Cystatin C as a Sensitive Marker of Nephrotoxicity in Children with Different Malignant Diseases During Induction Phase of Chemotherapy

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ABSTRACT

Background: Monitoring of kidney function is essential during the course of chemotherapy. Serum creatinine is of limited value in early detection of renal insufficiency. Cystatin C has proved to be a good marker for detection of early reduction in glomerular filtration rate (GFR).

Objectives: To assess serum cystatin C in children receiving chemotherapy and to compare its sensitivity with serum creatinine in detecting early reductions in GFR.

Methods: Serum cystatin C (Cc.), serum creatinine (Cr.) and corrected creatinine clearance (c.Cr.cl) levels were assessed in 34 children with different types of malignancy just before the start of chemotherapy and again in 33 of them one month later. Patients were compared to 14 healthy controls of matched age and sex.

Results: Before the start of chemotherapy, all patients when compared to controls had normal Cc [Median (IQR) = 0.57 (0.4-0.7) vs 0.4 (0.32-0.66) mg/L, p = 0.1], Cr. [Median (IQR) = 0.7 (0.6-0.8) vs 0.7 (0.57-0.8) mg/dl, p = 0.62], and c.Cr.cl [Median (IQR) = 115.35 (104.4-137.1) vs 115.8 (111.5-130.9) ml/min/1.73m², p = 0.76] respectively. One month after chemotherapy, patients showed a significant increase in their Cc levels (p < 0.001) and a significant decrease in their c.Cr.cl (p < 0.001). On the other hand their serum Cr. levels did not change significantly (p = 0.65). c.Cr.cl negatively correlated with both Cc and Cr (r = -0.622, p < 0.001), (r = -0.346, p = 0.045) respectively before the start of chemotherapy and also after chemotherapy (r = -0.577, p < 0.001) and (r = -0.45, p = 0.009), respectively. When pretreatment levels of Cc and Cr were used to predict patients who developed > 20% reduction in GFR after therapy, only Cc was statistically significant (p = 0.03). A cut off point of 0.57 mg/L with sensitivity of 77.8%, specificity of 63%, and an overall accuracy of 74% was suggested.

Conclusions: Children with malignant diseases develop significant reduction in their GFR during the induction phase of chemotherapy despite the fact that their serum creatinine level may not change significantly. Cystatin C as a more sensitive marker than creatinine in detecting mild reduction in GFR can be used to predict patients who will develop renal impairment during the induction phase of chemotherapy.

INTRODUCTION

It is well known that cytotoxic therapy may lead to altered renal functions(1). Since human kidneys are perfused with approximately 20% of the resting cardiac output, these organs are extensively exposed to systemic xenobiotics(2). Mechanisms of chemotherapy-induced renal dysfunction commonly include damage to vasculature or structures of the kidneys, hemolytic uremic syndrome (HUS) and prerenal perfusion defects(3).

Monitoring of kidney function is essential during the course of chemotherapy because altered renal functions may lead to impaired metabolism and accumulation of
chemotherapeutic agents and their metabolites\(^4\). It is well known that glomerular filtration rate (GFR) is the best overall estimate of renal function. The urinary clearance of an exogenous substance is accepted as the gold standard for estimation of GFR. However, because of the cost and inconvenience, serum creatinine and creatinine clearance are the most widely used measures of renal function\(^5\).

Serum creatinine is of limited value in early detection of renal insufficiency because it is well established that its concentration does not change significantly until inulin clearance is \(< 50 \text{ ml/min}/1.73 \text{ m}\)\(^2\)\(^6\). The measurement of corrected creatinine clearance (c.Cr.cl) involves a timed collection of urine, measurement of its volume, and determination of creatinine concentration in the urine and sera\(^5\).

Human cystatin C is a low molecular weight protein\(^7\). It is produced by all nucleated cells and its production rate is unaltered in inflammatory conditions\(^8\). As a small protein, it passes easily through the glomerular basement membrane and is then catabolized by the renal tubular cells\(^9\). It seems to be eliminated from the circulation almost exclusively by glomerular filtration which makes it a good indicator of GFR\(^10\). It is also age independent in children\(^11,12\). Cystatin C correlates well with GFR both in adults\(^13,14\) and in children\(^12,15\).

**AIM OF THE WORK**

This study was planned to assess the effects of anticancer drugs on GFR and to compare the sensitivity of cystatin C and creatinine in detection of mild reductions in GFR in children receiving chemotherapy.

**SUBJECTS AND METHODS**

This study included 34 children with different malignant disorders (22 males and 12 females) with their ages ranging from 2-14 years [median (IQR) = 5.5 (3.8-10) year]. They were recruited consecutively from the Hematology and Oncology Unit in Mansoura University Children’s Hospital in the period from January 2002 to September 2002. The study included 18 children with acute lymphoblastic leukemia (ALL), (10 males and 8 females), their ages ranged from 2-14 years [median (IQR) = 6 (3.5-10.25) year], 11 children with non Hodgkin lymphoma (NHL), (7 males and 4 females), their ages ranged from 4-13 years [median (IQR) = 5 (4-8) year] and 5 male children with neuroblastoma, their ages ranged from 2-6 years [median (IQR) = 3 (2.5-5.75) year].

ALL patients were diagnosed by bone marrow aspiration. Lymphoma and neuroblastoma patients were diagnosed by biopsy and pathological examination. Correct clinical staging was done by ultrasound, CT and/or MRI studies.

Before the start of therapy, patients having history of previous renal disease, history of recent intake of nephrotoxic drug, or elevated serum creatinine level were excluded from the study. Any patient with elevated serum uric acid level, elevated serum calcium level, elevated hematocrit, or with a radiological evidence suggestive of urinary tract obstruction at the time of sampling was also excluded from the study. Moreover patients with history of significant volume loss (severe gastroenteritis, massive hemorrhage) one week before time of sampling were similarly considered unsuitable to be enrolled in the study.
ALL patients received the induction phase of modified BFM 76/79 protocol (35 days therapy with Vincristine, Daunomycin, L-Asparaginase, Prednisone, intrathecal Methotrexate and intrathecal Ara-C)(16). NHL patients received the induction phase of COMP protocol (34 days therapy with Cyclophosphamide, Vincristine, Prednisone, intrathecal Methotrexate, and intravenous Methotrexate)(17). Neuroblastoma patients received the first cycle of OPEC II protocol (4 days therapy with Vincristine, Cyclophosphamide and Cisplatin)(18).

Sera were obtained from all patients (n = 34) before the start of chemotherapy. Sera were re-obtained from 33 patients after ending the induction phase of BFM 76/79 protocol for ALL patients, induction phase of COMP in NHL and 30 days after the start of therapy in Neuroblastoma patients. One NHL patient died during induction therapy. Serum was frozen and stored at -20°C until the time of assay. Repeated freeze and thaw of the stored serum was avoided. Stored serum was used to assay serum creatinine(19) and cystatin C levels by immunoagglutination reaction using DAKO Cystatin C PET Kit (DAKO A/S in Denmark). DAKO cystatin C PET Kit contains polystyrene particles of uniform size, chemically coupled with rabbit antibody against human cystatin C A reaction between these immunoparticles and cystatin C in a patient specimen results in the formation of agglutinates and a concomitant change in the absorbance signal. The cystatin C concentration of the patient specimen is determined by interpretation on a calibration curve(20). The minimum detectable serum level was 0.2 mg/L.

Twenty four hour urine was accurately collected. Pre- and post-treatment C.Cr.cl. were measured in the studied patients using the following equation:

\[
\text{C.Cr.cl (ml/min/1.73m²) = } \frac{(U \times V/P) \times 1.73/BSA}{1440}
\]

Where: \( U \) (mg/dl) = urinary creatinine concentration, \( V \) (ml/min) = total urine volume (ml) divided by the duration of urine collection (min). In 24 hours = 1440 min., \( P \) (mg/dl) = serum creatinine concentration, and BSA (m²) = body surface area(21). The degree of reduction of c.Cr.cl for each patient was calculated by the following equation:

\[
\text{Degree of reduction in c.Cr.cl } = 1 - \frac{A \text{ c.Cr.cl}}{B \text{ c.Cr.cl}}
\]

Where; \( A \text{ c.Cr.cl} = \text{c.Cr.cl} \text{ in patient after therapy} \) and \( B \text{ c.Cr.cl} = \text{c.Cr.cl} \text{ in patient before therapy} \). The resulting value from the equation was multiplied by constant (100) to be expressed as a percentage. Patients after therapy (n = 33) were reclassified into 2 subgroups according to the degree of reduction in their c.Cr.cl: 9 patients (27.3%) who did not develop reduction in their c.Cr.cl or developed mild reduction in c.Cr.cl up to 20% from pretreatment level, and 24 patients (72.7%) who developed > 20% reduction in their c.Cr.cl after treatment. Serum creatinine, cystatin C, and c.Cr.cl were also measured in 14 healthy controls of matched age and sex.

**Statistical methods:**

The data of the study were analyzed by SPSS (Statistical Package for Social Science) under windows (version 10). Data were found to have nonparametric
distribution by Kolmogorov Smirnov test. Data were expressed as median and interquartile range (IQR). Tests used included Mann-Whitney U test, Wilcoxon signed ranks test, and Spearman correlation test. To compare the sensitivity and specificity of cystatin C and creatinine in detection of mild reduction of post treatment c.Cr.cl, receiver-operator characteristic (ROC) graphics were used. P value < 0.05 was considered significant.

RESULTS

Before therapy, all patients had normal serum creatinine [median (IQR) = 0.7 (0.6-0.8) vs 0.7 (0.57-0.8) mg/dl respectively, p = 0.622], serum cystatin C [median (IQR) = 0.57 (0.4-0.7) vs 0.4 (0.32-0.66) mg/L respectively, p = 0.1], and c.Cr.cl. [median (IQR) = 115.35 (104.4-137.12) vs 115.85 (111.5-130.9) ml/min/1.73m² respectively, p = 0.76] when compared to controls [Table 1]. After therapy patients developed a significant drop in c.Cr.cl. (p < 0.001) and a significant increase in serum cystatin C (p < 0.001). On the other hand a non-significant change was found between pre- and post-treatment serum creatinine levels (p = 0.654) [Table 2].

Before therapy, c.Cr.cl. correlated significantly with both serum cystatin C (r = -0.622, p < 0.001) and serum creatinine (r = -0.346, p = 0.045). Again after treatment c.Cr.cl. was found to be significantly correlated with both serum cystatin C levels (r = -0.577, p < 0.001) and serum creatinine (r = -0.45, p = 0.009) [Table 3].

ROC analysis of pre-treatment serum creatinine and serum cystatin C was done to predict the degree of post-treatment reduction of c.Cr.cl. Only cystatin C was found to be statistically valid (p = 0.036). Basal cystatin C level > cut off point of 0.57 mg/L before therapy predicted patients who developed > 20% reduction in their c.Cr.cl after therapy with 77.8% sensitivity, 63% specificity and an overall accuracy of 74% [Table 4] and (Fig. 1).

Before therapy, ALL patients had normal serum creatinine [median (IQR) = 0.7 (0.6-0.8) vs 0.7 (0.57-0.8) mg/dl respectively, p = 0.357], serum cystatin C [median (IQR) = 0.55 (0.4-0.7) vs 0.4 (0.32-0.66) mg/L respectively, p = 0.338], and c.Cr.cl. [median (IQR) = 110.3 (101.95-129.25) vs 115.85 (111.5-130.9) ml/min/1.73m² respectively, p = 0.37] when compared to controls [Table 5]. After therapy, these patients developed a significant drop in c.Cr.cl. (p < 0.001), a significant increase in serum cystatin C (p < 0.001). On the other hand a non-significant change was found between pre- and post-treatment serum creatinine levels (p = 0.958) [Table 8].

Before therapy, NHL patients had normal serum creatinine [median (IQR) = 0.6 (0.6-0.7) vs 0.7 (0.57-0.8) mg/dl respectively, p = 0.609], serum cystatin C [median (IQR) = 0.65 (0.5-0.7) vs 0.4 (0.32-0.66) mg/L respectively, p = 0.107], and c.Cr.cl. [median (IQR) = 128.4 (114.7-143) vs 115.85 (111.5-130.9) ml/min/1.73m² respectively, p = 0.373] when compared to controls [Table 6]. After therapy, these patients developed a significant drop in c.Cr.cl. (p = 0.005) and a significant increase in serum cystatin C (p = 0.01). On the other hand a non-significant change was found between pre- and post-treatment
serum creatinine levels \( (p = 0.416) \) [Table 8].

Before therapy, neuroblastoma patients had normal serum creatinine [median (IQR) = 0.7 (0.6-0.8) vs 0.7 (0.57-0.8) mg/dl respectively, \( p = 0.687 \)], serum cystatin C [median (IQR) = 0.55 (0.51-0.74) vs 0.4 (0.32-0.66) mg/L respectively, \( p = 0.156 \)], and c.Cr.cl. [median (IQR) = 111 (99-135.5) vs 115.85 (111.5-130.9) ml/min/1.73m\(^2\) respectively, \( p = 0.622 \)] when compared to controls [Table 7]. After therapy, these patients developed a significant drop in c.Cr.cl. \( (p = 0.04) \). On the other hand a non-significant change was found between pre- and post-treatment serum creatinine levels \( (p = 1.00) \) [Table 8]. Although the increase in serum cystatin C in these patients after therapy was not statistically significant \( (p = 0.08) \) [Table 8]; yet the level of cystatin C in these patients after therapy was still significantly higher in comparison to controls \( (p < 0.001) \) [Table 7].

Before therapy, solid tumor (NHL and neuroblastoma) patients had normal serum cystatin C levels when compared to controls [median (IQR) = 0.6 (0.5-0.7) vs 0.4 (0.32-0.66) mg/L respectively, \( p = 0.06 \) [Table 9].

**Table 1: Comparison of the studied parameters between patients and controls\(^*\).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median (IQR)</th>
<th>Patients(^a) (n=34)</th>
<th>Patients(^b) (n=33)</th>
<th>Controls (n=14)</th>
<th>( p_1 )</th>
<th>( p_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.7 (0.6-0.8)</td>
<td>0.7 (0.6-0.8)</td>
<td>0.7 (0.57-0.8)</td>
<td></td>
<td>0.62</td>
<td>0.74</td>
</tr>
<tr>
<td>Cystatin C (mg/L)</td>
<td>0.57 (0.4-0.7)</td>
<td>1.1 (0.89-1.32)</td>
<td>0.4 (0.32-0.66)</td>
<td></td>
<td>0.10</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>c.Cr.cl. (ml/min/1.73m(^2))</td>
<td>115.35 (104.4-137.12)</td>
<td>84.6 (73.9-93.2)</td>
<td>115.85 (111.5-130.9)</td>
<td></td>
<td>0.76</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

\( \text{Patients}\(^a\) = \text{Patients before therapy} \)  
\( \text{Patients}\(^b\) = \text{Patients after therapy} \)  
\( p_1 = \text{Patients}\(^a\) vs controls \)  
\( p_2 = \text{Patients}\(^b\) vs controls \)  
\(^*\) Mann-Whitney test

**Table 2: Comparison of the studied parameters in patients before and after chemotherapy\(^*\).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>+ve Ranks</th>
<th>-ve Ranks</th>
<th>Ties</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before &lt; After</td>
<td>Before &gt; After</td>
<td>Before = After</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>31</td>
<td>0</td>
<td>2</td>
<td>0.654</td>
</tr>
<tr>
<td>Cystatin C (mg/L)</td>
<td>28</td>
<td>3</td>
<td>2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>c.Cr.cl. (ml/min/1.73m(^2))</td>
<td>1</td>
<td>31</td>
<td>1</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

\( \text{Wilcoxon signed ranks test} \)
Table 3: Correlation between corrected creatinine clearance and both creatinine and cystatin C before and after treatment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>c. Cr. cl (ml/min/1.73m²)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients a (n = 34)</td>
<td>Patients b (n = 33)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>Cystatin C (mg/L)</td>
<td>-0.622</td>
<td>&lt; 0.001</td>
<td>-0.577</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>-0.346</td>
<td>0.045</td>
<td>-0.45</td>
</tr>
</tbody>
</table>

Patients a = Patients before therapy
Patients b = Patients after therapy
* Spearman coefficient

Table 4: Validity of cystatin C and creatinine to predict patients with > 20% reduction of c. Cr. cl. after chemotherapy.

<table>
<thead>
<tr>
<th></th>
<th>Cystatin C (mg/L)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cut off point</td>
<td>0.57</td>
<td>0.65</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>77.8%</td>
<td>66.7%</td>
</tr>
<tr>
<td>Specificity</td>
<td>63%</td>
<td>46%</td>
</tr>
<tr>
<td>Overall accuracy</td>
<td>74%</td>
<td>50%</td>
</tr>
<tr>
<td>Significance</td>
<td>0.036</td>
<td>0.98</td>
</tr>
</tbody>
</table>

* ROC curve

Table 5: Comparison of the studied parameters between ALL patients and controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ALL a (n = 18)</th>
<th>ALL b (n = 18)</th>
<th>Controls (n = 14)</th>
<th>p1</th>
<th>p2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.7 (0.6-0.8)</td>
<td>0.7 (0.57-0.8)</td>
<td>0.7 (0.57-0.8)</td>
<td>0.35</td>
<td>0.61</td>
</tr>
<tr>
<td>Cystatin C (mg/L)</td>
<td>0.55 (0.4-0.7)</td>
<td>1.1 (0.94-1.4)</td>
<td>0.4 (0.32-0.66)</td>
<td>0.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>c. Cr. cl. (ml/min/1.73m²)</td>
<td>110.3 (101.95-129.25)</td>
<td>81.75 (73.95-89.6)</td>
<td>115.85 (111.5-130.9)</td>
<td>0.37</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

ALL a = ALL before therapy
ALL b = ALL after therapy
p1 = ALL a vs controls
p2 = ALL b vs controls
* Mann-Whitney test
Table 6: Comparison of the studied parameters between NHL patients and controls*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median (IQR)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NHL&lt;sup&gt;a&lt;/sup&gt; (n = 11)</td>
<td>NHL&lt;sup&gt;b&lt;/sup&gt; (n = 10)</td>
<td>Controls (n = 14)</td>
<td>p&lt;sub&gt;1&lt;/sub&gt;</td>
<td>p&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.6 (0.6-0.7)</td>
<td>0.6 (0.6-0.77)</td>
<td>0.7 (0.57-0.8)</td>
<td>0.609</td>
<td>0.93</td>
</tr>
<tr>
<td>Cystatin C (mg/L)</td>
<td>0.65 (0.5-0.7)</td>
<td>0.95 (0.89-1.1)</td>
<td>0.4 (0.32-0.66)</td>
<td>0.107</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>c.Cr.cl. (ml/min/1.73m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>128.4 (114.7-143)</td>
<td>90.8 (71.5-100.75)</td>
<td>115.85 (111.5-130.9)</td>
<td>0.373</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

NHL<sup>a</sup> = NHL before therapy  
NHL<sup>b</sup> = NHL after therapy  
p<sub>1</sub> = NHL<sup>a</sup> vs controls  
p<sub>2</sub> = NHL<sup>b</sup> vs controls  
* Mann-Whitney test

Table 7: Comparison of the studied parameters between neuroblastoma patients and controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median (IQR)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neuroblastoma&lt;sup&gt;a&lt;/sup&gt; (n = 5)</td>
<td>Neuroblastoma&lt;sup&gt;b&lt;/sup&gt; (n = 5)</td>
<td>Controls (n = 14)</td>
<td>p&lt;sub&gt;1&lt;/sub&gt;</td>
<td>p&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.7 (0.6-0.8)</td>
<td>0.6 (0.55-1.1)</td>
<td>0.7 (0.57-0.8)</td>
<td>0.687</td>
<td>0.893</td>
</tr>
<tr>
<td>Cystatin C (mg/L)</td>
<td>0.55 (0.51-0.74)</td>
<td>1.35 (0.79-1.5)</td>
<td>0.4 (0.32-0.66)</td>
<td>0.156</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>c.Cr.cl. (ml/min/1.73m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>111 (99-135.5)</td>
<td>76 (58-94.4)</td>
<td>115.85 (111.5-130.9)</td>
<td>0.622</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Neuroblastoma<sup>a</sup> = Neurob. before therapy  
Neuroblastoma<sup>b</sup> = Neurob. after therapy  
p<sub>1</sub> = Neuroblastoma<sup>a</sup> vs controls  
p<sub>2</sub> = Neuroblastoma<sup>b</sup> vs controls  
* Mann-Whitney test

Table 8: Comparison of studied parameters in ALL, NHL and neuroblastoma patients before and after treatment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Leukemia (n = 18)</th>
<th>NHL (n = 10)</th>
<th>Neuroblastoma (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.958</td>
<td>0.416</td>
<td>1.00</td>
</tr>
<tr>
<td>Cystatin C (mg/L)</td>
<td>&lt; 0.001</td>
<td>0.016</td>
<td>0.08</td>
</tr>
<tr>
<td>c.Cr.cl. (ml/min/1.73m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>&lt; 0.001</td>
<td>0.005</td>
<td>0.043</td>
</tr>
</tbody>
</table>

* Wilcoxon signed ranks test
Table 9: Comparison of cystatin C between patients with solid tumors [NHL & neuroblastoma] before treatment and controls (mg/L)*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median (IQR)</th>
<th></th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solid tumors (n = 16)</td>
<td>Controls (n = 14)</td>
<td></td>
</tr>
<tr>
<td>Cystatin C</td>
<td>0.6 (0.5-0.7)</td>
<td>0.4 (0.32-0.66)</td>
<td>0.064</td>
</tr>
</tbody>
</table>

* Mann-Whitney test

Fig. 1: ROC curve of the sensitivity and specificity of cystatin C

DISCUSSION

Patients with cancer are frequently at risk for renal dysfunction secondary to disease-related and iatrogenic factors. Renal dysfunction is a part of the pathophysiological process of certain malignant diseases, such as multiple myeloma and renal cell cancer. Patients with advanced cancer are at risk for disease-related postrenal obstructive uropathy. In addition, hypercalcemia, which is a consequence of some advanced tumors, can precipitate renal dysfunction. Tumor lysis syndrome, resulting from chemotherapy-induced cytolysis of certain malignancies with a high tumor burden, is also an important cause of renal failure during therapy. Moreover, the incidence of occurrence of sepsis-related hypotension
or the need for nephrotoxic antimicrobial therapy promotes renal impairment in these patients. Furthermore, renal failure may be a component of disease- or regimen-related hepatorenal failure or multiorgan failure\(^{(22)}\).

Although a variety of previous studies has focused on the renal effects of chemotherapy, most of these studies have focused on the long term nephrotoxic effects of chemotherapy\(^{(23)}\). In the current study, all patients had normal basal serum creatinine, cystatin C, and \(cCr.cl.\) when compared to controls. When these patients were reevaluated after receiving chemotherapy, they showed a significant drop in their \(cCr.cl.\) and a significant increase in their serum cystatin C levels. On the other hand a non-significant change was found between pre and post treatment serum creatinine levels. Plasma creatinine is a poor indicator of glomerular function, since it is an insensitive measure of early glomerular impairment and is dependent on non renal factors, especially the rate of creatinine production, which itself is dependent on muscle mass\(^{(24)}\). Decreased muscle mass secondary to disease or treatment related effects reduce creatinine production with a subsequent reduction in serum creatinine level. Reasons for low muscle mass in the patient with cancer include decreased nutritional intake, cachexia, decreased physical activity, and corticosteroid-induced myopathy\(^{(22)}\). The current work results are in agreement with previous studies reporting a poor value of creatinine in detection of chemotherapy related renal dysfunction\(^{(24,25)}\).

In the studied patients, prerenal causes of renal impairment were excluded, all patients were normotensive, none of them had gastroenteritis one week before sampling, and no increase in the hematocrit values were found (no hemococoncentration). Serum uric acid levels and serum calcium levels were also normal at the time of sampling. None of the patients had clinical manifestations suggestive of sepsis. Obstruction of the urinary tract was excluded in all patients by performing renal ultrasonography at the time of sampling. None of the patients received aminoglycoside antibiotics. As we excluded other possible causes of renal impairment, elevated cystatin C levels and decreased \(cCr.cl.\) in these patients are mostly due to the nephrotoxic effects of the cytotoxic therapy used. Our explanation is supported by the fact that a variety of the commonly used anticancer drugs have nephrotoxic effects. Reductions in GFR following treatment with cisplatin is common in adults\(^{(24)}\) and children\(^{(25)}\). Hematuria\(^{(26)}\) and acute renal failure\(^{(27)}\) are reported with methotrexate administration. Although these effects occur primarily with high dose methotrexate therapy; however, they can also occur with long term administration of conventional dose methotrexate\(^{(26)}\). Reduction of GFR in chronic Adriamycin nephrosis is due to glomerular sclerosis and tubulointerstitial injury\(^{(28-31)}\). Renal dysfunction secondary to haemolytic uremic syndrome (HUS) occurs in up to 10% of patients receiving mitomycin\(^{(32-34)}\). Signs of ifosfamide induced nephrotoxicity include increased serum creatinine and blood urea nitrogen levels, oliguria and proximal tubular wasting of electrolytes, glucose and amino acids\(^{(35)}\).

Cystatin C belongs to the family of
cysteine proteases\(^{36}\). In malignancy, an imbalance between cysteine proteases and their inhibitors is thought to facilitate tumor cell invasion and metastasis, thus, the extent of malignancy was thought to influence cystatin C concentration\(^{37}\). In the present study, we did not find a significant difference in the serum levels of cystatin C in the studied patients before start of therapy and controls. In the studied ALL patients, only one patient had high tumor burden in the form of hyperleukocytosis. In the solid tumor group, all the studied patients had advanced stages (III or IV) and so we compared their cystatin C levels before therapy with that of the controls and again we did not find significant difference, which means that serum cystatin C level is not related to the extent or to the stage of the malignant disease. Similar reports have been published and reported that serum cystatin C concentration in cancer patients receiving chemotherapy was not influenced by any variable other than GFR\(^{20,38}\). The structure of cystatin C gene and its promoter is of the “housekeeping type” which is compatible with stable production of cystatin C. It is mostly present in extracellular fluid, and cell damage caused by chemotherapy is not likely to influence its serum concentration\(^{36}\).

Although c.Cr.cl. correlated negatively with both serum creatinine and cystatin C levels before and after therapy, the correlation was more significant with cystatin C than with creatinine. Similar results have been reported in adult cancer patients\(^{38}\). The reported ratio of c.Cr.cl. to GFR estimated by inulin clearance increases with decreasing GFR to 1.7 at a GFR of 20 ml/min\(^{39,40}\), however, When GFR is moderately impaired or normal and urine collection errors were reduced, c.Cr.cl. was nearly equal to GFR, with a ratio of c.Cr.cl. to GFR of 1.15\(^{41,42}\). In the present work, the majority of patients had mild to moderate renal impairment, all patients were hospitalized, and urine collections were carefully monitored and done by trained persons; thus we can assure that measured c.Cr.cl. in these patients reflects the actual GFR of these patients to a large degree.

Biochemical evaluation of urine and serum concentration of urea, creatinine and uric acid are the commonly used parameters in assessment of renal function in children before the start of chemotherapy\(^{43}\). If these parameters were monitored during treatment with chemotherapy, acute impairment of kidney function can be diagnosed in time. However, it must be remembered that these parameters has certain fallacies in detecting mild and early reduction in renal function. A question therefore appears of whether in the initial phases of cytotoxic therapy another biochemical parameter should be monitored, which would signalize “finer” changes in the glomerular functions\(^{44}\). We performed ROC analysis to compare the sensitivity of basal serum cystatin C and creatinine levels in the studied children to predict the subsequent degree of reduction in c.Cr.cl. after receiving therapy. Our results revealed that only cystatin C was statistically valid to be used for prediction. Patients with basal serum cystatin C levels above 0.57 mg/L, developed reduction in their c.Cr.cl. of > 20% of pretreatment levels. This cut off point had 77.8%
sensitivity, 63% specificity, and over all accuracy of 74%.

In order to rule out the effect of the original disease on the results of this study, we reanalyzed the studied parameters in our patients after dividing them into 3 categories based on their diagnoses, ALL, NHL, and neuroblastoma patients. Statistical evaluation of the studied parameters in the 3 groups before and after therapy and in comparison to controls showed similar results to that of the whole number of patients. The only difference was found in neuroblastoma patients where cystatin C levels in this group did not change significantly after therapy. This could be easily explained if we take into consideration that this group had the smallest number of patients (n = 5). Among these 5 patients, 4 showed an increase in their cystatin C levels after therapy and only one patient did not have such an increase; we believe that if the number of this group was larger, the statistical difference would have become more evident. This suggestion is further confirmed by the fact that cystatin C levels in neuroblastoma patients after therapy was significantly higher than controls.

We can conclude that children with malignant diseases develop significant reduction in their GFR during the induction phase of chemotherapy despite the fact that their serum creatinine level may not change significantly. Cystatin C as a more sensitive marker than creatinine in detecting mild reduction in GFR can be used to predict patients who may develop significant renal impairment and thus may need more frequent assessment of their renal function during the induction phase of chemotherapy.

REFERENCES


