels exceeding 400 mg/dL, lead to visible turbidity and interference [14]. Lipemia usually poses maximum hindrance in assays that involve transmission of light as part of the detection mechanism, apart from causing volume displacement and adding to sample heterogeneity [8].

In view of this, we aimed to develop a method to resolve the headache of intractable lipemia. We hence developed a matrix synced diluent which could effectively remove lipemia induced turbidity while simultaneously ensuring that even after maximum dilution sample matrix remained unchanged. This was keeping in mind the disadvantage of commercially available diluents that do not offer matrix compatibility with serum, the latter being the commonest matrix for biochemical estimations. A serum sample containing albumin in sufficiently high concentration [> 3.5 g/dL], proved to be quite capable of overcoming the lipemia induced interference by its inherent property of binding free fatty acids, while maintaining matrix compatibility simultaneously. The advantage of lipemia dissolution by

albumin actually gives an edge over use of polar solvents like sodium chloride which do not work well in overcoming turbidity caused by the highly non-polar lipids viz triglycerides.

Using this matrix synced diluent, we aimed to study its ability to overcome interference due to lipemia induced artificially in patient sera. We chose to study the effect of lipemia on serum ALT and AST activities. This was a conscious choice as these enzyme activities are very commonly affected by lipemia [8].

Using the matrix synced diluent in two dilutions [1:3 and 1:4; sample to diluent], we obtained positive and significant correlations, between ALTand AST activities in non-lipemic serum and in lipemia-induced serum diluted with our matrix-synced diluent. Our matrix synced diluent proved to be quite capable of overcoming the interference induced by the injection of Intralipid solution (5 µL/250 µL serum), into the erstwhile non-lipemic patient sera, ensuring freedom from undue bias while at the same time proving to be capable of delivering the true value even with a high degree of dilution.

With regard to the important aspect of cost factor our matrix synced diluent being merely a patient sample in itself imposed no additional financial burden. This is in sharp contrast to use of ultracentrifugation which in contrast, is a much more expensive option and not feasible for many set-ups [13].

Apart from ALT and AST activities, lipemia can have a deleterious effect on the analytical sensitivity and specificity of various other biochemical parameters viz phosphorus, total bilirubin, uric acid, and total protein [15], while seemingly not affecting assays of glucose and albumin [16]. Thus, depending on the assay parameter, the dire necessity for overcoming lipemia is also enhanced.

CONCLUSIONS:

Our matrix-synced diluent with respect to ALT and AST activities, which are highly prone to give erroneous values in grossly lipemic samples. Such a diluent which poses no extra financial burden on the lab apart from ensuring effective dissolution of lipemia induced turbidity as well as matrix compatibility and resultant maintenance of result trueness even after high degree of dilution, can well be utilized to overcome a common and potentially intractable source of error in estimation of vital biochemical parameters.

Conflict of interest:

The authors declare they have no conflicts of interest.

Table 1: Serum activities of ALT [Alanine Aminotransferase] of recruited samples, after lipemia induction, followed by dilution with matrix-synced diluent in 1:3 and 1:4 ratio [sample to diluent]

Parameter with dilution	Sample number (n)	Ra; nge of activity (U/mL)	Mean ± S.D (U/mL)
ALT (1:3)	34	8 -542	69.85 ± 117.1
ALT (1:4)	53	6 -560	73.47 ± 111.7

Table 2: Serum activities of AST [Aspartate Aminotransferase] of recruited samples, after lipemia induction, followed by dilution with matrix-synced diluent in 1:3 and 1:4 ratio [sample to diluent]

Parameter with dilution	Sample number (n)	Range of activity (U/mL)	Mean ± S.D (U/mL)
AST (1:3)	57	12 -394	51.47 ± 72.75
AST (1:4)	77	10-392	59.38 ± 75.90

Fig 1A: Correlation of serum ALT levels in non-lipemic samples with levels in same lipemia-induced samples (1:3)

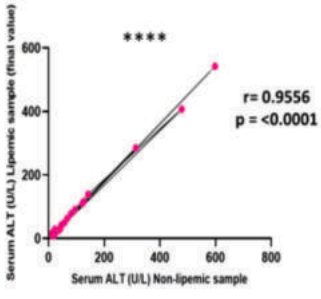


Fig 1B Correlation of serum ALT levels in non-lipemic samples with levels in same lipemia-induced samples (1:4)

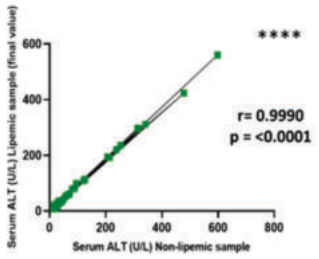


Fig 2A Correlation of serum AST levels in non-lipemic samples with levels in same lipemia-induced samples (1:3)

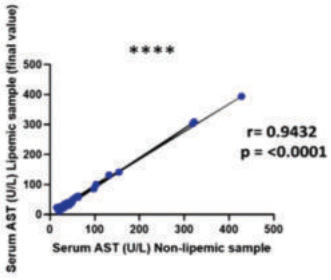
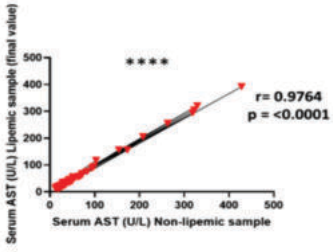


Fig 2B Correlation of serum AST levels in non-lipemic samples with levels in same lipemia-induced samples (1:4)



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