International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 6; Issue 12; December 2017; Page No. 8443-8444
DOI: http://dx.doi.org/10.24327/ijcar.2017.8444.1362

Research Article

POSSIBILITY OF CONJUGATED BILIRUBIN BEING MORE THAN TOTAL BILIRUBIN

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ARTICLE INFO

Article History:
Received 8th September, 2017
Received in revised form 5th October, 2017
Accepted 4th November, 2017
Published online 28th December, 2017

Key words:

Total; Conjugated; bilirubin; paraproteins; interference

ABSTRACT

Increase in serum conjugated bilirubin than total bilirubin is rare. Further investigations to identify the source of interference needs to be assessed for the interpretation and reporting. Our communication demonstrates that paraproteins specially IgM was the source of interference and should be thought of and analysed in case of such abnormal findings in estimation of bilirubin.

INTRODUCTION

Bilirubin is a product of heme catabolism. Normally, heme is catabolized to unconjugated bilirubin (indirect) in the reticuloendothelial system. Unconjugated bilirubin is bound to albumin in the plasma and transported bound to albumin to the liver and is conjugated with glucuronic acid in the hepatocytes; the conjugation is catalyzed by glucuronyl transferase. Conjugated bilirubin (direct) is secreted into the bile and enters the duodenum. In the small bowel, some of the bilirubin is hydrolyzed to yield unconjugated bilirubin and glucuronic acid. Most unconjugated bilirubin is excreted in the stool, but some is reabsorbed and returned to the liver for re-conjugation (enterohepatic circulation).Thus direct bilirubin is a component of total bilirubin and therefore should always be smaller in value. (1)

Nowadays, as a regular practice, clinical laboratory utilize automated systems using kits. Most methods of estimating the concentration of bilirubin in serum are based on the van den Bergh reaction in which bilirubin couples with diazotized sulphanilic acid to give red " azobilirubin," the reaction being facilitated by the addition of an activator or catalyst such as ethanol.(2)

Spurious results in the laboratory are a matter of concern not only to the laboratory personnal but also to the clinician. And more so, if concentration of conjugated bilirubin appears higher than that of total bilirubin.

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Though preanalytical and analytical errors need to be taken into consideration, interference by analytes plays a major role during sample processing and interpretation of results.

This interference in the analytes is defined as "the effect of a substance present in the sample that alters the correct value of the result for an analyte"(3)

Haemolysis, icterus, lipaemia and proteins are the most common interfering substances during investigations. (4)

Previous studies have shown that apart from other metabolites paraproteins caused spurious results on individual analytes including total bilirubin and conjugated bilirubin. (3,5,6,7)
This interference between measurements of distinct blood serum components can lead to a false interpretation of the results and a delay in disease recognition. Taking this into consideration the present study was carried to identify and interpret the variability of bilirubin values in serum sample.

MATERIAL AND METHOD

Conjugated and total bilirubin results for 2 patients serum samples from the Clinical Laboratory of tertiary care centre in central India during a six month period, were reviewed because of increased conjugated bilirubin concentrations than total bilirubin. Conjugated bilirubin and total bilirubin concentrations were routinely measured by the fully automated closed system Seimans autoanalyser. The method of estimation was based on the principle of diazo method. When the samples were retested (with and without dilution), the results were consistently present. In addition, serum samples from the two patients were further investigated to determine the cause of the interference by performing routine parameters of liver function tests (kit method), lipid profile (kit method) and paraproteins

(radial immunodiffusion). Visually, the patient samples showed no evidence of hemolysis or lipemia.

Institutional review was not required for this study because the study was performed using deidentified discarded blood samples that were obtained for patient care and not for research purposes.

RESULTS

Liver function test results (proteins, alkaline phosphatase, ALT, AST, triglyceride, cholesterol) of the two samples were within reference intervals. Serum concentration of IgA and Ig G were also found to be in the normal range. Serum IgM in both the samples was 780 and 1089 mg/dl respectively which was higher than the normal range (45-250 mg/dl)

DISCUSSION

Laboratory results and their interpretation is an essential element for correct decisions related to patient care and management. Many a times data obtained from the laboratory brings out a brain tossing experience to actually find out the proper interpretation of the results. Many of today's highly automated laboratory instruments have built-in mechanisms that help laboratories identify sample integrity, identify the test results that are outside specified reference range. Apart from preanalytical and analytical errors, as clinical biochemists interference is one of the key aspect which still needs to be evaluated in order to understand their potential clinical impact because dispensing correct reports is of utmost priority for any laboratory.

Estimation of serum bilirubin is a routine parameter which is carried out in laboratories. Interference in the levels is known to occur and haemolytic and lipemic samples are said to be associated with high levels of bilirubin.

However, as in our case the findings of increased conjugated bilirubin concentrations than total bilirubin with and without dilution and on successive rerunning further paved way to analyse the samples for other interfering substances.

Literature has suggested that lipids and monoclonal proteins (7-9) are known to interfere with the conjugated bilirubin assay. Of the monoclonal proteins, the predominant ones are IgM and IgG. These can interfere with all types of automated assays (spectrophotometric, immunonephelometric, immunoturbidimetric) including total bilirubin.(3)

Nowadays clinical chemistry assays are designed keeping in mind that proteins, normal or otherwise, should not interfere with the values assessed. However, it is said that when the monoclonal antibodies proliferate to a significant degree the "precipitation becomes relevant and influences the assay".

Interference rates in samples with monoclonal antibodies have been reported as high as 46%, depending on the concentration, methodology and isotype of the monoclonal protein, and the specific test and assay methodology being evaluated.(10)

Our study demonstrates that levels of IgM are increased in the serum. Artefactual hyperbilirubinaemia due to paraprotein interference in total bilirubin estimation but not conjugated bilirubin (11, 12) has been reported.

However less is available in literature (13) regarding levels of conjugated bilirubin more than total bilirubin. Hence, we would like to stress the fact that increase in conjugated bilirubin than total bilirubin in a sample must be cross checked with interference from paraproteins which could also pave the way for clinicians for further investigations in the patient.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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