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The Effect of Bilirubin on Laboratory Investigations on Serum Creatinine: A Comparison Study Between Jaffe Reaction and Creatinase Enzymatic Method With Creatinine in Phosphate Buffered Saline Solution and Serum

Ruchira Kumari Dissanayake[1], Kamburawala Kankanamgee Pramodya Tharaki Ranaweera[1], Pathiranage Dias[2], Athapaththu Mudiyanselage Buddhika Priyadarshani[1]

[1] University of Sri Jayewardenepura, Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Sri Lanka.
[2] University of Sri Jayewardenepura, Department of Statistics, Faculty of Applied Sciences, Sri Lanka.

Correspondence Author: Athapaththu Mudiyanselage Buddhika Priyadarshani
E-mail: priyadarshani@sjp.ac.lk
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ABSTRACT

Objective: To determine the creatinine concentration in phosphate buffered saline solution and serum with different bilirubin concentrations using Jaffe reaction and Creatinase method.

Methods: In Phase 1, creatinine and bilirubin concentrations in the dilution series were 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 mg/dL and 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30 mg/dL, respectively. Each creatinine concentration was spiked with eleven bilirubin concentrations used in Phase 1. In Phase 2, serum with creatinine values 0.51, 2.41 and 7.33 mg/dL were spiked with 11 bilirubin concentrations. The total bilirubin, creatinine by Jaffe reaction and Creatinase method were measured.

Results: In Phase 1, Jaffe reaction showed a significant underestimation up to creatinine concentration of 2 mg/dL at all bilirubin concentrations. From 3 mg/dL onwards, a significant overestimation was observed with high bilirubin concentrations. In Phase 2, Creatinase method gave no significant underestimation in serum with 0.51 mg/dL of creatinine. But Jaffe reaction showed a significant underestimation from bilirubin concentration of 7.18 mg/dL. In serum with 2.41 mg/dL creatinine, Creatinase and Jaffe methods gave significant underestimations from bilirubin concentrations of 9.05 and 5.64 mg/dL, respectively. In serum of 7.33 mg/dL creatinine, significant underestimations were given from bilirubin levels of 3.6 and 8.18 mg/dL by Creatinase and Jaffe methods, respectively.

Conclusion: In normal to moderately high bilirubin concentrations the Creatinase method is more accurate than the Jaffe method in all creatinine concentrations used. At high creatinine concentrations Creatinase method gave significant underestimations which increased with bilirubin concentration.

Keywords: Bilirubin, Creatinine, Jaffe reaction, Creatinase method

1. INTRODUCTION

Creatinine is a metabolic waste product of creatine phosphate which is a high energy molecule used in the muscle to provide energy (1). Serum creatinine measurement is used as a marker of renal function and in calculating the glomerular filtration rate (GFR) because creatinine is excreted solely through the kidneys. It is of utmost importance to assess the renal function accurately for the proper management of renal failure and it is used in grading of different stages of chronic kidney disease (CKD). Therefore, the accurate measurement of serum creatinine levels is of great importance.

Jaffe reaction is the most commonly used test method for serum creatinine estimation in the medical laboratories world-wide as it is both simple and cost effective. However, this method has several major drawbacks due to interferences from endogenous substances such as bilirubin, ketone bodies, proteins, glucose and exogenous substances including drugs (2). Out of these interferences the present study was designed to study the bilirubin interference since it is more frequently encountered in the clinical environment. In hyperbilirubinemic patients the Jaffe reaction has shown to give a significant underestimation of the serum creatinine value (3). As a result, accurate renal assessment has become impossible in patients with hepatorenal failure, multi-organ failure and neonatal jaundice with impaired renal activity (4).

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This problem can be overcome by using the Creatinase enzymatic test method. This is more specific since it uses enzymes to carry out specific reactions (5). However, this cannot be widely used due to its relatively high cost.

Therefore, the objective of this study was to compare these two test methods and obtain a better understanding in the error caused in the Jaffe reaction and to identify the minimum bilirubin concentration that gives interference to the serum creatinine measurement.

2. METHODS

This is an analytical cross-sectional study with laboratory investigations. The study was carried out under 2 distinct phases.

2.1. Phase 1

Phase 1 of this study was carried out to determine the effect of bilirubin on creatinine in phosphate buffered saline solution.

2.2. Reagent preparation

The creatinine dilution series was prepared with 20 mg/dL of creatinine standard solution. The final creatinine concentrations in phosphate buffered saline were; 0, 2, 4, 6, 8 10, 12,14,16,18 mg/dL. By using bilirubin standard solution of 60 mg/dL following bilirubin concentration gradient was prepared; 0, 6, 12, 18, 24, 36, 42, 48, 54 and 60 mg/dL.

2.3. Preparation of final matrix with creatinine and bilirubin

The first creatinine dilution of 0 mg/dL was taken and 0.5 mL from this concentration was pipetted into 11 labeled khan tubes which were covered with aluminum foil. Then, 0.5 mL from each bilirubin solution prepared in the dilution series was added to the above khan tubes and mixed well. This procedure was repeated with all the creatinine dilutions to give 110 khan tubes. Table 1 indicates the creatinine and bilirubin concentration of each tube.

2.4. Phase 2

Phase 2 of this study was carried out to determine the bilirubin interference in serum creatinine. Prior to start the study ethical approval was obtained from the Ethical Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka.

2.5. Preparation of serum pools

The serum specimens were collected in three groups depending on the serum creatinine values as (i) below 0.8 mg/dL, (ii) between 2 – 4 mg/dL (iii) above 6 mg/dL. Serum creatinine by Creatinase method and total bilirubin concentration of each pool was measured separately. Total bilirubin levels of serum pool 1, 2 and 3 were 0.45, 0.45 and 0.99 mg/dL, respectively.

The serum from each pool was placed on ice-packs within an insulating rigid foam box. The box was placed in an infant phototherapy incubator without a temperature regulator for 18 hours in order to photolyse the serum bilirubin. Ice packs were changed at every 6 hours to maintain serum pools at low temperature. The serum creatinine and total bilirubin values were then re-measured for all three pools to ensure that the serum creatinine values were unchanged and the total bilirubin concentrations were below 1.46 mg/dL (25 µmol/L). The serum pool 1, 2 and 3 gave serum creatinine values of 0.51, 2.41 and 7.33 mg/dL, respectively. The bilirubin concentrations of serum pool 1, 2 and 3 after phototherapy treatment were 0.05, 0.12 and 0.21 mg/dL, respectively which were well under the recommended maximum concentration (6).

2.6. Preparation of bilirubin dilution series

A series of bilirubin concentrations was prepared by mixing calculated volumes from bilirubin stock solution and 100 mM NaOH solution to get the bilirubin concentrations twenty-one times higher than what was required when mixed with the serum sample (7). This was done to minimize the dilution of the serum to a value below 5%.

The bilirubin stock solution was prepared with the concentration of 630 mg/dL as this concentration was required in order get the final dilution of the serum bilirubin to be no more than 5% (6). A series of bilirubin concentrations was prepared by using bilirubin stock solution with the concentrations of 0, 63, 126, 189, 252, 315, 378, 441, 504, 567 and 630 mg/dL.

2.7. Spiking of serum pools with bilirubin dilution series

All tubes used for mixing the solution were covered with aluminum foil to prevent exposure to sun-light. One milliliter from creatinine concentration of 0.51 mg/dL was pipetted into 11 khan tubes. Fifty microliters from each of the 11-bilirubin concentrations were added separately into each tube and mixed well. This was repeated for serum pools containing creatinine concentrations of 2.41 and 7.33 mg/dL.

2.8. Analysis of creatinine and bilirubin concentrations

Three reagent kits were used for the (i) creatinine enzymatic (ii) creatinine Jaffe reaction and (iii) total bilirubin assays and determination methods are as follows.

Creatinine enzymatic method: Creatinine is converted to sarcosine with the presence of creatininase and creatinase. Sarcosine is then converted to glycine, formaldehyde and hydrogen peroxide by sarcosine oxidase in the presence of oxygen. The hydrogen peroxide then reacts with 4-aminophenazone and 2,4,6-triiodo-3-hydroxybenzoic acid and form a quinone imine chromogen and this reaction

is catalyzed by peroxidase. The color intensity is directly proportional to the creatinine concentration and it can be measured by photometrically at 540 nm.

Creatinine Jaffe reaction: The reaction is based on the Jaffe method where creatinine forms a red color complex with an alkaline picrate solution. The intensity of the complex is measured at 510 nm.

Total bilirubin assay: The total bilirubin is coupled with p-nitrobenzenediazonium salt to form azobilirubin and the colour intensity is proportional to the concentration of total bilirubin.

An IndikoTM Clinical and specialty Chemistry System was used to analyze the samples. This is a fully automated, sample oriented and random-access analyzer. Prior to specimen analysis the analyzer was calibrated for all three test methods. All three test methods were calibrated using the same calibration fluid as specified by the manufacturers. Following the calibration of the analyzer, quality control samples were analyzed. The quality control samples were of two levels, normal and abnormal. Once the quality control results came out normal the specimens were analyzed. Once the analysis was completed the results were printed out.

2.9. Statistical analysis

The statistical software used was Minitab (Version 17.0) and the statistical method was Regression Analysis.

3. RESULTS

3.1. Phase 1

Creatinine concentrations of the final matrix prepared in Phase 1 was ranged from 0 to 9 mg/dL (see Table 1).

3.2. The effect of bilirubin on creatinine in phosphate buffered saline solutions: According to the Jaffe reaction

It could be observed that there was an underestimation of creatinine with increasing bilirubin concentrations up to the creatinine concentration of 2 mg/dL. After this point onwards an overestimation of creatinine concentration could be observed. To consider the underestimation as a significant value, the percentage of the difference between the creatinine measurement at 0 mg/dL and the given creatinine measurement should be more than 10% (8). The percentage of the difference was calculated by;

$$\frac{([\text{Creatinine}] \text{ at 0 mg/dL bilirubin} - [\text{Creatinine}] \text{ at X bilirubin concentration})}{[\text{Creatinine}] \text{ at 0 mg/dL bilirubin}} \times 100\%$$

X – Bilirubin concentration at which the interference is being analyzed.

The minimum bilirubin concentration which gives a significant underestimation for each of the creatinine standard solution is given in Table 2.

Table 1. *Preparation of final matrix with creatinine phosphate in buffered saline and bilirubin*

[Creatinine] (mg/dL) / [Bilirubin] (mg/dL)	0	2	4	6	8	10	12	14	16	18
0	0 / 0	1 / 0	2 / 0	3 / 0	4 / 0	5 / 0	6 / 0	7 / 0	8 / 0	9 / 0
6	0 / 3	1 / 3	2 / 3	3 / 3	4 / 3	5 / 3	6 / 3	7 / 3	8 / 3	9 / 3
12	0 / 6	1 / 6	2 / 6	3 / 6	4 / 6	5 / 6	6 / 6	7 / 6	8 / 6	9 / 6
18	0 / 9	1 / 9	2 / 9	3 / 9	4 / 9	5 / 9	6 / 9	7 / 9	8 / 9	9 / 9
24	0 / 12	1 / 12	2 / 12	3 / 12	4 / 12	5 / 12	6 / 12	7 / 12	8 / 12	9 / 12
30	0 / 15	1 / 15	2 / 15	3 / 15	4 / 15	5 / 15	6 / 15	7 / 15	8 / 15	9 / 15
36	0 / 18	1 / 18	2 / 18	3 / 18	4 / 18	5 / 18	6 / 18	7 / 18	8 / 18	9 / 18
42	0 / 21	1 / 21	2 / 21	3 / 21	4 / 21	5 / 21	6 / 21	7 / 21	8 / 21	9 / 21
48	0 / 24	1 / 24	2 / 24	3 / 24	4 / 24	5 / 24	6 / 24	7 / 24	8 / 24	9 / 24
54	0 / 27	1 / 27	2 / 27	3 / 27	4 / 27	5 / 27	6 / 27	7 / 27	8 / 27	9 / 27
60	0 / 30	1 / 30	2 / 30	3 / 30	4 / 30	5 / 30	6 / 30	7 / 30	8 / 30	9 / 30

Note: The final concentration of each analyte in the final tube is half the concentration of the initial solutions as they are diluted by 50% when mixing equal volumes of the two solutions. Each square in the above table gives the bilirubin and creatinine concentration in each individual tube.

Table 2. *The minimum bilirubin concentration causing significant underestimation in each creatinine concentration as determined by Jaffe reaction*

Creatinine concentration (mg/dL)	Minimum bilirubin concentrations which give significant underestimation in creatinine (mg/dL)
0	17.63
1	96.61
2	31.62
3	29.55
4	17.12
5	222.71
6	7.9
7	15.83
8	6.51
9	4.3

Excluding the bilirubin cut off points at creatinine concentrations of 5 and 6 mg/dL all other cut off points decreased with increasing creatinine concentration indicating that the error increases with the creatinine concentration.

3.3. The effect of bilirubin on creatinine in phosphate buffered saline solutions: According to the Creatinase enzymatic method

In the concentration series with no creatinine the enzymatic assay showed a reading of zero even with increasing bilirubin concentration. After this point the results indicated that, as the bilirubin concentration increases there is a slight underestimation in creatinine level. The underestimation increased with increasing bilirubin concentration. However, the rate at which the underestimation occurred, increased with the creatinine concentration itself. The minimum bilirubin concentration which gave a significant underestimation for each of the creatinine standard solution is given in Table 3. A specific pattern could not be observed in this sequence.

Table 3. *The minimum bilirubin concentration causing significant underestimation in each creatinine concentration as determined by Creatinase enzymatic method*

Creatinine concentration (mg/dL)	Minimum bilirubin concentrations which give significant underestimation in creatinine (mg/dL)
0	410.84
1	29.68
2	13.12
3	8.41
4	11.78
5	4.69
6	6.68
7	4.62
8	5.13
9	5.57

3.4. Comparison of Jaffe reaction and Creatinase enzymatic method in determination of creatinine concentration in phosphate buffered saline solutions

When comparing the two methods, Jaffe reaction showed underestimations relative to the enzymatic method at the initial creatinine concentrations. Jaffe reaction showed a significant underestimation in creatinine concentrations of 0, 1 and 2 mg/dL with all the bilirubin concentrations. From 3 mg/dL onwards, Jaffe reaction showed an overestimation with high bilirubin concentrations. The minimum bilirubin concentration required for overestimation varied with the creatinine concentration. According to the Creatinase method, as the bilirubin concentration increases, a significant underestimation was given by creatinine concentrations of above 2 mg/dL. The lowest bilirubin concentration needed for creatinine underestimation varied with the creatinine concentration. The underestimation observed with increasing bilirubin concentration increased when creatinine concentration of the standard solution increases. Comparison of these two methods can be done more accurately by plotting bias graphs. The creatinine values obtained by the Jaffe and enzymatic methods were used to calculate the bias and it was calculated according to the following equation.

Bias = [creatinine concentration by Jaffe reaction]–[creatinine concentration by Creatinase method]

The bias was then plotted against the bilirubin concentrations (see Figure 1). In these graphs the solution with 0 mg/dL creatinine (Figure 1 A) is the only concentration where the bias increases negatively. In the 1 mg/dl creatinine solution (Figure 1 B) the values indicated an initial negative bias but the negativity of the bias decreases as the bilirubin concentration increases. Even as the negativity of the bias reduces the bias still remains negative throughout the increasing bilirubin concentrations. However, after 1 mg/dL (Figure 1 C, D, E, F, G, H, I, J) the bias started giving positive values at high bilirubin concentrations. The bilirubin concentrations at which the bias becomes zero (i.e. the point at which the negative bias turns into a positive bias) in 2, 3, 4, 5, 6, 7, 8, and 9 mg/dL creatinine solutions are 20.79, 12.95, 10.47, 8.98, 8.808, 7.567, 8.55, 7.009, respectively. It can be seen that this value decreases with increasing creatinine concentration.

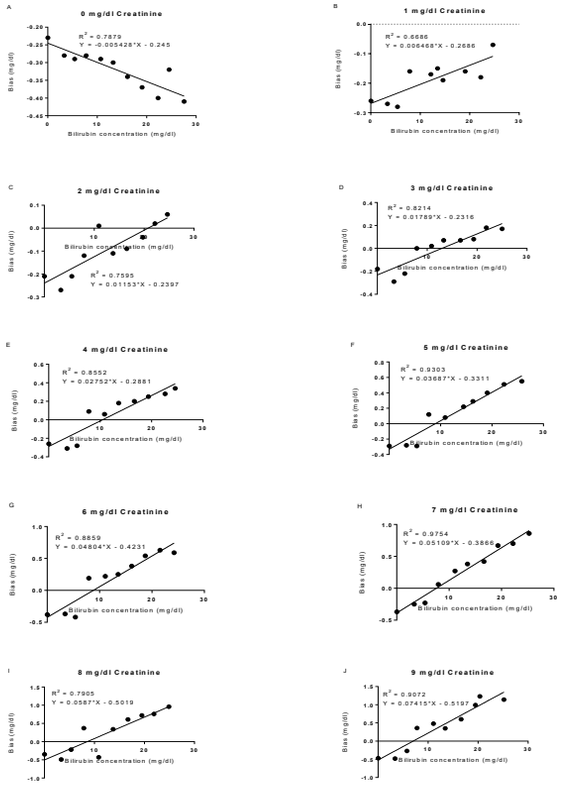

Figure 1. *Bias plot of creatinine. Bias plots compare Jaffe reaction and Creatinase enzymatic methods. Bilirubin concentrations used were 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 mg/dL. Creatinine concentration of the stock solutions ranged from 0 to 9 mg/dL*

3.5. Phase 2

3.5.1. The effect of bilirubin on serum creatinine concentrations

Three serum pools had creatinine concentrations of 0.51, 2.41 and 7.33 mg/dL as determined by Creatinase method and negligible bilirubin concentrations of 0.05, 0.12 and 0.21 mg/dL, respectively after phototherapy treatment. These bilirubin values were less than the recommended maximum concentration of 1.46 mg/dL prior to spiking the serum with bilirubin (6).

3.5.2. The effect of bilirubin on 0.51 mg/dL serum creatinine concentration

Creatinase enzymatic assay showed no significant underestimation with bilirubin concentrations used in the present study. However, underestimation became significant at the bilirubin concentration of 41.17 mg/dL. The Jaffe method on the other hand showed a significant underestimation of creatinine at bilirubin concentrations of 7.18 mg/dL.

3.5.3. The effect of bilirubin on 2.41 mg/dL serum creatinine concentration

At 2.41 mg/dL serum creatinine, the Creatinase enzymatic method showed a significant underestimation of creatinine at the bilirubin concentration of 9.05 mg/dL. The serum creatinine values given by the Jaffe method also have significant underestimations which increased with the increasing bilirubin concentration. According to the Jaffe reaction the significant underestimation started with the bilirubin concentration of 5.64 mg/dL.

3.5.4. The effect of bilirubin on 7.33mg/dL serum creatinine concentration

At 7.33 mg/dL creatinine level, according to the Creatinase method a significant underestimation of creatinine was observed from the bilirubin concentration of 3.6 mg/dL whereas in the Jaffe reaction a significant underestimation was observed at the bilirubin concentration of 8.18 mg/dL.

3.5.5. Comparison of Jaffe reaction and Creatinase enzymatic method in determination of serum creatinine concentrations of 0.51, 2.41 and 7.33 mg/dL

The bilirubin concentration where the underestimation is significant becomes lower with increasing creatinine concentration. However, in all three serum pools the Jaffe reaction gave a negative reading at all bilirubin concentrations with respect to the Creatinase enzymatic method. The bias between the enzymatic and Jaffe method was calculated for each bilirubin concentration in all three serum pools according to the following equation.

Bias = [Serum creatinine of Jaffe reaction] – [Serum creatinine of Creatinase method]

All three pools showed an increasing negative bias up to a given concentration of bilirubin (See Figure 2). After this point the bias decreases for serum pool 2 (2.41 mg/dL creatinine) and 3 (7.33 mg/dL creatinine). The data available is not enough to predict what happens in pool 1 (0.51 mg/dL creatinine) after the downward trend. The bilirubin concentration at which the trend in the bias changes (i.e. the bilirubin concentration at the maximum bias) is different for three pools. With increasing creatinine concentration, the bilirubin concentration at which the highest bias occurs reduces.

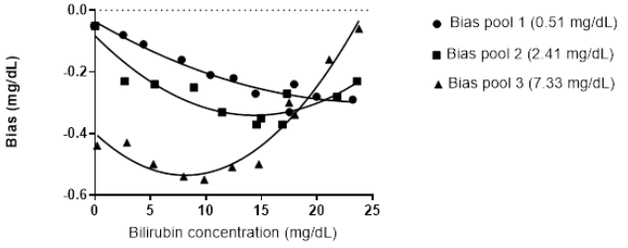

Figure 2. *Bias plot for creatinine with the bilirubin concentration*

3.5.6. Determination of the correction factor

Using the data collected in Phase 2 we were able to get equations for the bias of the Jaffe reaction relative to the enzymatic reaction. The bias can be added to the Jaffe reaction and thereby correction can be done. However, the data also showed that the bias varies with not only the bilirubin concentration but with the creatinine concentration itself as well. Hence, we got three separate equations for the three serum pools we used as follow.

Pool 1 Bias = (-0.034) + [-0.021x (bilirubin concentration)] + 0.000415 (bilirubin concentration)^2

Pool 2 Bias = (-0.078) + [-0.036x (bilirubin concentration)] + 0.001(bilirubin concentration)^2

Pool 3 Bias = (-0.399) + [-0.033x (bilirubin concentration)] + 0.002 (bilirubin concentration)^2

As a result, we could not calculate a single correction factor that could be applied for all creatinine concentrations.

4. DISCUSSION

Phase 1 was designed to give a broad view on all possible creatinine and bilirubin combinations. For a comprehensive study we required serum samples with different bilirubin and creatinine concentrations. This includes samples with high creatinine and bilirubin, high creatinine and low bilirubin, low creatinine and high bilirubin and low creatinine and low bilirubin. Due to the practical difficulty in obtaining such a large range of specimens covering the whole spectrum

we resorted to use an artificial matrix. Natural conditions in serum samples were stimulated as much as possible by adding Bovine serum albumin (BSA) and by maintaining the pH at 7.4. The creatinine and bilirubin concentrations used for Phase 1 were selected so as to cover all clinically significant values (9). Creatinine concentrations used ranged from 0 – 9 mg/dL.

Bilirubin can cause two kinds of interferences. A negative interference can be caused due to the conversion of bilirubin into biliverdin. A positive spectrophotometric interference can be caused as the absorbance of bilirubin, and the coloured complex produced by the Jaffe reaction have a similar wavelength (10,11).

In creatinine solutions of 0, 1 and 2 mg/dL the Jaffe reaction shows a significant underestimation with increasing bilirubin concentrations. This can be attributed to the negative interference being greater than the positive interference. At creatinine concentrations of 3, 4, 5, 6, 7, 8 and 9 mg/dL the Jaffe reaction shows a significant overestimation with increasing bilirubin concentrations. This can be due to the spectrophotometric interference caused by high bilirubin concentrations being greater than the negative interference. However, this does not explain why the spectrometric interference becomes greater than the chemical interference with increasing creatinine concentrations. Such an interaction in Jaffe reaction due to creatinine concentration itself has not been documented. Since the only changing factor between these solutions is creatinine concentration, we can assume that this trend is due a reaction involving creatinine molecules.

The enzymatic method has a non-significant underestimation at very low creatinine concentrations which slowly increases with increasing creatinine concentration. This tallies with a previous study (12). Our findings show that the underestimation increases with increasing creatinine concentration. In the enzymatic reaction the concentration is measured spectrophotometrically by measuring H_2O_2 produced in the reaction. The H_2O_2 can also oxidize bilirubin into biliverdin (5). However, it is mentioned that this error has been reduced by using efficient H_2O_2 acceptors (triiodo-hydroxy-benzoic acid), including potassium ferrocyanide and detergents (13). Our results indicate that these corrective measures are insufficient at very high bilirubin concentrations.

Both Jaffe and Creatinase enzymatic reaction show varying deviations with increasing creatinine concentrations. While no clear-cut answer can be given as to why this occurs, it can be assumed that it has to do with a chemical reaction involving creatinine molecules. Creatinine molecules may dimerize at high concentration which may have previously unidentified reaction with picric acid in the Jaffe reaction or with bilirubin itself. It may cause spectrophotometric variances as well. High creatinine concentrations may also have an allosteric effect on Creatinase causing an alteration in the reaction.

Although a wide concentration range was covered through Phase 1, for an experiment to be clinically applicable it should be done in a serum matrix. Hence, for Phase 2 we did a similar study using pooled serum of three creatinine concentrations. The three creatinine concentrations were 0.51, 2.41 and 7.33 mg/dL. These concentrations were chosen so as to have low, moderately high and very high creatinine concentrations. Bilirubin concentrations used to spike the serum pools were similar to that of Phase 1 (0 – 30 mg/dL). Prior to spiking the serum pools with bilirubin, any bilirubin already in the sera had to be removed. The guidelines suggested that the concentration of bilirubin before spiking should be less than 1.46 mg/dL in order to be considered negligible (6). Bilirubin breaks down under light of wavelength 420 – 510 nm (14). We used incubators used for neonatal phototherapy as they are specially designed to breakdown bilirubin. These incubators have light bulbs with similar wavelengths to that at which bilirubin break down takes place (14). The creatinine concentrations were also measured before and after incubation to ensure that the creatinine molecules did not undergo any breakdown.

According to the guidelines given by the Clinical Laboratory Standard Institute (CLSI) for interference testing, the serum matrix cannot be diluted more than 5% its initial concentration (6). Hence, we had to prepare a highly concentrated bilirubin stock solution of 630 mg/dL. The serum aliquots were then spiked at a ratio of 20:1 in order to fulfill the above criteria.

When analyzing the results of the Jaffe reaction it is obvious that it gives underestimations with increasing bilirubin concentrations at all creatinine levels as supported by most of the previous studies (15,16,12,17). This can be attributed to the negative interference caused by the conversion of bilirubin into biliverdin by NaOH (11).

However, when comparing this trend in the three serum pools, no significant pattern could be observed with the increasing creatinine concentration. These findings are somewhat contradictory to those of Phase 1 where high creatinine concentrations similar to the third serum pool (7.33 mg/dL) gave overestimations at high bilirubin concentrations. The only plausible reason for this change can be attributed to the difference in the serum matrix. This confirms that the Jaffe reaction is more prone to interferences caused by the matrix. The enzymatic reaction shows a minor underestimation in the first serum pool with low creatinine which can be considered as non-significant. The second and third serum pools also showed an underestimation with increasing bilirubin concentrations. This is compatible with the findings of Phase 1. This indicates that unlike the Jaffe reaction the enzymatic reaction is not prone to interferences from the matrix. When comparing the values, we can see that underestimation increases with increasing creatinine concentrations. This is similar to the findings of Phase 1. As mentioned above it can be suggested that this is due to a reaction involving creatinine molecules.

In all three serum pools at all bilirubin concentrations used the Jaffe reaction still gave an underestimation relative

to the enzymatic reaction. The Jaffe reaction gives an underestimation relative to the enzymatic reaction even at 0 mg/dL bilirubin concentrations. This is again validated in Phase 1. However, the difference between the values of Jaffe and enzymatic method at 0 mg/dL bilirubin remained constant in Phase 1 (0.2 ± 0.05). This difference seems to increase in Phase 2 (0.07, 0.19, and 0.58 for creatinine of 0.51, 2.41 and 7.33 mg/dL, respectively). A straight forward answer for this cannot be given. Since the only varying property between the three serum pools is the creatinine concentration it can be assumed that this is due to the creatinine concentration.

5. CONCLUSION

In a phosphate buffered saline (PBS) medium, at creatinine concentrations of ≤ 2 mg/dL the Jaffe reaction gives a significant underestimation relative to the enzymatic method. Hence, at these concentrations the Creatinase method is more accurate than the Jaffe method. At creatinine concentrations ≥3 mg/dL the Jaffe reaction gives a significant overestimation at high bilirubin concentrations. The exact bilirubin concentrations causing the interference depend on the creatinine concentration itself. In serum, Jaffe reaction gave a significant underestimation relative to Creatinase method at all creatinine concentrations. In both PBS and serum, Creatinase enzymatic method showed significant underestimations at high creatinine concentrations.

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