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FASTING AND POST PRANDIAL FUNCTIONING OF THE KIDNEY MEASURED BY SERUM CREATININE AND CREATININE CLEARANCE DATA DISTRIBUTION IN HEALTHY INDIVIDUALS ABOVE FORTY YEARS

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ABSTRACT The influence of diet and gender were analysed in fasting, 30-minute and 60-minute post prandial serum creatinine and creatinine clearance. As expected, gender differences were seen in all the three sets of serum creatinine values, with higher values in males. But, there were no gender differences seen in the three sets of creatinine clearance values. The lack of gender differences in creatinine clearance may be due to increased plasma creatinine compensated by increased concentration of urine creatinine as given by the equation, Creatinine clearance = UV/P. Fasting and 60-minutes serum creatinine and creatinine clearance data were found to be clearly positively skewed with outliers. These results indicated that the positive skewing and the presence of outliers may be early markers of kidney dysfunction. Such positive skewing was seen above 1.0 mg/dL serum creatinine. There were more outliers observed with 60-minute creatinine clearance, indicating the 60-minute post prandial creatinine clearance may be a better early marker for abnormal kidney function than the other parameters.

KEYWORDS : Creatinine, Creatinine clearance, Fasting, Postprandial

INTRODUCTION

There are different biochemical markers of renal functions, disease, or injury that are done in blood and urine samples. The markers of kidney functions may be endogenous substances (e.g. Creatinine and Urea) or exogenous substances (e.g. Inulin). Some markers of renal function are used to determine glomerular filtration rate (GFR), which are widely used as a robust indicator of renal function (1, 2). Urea is a poor marker of GFR, as it is produced at variable rates, undergoes marked reabsorption by the tubules, and its level is influenced by many other conditions, such as liver disease, and dietary intake of proteins (3). Serum or plasma creatinine is an endogenous substance used as a marker of glomerular filtration, tubular functions, to stage chronic kidney disease, along with urine albumin content if the abnormalities have persisted for longer than three months (4), and in acute kidney injury (5).

This study examined the influence of diet on serum creatinine and creatinine clearance by assaying fasting and post prandial serum creatinine in apparently healthy individuals above forty years of age.

METHODOLOGY

Serum creatinine was assayed from blood samples collected from apparently healthy individuals above forty years of age in the morning fasting, and 30-minute and 60-minute post-prandial (post breakfast) samples.

Serum creatinine and the calculated creatinine clearance data were analyzed for distributions and gender differences. The differences in the concentration serum creatinine in the fasting state and in the postprandial states were estimated among healthy individuals above forty years. Fasting, 30-minute and 60-minute serum creatinine samples were compared with each other.

Samples were collected in the Clinical Biochemistry Laboratory, Amala Institute of Medical Sciences. Participants were staff and their relatives in Amala Institute of Medical Sciences above forty years, selected at random, making sure that the number of males and females was the same. They were earlier given instructions for sample collection and evaluated for the inclusion and exclusion criteria. After inclusion, the participants filled out a questionnaire with personal information, weight, height, age, gender and other relevant information. Blood was then collected between 8 and 8.30 am in the morning, followed by postprandial samples after 30- and 60-minutes. The samples were centrifuged to separate serum at 3000 rpm for ten minutes and assayed for serum creatinine. Chemistry assay of (a) creatinine was assayed by creatininase enzymatic method in dry chemistry autoanalyzer, Vitros 5,1 FS from OCD, USA. An assay of creatinine in serum (6) was done by the enzymatic method which forms creatine by creatinine amidohydrolase, is converted to sarcosine by creatine amidino hydrolase. Sarcosine forms glycine, formaldehyde, and hydrogen peroxide with sarcosine oxidase. Hydrogen peroxide is assayed by peroxidase to form atomic oxygen which forms a colored product from a leuco-dye, whose color is measured at 670 nm by reflectance spectroscopy. The reportable range for creatinine in serum is 0.05 – 14 mg/dL and in urine is 1.05 - 346.5 mg/dL, respectively. Vitros chemistry products calibrator kit 1 for creatinine is certified by NIST (National Institute of Standards and Technology); Reference material SRM (Standard Reference Material) 914a. Within laboratory precision of Vitros 5,1 FS for serum creatinine was 1.8% CV at 1.2 mg/dL. The Reference range of S. Creatinine was 0.8 - 1.5 mg/dL in males and 0.7 - 1.2 mg/dL in females.

Equations and definitions for Creatinine clearance

Creatinine Clearance =

$$\frac{\text{Urinary concentration of creatinine (U)} \times \text{Volume of urine (V)}}{\text{Plasma concentration of creatinine (P)}}$$

Data Analysis by Statistical Methods Statistical analysis for calculating mean, SD and sample characteristics, distribution and the inferential statistics were done by SPSS software (7).

RESULTS

Distribution of fasting serum creatinine and creatinine clearance

Distribution studies of selected fasting, 30- and 60-minutes postprandial serum creatinine in apparently healthy individuals above forty years were examined. Data distribution was examined by mean±SD, median and Shapiro-Wilk tests (Table 1). The Shapiro-Wilk test indicated that the distribution of fasting S. Creatinine and fasting Creatinine clearance had a P value of <0.001 and 0.021, respectively, indicating that the distribution was positively skewed.

Table1. Distribution characteristics of fasting, and 30- and 60-minutes post prandial S. Creatinine and Creatinine Clearance.

Analytes	S. Creatinine (n = 54)		Creatinine Clearance (n = 54)	
Distribution characteristics	mean±SD, median	Shapiro-Wilk (P)	mean±SD, median	Shapiro-Wilk (P)
Fasting	0.79±0.19, 0.75	<0.001	79.99±44.45, 73.91	0.021

30-minutes	0.79±0.19, 0.80	0.003	80.90±44.13, 69.20	0.003
60-minutes	0.79±0.19, 0.80	0.006	92.06±121.39, 58.62	<0.001

The data distribution of the fasting sample was also evaluated graphically by Histogram and Box-Whisker plot (Fig. 1, Fig .2). These results indicated that most of the fasting S. Creatinine values were less than 1 mg/dL. The few values above 1 mg/dL were found to be positively skewed (Fig. 1). The fasting creatinine clearance data showed that the distribution was nearly platykurtic (Fig.1). Serum creatinine had two positive outliers.

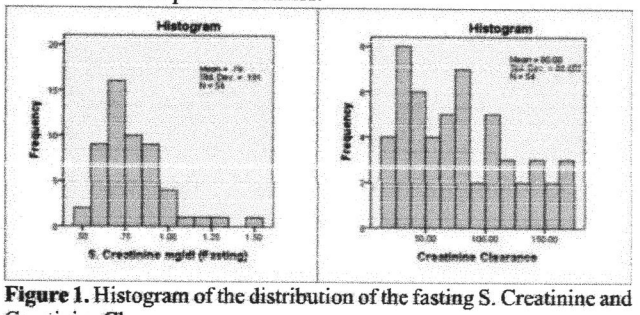

Figure 1. Histogram of the distribution of the fasting S. Creatinine and Creatinine Clearance.

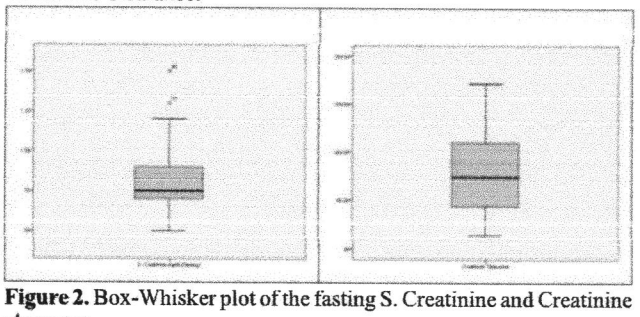

Figure 2. Box-Whisker plot of the fasting S. Creatinine and Creatinine clearance.

Distribution of 30-minute S. Creatinine and Creatinine clearance

The 30-minute S. Creatinine and 30-minute creatinine clearance were found to be positively skewed above 1 mg/dL (Fig. 3 and Fig. 4). Creatinine, Creatinine clearance were found to be having clear bimodal distribution

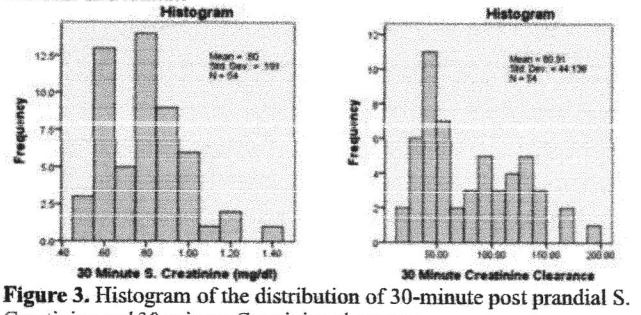

Figure 3. Histogram of the distribution of 30-minute post prandial S. Creatinine and 30-minute Creatinine clearance.

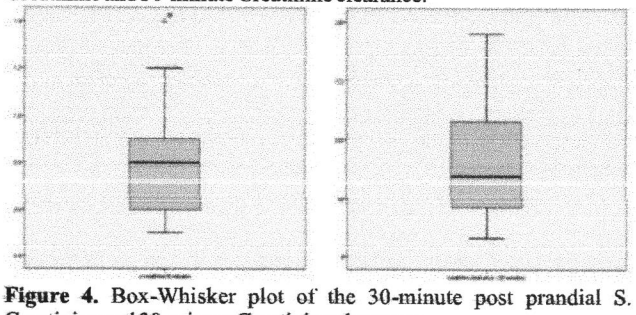

Figure 4. Box-Whisker plot of the 30-minute post prandial S. Creatinine and 30-minute Creatinine clearance.

Distribution of 60-minute post prandial S. Creatinine, and Creatinine Clearance

The 60-minute postprandial serum creatinine and creatinine clearance were positively

Skewed (Fig. 5 and 6).The Box-Whisker plot of S. Creatinine and Creatinine clearance showed positive skewing with one and three outliers, respectively (Fig. 6).

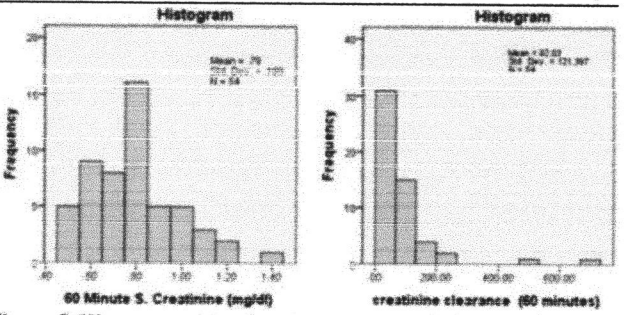

Figure 5. Histogram of the 60-minute post prandial S. Creatinine and 60-minute Creatinine clearance.

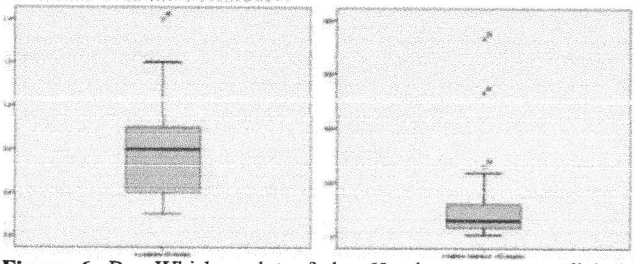

Figure 6. Box-Whisker plot of the 60-minute post prandial S. Creatinine and 60-minute Creatinine Clearance.

Gender differences between fasting, 30-miniute and 60-miniute postprandial S. Creatinine and Creatinine Clearance

There were gender differences in the fasting, 30-minute and 60-minute S. Creatinine values (Table 2). S. Creatinine values were higher in male samples than in female samples. But there were no gender differences seen in Creatinine clearance values (Table 2).

Table 2. Gender differences in S. Creatinine, Creatinine Clearance analytes with in fasting and post prandial samples.

Groups	Male n = 27 Mean± SD	Female n = 27 Mean± SD	Shapiro-Wilk (P) Male		Shapiro-Wilk (P) Female		Student t-test (P)/ Mann Whitney U test
			Before Log transformation	After Log transformation	Before Log transformation	After Log transformation	
Fasting S. Creatinine	0.92±0.18	0.66±0.84	<0.001	0.006	0.001	0.001	(<0.001)
30-minutes S. Creatinine	0.93±0.15	0.65±0.10	0.003	0.030	<0.001	0.001	(<0.001)
60-minutes S. Creatinine	0.93±0.16	0.65±0.11	0.017	0.074	0.002	0.003	(<0.001)
Fasting Creatinine Clearance	75.61±48.94	84.38±39.91	0.009	0.300	0.871	0.050	0.302
30-minutes Creatinine Clearance	79.24±44.55	82.57±44.49	0.110	0.020	0.557	0.081	0.711
60-minutes Creatinine Clearance	105.73±140.3	78.32±99.84	<0.001	<0.001	0.669	0.742	0.228

Differences in fasting, 30-minutes and 60-minutes post prandial serum Creatinine in healthy adults above 40 years of age.

Differences were calculated using student t test (Mann Whitney U test). There was no differences between fasting and 30-minute in post prandial serum creatinine levels in males and females (Table 3). But, there was a borderline increase in creatinine clearance in the female sample at 60-minute when compared to the fasting sample (P = 0.081). Similarly, there was borderline increase in 30-minute post prandial serum creatinine when compared to that of 60-minutes post prandial serum creatinine (P = 0.101)

Table 3. Differences in fasting, 30-minutes and 60-minutes post prandial serum Creatinine and Creatinine Clearance in healthy adults above 40 years of age. Differences were calculated using student T test (Mann Whitney U test).

Student t test/ Mann Whitney U test (P value)				
Groups	Serum Creatinine		Creatinine clearance	
	Male	Female	Male	Female

Fasting and 30-minutes post prandial	0.458	0.627	0.601	0.854
Fasting and 60-minutes post prandial	0.702	0.792	0.635	0.081
30-minutes post prandial and 60-minutes post prandial	0.810	0.985	0.959	0.101

DISCUSSION

The distribution characteristics of S. Creatinine and Creatinine Clearance from samples collected from apparently healthy individuals were analysed. Fasting, 30-minute post prandial, and 60-minute postprandial blood were collected. S. Creatinine was not normally distributed due to positive skewing and the outliers indicated that apparently healthy individuals had some higher values of S. Creatinine. This statement assumes that a normally distributed samples below 1.0 mg/dL might be healthier than the positively skewed samples and the outliers. Individuals with S. Creatinine between 1.0 and 1.4 mg /dL may be at a higher risk of kidney dysfunctions

The analysis of 30-minute S. Creatinine distribution by the two methods (Histogram and Box-Whisker plot) showed positive skewing, platykurtic or bimodal distribution, and the presence of outliers.

The platykurtic or bimodal form of the distribution may result from the fusion of different adjacent sub-group distributions, such as males and females, from the presence or absence of type 2 diabetes mellitus or impaired fasting and post-prandial glucose levels, or from age variations. These observations indicated that the sample may be further partitioned according to variations in GFR in the subgroups, as mentioned above. Such variations and increased dysfunction of the kidney may occur more with the male gender and with increase in age (1).

The distributions of fasting, 30- and 60-minutes S. Creatinine and Creatinine Clearance were not normally distributed due to positive skewing. These results indicated that S. creatinine may be a better marker for early kidney disease.

The higher S. creatinine in males in the fasting, 30-minutes, and 60-minutes samples may be the result of increased muscle mass in males. The lack of gender difference in the fasting, 30- and 60-minutes creatinine clearance may be due to increased plasma creatinine being compensated by increased concentration of urinary creatinine as given by the equation (UV)/P. There was gender difference seen in serum creatinine in the fasting, 30-minutes, and 60-minutes samples.

CONCLUSION

The data on serum creatine and creatinine clearance were evaluated in the fasting, 30- minutes, and 60-minutes samples of serum. S. creatinine and creatinine clearance may be used as early markers of kidney dysfunctions, as the data were positively skewed with outliers above 1 mg/dL. The increase in the concentration of glucose is damaging to the kidney and may be the cause of positive skewing in creatinine concentrations. There were no major changes in the fasting, 30-minutes and 60-minutes values of S. Creatinine. But there were differences in gender in the fasting, 30-minutes and 60-minutes S. Creatinine samples. But there were no gender differences in creatinine clearance. These results indicate that S. creatinine and creatinine clearance may be useful early markers of detection for kidney dysfunction.

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