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ESTIMATION OF THE LEVELS OF CHOLESTEROL AND UREA IN LIPEMIC SERUM SAMPLES BEFORE AND AFTER ULTRACENTRIFUGATION – A CROSS SECTIONAL STUDY

Biochemistry

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ABSTRACT

Introduction : Lipemia may be defined as the presence of high concentrations of emulsified fat or fatty substances in the blood. It makes the blood look turbid after a lipid rich meal.

Objectives : The objective of the present study was to estimate the levels of urea and cholesterol in lipemic serum samples before and after ultracentrifugation.

Materials & Methods : This study comprised of 30 lipemic serum samples. All the samples were analyzed for urea and cholesterol values before and after ultracentrifugation. The differences in the results were analyzed by using paired t test.

Results : It was observed that the mean and standard deviation of serum urea obtained after ultracentrifugation were lower than that obtained before ultracentrifugation. The p value was less than 0.05. Similarly, the mean and standard deviation of cholesterol values obtained after ultracentrifugation were found to be lower than that obtained before ultracentrifugation. The p value was less than 0.05.

Conclusion : Therefore the study concludes that ultracentrifugation helps to eliminate lipemia to a minor extent in urea and to a significant extent in cholesterol and thus produces reliable results.

KEYWORDS

Urea, Cholesterol, Lipemia, Ultracentrifugation.

INTRODUCTION

Laboratory errors may be classified as pre-analytical, analytical and post-analytical with respect to their source and time of presentation. Analytical interference is a deviation from the target value of the analyte caused by the presence of some endogenous and exogenous substances[1]. Endogenous factors are the ones that originate within the body. Few of them are hemolysis, bilirubin, lipemia etc. Common causes for lipemia are: Diet, alcohol ingestion, diabetes mellitus, chronic renal failure, pancreatitis, multiple myeloma and drugs such as protease inhibitors, oral contraceptives[2].

Lipemia is the presence of fats in the bloodstream in excess. It renders the blood a milky appearance. The interference caused by lipemia is fundamentally different from those associated with hemolysis and icterus. In human beings, the plasma normally contains 0.5 – 0.8 grams of lipid per 100 cubic centimeters (cc) and lipemia originally appears when values above these figures but below 1 gram per 100 cc are reached[3]. Several laboratory methodologies may suffer lipemia interference. Spectrophotometry is the most affected one. Lipoprotein particles absorb the light inversely proportional to the wavelength used in the analysis[4]. The interference from lipemia may be reduced by a number of ways including, the use of sample blank reading, kinetic analysis, changing the wavelength at which the reaction is read to the one at which there is minimal absorbance from the interferant and the use of commercial preparations that clear lipid content from serum[5].

Urea is the end product of protein metabolism which is synthesized in the liver through the urea cycle. Increase in the plasma urea concentration beyond the normal range indicates uremic state. Causes for uremia are pre-renal, renal and post renal which could be due to various abnormalities. Cholesterol is a lipid containing a steroid nucleus. It is an integral component of the biological membranes. It serves as the precursor for steroidogenesis. Accumulation of cholesterol beyond the normal range leads to cardiovascular disorders. The objective of the present study was to estimate the levels of urea and cholesterol in lipemic serum samples before and after ultracentrifugation.

MATERIALS AND METHODS

This study comprised of 30 lipemic serum samples which were obtained from the Dr. Prabhakar Kore Hospital, Belagavi, Karnataka, India.

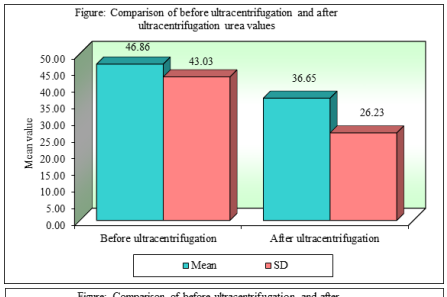
Visibly turbid serum samples were collected. They were first centrifuged at 3000 rpm for 15 minutes. Further they were divided into 2 aliquots. The first aliquot was used for estimating urea and cholesterol levels and the values were recorded. The second aliquot was subjected to ultracentrifugation at 40,000 rpm for 15 minutes. The supernatant thus obtained was discarded. Biochemical analysis of urea and cholesterol was performed on the infranatant and the values so obtained were recorded.

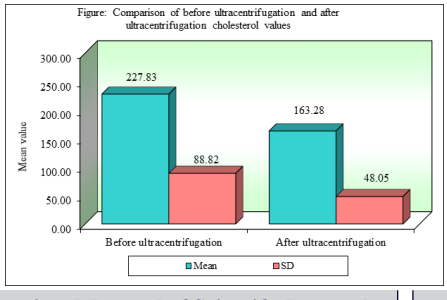
Urea was estimated by Glutamate Dehydrogenase (GLDH) – Urease method and cholesterol by Cholesterol oxidase - Peroxidase (CHOD - PAP)method using ErbaChem 7 Semiautoanalyzer.

The data obtained was analyzed statistically by computing descriptive statistics, the mean, standard deviation, paired t value and p value were calculated.

RESULTS

It was observed that the mean values for urea before and after ultracentrifugation were 46.86 mg/dl and 36.65mg/dl respectively. The p value was < 0.05. Also, the mean values for cholesterol before and after ultracentrifugation were 227.83 mg/dl and 163.28 mg/dl respectively and the p value was <0.05. This shows that there is a significant difference in the urea and cholesterol values obtained after ultracentrifugation.





DISCUSSION

Our study is in agreement with the study conducted by Calmarza P and Cordero J to see the difference in the levels of cholesterol, triglycerides, alanine transaminase, aspartate transaminase, urea, creatinine, iron, glucose etc. before and after ultracentrifugation. The greatest difference in the parameters analyzed was found for alanine transaminase, triglycerides and cholesterol. Glucose was the least affected parameter. Minor changes were found in the concentration of phosphorous, gamma glutamyltransferase, urea, creatinine, iron, total protein, uric acid, bilirubin, aspartate transaminase and calcium[2]. Sample turbidity from lipemia most significantly affects the photometric assays (end point, nephlometric or turbidimetric) due to light scattering and absorption of light by the lipids[6].

A study was conducted by Jabbar J, Raza SQ and Baig A to evaluate the effect of ultracentrifugation to eliminate lipid interference which suggested that, the removal of turbidity by ultracentrifugation is time saving, cost-effective and provides reliable results[7].

In a study done by Dimeski G and Jones BW to differentiate the effectiveness of lipid removal by high speed centrifuge with ultracentrifuge, the mean differences from the aliquots for the ultracentrifuged and high speed centrifuged sample pools were almost similar. The data obtained from the study confirmed that recentrifugation of the sample using high speed centrifuge is almost as effective as ultracentrifugation.

CONCLUSION

The study concludes that:

- The values of urea and cholesterol obtained after ultracentrifugation were lower.
- Therefore ultracentrifugation helps to eliminate lipemia to a minor extent for urea estimation and to a major extent for cholesterol and produces reliable results.

LIMITATIONS

The limitation of the present study was small sample size as lipemic samples are not routinely available.

CONFLICT OF INTEREST

None of the authors have potential conflict of interest

ETHICAL ISSUES

Ethical clearance was obtained from the institutional ethics committee.

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