Pre- and Post-Dialysis Correlations of Serum α-Amylase, Creatinine and Urea in Chronic Renal Failure Patients

M. F. Mahomoodally1*, H. Nugessur2

1Department of Health Sciences, Faculty of Science, University of Mauritius, Réduit, Mauritius,
2Ministry of Health and Quality of Life, Mauritius
*f.mahomoodally@uom.ac.mw

Abstract - Increased serum α-amylase level has been reported in chronic renal failure (CRF) patients undergoing haemodialysis even in the absence of pancreatic disease. Nonetheless, conflicting results and explanations for such increase persist in literatures. To this effect, the main endeavour of this study was to evaluate status of serum α-amylase in CRF patients, currently on hemodialysis (pre-dialysis and post-dialysis) and to delineate any correlations between urea, creatinine and α-amylase level in the same sub-groups. Fifty chronic renal failure patients, aged between 20-80 years undergoing routine haemodialysis, were recruited in the study. Blood samples were collected from each patient weekly for one month in which serum α-amylase, urea and creatinine levels were determined. Paired differences were evaluated with the paired samples t –test and statistical significance of the variables was established at the level \( p < 0.05 \). Linear regression models were also tested for any relationship between pre-dialysis and post-dialysis samples. The major finding of this study was the significant decrease \( (p < 0.05) \) in serum urea and creatinine levels in post-dialysis samples and a significant increase \( (p < 0.05) \) in post-dialysis serum α-amylase levels. However, there was no direct correlation between serum α-amylase and urea in both pre-dialysis \( (R = 0.09) \) and post-dialysis \( (R = -0.13) \) samples or creatinine in pre-dialysis \( (R = 0.26) \) and post-dialysis \( (R = 0.16) \) samples. This present study tends to show that serum α-amylase had a poor correlation with both urea and creatinine in pre-dialysis and post-dialysis samples. The obtained data corroborates to some extent with results of previous cross-sectional studies, to the effect that there is a significant increase that occurs in serum α-amylase and a significant decrease in both serum urea and creatinine in post-dialysis samples. Possible mechanisms for this increase are discussed herein.

Keywords - Dialysis; Serum; α-Amylase; Creatinine; Urea; Chronic Renal Failure

I. INTRODUCTION

Chronic kidney disease (CKD) is becoming a major and alarming public health burden worldwide. It is a progressive condition that results in significant morbidity and mortality [1-5]. In Mauritius the number of renal patients is increasing at a very alarming rate, and the number of patients undergoing dialysis was also increasing both in the five regional hospitals and in private centers from 2006 to 2009. Actually in Mauritius, patients undergo haemodialysis two to three sessions per week for duration of three hours. Once in a month, blood specimens are collected for pre-dialysis and post-dialysis and tested for urea and creatinine in order to ensure efficacy and adequacy of dialysis treatment. Presently in Mauritius, it is not a common practice to evaluate status of serum α-amylase for pre-dialysis and post-dialysis patients. However, a panoply of studies indicate a significant change in serum α-amylase in severe renal failure and in patients maintained on haemodialysis primarily due to the loss of nephrons, which results in decreased fractional reabsorption of α-amylase in the tubules. Nonetheless, there are some conflicting reports. Although amylase, being a pancreatic enzyme, has a relatively small molecular weight of about 50,000 Daltons, it is partially filtered at the renal glomerulus level and is reabsorbed in the tubule to some extent. Apart from being present in pancreatitis, high level of serum amylase is also found in ESRD patients [1-12,16-20].

In light of the above, it is obvious that conflicting data exist and there is a dearth of concrete evidence and correlation studies to suggest possible reasons for such an increase in amylase level. To this effect, the present study aimed to evaluate status of serum α-amylase in chronic renal failure patients currently on hemodialysis (pre-dialysis and post-dialysis) in Mauritius and to establish correlations, if any, with urea and creatinine in the same subgroups.

II. METHODOLOGY

During the study period, 50 chronic renal failure patients undergoing haemodialysis at the ‘Association pour l’Utilisation du Rein Artificiel à Maurice’ (A.U.R.A.M Trust) and Haemodialysis Unit (HDU) of S.S.R National Hospital were selected; 25 patients were selected from the A.U.R.A.M Trust and the other 25 patients from HDU.

Prior to blood sampling, an information sheet was provided to all participants to brief them the main aims of the study. Subsequently, each subject completed a consent form and a participant questionnaire for eligibility determination. The study was approved by the Ethics Committee of the Ministry of Health and Quality of Life, Mauritius. Pre-haemodialysis and post-
haemodialysis venous blood samples (5 ml) were collected using standard venipuncture technique into plain tubes from each selected patient weekly for one month. One blood sample was collected before dialysis and the other after dialysis. All the samples were coded to ensure confidentiality. Sera were separated immediately after centrifugation at -4 °C, 2000 g for 10 min and stored at -20 °C during the course of the study. The blood samples were tested for serum α-amylase, urea and creatinine using respective reagent kit and methodology. Serum α-amylase, urea and creatinine tests were performed on the automated chemistry analyser BTS-310 (BioSystems). It is a discrete analyser with a programme that enables the user to analyse α-amylase, urea and creatinine by their respective method and reagent kit. Serum α-amylase was determined by enzymatic method using 2-chloro-4-nitrophenyl-malto-trioside (CNP-G3); α-amylase catalyses the hydrolysis of 2-chloro-4-nitrophenyl-malto-trioside (CNP-G3) to 2-chloro-4-nitrophenol (CNP). The catalytic concentration was determined from the rate of 2-chloro-4-nitrophenol formation digitally from the absorbance curve and the rate of change was directly proportional to the amylase concentration in the sample. Reference range: serum or plasma amylase: up to 95 U/L.

Serum creatinine was determined using the BioSystem kit – Creatinine-Alkaline Picrate; the test was based on the principle that alkaline pH values, creatinine in blood samples reacts with Picric acid to produce a coloured alkaline picrate complex. The rate of creatinine-alkaline complex formation was measured digitally from the absorbance curve and the rate of change was directly proportional to the creatinine concentration in the sample. Reference range: serum or plasma creatinine: 53-124 µmol/L.

Serum urea was determined by enzymatic methods using urease- BioSystem kit- Urea- Color-urease. Urea in serum reacts with urease to produce a coloured complex known as Indophenol, which was measured digitally from the absorbance curve and the rate of change was directly proportional to the urea concentration in the sample. Reference range: serum or plasma urea: 2.5-7.5 mmol/L.

A. Data analysis

Statistical analysis was carried out using both SPSS 16.0 Inc software for windows Vista and Microsoft Office Excel 2007. Paired differences were evaluated using ANOVA and significant predictors of the paired difference (pre-haemodialysis minus post-haemodialysis values) of serum α-amylase, serum urea and serum creatinine levels were evaluated with univariate linear regression analysis. Statistical significance of the variables was established at the level p <0.05. Linear regression models tested for the relation between pre-dialysis and post-dialysis samples and these values were presented graphically.

III. RESULTS

A. Demographic details of patients

Out of 150 patients suffering from ESRF, fifty patients (50)- twenty nine (29) males and twenty one females (21)- were recruited from A.U.R.A.M Trust and HDU of SSRN Hospital, all undergoing haemodialysis. All the fifty (50) patients participated in the study after signing the participant consent form. The entire questionnaire was answered through a face-to-face interview.

<table>
<thead>
<tr>
<th>TABLE I CLINICAL CHARACTERISTICS OF PATIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemodialysis patients (n=50)</td>
</tr>
<tr>
<td>Mean age (years)</td>
</tr>
<tr>
<td>Gender (M/F)*</td>
</tr>
<tr>
<td>Ethnic group (H/C/M)*</td>
</tr>
<tr>
<td>Mean weight (Kg)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
</tr>
<tr>
<td>Diabetes (%)</td>
</tr>
<tr>
<td>Other health problems (%)</td>
</tr>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td>Alcohol consumption</td>
</tr>
</tbody>
</table>

*Mean ± SD; *(M/F: Males/Females); *(H/C/M: Hindu/Christian/Muslim)

The ages of the studied patients ranged from 20 to 80 years and the most patients undergoing dialysis were 40-50 or 60-70 years old. CRF is distributed in people of any age, from 20-80 years. There were only 4 patients aged 20-30 years, indicating that the frequency of young adults undergoing haemodialysis is less than those of middle age.

Out of the 50 patients under study, 78% were Hindu, 12% were Christian and 10% were Muslim. The numbers of Hindu, Christian and Muslim were 39, 6, and 5 respectively and among the sub-groups, the numbers of patients who were non-vegetarian were 37, 6 and 5 respectively. The weights of the patients ranged from 46-112 Kg with an average weight of 64.7 ± 12.7 Kg. All the 50 patients had full knowledge about dialysis and its complications. All of them had reported that they had changed their diet due to haemodialysis in terms of decreased intake of water, potassium, phosphorous and salt. 11 patients had recently been
undergoing dialysis (≤1 year) and one of them has been undergoing dialysis for the past 12 years. However, all patients were regularly undergoing haemodialysis at a frequency of three times per week. The duration of the dialysis session ranges from two and a half to three hours depending on the patients’ health and resistance.

The prevalence of both hypertension and diabetes among the population was 40%. However, 10% of the patients had neither hypertension nor diabetes. Moreover, 16 patients were suffering from eyes problems, cardiovascular disease, ulcers and gastritis and 11 patients were suffering from abdominal pain.

### TABLE 3 DISTRIBUTION OF DIFFERENT TREATMENTS TAKEN BY HYPERTENSIVE AND DIABETIC PATIENTS

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of patients</th>
<th>Drugs</th>
<th>Diet</th>
<th>Insulin Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension*</td>
<td>41</td>
<td>29</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>Diabetes*</td>
<td>24</td>
<td>7</td>
<td>5</td>
<td>12</td>
</tr>
</tbody>
</table>

*Denote number of patients suffering from CRF

Out of 41 patients who had hypertension, 29 patients were on hypertensive drugs and the rest followed a salt free diet. 24 patients had already been diagnosed of diabetes, 7 patients were on diabetic drugs, 12 patients were receiving insulin injection due to uncontrolled diabetes and poor drug compliances and the rest were on a controlled diet. All the patients were on drugs like calcium carbonate, fefol, vitamin B and 3 patients had to receive regular blood transfusion due to the low haemoglobin level.

**B. Preand post-dialysis correlations**

Levels of serum urea in pre-dialysis were higher than in post-dialysis and the distributions during the four weeks were almost the same for pre-dialysis and post-dialysis urea levels, respectively (Fig. 1). During the four weeks, there was a significant decrease (p < 0.05) in post-haemodialysis serum urea.

![Fig. 1 Comparison of serum urea among the population under study in the pre-dialysis and post-dialysis samples from week 1 to week 4 (p < 0.05)](image)

Levels of serum creatinine in pre-dialysis were higher than in post-dialysis and the distributions during the four weeks were almost the same for pre-dialysis and post-dialysis creatinine levels, respectively (Fig. 2). During the four weeks, there was a significant decrease (p < 0.05) in post-haemodialysis serum creatinine.
Fig. 2 Comparison of serum creatinine among the population under study in the pre-dialysis and post-dialysis samples from week 1 to week 4 (* p < 0.05)

Levels of serum α-amylase in pre-dialysis were lower than in post-dialysis and the distributions during the four weeks were almost the same for pre-dialysis and post-dialysis α-amylase levels, respectively (fig 3). During the four weeks, there was a significant increase (p < 0.05) in post-haemodialysis serum α-amylase.

Fig. 3 Comparison of serum α-amylase among the population under study in the pre-dialysis and post-dialysis samples from week 1 to week 4 (* p < 0.05)
Serum urea levels were significantly decreased ($p < 0.05$) in post-dialysis compared to pre-dialysis during the four weeks (Fig. 4).

![Fig 4. Comparison of mean levels of serum urea in pre-dialysis and post-dialysis among patients under study during the four weeks ($p < 0.05$)](image)

There was a significant decrease ($p < 0.05$) in serum creatinine in post-dialysis compared to that in pre-dialysis during the four weeks (Fig. 5).

![Fig 5. Comparison of mean levels of serum creatinine in pre-dialysis and post-dialysis among patients under study during the four weeks ($p < 0.05$)](image)
Serum α-amylase levels were significantly increased (p < 0.05) in post-dialysis compared to those in pre-dialysis during the four weeks (Fig. 6).

![Graph showing comparison of mean levels of serum α-amylase in pre-dialysis and post-dialysis among patients under study during the four weeks](image)

**Fig. 6** Comparison of mean levels of serum α-amylase in pre-dialysis and post-dialysis among patients under study during the four weeks (p < 0.05)

Fig. 7a-g shows results of the correlation studies. There is a poor positive linear correlation between serum α-amylase and urea in pre-dialysis, and a poor negative linear correlation between serum α-amylase and urea in post-dialysis. There is a poor positive linear correlation between serum α-amylase and creatinine in pre-dialysis, and a poor positive linear correlation between serum α-amylase and creatinine in post-dialysis. There is a moderate positive linear correlation between pre-dialysis and post-dialysis serum urea, and a high positive linear correlation between pre-dialysis and post-dialysis serum creatinine. There is a very high positive linear correlation between pre-dialysis and post-dialysis serum α-amylase.

![Graphs showing correlation studies](image)

**Fig. 7a** Linear regression model tested for the relation between serum α-amylase and serum urea in pre-dialysis; R = 0.09.
**Fig. 7b** Linear regression model tested for the relation between serum α-amylase and serum urea in post-dialysis; R = -0.13.
Fig. 7c. Linear regression model tested for the relation between serum α-amylase and serum creatinine in pre-dialysis; $R = 0.26$.

Fig. 7d. Linear regression model tested for the relation between serum α-amylase and serum creatinine in post-dialysis; $R = 0.16$.

Fig. 7e. Linear regression model tested for the relation between serum urea in pre-dialysis and post-dialysis; $R = 0.63$.

Fig. 7f. Linear regression model tested for the relation between serum creatinine in pre-dialysis and post-dialysis; $R = 0.85$.

Fig. 7g. Linear regression model tested for the relation between serum α-amylase in pre-dialysis and post-dialysis; $R = 0.98$. 
IV. DISCUSSION

CKD is an increasingly recognised health problem worldwide and affects over 5% of the Mauritian population. Indeed, progression of CKD to require renal replacement therapy like dialysis is associated with significant morbidity and mortality [1-5]. Therefore, there is a pressing need to detect early indicators of renal dysfunction, where therapeutic intervention is most effective. The number of renal patients in Mauritius is increasing at a very alarming rate, which has resulted in a marked increase in the number of patients undergoing haemodialysis in the regional hospitals and private centers across the island. The prime contributing factors have been the damage caused as a result of diabetes and hypertension [3,4]. Because of the important role of the kidney in regulating metabolism, the concentrations of many chemical constituents of the body are abnormal in patients with CRF and ESRD undergoing haemodialysis [24]. Hence, in this study, serum urea and creatinine were analysed to evaluate the effectiveness of the haemodialysis treatment. Additionally, serum α-amylase was analysed to establish correlation, if any, with serum urea and creatinine.

Result from the present study showed that both sexes were affected by chronic renal failure but a higher total number of males with ESRD were found undergoing haemodialysis. It has also been reported that CRF can be found in people of any age, from 20-80 years and this present finding is supported by both Anderson et al. [25] and Arora et al. [26] Most patients undergoing dialysis were between the ages of 40-50 years, which could be due to progressive physiological glomerulosclerosis that leads to a decrease in renal weight, and between the ages of 60-70 years, since the biologic process of aging initiates various structural and functional changes within the kidney. It is an established fact that renal mass progressively declines with advancing age [25,26]. Aging also results in concomitant progressive physiological decrease in muscle mass, thus decreasing daily urinary creatinine [5]. The weight of the patients ranged from 46 to 112 Kg with an average weight of 64.7±12.7 Kg. CKD affects all races, yet in the United States, a significantly higher incidence of ESRD exists in blacks as compared to whites; the incident rate for blacks was nearly 4 times that for whites [27]. It has also been found that the rate of ESRD among black patients exceeded those among white patients at all levels of baseline estimated glomerular filtration rate (eGFR) and similarly, mortality rates among black patients were equal to or higher than those among white patients at all levels of eGFR.

Patients (n=29) suffering from hypertension were on medications like amlopipine, enalapril and lasix. In the present case the angiotensin converting enzyme (ACE) inhibitor enalapril might have been one factor contributing to increased serum α-amylase observed in the present investigation. ACE inhibitors are known to increase the generation of bradykinin, a vasoactive substance that induces angioedema, which in turn could cause pancreatic duct obstruction followed by enzyme leakage [28]. One of the hypertensive patients was suffering from acute pancreatitis since the serum α-amylase levels in both pre-haemodialysis and post-haemodialysis were elevated more than threefold normal in association with abdominal pain [11]. Bradykinin and subsequent nitric oxide release associated with hypotension during haemodialysis together with underlying arteriosclerosis can induce mesenteric ischaemia represented by abdominal pain during the dialysis session, leading to acute pancreatitis. This is because reactive oxygen species such as superoxide released in the ischaemia/reperfusion process can result in pancreatic damage [28].

The major finding of this study was the significant decrease in serum urea and creatinine levels in post-dialysis and a significant increase serum α-amylase levels in post-dialysis. This is because during haemodialysis, urea and creatinine, being small molecules, flow through membranes into the sterile solution and is removed due to the counter-current flow of the blood and dialysate which maximises the concentration gradient of solutes between the blood and dialysate, which helps to remove more urea and creatinine from the blood [29]. Thus, this present study tends to show that the prescribed doses of dialysis therapy, i.e. intensity and frequency of haemodialysis, were successful to excrete urea and creatinine from blood. In addition, a recent publication by Stosovic et al. [30] revealed that there is a complex relationship between urea and mortality in hemodialysis patients such that patients with low or high urea levels exhibited higher mortality than those with medium levels, while both low and high levels of urea were independent predictors of all-cause mortality.

The significant increase in α-amylase was indeed in agreement with recent publication [21] since serum α-amylase levels above the upper normal limit in 50% before haemodialysis and 62% after haemodialysis were found. Values of serum α-amylase exceeding 3 times the upper normal limit were found in one patient both before and after dialysis and this phenomenon was attributed to a reduced glomerular filtration or subclinical pancreatic damage; but in all the 50 patients, the average increase in post-dialysis serum α-amylase was statistically significant. These results were also in fairly good agreement with those given by Eur [16] and Jiang et al.[11], who showed that high levels of serum amylase found in patients maintained on haemodialysis, both before and especially after dialysis, are often elevated within threefold normal in ESRD patients. However, the serum amylase value could be normal in individual cases in spite of the presence of severe ureamia.

In this present study, there was no direct correlation between serum α-amylase and serum urea both in pre-dialysis (R = 0.09) and post-dialysis (R = -0.13) or serum creatinine in pre-dialysis (R = 0.26) and post-dialysis (R = 0.16), and these results corroborate with the previous study done by Bardella et al. [31]. However, these data differed from another study carried by Ozmar [19], who concluded that serum α-amylase significantly correlated with creatinine level. Therefore, either the production of α-amylase might have been greatly reduced in these patients or α-amylase might be broken down in other organs, or possibly excreted by the intestinal tract. The data obtained from the present study also showed a moderate correlation between pre-dialysis
and post-dialysis serum urea (R = 0.63), a high correlation between pre-dialysis and post-dialysis serum creatinine (R = 0.85) but a very high correlation between pre-dialysis and post-dialysis serum α-amylase (R = 0.98).

A possible reason for an increased serum amylase level in renal failure had previously been discussed by Burtis et al. [12], who demonstrated that macroamylasemia is sometimes present in sera and can cause hyperamylasemia. Macroamylasemia is a benign condition characterised by abnormally large-sized serum amylase; it has also been reported to occur in 1–2% of the population. In macroamylasemia, a macromolecular complex consisting of amylase linked to immunoglobulins circulates in the plasma and usually causes hyperamylasemia with low or normal amylasuria. The clinical importance of macroamylasemia is the confusion of this condition with other causes of hyperamylasemia. In this condition, the serum amylase is abnormally large and is therefore not excreted in the normal way [12, 15].

The increase in serum amylase in post-dialysis was difficult to justify since the amylolytic activity in the dialysate fluid was not investigated, and the amylase molecule thus would not seem to have passed the cellulose membrane during ultrafiltration [32]. The type of semipermeable membrane used in the dialysis machine can also result in an increase in serum amylase in haemodialysis patients since the membrane can block the passage of macromolecular solutes and larger substances such as red blood cells, large proteins and amylase enzymes [18]. It is not only the size but also the shape of a molecule that determines whether it will pass through a dialysis membrane. Another study by Chen et al. [21] proved, for the first time, that hemodynamic change during haemodialysis and related factors are significantly associated with elevated serum α-amylase during haemodialysis. The complex hemodynamic, biochemical, and physiological alterations in uremia were speculated to cause excessive release of pancreatic enzymes beyond decreased renal clearance. The study of Chen et al. [21] also indicated an independent association of higher ultrafiltration (UF) volume with elevated serum amylase. The independent association of higher UF volume with elevated serum amylase may have two implications. First, greater intradialytic decrease in body weight or UF volume was known to be associated with greater blood pressure change during haemodialysis [33,34]. Therefore, the association of greater UF volume with elevated levels of serum pancreatic enzymes can be viewed as an indicator of greater hemodynamic change. Second, greater interdialytic body weight gain may be related to better nutritional status parameters [35]. Because plasma amylase levels were found to increase after feeding [36], greater UF volume may therefore be associated with elevated serum amylase levels through better enteral feeding and nutrition status, which may be supported by the higher pre-haemodialysis blood urea and creatinine.

In conclusion, the significant decrease in both serum urea and creatinine in post-dialysis proved that the frequency and length of haemodialysis treatment were efficient. This investigation indicated that in Mauritius there is a significant increase in serum α-amylase in patients undergoing dialysis. It may be suggested that the increased level of serum α-amylase in CRF might be due to decreased excretion of amylase by the kidneys. It is also plausible that there could be some subclinical pancreatic damage, and its genesis could also depend on the pharmacological treatment used (diuretics and hypertensive drugs) commonly adopted in these pathologies.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Bholah, Medical Specialist, Mrs Sooknah, officer in charge at A.U.R.A.M Trust, Mr Veerasamy, officer in charge at HDU of SSRN Hospital, nursing staff, and phlebotomists of A.U.R.A.M Trust and HDU.

REFERENCES
