ORIGINAL ARTICLE

MODIFICATION OF JAFFE'S KINETIC METHOD DECREASES BILIRUBIN INTERFERENCE: A PRELIMINARY REPORT

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ABSTRACT

The negative interference of bilirubin on serum creatinine determined by the kinetic alkaline picrate (Jaffe) reaction is the unresolved problem. 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 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. Though enzymatic methods are now routinely used as most accurate method but they are not acceptable due to cost constraints. 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 method so that it is amenable for use on the currently used analyzers.

KEY WORDS

True creatinine, Bilirubin, Negative interference, Jaffe's Kinetic method.

INTRODUCTION

The kinetic alkaline picrate (Jaffe's) method is used with various clinical laboratory instruments to determine serum creatinine. Over the years, bilirubin has caused significant problems in direct Jaffe assays but not in methods involving dialysis (1). Unlike end-point Jaffe methods, the kinetic method does not include protein precipitation or separation which also removes bilirubin (2). Thus when protein separation is omitted, bilirubin is present during the assay for creatinine. The decrease in apparent creatinine concentration is caused by both conjugated and unconjugated bilirubin (3). Different approaches have been taken to minimize this interference with varying degrees of success. Expert professional bodies have recommended that all creatinine methods should become traceable to a reference method based on isotope dilution-mass spectrometry (IDMS) (4). High performance liquid

chromatography, gas-chromatography with mass spectrometry methods have been developed as reference methods for creatinine estimation (5, 6) but the special instrumentation required for these methods is still an economic constraint in most of the laboratories in developing countries. Enzymatic systems using creatinine deaminase (7) which converts creatinine to N-methylhydantoin and ammonia (with various options for measuring ammonia) and creatininase (8), (with NADH measured at 340 nm after a creatine kinase reaction sequence) are being widely propagated as the most accurate routine methods which produce results that agree closely with IDMS (9,10). An alternative approach to manage bilirubin interference is to oxidise bilirubin to biliverdin using potassium ferricyanide prior to the Jaffe reaction (11), however, ferricyanide is destroyed over time if the reagent contains sodium dodecyl sulphate. Thus, ferricyanide must be separated from detergent to ensure protection from bilirubin interference (1).

Though the oxidizing properties of Sodium hydroxide (NaOH) have been known for years but it has never been used for removal of negative interference of bilirubin. Hence this study was planned in an effort to modify Jaffe's method of creatinine estimation so that it is compatible with present day analyzers and also takes care of bilirubin interference at the same time.

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In this study we compared the interference of bilirubin after pre-incubation of serum sample with 3 different concentrations of NaOH in Jaffe's reaction. The alkaline reagent R1 will convert any bilirubin present in the sample to biliverdin causing a decrease in absorbance at 505 nm, while we are trying to measure an increase in absorbance at 505nm due to picrate creatinine interaction.

MATERIALS AND METHODS

Serum samples from 250 patients with abnormal urea were selected and divided into 5 groups (50 each) based on bilirubin levels [Group A, bilirubin less than 1mg/dL, Group B- 1-5 mg/dL, Group C >5-15 mg/dL, Group D >15-25 mg/dL and Group E > 25 mg/dL]. The control group included 100 patients with normal urea levels. These were further subgrouped into Control Group I (50 patients with normal Bilirubin) and control group II (50 patients with bilirubin varying from 5-20 mg/dL). All the samples were analysed for serum creatinine in parallel, using 3 different concentrations of NaOH (95 mmol/L, 125 mmol/L and 150 mmol/L) in Jaffe's Kinetic reaction on Olympus AU 400 analyser, 95 mmol/L being the concentration of NaOH in Jaffe's kit being used in our clinical biochemistry laboratory. NaOH was treated as first reagent and sample was incubated with NaOH for 5 minutes before dispensing picric acid.

Twenty samples from each group were also run in parallel for estimation of serum creatinine by UV creatinine enzymatic assay (Randox). Statistical analysis was carried out using SPSS for windows 10.0 software (SPSS Inc., Chicago, IL, USA). The results are expressed as Mean $\pm$ SEM. Wilcoxon

signed Rank test was used for evaluation of paired samples. P-values of less than 0.05 were considered as significant.

RESULTS AND DISCUSSION

Serum creatinine estimation with 125 mmol/L of NaOH provided significantly higher values as compared to 95mmol/L in Group A and very highly significant values in Group B, C and D [Bilirubin concentration < 25 mg/dl]. In Group D & E, creatinine values with 150 mmol/L were significantly higher ($P<0.01$) as compared to 95 mmol/L NaOH. We compared the creatinine values obtained by 125 mmol/L NaOH and 150 mmol/L NaOH in Group D, as both the results were highly significant when compared to 95mmol/L NaOH. In Group D, creatinine results with 125mmol/L of NaOH were significantly higher than 150mmol/L ($P=0.015$).

In the control group I (with normal bilirubin and urea levels) there was minimal difference in creatinine estimation in all three groups but in the icteric samples the trend of serum creatinine was similar to study group B, C and D but with a lesser significance which could be attributed to otherwise normal serum creatinine in these patients.

The values of serum creatinine obtained by enzymatic methods showed significantly higher creatinine values in all the ranges of icteric sera as compared to the routinely used method in the lab (with 95 mmol/L NaOH). In control group I, (with normal values of creatinine, bilirubin and urea), the difference in creatinine values obtained by all methods was non-significant. However, in the icteric samples observed values with 125

Table 1: Comparison of Creatinine values obtained with different NaOH concentrations in Jaffe's Kinetic Reaction (Values are mean $\pm$ SD)

(n=50) in all groups	Urea levels (mg/dL)	Creatinine (mg/dl)		
		95 mmol/L NaOH	125 mmol/L NaOH	150 mmol/L NaOH
Group A (Bilirubin < 1 mg/dL)	117.29 $\pm$ 5.1	2.86 $\pm$ 0.42	3.26 $\pm$ 0.49 ($P=0.010$)	2.99 $\pm$ 0.48 ($P=0.042$)
Group B (Bilirubin 1-5 mg/dL)	115.32 $\pm$ 3.9	2.01 $\pm$ 0.17	2.92 $\pm$ 0.21 ($P<0.001$)	2.15 $\pm$ 0.19 ($P=0.051$)
Group C (Bilirubin >5- 15 mg/dL)	110.52 $\pm$ 5.32	1.86 $\pm$ 0.21	2.54 $\pm$ 0.20 ($P<0.001$)	1.99 $\pm$ 0.24 ($P= 0.058$)
Group D (Bilirubin >15- 25 mg/dL)	120.13 $\pm$ 4.09	1.87 $\pm$ 0.41	2.87 $\pm$ 0.42 ($P<0.001$)	2.52 $\pm$ 0.37 ($P=0.009$)
Group E (Bilirubin >25 mg/dL)	110.51 $\pm$ 5.46	1.83 $\pm$ 0.57	2.08 $\pm$ 0.39 ($P=0.058$)	2.69 $\pm$ 0.32 ($P=0.006$)
Control Group I (Urea< 50 mg/dL bilirubin<1 mg/dL)	30.12 $\pm$ 1.88	0.85 $\pm$ 0.05	0.90 $\pm$ 0.08 ($P=0.632$)	0.94 $\pm$ 0.09 ($P=0.51$)
Control group II (Urea <50 mg/dL) (Bilirubin 5-20 mg/dL)	36.23 $\pm$ 1.3	0.90 $\pm$ 0.08	1.20 $\pm$ 0.10 ($P=0.035$)	1.09 $\pm$ 0.16 ($P=0.054$)

Table 2: Comparison of creatinine values obtained by Jaffe's method and UV enzymatic method

(n=20) in all groups	Blood Urea levels	Serum Creatinine (mg/dl)			
		Enzymatic method	95mmol/L NaOH	125mmol/L NaOH	150mmol/L NaOH
Group A (bilirubin<1mg/dL)	121.29 ± 6.1	3.21 ± 0.61	2.86 ± 0.38 (P=0.043)	3.20 ± 0.38 (P=0.92)	3.05 ± 0.52 (p=0.27)
Group B (Bilirubin 1-5 mg/dL)	118.26 ± 4.3	3.02 ± 0.54	2.31 ± 0.44 (P=0.036)	2.97 ± 0.55 (P=0.88)	2.65 ± 0.43 (p=0.051)
Group C (Bilirubin >5- 15 mg/dL)	112.25 ± 4.52	2.84 ± 0.35	2.16 ± 0.39 (P=0.04)	2.76 ± 0.42 (P=0.90)	2.59 ± 0.45 (p= 0.058)
Group D (Bilirubin >15- 25 mg/dL)	125.43 ± 4.9	3.36 ± 0.53	2.13± 0.47 (P=0.015)	3.22 ± 0.59 (P=0.39)	3.01 ± 0.42 (p=0.109)
Group E (Bilirubin >25 mg/dL)	115.51 ± 6.2	2.74 ± 0.41	1.73 ± 0.52 (P=0.009)	2.38 ± 0.39 (P=0.048)	2.89 ± 0.47 (p=0.69)
Control Group I (Urea< 50 mg/dL bilirubin<1 mg/dL)	28.12 ± 2.05	0.88 ± 0.10	0.87 ± 0.09 (P=0.82)	0.90 ± 0.08 (P=0.65)	0.91 ± 0.09 (p=0.59)
Control group II (Urea <50 mg/dL) (Bilirubin 5-20 mg/dL)	30.29 ± 1.9	1.30 ± 0.20	0.99 ± 0.16	1.26 ± 0.10 (P=0.70)	1.19 ± 0.16 (p= 0.38)

P-values indicates comparison of creatinine values of the given Jaffe's reaction with enzymatic assay.

mmol/L NaOH and enzymatic method were very close and the difference was found to be non-significant. However, in the patients with very high bilirubin levels (>25 mg/dl), the results of enzymatic estimation had a better agreement with the results obtained by 150 mmol/L NaOH as compared to 125 mmol/L (Table 2).

The trend of creatinine values seen in this study with different concentrations of NaOH clearly indicate that in the absence of a reference method, this minor modification in Jaffe's kinetic reaction could solve a major problem of negative interference of bilirubin in creatinine estimation. This could be beneficial in biochemical assessment of patients with hepato-renal syndrome.

Though enzymatic assays are available for accurate determination of serum creatinine, however, the cost effectiveness of Jaffe's kinetic reaction as compared to enzymatic methods cannot be overlooked. The authors propose that for normal and mild to moderately icteric samples (bilirubin concentration below 25 mg/dl), NaOH solution used in Jaffe's kinetic reaction should have a concentration of 125 mmol/L to minimize the negative interference of bilirubin. However, if bilirubin concentration in the serum sample is more than 25 mg/dl, it is recommended that the sample should be pre-incubated with 150 mmol/L of NaOH instead of 125 mmol/L to completely oxidize bilirubin for minimal interference.

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