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A Comparative Study of Detection of Serum Total and Direct Bilirubin by Jendrassik & Grof and Automated Method

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Abstract

Bilirubin is one of the major bile pigments, clinically and biologically important among the bile pigments present in the mammals. The cross-sectional study was conducted in department of biochemistry in the collaboration with Department medicine in Civil Hospital Tarn Taran. Out of 90 patients, 54 were males and 36 were females. The comparison of Serum Total and Direct bilirubin by Jendrassik & Grof and Automated method was non-Significant with p value = 0.7788 and 0.5394 respectively. The comparison of Serum Total and Direct bilirubin by Malloy Evelyn and Automated method is Highly Significant with p value =0.0002 and 0.0003 respectively. The mean difference of malloy with jendrassik & grof and automated was 2.394 and 2.162 respectively and the mean difference of jendrassik & grof with automated method was -0.231. It was concluded that a good correlation is found between bilirubin detection by manual and automated method. Non-significant mean difference between Jendrassik & Grofand Automated makes it better than Malloy Evelyn.

Keywords: *Direct bilirubin, Jendrassik-Grof, Malloy-Evelyn, Fully-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 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 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

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 present study was aimed to estimate and compare total and direct bilirubin by manual methods with automated method.

Material and methods

The Study was conducted in Department of Biochemistry in the collaboration with Department of Medicine of Civil Hospital Tarn Taran. Ethical clearance was taken from institutional ethical committee. Proper informed consent was taken from all the participitants. Out of 90 patients, 54 were males and 36 were females. They was subjected to detailed history and examination, biochemical and special testing according to the pretested performa. Non probability convenient sampling was done. Study group consists of 60 hyperbilirubinemia patients whereas 30 healthy individuals having normal bilirubin levels were considered in control group. The study was completed in duration of 10 months.

Sample Collection and Processing

Venous blood was collected from median cubital vein under aseptic conditions. Patient was asked to make a fist so that the veins got more prominent. Keeping the arm straight, tourniquet was applied about 4-5 finger width above the selected site and the vein was re-examined. Skin at the venipuncture site was cleaned with sterile disinfectant before collecting blood sample.

Needle was inserted through the skin into lumen of the vein. After sufficient blood had been collected (5ml), tourniquet was released before withdrawing the needle. Needle was withdrawn gently and cotton swab was kept on puncture site of vein till bleeding stopped. Blood was transferred into plain vial and kept undisturbed till blood was clotted. After clotting, sample was centrifuged at 3000 rpm for 15 min and then serum was separated.

The data obtained was analyzed statistically by computing descriptive statistics, the mean, standard deviation and correlation coefficient. The difference between each method was also calculated 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. [9,10]

Results and observations

The study was carried out in Department of Biochemistry in Civil Hospital Tarn Taran Comparative study of serum total and direct bilirubin by Jendrassik& grof and Automated Methods. Showed about the male to female ratio 1.5:1 out of the total study population 54 were males and 36 were females.

The distribution of age group of participants involved in the studies. 41% participants were sharing age group of 18-30. 51% participants were sharing age group of 31-60 being maximum in number. On the other hand, age-group >60 were sharing 08% of the total number of participants. Moreover, The Mean±SD and range of age are 18.3±33.3

Table 1: Mean±Sd Of Total And Direct Bilirubin Levels Estimated By All The Three Methods In the Control Group

Method	Total BilirubinLevels		Direct BilirubinLevels	
	Range	***Mean±SD***	***Range***	***Mean±SD***
Malloy Evelyn Method (n=30)	*0.1-0.9*	*0.26±0.14*	*0.1-0.3*	*0.12±0.04*
Jendrassik &Grof method(n=30)	*0.2-1.6*	*0.58±0.27*	*0.1-0.6*	*0.2±0.10*
Automated method (n=30)	*0.3-1.4*	*0.78±0.28*	*0.1-0.5*	*0.22±0.11*

Table 2: Mean±Sd of Total and Direct Bilirubin Levels Estimated By All The Three Methods In Study Group

Method	Total Bilirubin Levels		Direct Bilirubin Levels	
	Range	***Mean±SD***	***Range***	***Mean±SD***
Malloy Evelyn Method (n=60)	*0.1-11.2*	*2.45±1.60*	*0.1-5.6*	*1.20±0.75*
Jendrassik &Grofmethod (n=60)	*0.2-31*	*6.58±4.0*	*0.1-18.1*	*4.77±2.20*
Automated method (n=60)	*0.3-22.2*	*4.71±3.76*	*0.1-12.1*	*2.53±1.85*

The comparison of total serum bilirubin and direct bilirubin by Malloy evelyn and Jendrassik & gr of method. The Mean±SD of total bilirubin by the Malloy Evelyn method and Jendrassik & grof method was found to be 2.45±1.60 and 6.58±4.0 mg/dl and mean±SDof direct bilirubinby the Malloy Evelyn method and Jendrassik & grof method was estimated as 1.20±0.75 and 4.77±2.20 respectively. P value was found to be ≤ 0.005 in both the cases (Total and Direct) making it to be highly significant.

Coefficient of correlation in total and direct bilirubin levels while comparing Malloy Evelyn and Jendrassik & grof method. r was found to be + 0.550 and + 0.384 in total and direct bilirubin level respectively. P value was found to be ≤ 0.001. Positive correlation has been found in between both the methods. Coefficient of correlation in total and direct bilirubin levels while comparing Jendrassik & grof and automated method. r was found to be +0.202 and+ 0.058 in total and direc t bilirubinlevel respectively. P value was found to be ≥0.5 which makes it non- significant.

Coefficient of correlation in total and direct bilirubin levels while comparing Malloy evelyn and automated method. r was found to be r= +0.025 and r= -0.050 in total and direct bilirubin level respectively. P Value was ≤0.0001 which is statistically significant Mean difference of Malloy Evelyn with Jendrassik & Grof and Automated method was statistically signif icant (p≤0.005). Automatedmethod with Jendrassik & Grof was (≥0.05) which is statistically non- signific ant.

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, [8,9] 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. [10,11] The mean difference of Malloy Evelyn with Jendrassik & Grof and Automated method was statistically signif icant (p≤0.005). Automatedmethod with Jendrassik & Grof was (≥0.05) which is statistically non- signific ant.

Conclusion

It was concluded that a good correlation is found between bilirubin detection by manual and automated method. Non-significant mean difference between Jendrassik & Grof and Automated makesit better than Malloy Evelyn.

References

1. Hemoglobin, Iron & Bilirubin; Bilirubin, Chapter 28, Tietz Fundamentals of Clinical chemistry, 6th ed. 520-521.
2. Fischbach F, Dunning MB, (2009), A manual of laboratory and diagnostic tests. 8th ed. Philadelphia: Lippincott Williams & Wilkin. 364.
3. Iyanagi, T., Emi, Y., & Ikushiro, S. I. (1998). Biochemical and molecular aspects of genetic disorders of bilirubin metabolism. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1407*(3), 173-184.
4. Doumas, B. T., Wu, T. W., & Jendrzejczak, B. (1987). Delta bilirubin: absorption spectra, molar absorptivity, and reactivity in the diazo reaction. *Clinical chemistry*, *33*(6), 769-774.
5. Heme synthesis & breakdown,

Hyperbilirubinaemia, Chapter 21; Text book of Biochemistry D Vasudevan, 7th ed. 279.

6. Ameri, M., Schnaars, H., Sibley, J., & Honor, D. (2011). Comparison of the vanadate oxidase method with the diazo method for serum bilirubin determination in dog, monkey, and rat. *Journal of Veterinary Diagnostic Investigation*, *23*(1), 120-123.
7. Doumas, B. T., & Wu, T. W. (1991). The measurement of bilirubin fractions in serum. *Critical reviews in clinical laboratory sciences*, *28*(5-6), 415-445.
8. Rehak, N. N., Cecco, S. A., & Hortin, G. L. (2008). Photolysis of bilirubin in serum specimens exposed to room lighting. *Clinica chimica acta; international journal of clinical chemistry*, *387*(1-2), 181.
9. Kazmierczak, S. C., Robertson, A. F., Briley, K. P., Kreamer, B., & Gourley, G. R. (2004). Transcutaneous measurement of bilirubin in newborns: comparison with an automated Jendrassik–Grof procedure and HPLC. *Clinical chemistry*, *50*(2), 433-435.
10. de Cordova, C. M. M., Nogara, M. S., & de Cordova, M. M. (2009). Interference on the laboratory measurement of bilirubin: the effect of in vitro interactions. *Clinica chimica acta; international journal of clinical chemistry*, *407*(1-2), 77-79.
11. Fossati, P., Ponti, M., Prencipe, L., & Tarenghi, G. (1989). One-step protocol for assays of total and direct bilirubin with stable combined reagents. *Clinical chemistry*, *35*(1), 173-176.