Section: Miscellaneous

Original Research Article

CHANGES IN SERUM CREATININE AND CREATININE CLEARANCE IN FASTING VERSUS POSTPRANDIAL STATE AMONG APPARENTLY HEALTHY STAFF MEMBERS ABOVE FORTY YEARS IN A TERTIARY CARE HOSPITAL IN KERALA, INDIA

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Abstract

Background: The renal markers help in determining the glomerular filtration rate and thereby the renal function. Creatinine is an endogenous marker of renal function. The objective is to assess the influence of diet on serum and urine creatinine and creatinine clearance. This is done by assaying serum creatinine in the fasting state and post-prandial state among apparently healthy individuals above forty years. The aim is to study the changes in selected renal function tests in the fasting and post prandial state among healthy individuals above 40 years of age. **Materials and Methods:** This observational cross-sectional study was conducted among Amala Institute of Medical Sciences staff, in Thrissur, Kerala. Questionnaires were filled by the participants related to their personal and medical history and adequate instructions were given about sample collection on the previous day. Blood and urine samples for creatinine were collected during fasting and analyzed for 30 minutes and 60 minutes postprandial. An assay of serum creatinine was done by the creatinine amidohydrolase method in a dry chemistry auto analyzer, Vitros 5, 1 FS from OCD, USA; GFR was calculated from serum creatinine by the Cockcroft-Gault method. Creatinine clearance was calculated from serum creatinine, urine creatinine, and volume of urine. SPSS software was used for statistical calculations. **Result:** Fasting, 30-minute, and 60-minute serum creatinine and creatinine clearance did not have Gaussian distribution in most of the samples due to bimodal distribution and positive skewing. These results indicated that serum creatinine values were higher in some healthy individuals. **Conclusion:** These results indicated that in apparently healthy individuals several samples were falling in the positive outlier region of distribution, with a higher risk of kidney dysfunction. Males have greater serum creatinine values than females. Lack of gender differences is seen in creatinine clearance which may be due to increased plasma creatinine compensated by increased concentration of urine creatinine.

INTRODUCTION

The kidney performs several functions that may be evaluated to understand the healthy performance of the kidney and its variations in disease.[1] Different biochemical markers of renal function, disease, or injury are found in blood and urine samples. The markers of kidney functions may be (a) endogenous substances (e.g. creatinine and urea) or (b) exogenous substances (e.g. Inulin). Some markers of renal function are used to determine GFR, which is 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] Creatinine is an endogenous substance used to stage CKD, along with urine albumin content if the abnormalities have persisted for longer than three months,[4] and in AKI.[5] Exogenous substances, such as inulin and radio isotopic markers, give an accurate estimation of GFR,[6,7] but have disadvantages: they are time-consuming procedures, are not routinely available, and have possible radiation exposure. An endogenous marker that can circumvent these limitations is desirable

Although serum creatinine has been extremely helpful as a marker in kidney function testing, it is

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affected by a variety of causes and is less sensitive than anticipated to changes in renal functions. The condition of the kidney's baseline state of functioning in a healthy person is that of fasting and normal hydration. In contrast, a stressful situation like a meal (postprandial) may or may not have affected kidney function, but it is likely to show early changes in renal function. The methods of assay for S. Creatinine and U. Creatinine are Jaffe's method and creatininase methods. Substances causing positive creatinine interference in the Jaffes reaction are ascorbic acid (1), pyruvate (1), protein (1), glucose (1), creatine (1), various cephalosporins (1), acetoacetate (1) and fluorescein (1). Substances causing negative creatinine interference in the Jaffe reaction are dopamine/LDOPA/methyldopa (1), bilirubin (1), and hemoglobin F (1). In addition to these multiple methodological interference and deficiencies, there is a further limitation of using S. Creatinine to determine GFR. To overcome these problems, the methods used in this study for the evaluation of glomerular filtration were serum creatinine, creatinine clearance, and estimated GFR from serum creatinine using the Cockcroft-Gault equation.[8,9]

MATERIALS AND METHODS

This observational cross-sectional study was conducted among 54 selected staff in Amala Institute of Medical Sciences, Kerala. All data were collected between 1st May 2021 and 31st December 2023.

The Institutional Ethics Committee approved the study protocol, Amala Institute of Medical Sciences, Thrissur, Kerala, India (Ref No:17/IEC/21/AIMS-11), dated 26-04-2021

Blood and urine samples were collected in the Clinical Biochemistry Laboratory, Amala Institute of Medical Sciences, Thrissur, Kerala. The participants selected were healthy individuals. Participants were above forty years old and were staff from Amala Institute of Medical Sciences. They were selected using simple random sampling. The number of males and females selected are equal.

Inclusion Criteria

Apparently healthy males and females above 40 years will be included in the study.

Exclusion Criteria

Involved those with kidney disease, lifestyle diseases (like diabetes mellitus, hypertension), and medications that may influence serum creatinine.

Questionnaires were filled out by the participants related to personal history like age, gender, weight, height, and family history of lifestyle diseases. Participants were asked to follow a regular diet on the previous week. Proper instructions regarding sample collection were also given on the previous day. They were instructed to fast from previous night 8 pm. Blood and urine were collected on the next day between 8 and 8.30 am in a fasting state. The third voided morning fasting sample of urine formed for 30 minutes was collected before breakfast and the blood sample was also collected. Another sample of blood and urine formed for 30 minutes is collected 30 minutes post-breakfast. Following this, a second post-breakfast sample of blood and thereby urine also was collected at 60 minutes.

The samples were centrifuged to separate serum at 3000rpm for 10 mins and creatinine assays were done. These samples of urine and blood were analyzed for the following: 1) serum and urinary creatinine, volumes of urine, and calculations of creatinine clearance.2) The Cockcroft-Gault equation is used for correction of the influence of physical parameters of body and gender on the analytes and their estimates on glomerular filtration rate. Chemistry assay of (a) creatinine is assayed by creatinine amidohydrolase enzymatic method in dry chemistry autoanalyzer, Vitros 5, 1 FS from OCD, USA (10). An assay of creatinine in serum and urine is done by an enzymatic method which forms creatine by creatinine amidohydrolase, which 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 coloured product from a leuco-dye, whose colour is measured at 670 nm by reflectance spectroscopy. The Reference range of S. Creatinine is 0.07-0.13mmol /litre in males and 0.061-0.106 mmol/L in females and 24-hour urine creatinine is 4420-17680 mmol/day(10).

The reportable range for creatinine in serum is 0.0044 – 1.23 mmol/L and in urine is 0.092 -30.63 mmol/L, 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 is 1.8% CV at 0.106 mmol/lL and that of urine is 2.3% CV at 6.71mmol/L

Equations and definitions for Creatinine clearance and estimated GFR

Creatinine Clearance=

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

Estimated GFR =

$$\frac{(140 - \text{age in years}) \times \text{weight in kg} \times K}{72 \times \text{S. Creatinine (mg/d L)}}$$

K = 0.85 for women and 1 for men.

Statistical analysis for calculating mean, SD, sample characteristics, distribution, and inferential statistics was done by SPSS software

RESULTS

Distribution studies of fasting basal state functioning of the kidney as measured by fasting serum creatinine, creatinine clearance, and GFR calculated from fasting serum creatinine. Distribution studies of selected fasting,30 and 60-minute postprandial basal state analytes of kidney function in apparently healthy individuals were examined. Data distribution

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was examined by mean±SD, median, and Shapiro-Wilk tests [Table 1]. The Shapiro-Wilk test indicated that the distribution of fasting serum creatinine and fasting Creatinine clearance had a P value of <0.001 and 0.021 respectively indicating that the distribution was not Gaussian or normal. The fasting estimated GFR calculated from serum creatinine, urine creatinine, and volume of urine, had normal Gaussian distribution [Table 1]. These results indicated that most of the fasting serum creatinine values were less than 0.88mmol/L. The few values above 0.88mmol/L were found to be positively skewed [Figure 1B]. The fasting creatinine clearance data showed that the distribution was nearly platykurtic [Figure 1A]. GFR calculated from fasting serum creatinine appeared to have bimodal distribution [Figure 1C].

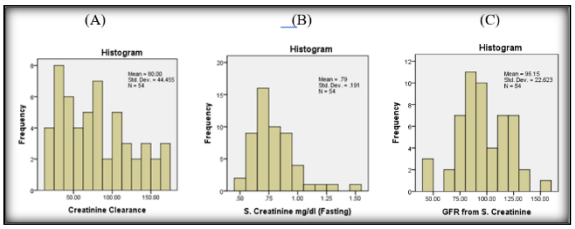

Figure 1: Histogram of the fasting basal state functioning of the kidney as measured by fasting S. Creatinine (A), Creatinine clearance (B), and GFR calculated from fasting S. Creatinine (C).

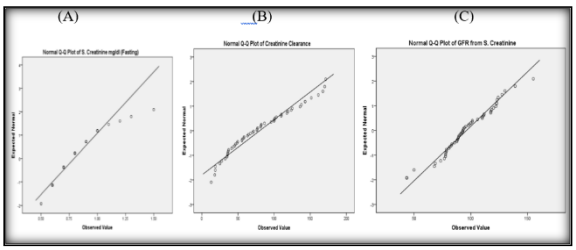

Figure 2: Normal Q-Q plot of the fasting basal state functioning of the kidney as measured by fasting S. Creatinine (A), Creatinine clearance (B), and GFR calculated from fasting S. Creatinine (C).

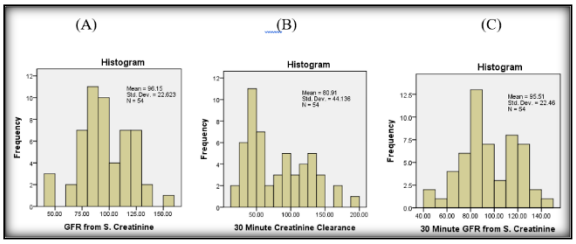

Figure 3: Histogram of the distribution of 30-minute post prandial basal state functioning of the kidney as measured by 30-minute S. Creatinine (A), 30-minute Creatinine clearance (B) and GFR calculated from 30-minuteS.Creatinine (C)

**Normal Q-Q plot of fasting serum creatinine was also found to be deviating from the straight-line normal distribution at concentrations above 1.0 mg/dL [Figure 2A]. The values below 0.88mmol/L overlapped the straight-line normal distribution curve. However, the QQ plot of fasting creatinine clearance and calculated GFR nearly overlapped the straight-line normal distribution curve, except for the mild deviations that represented the bimodal distributions [Figure 2B and Figure 2C].

**Distribution of 30-minute basal state functioning of the kidney as measured by 30-minute S. Creatinine, Creatinine clearance, and GFR calculated from 30 minutes creatinine. The 30-minute S. Creatinine and 30-minute creatinine clearance were found to be positively skewed above 0.88 mmol/L [Figure 3A and Figure 3B]. The 30-minute calculated GFR was found to behave clear bimodal distribution [Figure 3C].

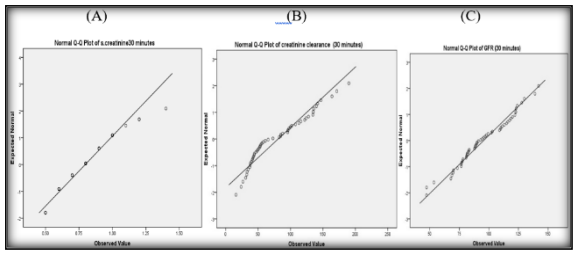

Figure 4: Normal Q-Q plot of the 30-minute post prandial basal state functioning of the kidney as measured by 30-minute S. Creatinine (A), 30-minute Creatinine clearance (B), and GFR calculated from 30-minute S. Creatinine (C).

**The 30-minute calculated GFR showed deviation above and below the normal straight line of the normal Q-Q plot, indicating that there is a bimodal distribution [Figure 4C].

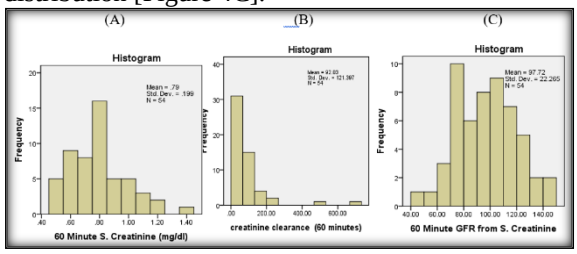

Figure 5: Histogram of the 60-minute postprandial basal state functioning of the kidney as measured by 60-minute S. Creatinine (A), 60-minute Creatinine clearance (B) and GFR calculated from 60-minute S. Creatinine (C).

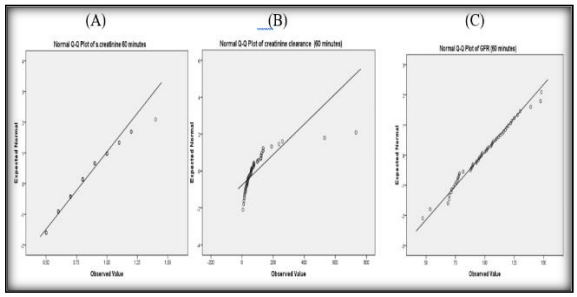

Figure 6: The normal Q-Q plot of 60-minute S. Creatinine showed a deviation from normal distribution above 0.88mmol/L (6A). Normal Q-Q plot of 60-minute Creatinine clearance showed a marked deviation from normal distribution straight line almost at all points (Fig.6B). But the calculated GFR was nearly overlapping the straight-line normal Q-Q plot (6C).

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**The 60-minute post-prandial serum creatinine and creatinine clearance were positively skewed [Figure 5B and 5C]. The 60-minute GFR was normally distributed with a mild bimodal tendency [Figure 5A].

**The normal Q-Q plot of 60-minute postprandial S. Creatinine showed a deviation from normal distribution above 1 mg /dL [Figure 6A]. Normal Q-Q plot of 60-minute Creatinine clearance showed a marked deviation from normal distribution straight line almost at all points [Figure 6B]. But the calculated GFR was nearly overlapping the straight-line normal Q-Q plot [Figure 6C].

Table 1: Distribution characteristics of the fasting and postprandial basal state functioning of the kidney.

Analytes	S. Creatinine (n = 54)		GFR (n = 54)		Creatinine Clearance (n = 54)	
Distribution characteristics	**mean±SD, median**	**Shapiro Wilk (P)**	**mean±SD, median**	**Shapiro Wilk (P)**	**mean±SD, median**	**Shapiro Wilk (P)**
Fasting	0.79±0.19, 0.75	< 0.001	96.14±22.62, 93.06	0.377	79.99±44.45, 73.9	0.021
30-minutes	0.79±0.19, 0.8	0.003	95.50±22.45, 91.98	0.377	80.90±44.13, 69.20	0.003
60-minutes	0.79±0.19, 0.80	0.006	97.71±22.26, 97.08	0.828	92.06±121.39, 58.6	< 0.001

DISCUSSION

This study analyses the characteristics of selected kidney function tests from samples collected from apparently healthy individuals. Fasting, 30-minute postprandial, and 60-minute postprandial blood and urine samples were collected. The distributions of fasting, 30- and 60-minute Serum Creatinine, and Creatinine Clearance were not normally distributed due to positive skewing and the outliers indicated that healthy individuals had some abnormal values of S. Creatinine. The Shapiro-Wilk test of distribution of fasting S. Creatinine and fasting Creatinine clearance had a P value of <0.001 and 0.021. This indicates there is a significant difference in the distribution of serum creatinine and creatinine clearance values. This statement assumes that a normally distributed sample below 0.088 mmol/litre might be healthier than the positively skewed samples and the outliers. Individuals with S. Creatinine between 0.088 and 0.123 mmol/L may be at a higher risk of kidney dysfunctions.

Fasting calculated GFR and 30-minute calculated GFR showed bimodal distribution. These observations indicated that the sample may be further partitioned according to variations in GFR in the subgroups. Such variations and increased dysfunction of the kidney may occur more with the male gender and with an increase in age. However the calculated GFR appeared to be normally distributed in fasting,30-minutes and 60-minutes samples. The normal distribution of calculated GFR may be due to adjustments seen in GFR with body weight, dilutions in the urine sample, and age.

CONCLUSION

Early kidney dysfunction is evaluated by serum Creatinine, calculated GFR, and creatinine clearance. The data on these 3 parameters were evaluated in the fasting, 30-minute, and 60-minute samples of serum and urine. That showed serum creatinine and creatinine clearance can be used as early markers of kidney dysfunctions as the data were positively skewed with outliers above 0.088mmol/L. However, calculated GFR cannot be used as an early marker because their distribution is normal. These results indicate that S. creatinine and creatinine clearance are useful early markers of detection of kidney dysfunction.

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