International Journal of Biochemistry Research & Review
13(1): 1-5, 2016, Article no.IJBCRR.28150
ISSN: 2231-086X, NLM ID: 101654445

SCIENCEDOMAIN *international*
www.sciencedomain.org

Diagnostic Significance of Icteric Index

Usha Sachidananda Adiga[1*]

[1]Department of Biochemistry, Karwar Institute of Medical Sciences, Karwar, Karnataka, India.

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/IJBCRR/2016/28150
Editor(s):
(1) Richard A. Manderville, Departments of Chemistry and Toxicology University of Guelph, Canada.
Reviewers:
(1) Bruna Maria Roesler, State University of Campinas, Campinas, Sao Paulo, Brazil.
(2) Rupali Sengupta, SNDT University, Mumbai, India.
(3) S. Sundaresan, SRM University, Kattankulathur, Tamil Nadu, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/15640

Original Research Article

Received 4th July 2016
Accepted 28th July 2016
Published 3rd August 2016

ABSTRACT

Background: The pre-analytical errors occur commonly due to the presence of interfering substances like hemoglobin, bilirubin, lipids and paraproteins. Analyzer has the capability to detect them and produce semi quantitative unit less index values, collectively known as serum indices. Interference with bilirubin is estimated as icteric index. Aim of the study is to assess the icteric index of serum samples and to find the correlation with total bilirubin levels. Objective is also to find out whether icteric index can be used as a initial biomarker to decide when total bilirubin estimation is essential.
Methodology: The study was conducted in the Biochemistry Department of Karwar Institute of Medical Sciences, Karwar. Icteric index was measured in 779 patients' blood samples in the year 2015 using XL-640,Transasia analyzer at 480 nm (primary wavelength) and 505 nm (secondary wavelength). Total bilirubin was also estimated for these blood samples. Receiver operating characteristic curve (ROC) analysis was carried out to determine area under the curve (AUC).
Results: Area under the curve for ROC was 0.814. Sensitivity of the test was 92.4% and specificity was more than 93.2%. Positive predictive value of icteric index is 96.1% and negative predictive value is 82.2%. LR+ is 13.6 and LR- is 0.08. The cut off value of icteric index above which bilirubin levels are considered abnormal (> 1mg/dl) was 58.
Conclusion: The icteric index is a cost effective test and can be safely utilized by laboratories as a screening tool for hyperbilirubinemias.

**Corresponding author: E-mail: ushachidu@yahoo.com;*

Keywords: Icteric index; semi quantitative test; diagnostic importance.

1. INTRODUCTION

Major endogenous compounds that interfere with most laboratory results are hemoglobin, bilirubin, lipids, and paraproteins [1–3]. Assay interference by endogenous or exogenous substances has to be assessed to minimize analytical errors. These substances can influence patients investigation reports and are dangerous [1,4,5]. These interfering substances are potential sources of biological and analytical biases. This ultimately compromises the reliability of testing. It makes the systematic identification of unsuitable specimens virtually unavoidable for preventing unreliable or misleading test results. Recognition of interfering substance by a specific color or turbidity of the specimen is 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, hyper bilirubinemia and turbidity came in to picture. Serum indices (SI) is a specific tool which makes laboratory personnels aware of interferences, helps to increase the quality of the sample, minimize aberrant test results.

Elevated concentrations of bilirubin are an important source of endogenous interference. Such an elevation can be found in various clinical conditions including acute and chronic liver disease, biliary cirrhosis, alcoholism or as a physiological response to many drugs. Using serum bilirubin as screening test for liver disease is not cost effective [6]. Despite this fact, 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 the study is to assess the icteric index of serum samples and to find the correlation with total bilirubin levels. Main objective is to find out whether icteric index can be used as a preliminary biomarker to decide when total bilirubin estimation 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 year 2015, 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. The extent of interference used to be done by visual assessment in our laboratory, which was not accurate. Visual detection is subjective and is unreliable since it may over- or under-estimate the actual amount of substance in the specimen. Therefore an automated serum index detection by photometric method has been implemented. Transasia XL - 640, automated clinical chemistry analyzer in our laboratory was used to measure the degree of icterus.

2.1 Working Principle

The assay is based on measurements of absorbance values, of diluted samples at different specific bichromatic wavelength pairs. It provides a semi-quantitative representation of levels of bilirubin in serum or plasma specimens. The XL-640 analyzer takes an aliquot of specimen under testing 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 readings 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 drawing ROC curve.

3. RESULTS

Receiver operating characteristic (ROC) was constructed. The correlation coefficient (R2) is calculated to find the correlation between total bilirubin and icteric index which is 0.574. Sensitivity of the test was 92.4 percent and specificity was more than 93.2 percent. Positive predictive value of icteric index is 96.1% and negative predictive value is 82.2%. The cut off value of icteric index above which bilirubin levels are considered abnormal (> 1 mg/dl) was 58. The details of calculation are as follows;

Table 1. Showing distribution of patients with respect to disease and diagnostic test

	Jaundice present	Jaundice absent
Icteric index +ve	True positive (TP) N=463	False positive (FP) N=19
Icteric index –ve	False negative(FN) N=38	True negative(TN) N=259

Sensitivity, specificity, positive predictive value (PPV),negative predictive value (NPV), positive and negative likelihood ratios(LR+ and LR-) are calculated using following formulas;

Sensitivity = TP/ TP+FNX 100
Specificity = TN/ TN+FPX 100
Positive predictive value = TP/TP+FPX100
Negative predictive value = TN/TN+FNx100
Positive likelihood rtio (LR+) = sensitivity/1-specificity
Negative likelihood ratio (LR-) = 1-sensitivity/ specificity
Accuracy = sensitivity x specificity /100
False positivity = FP/FP+TN X100
False negativity = FN / TP+FNX100

Table 2. Representing calculated parameters

Parameter	Calculated value
Sensitivity	92.4%
Specificity	93.2%
Positive predictive value	96.1%
Negative predictive value	82.2%
Positive likelihood ratio	13.6
Negative likelihood ratio	0.08
Accuracy	86.1%
False positivity	6.8%
False negativity	7.6%

4. DISCUSSION

This study shows that icteric index is an important semi quantitative test to decide whether to perform the measurement of total bilirubin or not. The study shows an acceptable correlation between the icteric index and total bilirubin results (R2 =0.574). The icteric index is capable to detect patients with total bilirubin values above the reference range [10]. As ROC curve follows the left hand border and top border of ROC space, it implies that the test is accurate. A perfect test will have area under the curve (AUC) 1. The test is considered excellent if area under the curve lies between 0.90-1.Since AUC is 0.814, it implies that our test has good accuracy (0.80-0.90).

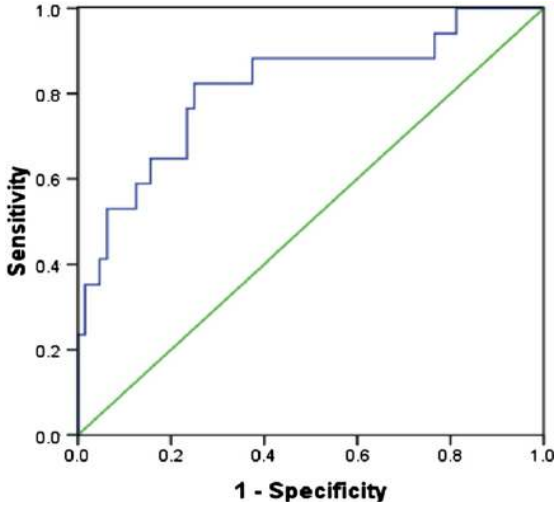

Fig. 1. ROC curve for diagnostic accuracy of icteric index (y = 0.18Ln(x) + 0.99)
R2 = 0.5737
Area under curve = 0.814

As sensitivity as well as specificity obtained is more than 92% and 93% respectively, it can be confidently said that the test (icteric index) identifies true positives and true negatives with good accuracy. The accuracy calculated by using the formula was 86.1% which is acceptable for any diagnostic test. Our results are in accordance with study by Salinas and colleagues, which show a higher sensitivity (94%) and specificity (98%) [11]. Application of icteric index is that it is a diagnostic tool. The icteric index may be useful as it is a valid and reliable test, and may give an important insight into clinical laboratory appropriate ness in decision - making clinically.

The ability of the test to predict diseased (jaundiced) is high (PPV=96.1%) as compared to its ability to diagnose people without disease (NPV=82.2%). Likelihood ratios calculated express the odds of a diagnostic test results that would be expected in a patient with disease or without disease. LR+ value observed suggests that 13.6 times jaundiced patients will have high icteric index values as compared to normal people. Likelihood ratios above 10 and below 0.1 are considered to provide strong evidence to rule in and rule out diagnoses respectively.

The interference caused by bilirubin during analysis could be due to 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 the concentration of the bilirubin. Thus, it mainly interferes in spectrophotometric assays [12]. Another way of interference with analysis is due to the chemical interference. Bilirubin may interact chemically with test reagents. 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 [13]. Bilirubin also causes underestimation phosphorus levels when measured by a UV method for the detection of phosphate [14].

We have noted high icteric index values for samples with normal bilirubin. False positive results (6.8%) observed in our study could be probably caused by interference of other yellow pigments such as carotene or haemoglobin.

The icteric index has several advantages: it is simple and can be measured quickly (0.18 min) as observed in our own study [15]. It does not have problems involving reagent presentation and their stability. 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 neonates and childrens' samples. Therefore, it can become the first marker when a total bilirubin test is requested in every health care 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.

5. 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.

ACKNOWLEDGEMENTS

Sincere thanks to Dr Poornima RT, HOD Biochemistry for the support.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Kroll MH, Elin RJ. Interferences with clinical laboratory analyses. Clin Chem. 1995;40(11 pt 1):1996–2005.
2. Behrendt H. Chemistry of erythrocytes. Springfield, IL: Charles C Thomas. 1957;227.
3. Caraway WT. Chemical and diagnostic specificity of laboratory tests, effect of hemolysis, lipemia, anticoagulants, medications, contaminants and other variants. Am J Clin Pathol. 1962;37:445–64.
4. Plebani M, Carraro P. Mistakes in a stat laboratory: Types and frequency. Clin Chem. 1997;43(8 pt 1):1348–51.
5. Witte DL, VanNess SA, Angstadt DS, Pennell BJ. Errors, mistakes, blunders, outliers, or unacceptable results: How many? Clin. Chem. 1997;43(8):1352–6.
6. Dufour DR, Lott JA, Nolte FS, et al. Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis, and monitoring. Clin Chem. 2000;46:2050-68.
7. Salinas M, Lo´pez-Garrigo´s M, Diaz J, et al. Regional variations in test requiring patterns of general practitioners in Spain. Ups J Med Sci. 2011;116:247-51.
8. Quinn PG, Johnston DE. Detection of chronic liver disease: Costs and benefits. Gastroenterologist. 1997;5:58-77.
9. Guder WG, Fonseca-Wollheim FD, Heil W, et al. The haemolytic, icteric and lipemic sample. Recommendations regarding their recognition and prevention of clinically relevant interferences. J Lab Med. 2000;24:357-64.
10. Pincus MR, Tierno P, Dufour DR. Evaluation of liver function. In: McPherson RA, Pincus MR, eds. Henry's Clinical

Diagnosis and Management by Laboratory Methods. 21st edn. Philadelphia, Pa: Elsevier Saunders. 2007;263.
11. Salinas M, Maite LG, Javier L, Mercedes Guiterrez, Lucia Flors, Carlos LS. Diagnostic accuracy of icteric index to detect abnormal total bilirubin. J Clin Pathol. 2012;65:928-933.
12. Guder WG, Berlin F, Heil W, Schmitt YM, Weisser H, Zawta B. The haemolytic, icteric and lipemic sample. Recommendations regarding their recognition and prevention of clinically relevant interferences. J Lab Med. 2000;24(8):357-64.
13. Bertholf RL, Johannsen LM, Bazooband A, Mansouri V. False-positive acetaminophen results in a hyperbilirubinemic patient. Clin Chem. 2003;49(4):695-8.
14. Duncanson GO, Worth HGJ. Pseudohypophosphataemia as a result of bilirubin interference. Ann Clin Biochem. 1990;27(3):253-7.
15. Usha Adiga. Interference of serum indices in analyzer function. IOSR-JBB. 2016;2(1): 59-61.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/15640