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# COMPARATIVE RELIABILITY OF DIFFERENT METHODS OF SERUM CREATININE ESTIMATION IN JAUNDICE PATIENTS FOR MINIMISINGNEGATIVE INTERFERENCE OF BILURIBIN

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## ABSTRACT

One hundred and twenty three (123) blood samples from icteric patients, received for serum creatinine estimation were divided into different groups depending on bilirubin concentration -- Group I (1-5 mg/dl), Group II (5 -15 mg/dl), Group III (15-25 mg/dl), Group IV (>25 mg/dl). Twenty (20) blood samples from non-icteric patients within the normal reference range of bilirubin (0.2 - 0.8 mg/dl) were taken as controls, group V. All these samples were estimated for serum creatinine by four methods (1) Jaffe's kinetic method, (2) pre-incubation with NaOH method, (3) addition of SDS method and (4) enzymatic method (reference method). A significant increase in serum true creatinine was founded by pre-incubation with 200mmol/L NaOH and the addition of SDS method for bilirubin concentration > 15 mg/dl ($p < 0.01$) and mild to moderate increase for bilirubin concentration 1-15 mg/dl. From this study, it was found that increased concentration of bilirubin interfered in creatinine value measurement except in Jaffe's kinetic method.

**KEY WORDS: Glomerular Filtration Rate, Isotope Dilution Mass Spectrometry, Serum Bilirubin, Serum Creatinine, Jaffe's kinetic method.**

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## INTRODUCTION

Creatinine is the anhydride of creatine and a product of endogenous metabolism. Creatinine (MW113 Da) is present in all body fluids and secretions and is freely filtered by the glomerulus. Creatinine has been used as one of the endogenous markers of glomerular filtration rate [1] . Serum creatinine has also been used in MELD score (Model for End-stage Liver Disease) along with serum bilirubin and INR (International Normalised Ratio) of prothrombin time, to categorize the severity of liver disease in cirrhosis and to determine the prioritization for liver transplantation Although manual methods have traditionally been equilibrium methods, with 10 to 15 minutes allowed for color development at room temperature, kinetic assays were developed in a quest both for specificity and for faster and automated analysis. But the serum creatinine measurement by Jaffe's kinetic method in icteric samples is a major problem due to negative bilirubin interference. Expert professional bodies have recommended that all creatinine methods should become traceable to a reference method based on Isotope dilution-mass spectrometry (IDMS) [2]. High performance liquid chromatography (HPLC), Gas chromatography with mass spectrometry (GCMS) methods have been developed as reference methods for creatinine estimation [3-5] but the special instrumentation required for these methods is expensive. Enzymatic methods using creatinine deaminase, creatinase and creatininase are being widely propagated as the most accurate routine methods which produce results that agree closely with isotope dilution mass spectrometry (IDMS) [5-9]. An alternative approach to manage bilirubin is to oxidize bilirubin to biliverdin using potassium ferricyanide prior to the Jaffe reaction [1,10,11]. However, an increase in potassium ferricyanide concentration resulted in false positive creatinine values [10]. The addition of buffering ions such as borate and phosphate, together with surfactant like sodium do-decyl sulphate(SDS) to the reagent was more appropriate to minimize bilirubin interference than reagent containing potassium ferricyanide [1,10,12]. Incorporation of Sodium do-decyl sulphate(SDS) in the reagent will help to dissociate bilirubin from albumin [10,13]. Another important approach to minimize bilirubin interference is to oxidize bilirubin to biliverdin using NaOH, by its oxidizing property [14-16]. 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 absorbance at 505nm due to picrate creatinine interaction.[11,14,15]. Hence, in this study we used the above two methods with minor modifications i.e., effect of SDS in the reagent and pre incubation of the icteric sample with NaOH; while we compared the values with enzymatic method involving creatinase and creatininase which is taken as reference method traceable to IDMS.

## MATERIALS AND METHODS

All icteric samples which were received for the estimation of creatinine at clinical chemistry laboratory, Gandhi Hospital (a tertiary level teaching hospital), Secunderabad were included in this study. The lipaemic samples, lysed samples, samples with high glucose concentration were excluded from the study. All the blood samples were analysed for bilirubin, creatinine and glucose. Bilirubin was estimated by Malloy Evelyn method [17], creatinine by Jaffe's, and glucose by God Pod method. About 123 samples were analysed on AGAPPE –MISPA EXCEL semi-auto analyzer (Agappe Diagnostics Ltd, Ernakulam, Kerala, India)

The test samples were stratified into 4 groups based on their bilirubin values.

Group I (n = 35): Bilirubin 1-5 mg/d
Group II (n = 35): Bilirubin 5-15 mg/dl
Group III (n = 30): Bilirubin 15-25 mg/dl
Group IV (n = 23): Bilirubin > 25 mg/dl
Group V (n=20): A control group of samples having bilirubin levels within normal reference range (0.2 to 0.8 mg/dl)

Each of these samples was estimated for creatinine by

1. Jaffe's kinetic method using 200 mmol/L of NaOH which is followed in our laboratory routinely [18].
2. Pre-incubation of sample in 200mml/L NaOH method [18].
3. Addition of surfactant, SDS to the Jaffe reagent method [12].
4. Enzymatic method using creatinase and creatininase which is taken as reference method traceable to IDMS method [19].

The data obtained were statistically analyzed by statistical package SPSS version 16.0. The p value of <0.05 was considered as significant. The values were analyzed in terms of mean, standard deviation, and paired sample test and Pearson's Correlation were used for testing statistical significance.

## RESULTS

In the present study we have estimated serum creatinine in 123 icteric samples by four different methods, out of which enzymatic method was taken as reference method which is traceable to IDMS method. Icteric samples (123) were categorized depending on the bilirubin concentration (Table 1) into Group I, II, III, IV and 20 samples with total serum biluribin < 1 mg/dl (Group V) were taken as Control samples.

**Table 1**
***Group categorization of samples tested for biluribin***

| Category | No. of Samples | Bilirubin value of the group | Mean total serum bilirubin value |
|---|---|---|---|
| Group – I | 35 | 1-5 mg/dl | 3.167±1.334 mg/dl |
| Group – II | 35 | 5-15 mg/dl | 9.691±3.311 mg/dl |
| Group – III | 30 | 15-25 mg/dl | 18.960±3.041 mg/dl |
| Group – IV | 23 | > 25 mg/dl | 28.193±2.547 mg/dl |
| Group – V (controls) | 20 | 0.2 – 0.8 mg/dl | 0.547±0.189 mg/dl |

In Group I (Table-2) the values of serum creatinine obtained by enzymatic method were slightly higher with mean as 1.416 ± 0.961 than our routine laboratory method (Jaffe's) with a mean as 1.182 ± 0.923. The values of serum creatinine obtained by pre-incubation with 200mmol/L NaOH and addition of SDS to Jaffe reagent method were also mildly higher and closer to the values obtained by enzymatic method with a mean as 1.405 ± 0.970 and 1.404 ± 0.958 respectively.

**Table 2**
***Comparison and correlation of creatinine values obtained by four methods in icteric samples with total serum biluribin 1 – 5 mg/dl***

| Method | Mean ± S.D mg/dl | P - Value (2-tailed) | Pearson's Correlation (r - value) |
|---|---|---|---|
| Jaffe's kinetic method (ourlab. routine method) | 1.182 ± 0.923 | 0.000* | 0.973 |
| Pre-incubation method | 1.405 ± 0.970 | 0.357 | 0.997 |
| Addition of SDS method | 1.405 ± 0.958 | 0.294 | 0.998 |
| Enzymatic method (reference method) | 1.416 ± 0.961 | | |

The serum Creatinine levels obtained by pre-incubation method and addition of SDS method showed positive correlation with enzymatic method value, which was highly significant statistically as evident by r values of 0.997 and 0.998 respectively using Pearson's formula. In this group, a significant p-value (0.000) was observed with routine Jaffe's kinetic method only and not with other methods. In Group II (Table-3) also the values of serum creatinine obtained by enzymatic method, pre-incubation method, addition of SDS method were moderately higher with means as 1.498 ± 0.696, 1.473 ± 0.688, 1.495 ± 0.714 than routine Jaffe's kinetic method with mean as 1.052 ± 0.676.

**Table 3**
***Comparison and correlation of creatinine values obtained by four methods in icteric samples with bilirubin 5 – 15 mg/dl (n=35)***

| Method | Mean ± S.D mg/dl | P - Value (2-tailed) | Pearson's Correlation (r - value) |
|---|---|---|---|
| Jaffe's kinetic method (our lab. routine method) | 1.052 ± 0.676 | 0.000* | 0.888 |
| Pre-incubation method | 1.473± 0.688 | 0.041* | 0.995 |
| Addition of SDS method | 1.495± 0.714 | 0.723 | 0.996 |
| Enzymatic method (reference method) | 1.498 ± 0.696 | | |

The serum Creatinine levels obtained by pre-incubation method and addition of SDS method were moderately higher and showed positive correlation with enzymatic method value, which was highly significant statistically as evident by r values of 0.995 and 0.996 respectively using Pearson's formula. Compared to pre-incubation method (1.473 ± 0.688), addition of SDS method (1.495 ± 0.714) is more correlated with enzymatic method (1.498 ± 0.696). In group II, the p-value was significant for routine Jaffe method and pre-incubation method. In Group III (Table-4), the values of serum creatinine obtained by routine Jaffe's kinetic method were much lower than other three methods with a mean as 0.961 ± 0.537. There was not much difference in the values obtained by other 3 methods with the mean as 1.447 ± 0.692; 1.460 ± 0.690; 1.470 ± 0.695.

**Table 4**
***Comparison and correlation of creatinine values obtained by four methods in icteric samples with bilirubin 15 –25 mg/dl(n = 30)***

| Method | Mean ± S.D mg/dl | P - Value (2-tailed) | Pearson's Correlation (r - value) |
|---|---|---|---|
| Jaffe's kinetic method (our lab. Routine method) | 0.961 ± 0.537 | 0.000* | 0.902 |
| Pre-incubation method | 1.447 ± 0.692 | 0.160 | 0.992 |
| Addition of SDS method | 1.460 ± 0.690 | 0.559 | 0.991 |
| Enzymatic method (reference method) | 1.470 ± 0.695 | | |

The serum Creatinine levels obtained by pre-incubation method and addition of SDS method showed positive correlation with enzymatic method value, which was highly significant statistically as evident by r values of 0.992 and 0.991 respectively using Pearson's formula. The trend in the creatinine values obtained in Group – III was same as in Group – II, i.e., addition of SDS method Values are morecloser to enzymatic method

than pre-incubation method. In Group IV (Table-5) the serum creatinine values obtained by our routine laboratory method with a mean as 0.701 ± 0.428 were very much low than true creatinine obtained by enzymatic method with a mean as 1.366 ± 0.705.The values obtained by pre-incubation method and addition of SDS method were very close to that of enzymatic method with a mean 1.300 ± 0.694 and 1.335 ± 0.734respectively.

**Table 5**

***Comparison and Correlation of creatinine values obtained by four methods in icteric samples with bilirubin > 25 mg/dl(n = 23).***

| Method | Mean ± S.D mg/dl | P - Values (2-tailed) | Pearson's Correlation (r - value) |
|---|---|---|---|
| Jaffe's kinetic method (our lab. Routine method) | 0.701 ± 0.428 | 0.000* | 0.882 |
| Pre-incubation method | 1.300± 0.694 | 0.003* | 0.991 |
| Addition of SDS method | 1.335 ± 0.734 | 0.125 | 0.993 |
| Enzymatic method (reference method) | 1.366 ± 0.705 | | |

The serum Creatinine levels obtained by pre-incubation method and addition of SDS method significantly higher than routine lab method and showed positive correlation with enzymatic method value, which was highly significant statistically as evident by r values of 0.991 and 0.993 respectively using Pearson's formula. In Group V (Table-6) the difference in the creatinine values obtained by all the four methods was non-significant. The means were very close to routine laboratory method.

**Table 6**

***Comparison and correlation of creatinine values obtained by four methods in control samples with bilirubin 0.2 to 0.8 mg/dl (n = 20).***

| Method | Mean ± S.D mg/dl | P - Values (2-tailed) | Pearson's Correlation (r - value) |
|---|---|---|---|
| Jaffe's kinetic method (our lab. Routine method) | 0.755 ± 0.264 | 0.010* | 0.932 |
| Pre-incubation method | 0.806 ± 0.231 | 0.461 | 0.972 |
| Addition of SDS method | 0.805 ± 0.248 | 0.321 | 0.979 |
| Enzymatic method (reference method) | 0.816 ± 0.248 | | |

The difference in the serum creatinine values obtained by four different methods in control group samples where bilirubin is 0.2 to 0.8 mg/dl was not significant. When the three methods were compared with enzymatic method they were statically significant as evident by r values of 0.932, 0.972 and 0.97997 respectively.

## DISCUSSION

The most commonly used method for serum creatinine estimation in clinical laboratory is Jaffe's Kinetic method which is non-specific. Many compounds produce a Jaffe like chromogen which include protein, glucose, ascorbic acid, ketone bodies, pyruvate etc. and are responsible for false positive values of creatinine (positive interferants) [1,20,21]. In the present study, serum creatinine was estimated by 4 different methods in 123 icteric samples ranging from 1 to > 25mg/dl of bilirubin which were divided into 4 groups depending on the bilirubin concentration. Twenty (20) control samples with normal bilirubin reference range were also taken and estimated the creatinine concentration. The value of serum creatinine obtained by enzymatic method (reference method) showed higher creatinine values in all the ranges of icteric samples (Tables 2-5) as compared to Jaffe's kinetic method (routinely used in our laboratory). The creatinine value obtained by pre-incubation with 200 mmol/L NaOH solution (true creatinine) was found to be increased than creatinine obtained without pre-incubation (interference Creatinine). Similar findings were reported by Maizy [20]; Jacobs and Lumsden, [21]. Sah et al., [17] in their study on evaluating serum true creatinine concentration by Jaffe's kinetic method found variable result for different concentrations of bilirubin. For bilirubin concentration <1 mg/dl, there was just little variation in serum true creatinine mean value. This may be due to light yellow color of serum due to low concentration of bilirubin present. Our observation showed a mild increase in mean serum true concentration by pre-incubation method for samples with total serum biluribin concentration 1-5 mg/dl and 5-15 mg/dl as compared to creatinine values obtained by routine lab. method. The values were correlated to that of enzymatic method with significant positive 'r' value of 0.997 and 0.995 respectively. The same trend was followed by the addition of SDS method for bilirubin concentration 1-5 mg/dl and 5-15 mg/dl with statistically significant 'r' values of 0.998 and 0.996 respectively when correlated with values obtained by enzymatic method. Similarly for bilirubin concentration of 15-25 mg/dl we observed moderate the values obtained by addition of SDS method (1.4603 ± 0.68957) were closely related to enzymatic method values (1.4703 ±0.69453) than values obtained by pre-incubation method (1.4473± 0.69237). In the present study, we found that with bilirubin concentration >25 mg/dl the serum creatinine values obtained by routine lab method were very low. This type of findings were due to dark yellow color of serum containing high bilirubin concentration. We observed great significant increase in the serum true creatinine concentration by both pre-incubation method and addition of SDS method. Similar findings were reported earlier in the literature. Lolekha and Sritong [10] found that pre-incubation of the sample with alkaline buffer containing SDS (140 mmol/L) for 5-10 minutes before the Jaffe reaction can correct the unconjugated bilirubin interference up to 684 µmol/L and also

mentioned that surfactant in the reagent will help to dissociate bilirubin from albumin and the dissociated bilirubin gets oxidized to biliverdin with the oxidizing reagent NaOH. According to Cayman's creatinine (serum) assay, addition of surfactant like SDS or Brij 35, together with buffering ions such as borate and phosphate has been used to minimize the effects of bilirubin interference. In the present study, we have observed that, for the bilirubin concentrations>15mg/dl addition of SDS to the Jaffe reagent method was more appropriate and the difference in the creatinine values obtained by enzymatic method were not significant compared to pre-incubation method with 200mmol/L of NaOH 10 minutes prior to occurrence of Jaffe's reaction. Vaishya et al [14] observed that there is a little variation in creatinine estimation for normal bilirubin concentration and for bilirubin concentration 5.0 – 25 mg/dl, there was great interference of bilirubin in serum creatinine estimation. They propose that for bilirubin concentration below 25 mg/dl, the sample should be pre-incubated with 125 mmol/L NaOH and if bilirubin concentration is more than 25 mg/dl, it is recommended that the sample should be pre-incubated with 150mmol/L of NaOH to minimize the negative interference of bilirubin. Srisawasdi et al [22] in their study revealed that, the addition of SDS to the alkaline – picrate reagent was shown to be effective in reducing bilirubin and protein interferences. According to Peake and Whiting [2] , all methods for measuring serum creatinine should have their calibration traceable to an IDMS reference measurement procedure, with low combinations of bias and imprecision. So, in the present study the trend of creatinine values seen with pre-incubation method and addition of SDS method clearly indicate that in the absence of a reference method, these minor modifications in Jaffe's kinetic reaction could minimize a major problem of negative interference of bilirubin in creatinine estimation. This could be beneficial in biochemical assessment of patients with hepato-renal syndrome (MELD Scores); CAPD patients (creatinine clearance) to monitor the nutritional status (creatinine kinetic lean body mass) and staging of CKD (GFR estimation). 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. Bilirubin is the major specific chromogen present in serum which decreases the optical density during creatinine estimation due to its similar absorbance with colored incubation product of creatinine with Jaffe's reagent. Thus it gives false negative values of creatinine (negative interferants) [20,21,23]. Only 80% of the color developed during performing test is due to serum creatinine [21,22]. It has been recognized for many years that bilirubin has caused significant problems in direct Jaffe assays but not in methods involving dialysis [16]. This interference is evident with both conjugated and unconjugated bilirubin and is directly related to bilirubin concentration is independent of creatinine concentration [10,11] . Unlike end-point Jaffe methods, the kinetic methods do not include protein precipitation or separation which also removes bilirubin [24,10]. Thus when protein separation is omitted, bilirubin is present during the assay for serum creatinine. A significant increase in serum true creatinine was found by pre-incubation with 200mmol/L NaOH and addition of SDS method for bilirubin concentration > 15 mg/dl ($p < 0.01$) and mild to moderate increase for bilirubin concentration 1-15 mg/dl. From this study, it was found that increased concentration of bilirubin interfered in creatinine value measurement except in Jaffe's kinetic method.

## CONCLUSION

A significant increase in serum creatinine was found by pre-incubation with 200mmol/L NaOH and addition of SDS method for bilirubin concentration >15mg/dl ($p<0.01$) and mild to moderate increase for bilirubin concentration 1-15 mg/dl.This significant increase in serum true creatinine is due to oxidizing property of aqueous NaOH solution which oxidizes bilirubin to biliverdin causing a decrease in absorbance at 505nm, while we are measuring an increase in absorbance at 505nm due to picrate, creatinine interaction.The surfactant SDS dissociates all the bilirubin from albumin and together with buffering ions in the Jaffe reagent minimizes the effect of bilirubin interference.Hence, these minor modifications could be beneficial in minimizing negative bilrubin interference in serum creatinine estimation by Jaffe's method in icteric serum samples.

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## CONFLICT OF INTEREST

Results from Enzymatic methods for estimation of serum creatinine agree closely with IDMS a reference method. But the special instruments required make these methods expensive. Hence in this study we used the above two modifications that is effect of SDS in the reagent and pre-incubation of the icteric sample with NaOH to minimize the negative interference of bilirubin during creatinine estimation by kinetic Jaffe's method.

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