Serum Indices – A tool to Measure Interfering Substances in Blood Samples

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ABSTRACT

Objectives: Aim of the study was to evaluate the degree of hemolysis, turbidity, and interference by bilirubin by measuring hemolytic, lipemic and icteric indices of the received blood samples in the clinical biochemistry laboratory for routine investigations. Methods: The study was carried out in the Clinical Biochemistry laboratory in January 2016. Total of 695 blood samples were collected and evaluated in autoanalyzer, transasia XL-640. Serum indices (SI) values were assayed and categorized based on their concentration. Percentage of the sample in each category was calculated. Results: Percentage of non-hemolyzed samples was minimum (0.58%), a majority of the samples were non-lipemic (68.3%), and 37% of samples were non-icteric, suggesting that maximum interference is due to hemolysis. The study results quantified the extent of interference by each interfering substance. Conclusion: Estimation of serum indices in an automated analyzer overcomes the limitations of visual estimation of interfering substances. It also provides a more objective and accurate estimation of the interfering substance.

Key Words: *interference in the analysis, hemolytic index, lipemic index, icteric index*

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INTRODUCTION

Studies have reported hemoglobin, bilirubin, lipids, and paraproteins are the four major endogenous compounds that interfere with laboratory results [1, 2]. Interference in assay by these endogenous or exogenous substances is an underestimated problem with potential detrimental effects on the patient [3, 4]. These interfering substances could be attributed to biological and analytical biases which ultimately lead to a compromise in the reliability of testing. This suggests that unsuitable specimens should be systematically identified so as to avoid unreliable or misleading test results. Recognition by color or turbidity of the specimen is proved to be unreliable. It is difficult to interpret the effect of these components on the report, because each sample needs to be visualized immediately after centrifugation. So automated determination of potential interference of hemolysis, hyperbilirubinemia, and turbidity came in to the picture. Serum indices (SI) is a tool which guides laboratory professionals about interferences, increases the quality of the sample, and minimizes aberrant test results.

Following a damage to cell membrane, hemoglobin and other intracellular components of RBCs are released into the surrounding plasma, which is termed as hemolysis [5]. It is one of the most common reasons for blood specimen rejection. It has been reported that hemolysis accounts for 40%–70% of unsuitable specimens sent to the clinical laboratory [5, 6]. The degree of unsuitability varies based on different methods used for analysis of hemolysis and also on various cut-off thresholds for analytical interference.

Prevalence of hemolysis was evaluated earlier by visual assessment which is subjective or by manual spectrophotometric techniques for analyzing free hemoglobin [7-14]. But now, it has been suggested that the estimation of hemolysis index (HI) in automated analyzers is a more efficient method for the detection of hemolysis. The hemolysis index, H, is semiquantitative and is linear up to1000 mg/dl.

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Interference by lipemia is due to scattering of light and disturbance in the transmission of light through the reaction mixture. A number of lipid constituents cause scattering of light in lipemia and produce a turbidity. No measurement may be possible at high turbidity because of 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 a serious problem in the analytical process. Hence, it is very much essential to assay the lipemic index.

The icteric index is the quantification of interference by bilirubin. It is measured in autoanalyzer without any reagent cost [15].

Objectives

The aim of the study was to evaluate the degree of hemolysis, turbidity, and interference by bilirubin by measuring hemolytic, lipemic and icteric indices of the received samples in the clinical biochemistry laboratory for routine clinical investigations.

METHODOLOGY

The study was carried out in the Department of Biochemistry, Karwar Institute of Medical Sciences. A total of 695 patient samples were analyzed in the month of January 2016, which consisted of 268 males and 271 female patients. Blood samples were collected in the clinical laboratory in EDTA bottles, vacutainers or sometimes in syringes in the government hospital settings.

Serum indices estimation was carried out by automated photometric method. Automated chemistry analyzer, Transasia XL -640 was used to measure the degree of interference. The serum indices were assayed on fresh samples along with requested investigations.

The principle of the assay is based on the calculations of absorbance at different bichromatic wavelength pairs of diluted samples. This provides a semi-quantitative representation of the degree of interference in serum and plasma samples. An aliquot of the patient specimen is diluted with saline (0.9% sodium chloride) by the chemistry analyzer to measure the absorbance for hemolysis at 570 nm and 600 nm, absorbance for lipemia at 660 nm and 700 nm, and the absorbance for icteric index at 480 nm and 505 nm, wavelengths being primary and secondary wavelengths respectively. Serum indices were calculated from these absorbance values by the instrument.

Approval from the institutional ethics committee was obtained to conduct this retrospective study.

RESULTS

The degree of hemolysis was denoted by HI, ranging from H0-H4. Lipemia was graded as L^- to L^{++++}. Samples were categorized into different grades based on their lipemic indices and represented as a percentage. Icterus was graded as I^- to I^{++++}. Samples were categorized in to different grades based on their icteric indices and represented as a percentage.

It was found that the majority of the samples were lysed to H1(52.7%) and H2(31.36%) (small to an intermediate degree). Non-hemolyzed samples percentage was minimum (0.58%) whereas marked hemolysis was 4.31% (Table 1).

Majority of patients' specimen (68.23%) had LI <10, that is L. A considerable proportion of samples (28.3%) were in L^+ range. The degree of lipemia was highest in 0.98% patients (Table 2). 37% of samples were non-icteric (I^-) whereas 18% of samples had a high icteric index (I^{++++}) (Table 3).

Table 1: Classification of Hemolytic Index

Degree of hemolysis	Range	Percentage of samples
H0	0-09	0.58
H1	10-199	57.4
H2	200-299	31.36
H3	300-399	6.33
H4	>400	4.31

Table 2: Categorization of Lipemic Index.

Lipemic index grading	Range	Percentage of samples
L-	<10	68.23
L^+	10-20	28.31
L^{++}	20-30	2.1
L^{+++}	30-40	0.37
L^{++++}	>40	0.99

Table 3: Categorization of Icteric index.

Degree of hemolysis	Range	Percentage of samples
I-	<50	37.22
I^+	50-60	17.97
I^{++}	60-70	15.27
I^{+++}	70-80	11.68
I^{++++}	>80	17.84

DISCUSSION

A majority of patients' samples had mild to moderate hemolysis with a minimal massive hemolysis. Percentage of non-hemolyzed samples were negligible. Thus, hemolysis was one of the major problems that has to be dealt to ensure accurate results.

A review by Lippi et al reported that improper specimen collections or handling may affect the specimen and cause

in vitro hemolysis [16]. In vitro hemolysis makes both outpatient and inpatient samples unsuitable for analysis. Hemolyzed specimens account for 40–70% of unsuitable specimens and hemolysis is one of the leading causes of assay interference [17, 18].

A mild hemolysis can even cause clinically significant variations in serum electrolytes, LDH and AST values, questioning the reliability of testing. Lysed RBCs release potassium, LDH, AST, magnesium and other components into plasma giving a pseudo-elevation. Analyte values which are falsely elevated in hemolysis are acetaminophen, ALT, NH3, AST, Phosphorus, CK, Potassium, Iron, UIBC, and cardiac troponin [19]. Hemoglobin also poses optical interference in addition to chemical interference with reagents or analytes.

Erroneous reports due to interferences may mislead patients' diagnosis and it may also question the authenticity of the clinical laboratory. This necessitates a corrective measure to be taken so as ensure accurate reports. Investigation has to be carried out to determine the underlying causes of erroneous reports. Technical or quality management system shall be identified and planned accordingly. The action plan needs to be planned, developed, implemented and monitored so as to reduce such nonconformities.

A significant proportion of our laboratory specimen (68.23%) had a LI <10, that is L^-. A majority of patients (28.3%) were in L^+ range. Degree of lipemia was highest ((L^{++++}) in 0.98% of samples.

Degree of lipemia depends on triglyceride concentration and it becomes visible if the triglyceride concentration in patients is above 3.4 mmol/L [20]. Visual detection of lipemia is difficult in whole blood sample to perform and it can be detected at a greater concentration of triglycerides (>11.3 mmol/L) [21].

Lipoproteins present in the blood specimen absorb light, the concentration of which is inversely proportional to the wavelength and decreases from 300 to 700 nm [15]. The methods that are based on lower wavelengths are more affected by lipemia, because the absorbance is maximum in that part of the spectra. Most of the clinical chemistry methods like transaminases and glucose use the reaction producing NAD(P)H as an indicator reaction for estimation of the concentration of an analyte. The change of absorbance is measured at 340 nm, and majority of the methods are affected by lipemia.

Advantages of automated estimation of LI is low cost, faster, enhanced reproducibility and minimum turnaround time.

The analytical interference by bilirubin may be caused due to spectral interference. The spectral properties of bilirubin absorbance in the wavelength between 340 and 500 nm. Bilirubinemia, based on its concentration, proportionately induces high background absorbance. Thus, it mainly interferes in spectrophotometric assays [15]. Another way of interference with the analysis is due to the chemical interference. Bilirubin may interact chemically with test reagents. Since bilirubin reacts with peroxidase-catalyzed reactions, H_2O_2 generated during the chemical reaction is utilized by bilirubin, thereby causing spuriously low results of creatinine, glucose, cholesterol, triglyceride and uric acid [22]. Bilirubin also causes underestimation in phosphorus measurement that uses a UV method for the detection of phosphate by formation with phosphomolybdate [23]. We have noted high icteric index values for samples with normal bilirubin. False positive results might be attributed to interference of other yellow pigments such as carotene or hemoglobin. The icteric index is technically simple and rapidity of measurement (less than 20 s) offer an additional advantage. No reagent presentation, preparation or stability, zero reagent cost are the added advantages of automated SI detection.

CONCLUSION

Automated serum indices assay is the systematic way of ensuring authenticity of the reports. It overcomes the inherent limitations of visual detection of interfering substances. It also provides a more objective and accurate assessment of interfering substances. SI values guide us to take preventive and corrective actions. This avoids misinterpretation of results due to hemolytic, lipemic or icteric interferences.

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