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

OTILIA MICLE\textsuperscript{1}, SIMONA IOANA VICAS\textsuperscript{2*}, IOANA RATIU\textsuperscript{3}, LAURA VICAS\textsuperscript{4}, MARIANA MURESAN\textsuperscript{1}

\textsuperscript{1}University of Oradea, Faculty of Medicine and Pharmacy Oradea, Department of Preclinical Disciplines, Oradea, Romania
\textsuperscript{2}University of Oradea, Faculty of Environmental Protection, Oradea, Romania
\textsuperscript{3}University of Oradea, Faculty of Medicine and Pharmacy Oradea, Department of Internal Medicine, Oradea, Romania
\textsuperscript{4}University of Oradea, Faculty of Medicine and Pharmacy Oradea, Department of Pharmacy, Oradea, Romania

\*corresponding author: sim_vicas@yahoo.com

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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 cystatine 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 constantă 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 cronnică, 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 cronnică. Serul obținut a fost utilizat pentru determinarea cantitativă a creatininei, ureii și cistatinei C înainte și după dializă. Valoarea a fost comparată cu cele obținute de la un număr de 75 de subiecți sănătosi. Cistatina C a fost determinată printr-o metodă imunoturbidimetrică. Concentrația serică a cistatinei C la pacienții înainte de dializă (6,237 ± 1,156 mg/L) a fost semnificativ crescută comparativ cu marțorul (0,787 ± 0,092 mg/L), (p < 0.001). După dializă, concentrația serică a cistatinei C a rămas, de asemenea crescută (6,334 ± 1,610 mg/L). Cistatina C serică la pacienții cu insuficiență renală cronnică nu a înregistrat scăderi ale concentrației după dializă. Prin urmare, cistatina C serică nu poate fi utilizată pentru a monitoriza eficiența dializelor.

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 ± 10.5 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 before and after undergoing hemodialysis and to assess the usefulness of this molecule toxin removal.

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>
<thead>
<tr>
<th>Patients (n)</th>
<th>Control</th>
<th>Before dialysis</th>
<th>After dialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>75</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>a) Clinical characteristics*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.5 ± 10.5</td>
<td>50.1 ± 11.87</td>
<td></td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>22.85 ± 1.47⁷</td>
<td>25.86 ± 6.35⁷</td>
<td></td>
</tr>
<tr>
<td>b) Biochemical parameters*</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Urea (g/L)</td>
<td>0.29 ± 0.09⁷</td>
<td>1.38 ± 0.40⁸</td>
<td>0.45 ± 0.16⁸</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.85 ± 0.19⁷</td>
<td>8.74 ± 2.59⁸</td>
<td>3.73 ± 1.39⁹</td>
</tr>
<tr>
<td>Cystatin C (mg/L)</td>
<td>0.79 ± 0.09⁷</td>
<td>6.22 ± 1.16⁷</td>
<td>6.33 ± 1.61⁷</td>
</tr>
<tr>
<td>c) Cause of chronic renal disease of haemodialysed patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubulointerstitial nephropathy (TN)</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic nephropathy (DN)</td>
<td>12</td>
<td></td>
<td></td>
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<tr>
<td>Chronic glomerulonephritis (CG)</td>
<td>15</td>
<td></td>
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<tr>
<td>Polycystic renal disease</td>
<td>2</td>
<td></td>
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</tr>
<tr>
<td>Tubulointerstitial nephropathy (TN) + Diabetic nephropathy (DN)</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>1</td>
<td></td>
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</tr>
</tbody>
</table>

*Different letters superscripts in the same row indicate statistically significant differences (p ≤ 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).

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 1.
Serum Creatinine (A), Urea (B) and Cystatin C (C) in Pre- and Post-dialysis groups compared to control group

Figure 2.
Correlation of cystatin C with urea and creatinine before and after dialysis
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 creatinine 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 considerably 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 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-dialized 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-dialized 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.

References


2. Campo A., Lanfranco G., Gramaglia L., Goia F., Cottino R., Giusto V., Could plasma cystatin C be useful as a marker of hemodialysis low molecular