Interference of Bilirubin in Creatinine Value Measurement by Jaffe Kinetic Method

Siddhartha Shankar Chaudhary,[1*] Jay Prakash Shah,[2] Ram Vinod Mahato[3]

BACKGROUND: Creatinine measurement in icteric sample is a major but unresolved problem. Bilirubin causes negative interference in creatinine value measurement using general techniques. The objective of this study was to find differences in creatinine value by Jaffe Kinetic method pre-incubation and without pre-incubation with NaOH.

METHODS: This was a cross sectional, descriptive study carried out in 71 samples with different level of bilirubin concentration. We took blood samples of 71 different patients, 48 males and 23 females, from two different hospitals of Kathmandu village. Both creatinine and bilirubin concentration in serum samples were measured by using Staxfax 3300 semi auto analyzer in the hospital. In the laboratory creatinine value was measured by kinetic method and bilirubin measured by Jendrassik/ Grof method using commercial kits. Statistical analysis of quantitative data was done by using SPSS version 16.0.

RESULTS: The results shows differences in creatinine values with respect to methods and extent of bilirubin concentration. It was found that the creatinine obtained by pre-incubation with NaOH has greater value than without pre-incubation (i.e. by direct estimation using working reagent). It was also shown that the high bilirubin cause the interference in greater extent. The significant interference was seen in the sample with bilirubin concentration greater than 20 mg/dl i.e. creatinine value after treatment with NaOH prior to dispensing picric acid is significantly increases, $P<0.01$ at 99% confidence level.

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.

Key Words: Creatinine. True creatinine, Bilirubin total, Bilirubin direct (T/D), out value

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 [1]. 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.

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 liver and kidney disease that occur in the same patient [2]. Creatinine estimation in such condition should be done with great care since bilirubin has negative interference on creatinine measurement by Jaffe Kinetic method.

The exact mechanism of bilirubin interference is not known but the color of bilirubin effects on spectrum absorption with yellow color of picrate used in creatinine measurement [3]. In the case when creatinine has to be measured in icteric sample (high bilirubin) then color produced by bilirubin should be removed or minimized. This can ordinarily be done by oxidation of bilirubin to biliverdin by oxidizing agents. In this study oxidation of bilirubin is carried out by pre-incubation with NaOH before estimation of creatinine [4].

[1]Department of Medical Biochemistry, Nobel College, Sinamangal, Kathmandu, Nepal. [2]School of Health and Allied Sciences, Pokhara University, Pokhara. [3]Central Campus of Technology, Hattisar, Dharan.

Correspondence to: Department of Medical Biochemistry, Nobel College, Sinamangal, Kathmandu, Nepal.Email: ch_siddharth07@yahoo.com, Mobile: +977 9841585427

Methods

The laboratory based, descriptive, cross-sectional study was carried out in 71 serum samples taken from patients visiting two different hospital in Kathmandu valley. The serum samples contain different concentration of bilirubin. Study population were jaundiced and non-jaundiced patients attending OPD and wards of the hospitals. The study was conducted for three months from August 2010 to November 2010. Laboratory investigation of the samples were carried out by using Staxfax 3300 semi auto-analyzer..

Blood samples were collected purposively both icteric and normal samples during the study period. Approval from the institution and patients' consent were taken before conducting study and sample collection. Biochemical parameters were creatinine and bilirubin serum concentration and variables were age and sex of the patients.

The estimation of biochemical parameters were carried out by using Staxfax 3300 semi auto-analyzer. Venous blood was collected by using tourniquet and was kept in a test tube. The clotted blood sample was quickly centrifuged and separated serum was used for the estimation of creatinine and bilirubin value. CREST BIOSYSTEMS, Jaffe Kinetic method, creatinine kit was used for serum creatinine measurement.

SERUM CREATININE MEASUREMENT BY JAFFE KINETIC METHOD:

Creatinine reacts with picric acid in an alkaline medium (i.e. alkaline picrate) to form an orange colored complex. The intensity of the color formed during the fixed time is directly proportional to the amount of creatinine present in the sample. And creatinine is measured kinetically at 490nm (490-510).

Creatinine+ Alkaline picrate → Orange colored complex

SERUM BILIRUBIN (TOTAL/DIRECT) MEASUREMENT BY MODIFIED JENDRASSIK/ GROF METHOD:

Bilirubin reacts with diazotized suphanilic acid (DSA) to form a red azo dye. The absorbance of this dye at 546 nm is directly proportional to the bilirubin concentration in the sample. Water soluble bilirubin glucuronides react directly with DSA whereas the albumin conjugated indirect bilirubin will only react DSA in the presence of an accelerator: total bilirubin = direct + indirect

Sulphanilic acid + Sodium nitrite → DSA

Bilirubin + DSA → Direct Azobilirubin

Bilirubin + DSA + Accelerator → Total Azobilirubin

Statistical analysis of data was carried out by using statistical package SPSS version 16.0 and Microsoft excel.

Results

The analysis of the data showed that the mean concentration of bilirubin was found to be 5.728 and that of creatinine 1.048. The mean difference of creatinine (without pre incubation) and true creatinine (pre incubation with NaOH) was found to be statistically significant, i.e $P<0.01$ [Student's paired t-test (1 tail)]. The creatinine value was found to be decreased in procedure without pre incubation.

The maximum and minimum bilirubin values were 27.10 and 0.70 mg/dl respectively. Thus this study covers a required range of icteric sample. The minimum and maximum creatinine value obtained were 0.00-3.90 mg/dl in this study.

Discussion

In the present study we find that bilirubin interfere in the estimation of creatinine by Jaffe Kinetic method. For normal bilirubin also creatinine is found to be slightly decreased but is not significant. 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) (Table1). Similar findings were reported by R.Vaishya, S.Arora et al, 2010.

The mean value of creatinine was different for pre-incubation with NaOH and without pre-incubation (Table 2). Little variation in creatinine estimation for normal bilirubin concentration. For bilirubin concentration <1mg/dl there was just little variation in creatinine value.

Table 1. Mean, median, S.D, min/maximum value of bilirubin and creatinine of total (71) samples

	Bilirubin (mg/dl)	Creatinine (without pre-incubation (mg/dl)	Creatinine (pre-incubation with NaOH) (mg/dl)	t-test (1-tail)
Mean	5.72	1.04	1.3	
Median	2.80	0.9	1.1	
SD	6.42	0.67	0.67	<0.001
Minimum	0.70	0.0	0.6	
Maximum	27.10	3.9	4.1	

Table 2. Different bilirubin concentrations and their respective findings

Bilirubin (mg/dl)		Creatinine without pre-incubation (mg/dl)	Creatinine with NaOH pre-incubation (mg/dl)	t-test (1-tail)*
	N	22	22	<0.001
< 1	Mean	0.86	0.97	
	S.D	0.21	0.23	
	N	24	24	<0.001
1-5	Mean	1.19	1.37	
	S.D	0.74	0.75	
	N	12	12	<0.001
5-10	Mean	1.15	1.44	
	S.D	0.56	0.53	
	N	5	5	<0.01
10-15	Mean	1.74	2.16	
	S.D	1.20	1.13	
	N	5	5	<0.01
15-20	Mean	1.05	1.62	
	S.D	0.72	0.70	
	N	3	3	<0.01
> 20	Mean	0.0	1.0	
	S.D	0.00	0.28	

*Value of Student's paired t-test between creatinine (without pre incubation) and true creatinine (pre incubation with NaOH).

Similarly for bilirubin concentration 1-5mg/dl and 5-10mg/dl there was mild increase in creatinine value (pre incubation with NaOH) and moderate increase for bilirubin concentration of 10-20mg/dl (in two categories 10-15 and 15-20 mg/dl). The significant increase in creatinine concentration after pre-incubation was found for bilirubin concentration >20mg/dl.

In this study bilirubin concentration >20mg/dl gave "out value" when creatinine is measured without pre incubation with NaOH. Though it is different from R.Vaishya, S.Arora et al but satisfied with other researchers: bilirubin- no significant interference up to 20mg/dl (Beckmen coulter).

The sample size in this study was 71, may not cover the large number of icteric samples but, this sample size was sufficient to give knowledge of bilirubin interference in creatinine estimation. Due to the scarcity of enough icteric samples this study did not contain large number of samples with bilirubin concentration higher than 20 mg/dl.

This study was carried out to remove bilirubin interference only but there might be other interferents like acetoacetate or lipids in the same sample that could cause the similar negative interference in creatinine estimation in Jaffe Kinetic method [5].

Creatinine estimation is primarily used indicator for renal function. As shown by the results of this study creatinine estimation by normal method may give false value of creatinine [6]. Hence care should be taken before creatinine value measurement in icteric sample particularly bilirubin > 5 mg/dl.

Conclusion

According to this study pre-incubation with NaOH helps to reduce this negative interference

of bilirubin in creatinine value measurement by Jaffe Kinetic Method.

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