

Icteric index and its significance

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Abstract

Background: Analyzer has the capability to detect hemolysis, icterus, and lipemia in samples and produces semi quantitative unit less index values for hemoglobin, bilirubin, or intralipid (serum indices), but these serum index functions are not intended for diagnostic purposes. With increasing amounts of the specific interfering compounds, increasing index values are generated, which are linearly correlated with the amount of interferent.

Aim of our study is to find the icteric index of serum samples and to find its correlation with total bilirubin levels.

Methodology: The study was conducted in the Clinical Biochemistry Department. A total of 779 patients blood samples were collected in the month of January 2016. The XL-640, Transasia analyzer measures the absorbances for icteric index at 480 nm (primary wavelength) and 505 nm (secondary wavelength). From these absorbance values the instrument calculates the serum index value for interference by bilirubin. Total bilirubin was also estimated for the above mentioned blood samples.

Results: Pearson's correlation coefficient (r) is calculated to find the correlation between total bilirubin and icteric index which is 0.743. This suggests a strong positive correlation between the two. Higher the serum bilirubin levels, greater was the icteric index.

Conclusion: The icteric index is a semi quantitative test, it can't be used for decision-making but to detect patients with total bilirubin values above the reference range, it can be safely utilized by laboratories.

Keywords: bilirubin, interference in analysis, icteric index

1. Introduction

1. Studies have reported four major endogenous compounds that often interfere with most laboratory results are hemoglobin, bilirubin, lipids, and paraproteins [1-3]. Authors have reported on improvements in process performance to reduce analytical errors, assay interference by endogenous or exogenous substances is an under rated problem with potential detrimental effects for the patient [1, 4, 5]. Each of these interfering substances is a potential source of biological and analytical biases which ultimately compromises the reliability of testing and makes the systematic identification of unsuitable specimens virtually unavoidable for preventing that unreliable or misleading test results. Recognition by a colour or turbidity of the specimen is proved to be unreliable. It is very difficult to predict the effect of these components on report because each sample has to be visualized immediately after centrifugation. So automated determination of potential interference of hemolysis, hyperbilirubinemia and turbidity came in to picture. Serum indices (SI) is a tool which makes laboratory professionals aware of interferences, helps to increase the quality of the sample, minimize aberrant test results.

Elevated concentrations of bilirubin is an important source of endogenous interference. Such elevations can be found in a variety of conditions including acute and chronic liver disease, biliary cirrhosis, alcoholism or as a physiological response to many drugs. Using total bilirubin as screening test for liver disease is not cost effective [6] inspite of which total bilirubin is over requested [7]. This test should be restricted to high risk patients [8]. Icteric index is the quantification of

interference by bilirubin. It is measured in autoanalyzer without any reagent cost [9].

Aim of our study is to assess the icteric index of serum samples and to find the correlation with total bilirubin levels. Our objective is to find out whether icteric index can be used as a preliminary biomarker to decide when total bilirubin is essential.

2. Methodology

The study was conducted in the Department of Biochemistry, Karwar Institute of Medical Sciences. A total of 779 patient samples were collected in the month of January 2016, out of which 370 males and 409 female patients. Institutional ethics committee permission was obtained to conduct the study. Blood samples were collected in the clinical laboratory in EDTA bottles, vacutainers or sometimes in syringes. Frequently samples were giving erroneous results due to icteric interference. We used to assess the extent of interference by visual assessment which was not accurate. Visual detection is subjective and therefore mostly unreliable since it may over- or under-estimate the actual amount of colour in the specimen. An automated serum index detection by photometric method has been implemented. We used Transasia XL -640, automated clinical chemistry analyzer in our laboratory that measures the degree of icterus.

3. Principle of assay

The assay is based on calculations of absorbance measurements, of diluted samples at different bichromatic wavelength pairs to provide a semi-quantitative representation of levels of bilirubin in serum and plasma

samples. The XL-640 analyzer takes an aliquot of the patient specimen and dilutes it with saline (0.9% sodium chloride) to measure the absorbances for icteric index at 480 nm (primary wavelength) and 505 nm (secondary wavelength). From these absorbance values the instrument calculates the serum index value for interference by bilirubin. Total bilirubin was estimated for the above mentioned blood samples. Statistical analysis was done by descriptive statistics.

4. Results

Icterus was graded as I- to I++++. Samples were categorized in to different grades based on their icteric indices and represented as percentage (Table 1). A comparative study of icteric indices was done among males and females and percentage was expressed (Table 2). Gender wise percentage distribution of Icteric index is represented in figure 1. Pearson's correlation coefficient (r) is calculated to find the correlation between total bilirubin and icteric index which is 0.743. This suggests a strong positive correlation between the two.

Table 1: Icteric index in patient samples in the month of January 2016

Grading of icteric index	Range	Total	Percentage (%)
I-	<50	290	37.22
I+	50-60	140	17.97
I++	60-70	119	15.27
I+++	70-80	91	11.68
I++++	>80	139	17.84
		779	

Table 2: Icteric index in patient samples in the month of January 2016

Grading of icteric index	Range	Males	Females	Total	% of males	% of females
I-	<50	112	178	290	38.6	61.4
I+	50-60	69	71	140	49.3	50.7
I++	60-70	47	72	119	39.5	60.5
I+++	70-80	47	44	91	51.6	48.4
I++++	>80	95	44	139	68.3	31.7
		370	409	779		

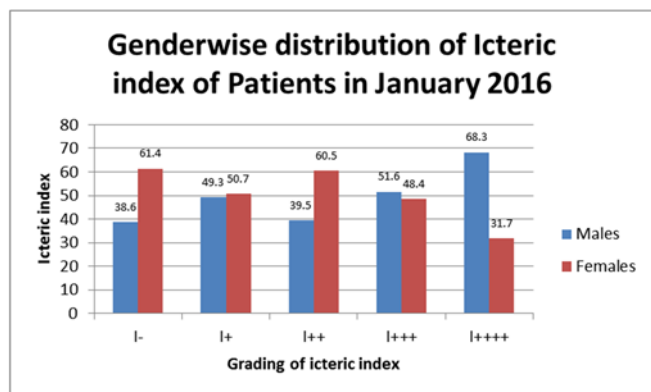


Fig1: Gender wise distribution of icteric index in January 2016

5. Discussion

This study is to show the value of the icteric index as a front-line semi quantitative test to decide on performing the measurement of total bilirubin when the latter is requested. Our study shows an excellent correlation between the icteric index and total bilirubin results. The icteric index is able to detect patients with total bilirubin values above the reference range [10]. This is the first application of the icteric index for diagnostic purposes. The icteric index may represent a valid and reliable test, and may give an important insight into clinical laboratory appropriateness in clinical decision-making.

The analytical interference by bilirubin can be caused by spectral interference. The spectral properties of bilirubin absorbance in the wavelength between 340 and 500 nm. Bilirubinemia induces high background absorbance which is proportional to its concentration. Thus, it mainly interferes in spectrophotometric assays [11]. Another way of interference with analysis is due to the chemical interference. Bilirubin may interact chemically with test reagents. Since bilirubin reacts with peroxidase catalyzed reactions, H₂O₂ generated during the chemical reaction is utilized by bilirubin, thereby causing spuriously low results of creatinine, glucose, cholesterol, triglyceride and uric acid [12]. Bilirubin also causes underestimation in phosphorus measurement that uses a UV method for the detection of phosphate by formation with phosphomolybdate [13].

We have noted high icteric index values for samples with normal bilirubin. False positive results were probably caused by interference of other yellow pigments such as carotene or haemoglobin. However, as false positive results, bilirubin is still measured in those samples. As a consequence, in the case of haemolysis or a high carotene concentration, correct total bilirubin values are reported.

The icteric index has several advantages: it is technically simple and fast to measure (less than 20 s); it lacks problems involving reagent presentation, preparation or stability; and the reagent cost is zero. Furthermore, if bilirubin test measurement is avoided, more sample will be available to process the other requested tests in the case of small specimens such as children's samples. Therefore, it can become the first marker when a total bilirubin test is requested in every healthcare setting. This index can be used as a screening tool to avoid total bilirubin measurement in a substantial number of cases. This is especially important in a patient population such as the one reported in our study for which, unfortunately, bilirubin is inappropriately requested as a screening test for chronic liver disease, as shown by the fact that 95% of the test values were in the reference range.

6. Conclusion

The icteric index is a semi quantitative test, as it is not used for decision-making but to detect patients with total bilirubin values above the reference range, it can be safely utilized by laboratories. It can reduce the cost that incur to the health care sector due to the inappropriate request of bilirubin test. However it is not an alternative to bilirubin estimation.

7. Acknowledgements: Special thanks to tutors and technicians.

8. References

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