

CYSTATIN C, A LOW MOLECULAR WEIGHT PROTEIN IN CHRONIC RENAL FAILURE

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Abstract

Cystatin C is a protein with a low molecular weight of 13 kDa, a member of the cysteine proteinase inhibitor family, produced at a constant rate by all nucleated cells. There are few studies of serum cystatin C levels in dialysis patients. The aim of this study was to measure the cystatin C levels in patients with chronic renal failure, before and after undergoing haemodialysis, and to assess the usefulness of the test in these patients. Our research was performed on 48 patients (23 females and 25 males) with chronic renal failure. The serum obtained was used for the evaluation of creatinine, urea and cystatin C before and after dialysis. The values were compared to those obtained from a number of 75 healthy individuals. Cystatin C was determined using an immunoturbidimetric assay. Cystatin C levels in pre-dialyzed patients (6.237 ± 1.156 mg/L) were significantly increased compared to control patients (0.787 ± 0.092 mg/L), ($p < 0.001$). After dialysis, cystatin C levels remained also increased (6.334 ± 1.610 mg/L). Cystatin C levels in chronic renal failure patients did not decrease after dialysis. Therefore, serum cystatin C cannot be used for monitoring dialysis efficiency.

Rezumat

Cistatina C este o proteină cu o greutate moleculară mică (13 kDa) și face parte din familia inhibitorilor cistein proteinazei, produsă constant de către toate celulele nucleate. Există puține studii referitoare la cistatina C serică la pacienții dializați. Scopul acestui studiu a fost de a determina concentrația serică a cistatinei C la pacienții cu insuficiență renală cronică, înainte și după hemodializă și evaluarea utilității testului la acești pacienți. Studiul a fost efectuat pe 48 de pacienți (23 femei și 25 bărbați) cu insuficiență renală cronică. Serul obținut a fost utilizat pentru determinarea cantitativă a creatininei, ureei și cistatinei C înainte și după dializă. Valorile au fost comparate cu cele obținute de la un număr de 75 de subiecți sănătoși. Cistatina C a fost determinată printr-o metodă imunoturbidimetrică. Concentrația serică a cistatinei C la pacienți înainte de dializă ($6,237 \pm 1,156$ mg/L) a fost semnificativ crescută comparativ cu martorul ($0,787 \pm 0,092$ mg/L), ($p < 0.001$). După dializă, concentrația serică a cistatinei C a rămas, de asemenea crescută ($6,334 \pm 1,610$ mg/L). Cistatina C serică la pacienții cu insuficiență renală cronică nu a înregistrat scăderi ale concentrației după dializă. Prin urmare, cistatina C serică nu poate fi utilizată pentru a monitoriza eficiența dializei.

Keywords: cystatin C, haemodialysis, urea, creatinine

Introduction

Chronic kidney disease affects millions of adults and its prevalence is rising, mostly in the elderly [5]. Residual renal function is known as a significant factor which influences morbidity, mortality and quality of life in patients submitted to dialysis [20, 25, 27]. In many studies, during the last decade, it was observed a correlation between serum cystatin C and glomerular filtration rate [1, 11].

Cystatin C is a non-glycosylated protein with a low molecular weight (13 kDa) and positively charged having an isoelectric point of 9.3 [12]. It is a member of the cysteine proteinase inhibitor family [22], is produced at a constant rate by all nucleated cells [23]. It is distributed mainly extracellularly [9]

and is present in a number of body fluids such as blood plasma, cerebrospinal fluid, urine, saliva, seminal plasma, amniotic fluid, tears, milk [13].

Studies showed that there are differences in the extracellular levels of cystatin C such as micromolar levels in cerebrospinal fluid, semen, while serum, saliva, and tears have a much lower concentration of cystatin C [15]. The production is constant, but it may be altered by inflammation or thyroid pathologies [20, 29]. Glucocorticoid therapy, in different diseases, remains controversial on its impact on the production of cystatin C [10, 24].

Several studies demonstrated an increased cystatin C level in sera, pleural effusions, and ascetic fluids collected from cancer patients [16].

In the kidney, it is freely filtered and catabolized in the proximal tubule without being secreted [18]. Cystatin C is suggested to be a better marker of kidney function than creatinine because of its independence of age, gender and body mass index [8]. There are few studies of serum cystatin C levels in dialysed patients. Al-Maliki *et al.* [1] have suggested that its size (13.2 kDa) should make it dialyzable and a better marker for “middle molecule” toxin removal.

The aim of our study was to measure the cystatin C levels in patients with chronic renal failure (CRF), before and after undergoing hemodialysis and to establish a possible correlation with serum urea and creatinine. We also assessed the usefulness of this parameter in these patients.

Materials and Methods

Our research was performed on patients with chronic renal failure submitted to dialysis in the “Renamed” Centre of Haemodialysis from Oradea, Romania. The patients selected were all known to be functionally anephric with residual urine volumes of 0 to 250 mL/day. They received conventional haemodialysis (CHD) 12 hours a week in three sessions of four hours. The dialysis was performed by using high-flux polysulphone membrane dialyzers. The blood flow varied between 250-400 mL/min. The normalized treatment ratio Kt/V (Kt/V = the ratio of the urea clearance x time product to total body water) average was 1.58. Average age of dialysis in the studied patients was 61 months, with extremes 11-240 months.

The total number of patients included in this study was 48 (23 females and 25 males), with an average age of 59.5 ± 13.8 and 75 healthy controls (age 50.1 ± 11.9). Patients were excluded if they were younger than 18 years, mentally disabled, with an organ transplant within the previous year or acute obstructive uropathy.

All blood samples were taken before and after the second HD session of the week, according to the guidelines for HD adequacy [14] using Venosafe® serum gel tubes (Terumo, Europe). The serum obtained was used for the estimation of creatinine, urea and cystatin C on the same day. The values were compared to those obtained from a number of 75 healthy individuals. All measurements were performed on an Advia 1200 Chemistry System (Siemens, Germany). Serum creatinine was measured using an automated Jaffe's assay from DiaSys (DiaSys Diagnostic Systems, Germany) according to the manufacturer's procedure. Cystatin C was measured in serum samples using the particle-enhanced turbidimetric immunoassay (PETIA) from DiaSys (DiaSys Diagnostic Systems, Germany) as per the application note provided by the manufacturer.

Urea was determined using an urease – GLDH, enzymatic UV assay from DiaSys (DiaSys Diagnostic Systems, Germany).

The written informed consent was obtained from all participants, prior to enrolment and the study was approved by the institutional Ethics Committee of the University of Medicine and Pharmacy of Oradea.

Statistical analysis

The data were expressed as mean $\pm$ SD. p-values < 0.05 were considered significant. Two-way ANOVA with Bonferroni post-test was performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA.

Results and Discussion

The total number of studied subjects was 123, including 48 patients submitted to dialysis and 75 healthy subjects in the control group. The clinical characteristics, biochemical parameters and aetiology of haemodialyzed patients and control group are presented in Table I.

Table I
Baseline characteristics of studied groups

	Control	Before dialysis	After dialysis
Patients (n)	75	48	48
a) Clinical characteristics*			
Age (years)	59.5 ± 10.5	50.1 ± 11.87	
BMI (Kg/m ²)	22.85 ± 1.47^a	25.86 ± 6.35^b	
b) Biochemical parameters*			
Urea (g/L)	0.29 ± 0.09^a	1.38 ± 0.40^b	0.45 ± 0.16^a
Creatinine (mg/dL)	0.85 ± 0.19^a	8.74 ± 2.59^b	3.73 ± 1.39^c
Cystatin C (mg/L)	0.79 ± 0.09^a	6.22 ± 1.16^b	6.33 ± 1.61^b
c) Cause of chronic renal disease of haemodialysed patients			
Tubulointerstitial nephropathy (TN)			15
Diabetic nephropathy (DN)			12
Chronic glomerulonephritis (CG)			15
Polycysticrenal disease			2
Tubulointerstitial nephropathy (TN) + Diabetic nephropathy (DN)			3
Amyloidosis			1

*Different letters superscripts in the same row indicate statistically significant differences ($p \leq 0.05$). Values with same superscript are not different (Two-way ANOVA – Bonferroni multiple comparisons)

The serum creatinine levels in the pre-dialysis samples varied from 3.87 to 16.21 mg/dL, with an average of 8.74 ± 2.59 mg/dL and post-dialysis varied from 1.78 to 6.58 mg/dL, with an average of 3.73 ± 1.39 mg/dL (Figure 1A). The mean serum urea level decreased from a pre-dialyzed value of 1.38 g/L to a post-dialysis value of 0.45 g/L (Figure 1B).

Cystatin C levels in pre-dialyzed patients (6.22 ± 1.16 mg/L) were significantly increased compared to the healthy subjects (control) (0.79 ± 0.09 mg/L), ($p < 0.001$). After dialysis, cystatin C levels remained also increased (6.33 ± 1.61 mg/L) (Figure 1C).

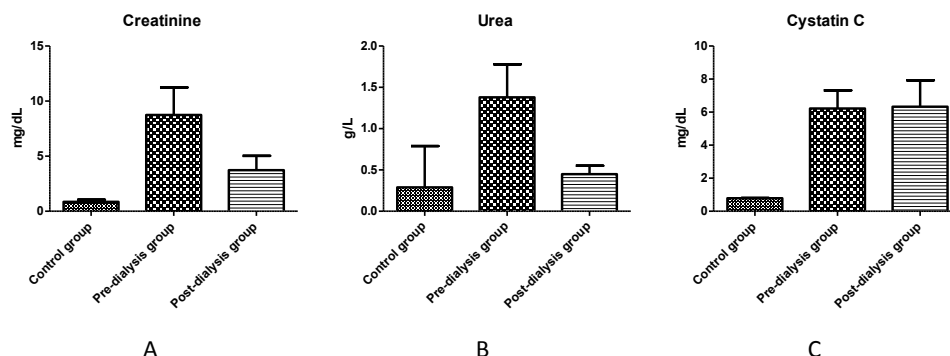


Figure 1.

Serum Creatinine (A), Urea (B) and Cystatin C (C) in Pre- and Post-dialysis groups compared to control group

Correlations were noticed between creatinine and urea (Pearson coefficient = 0.5764) and also between creatinine and cystatin C (Pearson coefficient = 0.5650) in pre-dialyzed patients. There was a significant correlation between

creatinine and urea in post-dialyzed patients (Pearson coefficient = 0.6201) (Table II) (Figure 2). Before dialysis, a weak correlation was observed between cystatin C and urea (Pearson coefficient = 0.0058) (Table II) (Figure 2).

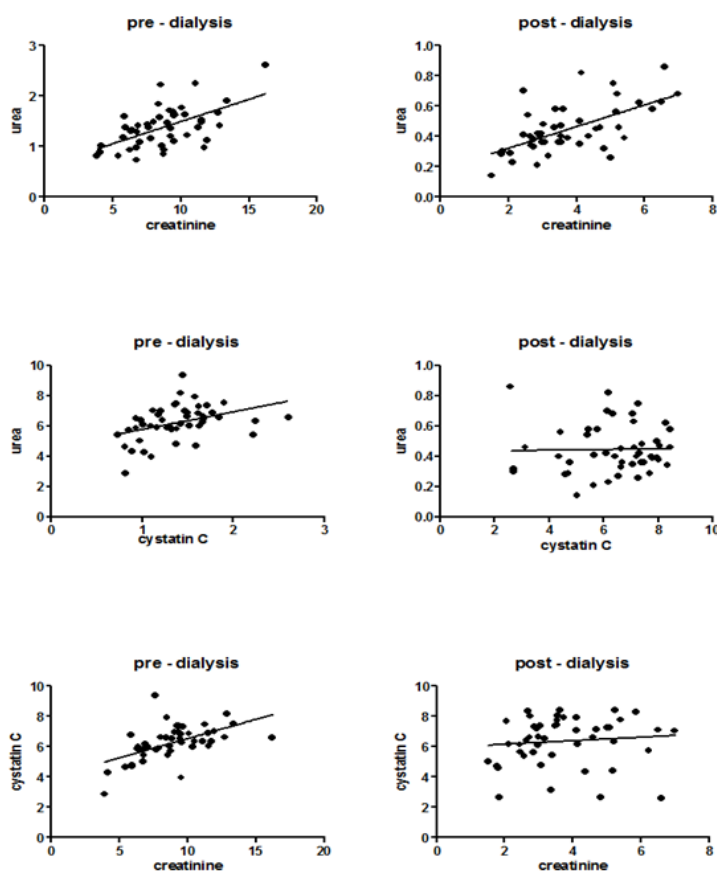


Figure 2.

Correlation of cystatin C with urea and creatinine before and after dialysis

Table II

Pearson correlation coefficient (r) and p value of creatinine with urea and cystatin C and cystatin C with urea between pre-dialysis and post-dialysis groups

	Pre-dialysis Creatinine		Post-dialysis Creatinine		Pre-dialysis Cystatin C		Post-dialysis Cystatin C	
	Pearson r	p value	Pearson r	p value	Pearson r	p value	Pearson r	p value
Urea Pre-dialysis	0.5764	< 0.0001	-	-	0.3962	0.0058	-	-
Urea Post-dialysis	-	-	0.6201	< 0.0001	-	-	0.0203	0.8926
Cystatin C Pre-dialysis	0.5650	< 0.0001	-	-				
Cystatin C Post-dialysis	-	-	0.1009	0.4996				

The purpose of this study was to evaluate the cystatin C concentration in patients with chronic renal failure, submitted to haemodialysis and to investigate the relationship between cystatin C and creatinine and urea serum levels.

Glomerular filtration rate is the main indicator of kidney function. It is defined as the clearance of a substance carried in the plasma that is not metabolized outside the kidney, filtered freely through the glomerular membrane [7].

Serum creatinine with a molecular weight of 113 Da and urea, molecular weight of 60 Da, are small molecules that are used to monitor renal function in patients with chronic renal disease [3, 4].

In our study a strong correlation was observed between creatinine and urea before haemodialysis (Pearson coefficient = 0.5764). We have found a significant correlation between creatinine and urea in post-dialyzed patients (Pearson coefficient = 0.6201). Serum creatinine levels are depending on muscle mass, age, and race [26].

Dharnidharka *et al.* demonstrated that cystatin C is a better marker of glomerular filtration rate than serum creatinine because its plasma level is not influenced by age and sex [8].

Huang *et al.* showed that cystatin C levels decrease over the course of dialysis and that the reduction ratio is related directly to the normalized liters of blood processed but indirectly by ultrafiltration [15]. They suggest that the different volumes of distribution and the different equilibration times between compartments for cystatin C, creatinine, and urea explain their findings [15].

Krishnamurthy *et al.* obtained significantly higher serum cystatin C levels in the post-dialysis samples as compared with the pre-dialysis ones [17]. Similar results have been reported by Montini *et al.* in uremic children undergoing peritoneal dialysis [21]. Serum creatinine levels declined significantly following dialysis, while cystatin C levels did not. Cystatin C concentrations were considerable higher after dialysis.

A study of Campo *et al.* showed that only MCD (mixed convective dialysis) clears cystatin C, but not bicarbonate haemodialysis (BHD) [2]. Montini

et al. underlined that the high concentration of serum cystatin C after dialysis it is due to the influence of the nature of the dialyzing membrane and due to the composition of the dialyzing fluid [21, 28].

Another factor which can impede the filtering of cystatin C, a low molecular weight protein, is that it has a strong cationic character [1].

It was found a significant positive correlation between creatinine and cystatin C (Pearson coefficient = 0.5650) in pre-dialyzed patients. These results are similar to those obtained by other researchers [6, 11]. A weak correlation between cystatin C and urea was observed in the patients group, before dialysis (Pearson coefficient = 0.0058).

Conclusions

In chronic renal failure patients cystatin C level did not decrease after dialysis. Therefore, serum cystatin C cannot be used to monitor dialysis adequacy. It was found a significant positive correlation between creatinine and cystatin C in pre-dialyzed patients.

This study showed that serum cystatin C levels in functionally anephric patients undergoing haemodialysis remained as increased as before dialysis. In contrast, serum urea and creatinine levels registered a decrease, which demonstrated the effectiveness of dialysis.

Further studies concerning serum cystatin C levels crossing through dialysis membranes and its existence in dialysate are indicated. Additional researches on dialysis patients who have residual renal function are necessary.

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