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Comparison of Direct Bilirubin Estimation by Three Different Methods

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ABSTRACT

Background: Bilirubin is one of the major bile pigment, clinically and biologically important among the bile pigments present in the mammals. The objective of this study was to compare direct bilirubin concentration determined by fully-automated, semi-automated and manual methods in serum samples as well as to correlate these values. **Methods:** All the serum samples were analysed by above mentioned three methods and the difference between the results from these three methods were analysed using t-test and the correlation coefficient was also calculated. **Results:** The comparison of results measured by manual and fully-automated method showed a good correlation ($r = 0.993$, $p < 0.001$) when compared with fully-automated and semi-automated method ($r = 0.975$, $p < 0.001$). The difference in direct bilirubin values between fully-automated and semi-automated was more (1.06 ± 0.77 mg/dL) when compared with the difference between fully-automated and manual method (0.48 ± 0.36 mg/dL). **Conclusion:** We concluded that the measurement of direct bilirubin with manual method (Malloy-Evelyn) provides better agreement with fully-automated method than does a semi-automated method and manual method provides the advantage of less costly and more reagent shelf life.

Keywords: Direct bilirubin, Jendrassik-Grof, Malloy-Evelyn, Fully-autoanalyzer, Semi-autoanalyzer.

INTRODUCTION

Bilirubin is the orange-yellow pigment derived from senescent red blood cells. Following formation in the reticuloendothelial cells, bilirubin is transported and biotransformed mainly in the liver and excreted in bile and urine.[1] There are two forms of bilirubin in the body- Unconjugated or indirect bilirubin and conjugated or direct bilirubin. The unconjugated form is protein bound and insoluble in water while the conjugated form circulates freely in the blood and was transformed into water soluble bilirubin in the liver by conjugating with glucoronic acid and is excreted into the bile.[2,3] Apart from conjugated and unconjugated bilirubin, there is delta bilirubin which arises through a non-enzymatic covalent coupling reaction between glucoronated bilirubin and albumin which is non-toxic and excreted neither in urine nor in bile but is slowly metabolised with a half-life of 20 days.[4]

Measurement of total bilirubin and determination of direct and indirect fractions are important in routine screening and also for the differential diagnosis of jaundice. Depending upon the nature of bilirubin elevated, the condition may be grouped into conjugated and unconjugated hyperbilirubinaemia.[5] The accurate determination of the types and amount of bilirubin fractions in the body fluids, especially serum is important for diagnostic purpose and therapeutic monitoring.6 However considerable variability in results for the identical specimen is often encountered from laboratory to laboratory. A lack of pure standard for conjugated bilirubin, the presence of interfering substances and the large dynamic range necessary for bilirubin assays to be clinically useful add to the difficulties in its accurate measurement.

Bilirubin and related compounds are measured in the body fluids by various spectrophotometric, chromatographic and electrophoretic methods. Currently most clinical laboratory rely on automated analyzers for rapid bilirubin determination in multiple samples.[7] The most widely used chemical method for determination of total bilirubin and conjugated bilirubin concentration in serum is the diazo method, in which the colour of azobilirubin formed by the reaction of the porphyrin rings of bilirubin with a diazo compound is spectrophotometrically measured.[6]

The aim of this study was to explain the relationship between the three methods for bilirubin estimation with the possibility of replacing the Semi-automated method by the manual method of Malloy and Evelyn.

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MATERIALS AND METHODS

The present study was conducted between October 2016 to December 2016. The study comprised of 50 leftover serum samples. Serum samples were collected from the Biochemistry laboratory of

Raipur institute of medical sciences and hospital, Bhansoj road, Raipur, chattisgarh, India. All the serum samples were analysed by Fully-automated, Semi-automated and Manual method. The direct bilirubin estimation by fully-automated is modification of the Doumas reference method,[8] which is a modification of the diazo method described by Jendrassik-Grof in 1938.[9] Semi-automated estimation of direct bilirubin is based on Diazo-method of Pearlman and Lee,[10] whereas the Malloy and Evelyn method is used for manual estimation.

The instruments used for fully-automated, semi-automated and manual analysis were XL 200^{R} Clinical Chemistry analyzer by Erba manheim, Erba Chem 5 V2 and EI spectrophotometer respectively. Semi-automated analysis was done by using commercially available Erba kit whereas for manual analysis reagents were prepared in the lab.

The data obtained was analysed statistically by computing descriptive statistics, the mean, standard deviation and correlation coefficient. The difference between each method was also calculated. The results were considered statistically significant whenever $p \leq 0.05$.

RESULTS

The range of direct bilirubin values in fully-automated, semi-automated and manual methods were 0.72-13.03 mg/dL, 0.4 - 9.36 mg/dL and 0.6 - 11.26 mg/dL respectively whereas the mean ± SD were 3.15 ± 2.30, 2.08 ± 1.66 and 2.67 ± 2.04 respectively.

Correlation between the direct bilirubin values were statistically significant between fully-automated and semi-automated method with r = 0.975 and p value < 0.001. The direct bilirubin values were more strongly correlated with fully-automated and manual method which is indicated by r = 0.993 and p value < 0.001 when compared to semi-automated and manual method. Correlation coefficient (r) of direct bilirubin values between semi-automated and manual method was 0.0984 and p value was < 0.001. Difference between the direct bilirubin values obtained from fully-automated and semi-automated was 1.06 ± 0.77 mg/dL, while difference between direct bilirubin values obtained from fully-automated and manual was 0.48 ± 0.36 mg/dL. Difference between direct bilirubin values obtained from semi-automated and manual method was 0.58 ± 0.50 mg/dL.

DISCUSSION

Measurement of bilirubin in serum is invaluable in the diagnosis and treatment of hepatic dysfunction, hemolysis and newborn jaundice. Accurate determination of bilirubin in serum appears to be more difficult than for any other substances because of its sensitivity to many factors such as light, oxygen, haemoglobin concentration, pH, high affinity to protein, the technique used to obtain blood sample,[11-13] types of autoanalyzer and method employed. A lack of pure standard for conjugated bilirubin also adds to the difficulties in its accurate measurement. There are several methods for the determination of bilirubin. The most widely used method in clinical laboratories is based on the colorimetric method using diazotization reaction as it is cheap, easy and convenient to apply to use with automated analyzers.[14,15]

This study which aimed to compare direct bilirubin concentration determined by Fully-automated, Semi-automated and manual method showed a good correlation between fully-automated and manual method's values of direct bilirubin when compared to that of fully-automated and semi-automated method. The possible cause for this could be because of more interference in semi-automated analysis than in the manual analysis.

CONCLUSION

The manual method gives similar results compared to semi-automated &fully-automated methods. Manual method provides the advantages of less costly and reagents can be prepared easily with more shelf life. Hence, any of the method can be used for the direct bilirubin estimation depending upon the availability of the reagent & instruments. The manual method for direct bilirubin analysis can be used in the primary health centre as well as urban health centre where the semi-automated & fully-automated implementation is difficult.

These findings are of clinical significance to laboratory technicians as they deal with direct bilirubin measurement in the different levels of laboratory using different instruments as well as methods. Hence our study adds to the knowledge about variation in the direct bilirubin analysis by different methods.

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