Bilirubin Interference in Serum Creatinine Estimation by Jaffe's kinetic Method and Its Rectification in Three Different Kits

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Abstract The negative interference by bilirubin in serum creatinine estimation by Jaffe's kinetic method is well known. Several approaches have been suggested to overcome this interference. In this article three different creatinine kits (Jaffe's kinetic method) have been tested for bilirubin interference and its rectification using two simple approaches. The performance of three kits (A, B and C) supplied by three different manufacturers was tested using IQC and EQAS sera and pooled serum with added bilirubin. To overcome the bilirubin interference two approaches viz. NaOH preincubation and TCA precipitation were used. Bilirubin did interfere in creatinine estimation after a certain level (2.3 mg/dl). However, both NaOH preincubation and TCA precipitation approach rectified this interference. The performance of kit A was better than kit B and C. All the three kits showed bilirubin interference upon increasing the bilirubin concentration but kit A performed better than kit B and C. However, NaOH incubation and TCA precipitation methods overcame this interference to a great extent.

Keywords Bilirubin · Creatinine · Interference

Introduction

The negative interference by bilirubin in estimation of serum creatinine by Jaffe's method is well known [1–3]. Several approaches have been suggested to overcome this

interference [4–10]. Interestingly, the manufacturers of creatinine kits (Jaffe's kinetic method) do not mention about bilirubin interference in their package inserts. Therefore, when it comes to select a kit for serum creatinine estimation it becomes imperative to check it for bilirubin interference as it is important to assess the quality of the kit we use [11]. In this article the performance of three kits from three different manufacturers has been mentioned using deproteinization of serum by trichloroacetic acid (TCA) [8] and preincubation with sodium hydroxide (NaOH) [10] which are the two most convenient approaches to overcome the bilirubin interference.

Materials and Methods

The performance of three creatinine kits (Jaffe's kinetic method) A, B and C from three different manufacturers was tested. Kit A and B used two reagents viz. NaOH and picric acid which were to be mixed in equal volumes before use whereas kit C had a single reagent containing both NaOH and picric acid and was ready to use. The performance of these three kits was checked using Internal Quality Control (IQC) sera (Biorad) and External Quality Control sera (EQAS) provided by Christian Medical College, Vellore, India as neither the reference method (IDMS) nor the enzymatic method for creatinine estimation was available with us. In addition, bilirubin was added to pooled serum (from left over sera) to make bilirubin concentration 2, 4 and 8 mg/dl. Appropriate calculation was made to compensate the dilution due to addition of bilirubin. To assess the rectification of bilirubin interference two simple approaches were used: (1) Preincubation with NaOH—The serum was incubated with NaOH reagent provided with the kits for 5 min at room temperature and

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then picric acid was added and the reaction rate was monitored as specified in the package insert. In case of kit C which contained a single reagent, the serum was incubated with 125 mmol/L NaOH in the ratio of 1:1 for 5 min at room temperature and aliquots was taken to estimate creatinine. The results were multiplied by 2 (the dilution factor). (2) TCA precipitation—The serum was mixed with 0.55 M TCA in the ratio of 2:1 and centrifuged for 10 min after standing at room temperature for 10 min. The supernatant was used for creatinine estimation and the results were multiplied by 1.5 (the dilution factor). All samples were analysed in triplicates.

Results

Perusal of Table 1 shows that the values of creatinine estimated directly in serum by brand A and C at bilirubin level up to 2.3 mg/dl were close to target values (bias ranged from 0 to 0.1 mg/dl by A and 0 to 0.23 mg/dl by C; Table 2). However, brand B gave relatively more bias ranging from 0 to 0.4 mg/dl. As the bilirubin levels increased the creatinine was underestimated by all the three kits and the least values were given by brand B. The measured bias (mg/dl) ranged from −0.3 to −0.93, −0.6 to −1.53 and −0.5 to −1.03 for brand A, B and C respectively. In pooled serum with added bilirubin also all the three kits underestimated creatinine with increase in bilirubin concentration (Table 3). However, the preincubation with NaOH and TCA precipitation resulted in bringing the creatinine values close to the target values and the measured bias also decreased with least bias given by brand A (+0.1 to −0.23 by NaOH preincubation and +0.15 to −0.37 by TCA precipitation). Preincubation with NaOH and TCA precipitation resulted in definite improvement in creatinine values in pooled serum with added bilirubin also as was noticed with IQC and EQAS sera.

Discussion

Out of the three brands tested the brand A showed best performance followed by brand C but only up to bilirubin level 2.3 mg/dl. NaOH preincubation and TCA precipitation resulted in definite improvement in creatinine estimates in icteric sera. However, the overall response of preincubation with NaOH seemed to be better than TCA precipitation procedure to overcome bilirubin interference. Moreover, TCA precipitation approach appears to be less comfortable, more time consuming and error prone due to dilution step. Though the influence of the interfering substances has been found to be less frequent with enzymatic

Table 1 Serum creatinine (mg/dl) at different bilirubin levels in IQC and EQAS sera (values are mean of triplicate determination)

Sample	Bilirubin (mg/dl)	Creatinine (mg/dl) Target values	BRAND 'A'			BRAND 'B'			BRAND 'C'		
			Direct serum	NaOH preincubation	TCA precipitation	Direct serum	NaOH preincubation	TCA precipitation	Direct serum	NaOH preincubation	TCA precipitation
Biorad level 1	0.9	2.53	2.47	2.6	2.68	2.2	2.6	2.65	2.3	2.6	2.78
Biorad level 2	4.2	6.23	5.3	6.0	5.86	4.7	5.3	5.1	5.2	5.4	5.6
EQAS 1	1.6	3.6	3.5	3.57	3.67	3.2	3.5	3.54	3.6	3.6	3.5
EQAS 2	2.3	0.8	0.8	0.92	0.9	0.8	0.9	0.9	0.8	0.8	0.7
EQAS 3	8.3	3.9	3.6	4.0	4.05	3.3	3.4	3.45	3.4	3.6	3.9

Table 2 Measured bias in creatinine values (mg/dl) at different bilirubin levels

Bilirubin concentration (mg/dl)	BRAND 'A'			BRAND 'B'			BRAND 'C'		
	Direct serum	NaOH preincubation	TCA precipitation	Direct serum	NaOH preincubation	TCA precipitation	Direct serum	NaOH preincubation	TCA precipitation
0.9	−0.06	+0.07	+0.15	−0.33	+0.07	+0.12	−0.23	+0.07	+0.25
1.6	−0.1	−0.03	+0.07	−0.40	−0.10	−0.06	0	0	−0.10
2.3	0	+0.10	+0.10	0	+0.10	+0.10	0	0	−0.05
4.2	−0.93	−0.23	−0.37	−1.53	−0.93	−1.13	−1.03	−0.83	−0.63
8.3	−0.3	+0.1	+0.15	−0.6	−0.5	−0.45	−0.5	−0.3	0

Table 3 Serum creatinine levels (mg/dl) in pooled serum with added bilirubin (values are mean of triplicate determinations)

Bilirubin level (mg/dl)	BRAND 'A'			BRAND 'B'			BRAND 'C'		
	Direct serum	NaOH preincubation	TCA precipitation	Direct serum	NaOH preincubation	TCA precipitation	Direct serum	NaOH preincubation	TCA precipitation
1.2	1.38	1.44	1.40	1.40	1.5	1.5	1.30	1.6	1.35
2.0	1.35	1.46	1.57	1.38	1.4	1.6	1.28	1.4	1.20
4.0	1.1	1.5	1.51	1.16	1.4	1.6	1.1	1.4	1.20
8.0	1.0	1.5	1.5	1.0	1.5	1.6	0.9	1.2	1.20

procedure than with Jaffe's kinetic method [12] yet the Jaffe's method has the benefit of cost effectiveness. Moreover, the enzymatic methods do not show complete specificity to creatinine [13]. Therefore, the Jaffe's kinetic method is still a method of choice as the bilirubin interference can be reduced by using NaOH preincubation and TCA precipitation approaches. It is also important to check the creatinine kits before use for bilirubin interference and creatinine estimation in icteric sera should be done using modified procedures.

Compliance with Ethical Standards

Conflict of interest None.

Ethical Approval Not required as this study does not contain any studies with human participants or animals performed by the author.

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