ORIGINAL ARTICLE

EFFECT OF HEMODIALYSIS ON CIRCULATING CYSTATIN C LEVELS IN PATIENTS WITH END STAGE RENAL DISEASE

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ABSTRACT

Plasma cystatin C is an emerging parameter to assess kidney function. Its utility in assessing the adequacy of hemodialysis in patients with end-stage-renal disease has however not been established with certainty. This study was therefore carried out to assess the usefulness of serum cystatin C estimation in patients undergoing low flux membrane hemodialysis. Serum creatinine and cystatin C were estimated in 20 patients before and after undergoing hemodialysis. The mean serum creatinine decreased from a pre-dialysis value of 7.72 mg/dL to a post-dialysis value of 2.90 mg/dL. On the contrary, the mean serum cystatin C levels were found to increase from a pre-dialysis value of 5.97 mg/L to a post-dialysis value of 8.25 mg/L. Therefore, serum cystatin C cannot be used to monitor dialysis adequacy. It however, serves as a surrogate marker of the inadequacy of low flux membrane bicarbonate hemodialysis in clearing low molecular weight proteins from the circulation.

KEY WORDS

Hemodialysis, Pre-dialysis, Post-dialysis, Cystatin C, Creatinine.

INTRODUCTION

Dialysis efficiency greatly influences the well being, outcome and survival of patients with end-stage renal disease. Therefore a close monitoring and follow up is essential, so as to achieve the best outcome. Presently, the efficacy of dialysis is assessed by estimating serum creatinine levels before and after each session of dialysis. However serum creatinine levels are influenced by inter-individual variability related to age and gender (1). In addition plasma creatinine is also affected by body composition and dietary factors. For example intake of meat can increase the plasma creatinine because it contains

creatine which can be converted to creatinine by cooking (2). Numerous drugs such as trimethoprim-sulfamethoxazole (3) and endogenous substances such as ketone bodies (4) also interfere with the measurement of creatinine by the Jaffe's technique, leading to falsely high or low creatinine values. Furthermore, secretion or reabsorption of creatinine in renal tubules could lead to under or overestimation of glomerular filtration rate (5, 6).

Currently the determination of serum cystatin C has been proposed as an additional parameter for assessment of renal function (7). Cystatin C is a single non- glycosylated polypeptide chain consisting of 120 amino acid residues with a molecular mass of 13 kDa (8). Low molecular weight proteins are now recognized as a distinct class of uremic toxins and numerous compounds in this category have been identified including cystatin C (9). Serum cystatin C produced at a constant rate by all nucleated cells and freely filtered by the glomerulus, is neither secreted nor reabsorbed and has been known to be unaffected by non- renal factors (10). Therefore serum cystatin C is known to be an excellent surrogate marker of glomerular filtration rate. Meta analysis by Dharnidharka et

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al (7) and a recent publication by Roos et al (11) provide evidence that cystatin C has superiority over serum creatinine as a marker of renal impairment.

To the best of our knowledge, there are no studies from South India comparing the levels of serum cystatin C and serum creatinine in patients undergoing hemodialysis. Serum creatinine is the only parameter still widely used to assess the efficacy of dialysis. The usefulness of serum cystatin C levels as an indicator of the efficacy of hemodialysis is not known with certainty. The present study was therefore undertaken in order to evaluate the potential clinical utility of serum cystatin C determination in patients undergoing hemodialysis.

MATERIALS AND METHODS

The study was approved by Human Ethics Committee of the PSG Institute of Medical Sciences and Research and oral consent was obtained from all the participants. Patients with end-stage-renal-disease who were on maintenance hemodialysis, in the Department of Nephrology at PSG Hospitals in Coimbatore, India, during the period of January 2009 to February 2009 formed the subjects of the study. The mean age of the patients were 49.75 years, range 25 – 64, with 15 males and 5 females.

A low flux polysulfone membrane was used for bicarbonate hemodialysis. The duration of dialysis was 3.5 – 5 hours; mean heparinization dose 4231 ± 1113 IU; temperature of the dialysis bath, 35.5 – 36.6°C. The dialysis bath consisted of bicarbonate, 33–36 mmol/L; sodium, 138 – 144 mmol/L; potassium, 2 mmol/L; and calcium, 1.25 – 1.7 mmol/L. Samples of blood were collected from the patients before and after hemodialysis using vacutainers (BD, USA) containing clot activator. The serum obtained was used for the estimation of creatinine and cystatin C on the same day. Serum creatinine was measured using an automated Jaffe's assay on the Olympus AU 400 (Olympus Optical, Tokyo, Japan) analyzer according to the manufacturer's procedure. Cystatin C was measured in serum samples with latex particle – enhanced turbidimetric immunoassay from Gentian (Gentian AS, Moss, Norway) performed on an Olympus AU 640 (Olympus Optical, Tokyo, Japan) as per the application note provided by the manufacturer.

The efficacy of dialysis was assessed by calculating the reduction ratio for serum creatinine as shown below: Creatinine reduction ratio (CrRR) = $100 \times (1 - C_i / C_o)$ where C_i & C_o represent postdialysis and pre-dialysis serum creatinine levels. The same formula was also used for the calculation of serum

cystatin C reduction ratio (CyRR) (12).

MedCalc statistical software 10.3.0.0 was used for the analysis of the results. Results are expressed as mean $\pm$ SD and are shown in the form of a histogram. The student's 't' test was used for comparing the serum creatinine and serum cystatin C before and after dialysis. P value <0.05 was taken as significant. The dot and line diagram was used to depict the changes in the individual values of serum creatinine and serum cystatin C in pre and post dialysis samples.

RESULTS AND DISCUSSION

The results are as shown in Table 1 and the Figures 1, 2A and 2B. The serum creatinine levels in the pre-dialysis samples varied from 4.49 to 12.24 mg/dL, with a mean and SD of 7.72 ± 2.22 mg/dL and for the post-dialysis it varied from 1.64 to 5.73 mg/dL, with a mean and SD of 2.90 ± 1.11 mg/dL. The serum cystatin C levels in the pre-dialysis samples varied from 2.89 to 7.73 mg/L, with a mean and SD of 5.97 ± 2.10 mg/L and for the post-dialysis it varied from 4.6 to 11.49 mg/L, with a mean and SD of 8.25 ± 1.85 mg/L.

Table 1: Serum creatinine and cystatin C levels in patients undergoing hemodialysis

	Pre- dialysis	Post -dialysis	%
No of Patients (n)	20	20	
Age(yrs)	49.75 ± 13.84	49.75 ± 13.84	
Creatinine (mg/dL)	7.72 ± 2.22	$2.90 \pm 1.11^*$	
Cystatin C (mg/L)	5.97 ± 2.10	$8.25 \pm 1.85^*$	
CrRR			(+) 62.5
CyRR			(-) 38

Values are mean $\pm$ SD; * $P < 0.0001$

The results showed that the process of dialysis leads to a significant fall in mean serum creatinine. In contrast to this, the serum cystatin C levels were significantly higher in the post-dialysis samples as compared with the pre-dialysis ones. The rise in serum cystatin C following hemodialysis was observed in all the patients taken up for the study. This was in spite of the concomitant fall in serum creatinine, which is an accepted index for the adequacy of dialysis. The calculated parameters namely CrRR and CyRR reflect the differential behavior of the two molecules towards the process of dialysis. While the mean CrRR was + 62.5%, the mean CyRR was – 38% (the negative sign is indicative of the rise in cystatin C following hemodialysis, as shown in Table 1).

The two parameters studied to evaluate the efficacy of dialysis

Fig 1

namely serum creatinine and serum cystatin C showed conflicting results. Similar results have been reported by Montini et al (13) in uremic children undergoing peritoneal dialysis. Serum creatinine levels fell significantly following dialysis, while cystatin C levels did not. Cystatin C levels were actually significantly higher after dialysis.

The rise in the serum cystatin C following dialysis could be attributed to several factors such as the nature of the dialyzing membrane and the composition of the dialyzing fluid (13). When dialysis is carried out using low flux membrane, the pore size is smaller than 1.5 nm which does not permit the removal of low molecular weight proteins such as cystatin C. Another factor to be considered is the electro-static interaction between micro-proteins and other plasma proteins adsorbed onto the dialyzer membranes. Cystatin C is strongly cationic and the charged nature of the molecule might hinder its filtration (14). The rise in serum cystatin C could also be attributed to the effect of hemoconcentration which occurs during dialysis. The fall in serum creatinine despite such changes is because of the magnitude of reduction of this metabolite during dialysis. In conclusion, the results of the study have shown that serum cystatin C cannot be used to monitor adequacy of hemodialysis. However, it serves as a surrogate marker of the inadequacy of dialysis, more so when low flux membranes are used. Cystatin C is considered to be a prognostic biomarker of risk for death and cardio-vascular disease (15). Routine estimation of serum cystatin C in patients undergoing hemodialysis would help to monitor the overall clinical status of the patient.

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Fig 2: Levels of serum Creatinine (A) and Cystatin C (B) in Pre- and Post-hemodialysis

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