ORIGINAL ARTICLE

Modification of Jaffe's Kinetic Method for Serum Creatinine Estimation to Decrease Bilirubin Interference

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ABSTRACTS

BACKGROUND: Bilirubin causes negative interference in creatinine value measurement using general techniques.The objective of this study was to find a method of serum creatinine estimation in which interference of bilirubin is minimized. **METHODOLOGY:** The study was conducted in the Clinical Chemistry Laboratory SSG Hospital, Vadodara. 60 samples with different levels of serum bilirubin were grouped as group I (1-5mg/dl), group II (5-15mg/dl), group III (>15mg/dl). Both creatinine and bilirubin concentration in serum samples were measured by using semiautoanalyzer in laboratory. The creatinine value was measured by two variations of Jaffe's kinetic Method A (preincubation with NaOH) & Method B (without preincubation) and Total bilirubin measured by Diazo method using commercial kits. **RESULTS:** The mean value of creatinine was different for preincubation with NaOH and without preincubation. Results of the Method-A (with NaOH Preincubation) & B (without NaOH Preincubation) were compared in all the 3 groups and are as follows. The mean difference was higher at higher bilirubin level & difference was statistically significant at bilirubin >5mg/dl. **CONCLUSION:** This shows that the bilirubin has negative interference in creatinine value measurement by ordinary laboratory practices and interference increases with higher concentration of bilirubin in blood sample. We conclude that preincubation with NaOH helps to reduce this negative interference of bilirubin in creatinine value measured by Jaffe Method in icteric serum sample.

Keywords: Preincubation, bilirubin, Negative interference, Jaffe's Kinetic method

INTRODUCTION

Creatine is present as creatine phosphate, reserved energy in muscle. Creatine is produced in kidney, liver and pancreas by enzymatic reactions. Thus produced creatine is transported to different organs such as brain and muscles where it is phosphorylated to form creatine phosphate and stored as reserved energy source. Creatinine is a breakdown product of creatine phosphate and produced in a fairly constant rate by the body depending on muscle mass, age, sex, diet and exercise.

It is a waste product excreted through urine hence used as a helpful parameter to measure clearance test of GFR. Generally it is fairly constant but also found increased with certain diets. The serum creatinine level is abnormal in cases of muscle and kidney diseases. Its estimation occurs as an important biochemical parameter in clinical laboratory tests.[1] Serum creatinine has also been used in MELD score (Model for End-stage Liver Disease) along with serum bilirubin and bilirubin is a yellow pigment formed inside the body by the metabolism of heme. It is formed by the enzymatic reduction of biliverdin to bilirubin. It is also an important biochemical parameter particularly of liver function tests. These tests are intertwined in combined hepatic and renal test. The combined liver and kidney function test provide the knowledge of liver and kidney disease that occur in the same patient. Creatinine estimation in such condition should be done with great care since bilirubin has negative interference on creatinine measurement by Jaffe's Kinetic method.[2]Though high performance liquid chromatography and gas chromatography with mass spectroscopy have been proposed to be gold standards for creatinine estimation but they are not

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readily available in most of the clinical chemistry laboratories due to economic and technical constraints. Most of the present day analyzers use Jaffe's kinetic method without deproteinization. Hence this study was planned to find out a possible solution to the problem of bilirubin interference by a minor modification in the commonly used Jaffe's method so that it is amenable for use on the currently used analyzers.[3]The exact mechanism of bilirubin interference is not known but the color of bilirubin affects on spectrum absorption with yellow color of picrate used in creatinine measurement.In the case when creatinine has to be measured in icteric sample (high bilirubin) then color produced by bilirubin should be removed or minimized.In this study oxidation of bilirubin is carried out by pre-incubation with NaOH before estimation of creatinine.[4]

The important approach to minimize bilirubin interference is to oxidize bilirubin to biliverdin using NaOH, by its oxidizing property. Pre incubation of icteric sample with NaOH before Jaffe's reagent addition, will convert any bilirubin present in the sample to biliverdin causing a decrease in absorbance at 505nm, while we are trying to measure an increase in 505nm due to picrate creatinine interaction.[2]

MATERIAL AND METHOD

The study was conducted in the Clinical Chemistry Laboratory,SSG Hospital,Vadodara.60 samples with different levels of serum bilirubin were grouped as group I (1-5mg/dl),groupII(5-15mg/dl), group III (>15mg/dl). In group III with Method –A the Mean & SD are 1.64&0.98 (0.66-2.62) and with Method-B the Mean & SD are 0.84 &1.01 (0.17-1.85) and the p-value (P<0.0001) Showed very high statistically significant difference. Serum creatinine level estimation by absorbance at Both creatinine and bilirubin concentration in serum samples were measured by using Semiautoanalyzer in laboratory.The lipaemic samples, lysed samples, samples with high glucose concentration were excluded from the study.The creatinine value was measured by two variations of Jaffe's kinetic Method A (preincubation with NaOH) & Method B (without preincubation) and Total bilirubin measured by Diazo method using commercial kits.

RESULT

Results were tabulated and Mean & SD in all the 3 groups were calculated. The mean value of creatinine was different for preincubation with NaOH and without preincubation. Results of the Method-A & B were compared in all the 3 groups and are as follows. In group I, with Method –A the Mean & SD are 1.0 & 0.43 (0.57-1.43) and with Method-B the Mean & SD are 0.94 & 0.41 (0.53-1.35) and the p-value (P<0.01) showed statistically significant difference. In group II with Method –A the Mean & SD are1.27&1.05(0.22-2.32) and with Method-B the Mean & SD are 0.92 &1.06 (0.14-1.98) and the p-value (P<0.001) showed very high statistically significant difference.

Group	METHOD[A] With NaOH Preincubation			METHOD [B] Without NaOH Preincubation			Mean Difference	P-value
	Mean	SD	Range	Mean	SD	Range		
Group I **(1-5mg/dl)**	1.0	±0.43	0.57-1.43	0.94	±0.41	0.53-1.35	0.06	P <0.01
Group II **(5-15mg/dl)**	1.27	±1.05	0.22-2.32	0.92	±1.06	0.14-1.98	0.35	P <0.001
Group III **(>15mg/dl)**	1.64	±0.98	0.66-2.62	0.84	±1.01	0.17-1.85	0.79	P <0.0001

Method A (with NaOH Preincubation) gives higher results compared to Method B (without NaOH Preincubation). The mean difference was higher at higher bilirubin level & difference was statistically significant at bilirubin >5mg/dl.

DISCUSSION

In the present study we find that bilirubin interfere in the estimation of creatinine by Jaffe's Kinetic method. The creatinine value obtained by pre incubation with NaOH (i.e true creatinine) is found to be increased than creatinine obtained without pre incubation (creatinine) .The mean value of creatinine was different for pre-

incubation with NaOH and without pre-incubation. Little variation in creatinine estimation for normal bilirubin concentration. Serum creatinine level estimation by Method A (with NaOH Preincubation) gives higher results compared to Method B (without NaOH Preincubation). The mean difference was higher at higher bilirubin level & difference was statistically significance at bilirubin >5mg/dl.

The sample size in this study was 60, may not cover the large number of icteric samples but, this sample size was sufficient to give knowledge of bilirubin interference in creatinine estimation. Creatinine estimation is primarily used as indicator for renal function. As shown by the results of this study creatinine estimation by normal method may give false value of creatinine. Hence care should be taken before creatinine value measurement in icteric sample particularly bilirubin > 5 mg/dl.

CONCLUSION

This shows that the bilirubin has negative interference in creatinine value measurement by ordinary laboratory practices and interference increases with higher concentration of bilirubin in blood sample.

We conclude that preincubation with NaOH helps to reduce this negative interference of bilirubin in creatinine Value measured by Jaffe's Method in icteric serum sample.

REFERENCES

1. Chaudhary SS,Shah JP,Mahato RV.Interference of bilirubin in creatinine value measurement by jaffe's kinetic method.ACCLM,Nepal;2015;25-28.
2. Cheemalavagupali R,Manchala S.Comparative Reliability of different method of serum creatinine estimation in jaundice patients for minimizing negative interference of bilirubin.Int J Pharm BioSci,Telangana;2016 July;355-360.
3. ViaishyaR,Aroras,SinghB,MallikaV.Modification of Jaffe's kinetic method decreases bilirubin Interference.IJCB,New Dehli;2010;64-66.
4. O'LearyN,PembrokeA,Duggan PF.A Simplified Procedure for eliminating the negative interference of bilirubin in the Jaffe's reaction for creatinine.Clin.Chem,Ireland;1992;1749-1751.