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Utility of Cystatin C to monitor renal function in Duchenne muscular dystrophy

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Abstract

Introduction: Creatinine as a marker of renal function has limited value in Duchenne muscular dystrophy (DMD) because of reduced muscle mass. Alternative methods of assessing renal function are sorely needed. Cystatin C, a nonglycosylated protein unaffected by muscle mass, is potentially an ideal biomarker of nephrotoxicity for this population but requires validation.

Methods: 75 subjects were recruited: 35 DMD (mean age 10.8 ± 5.4 years, corticosteroids n = 19, ambulatory n = 26), 29 healthy controls, 10 with renal disease, and one DMD with renal failure.

Results: Cystatin C levels in DMD were normal irrespective of age, ambulation or corticosteroid treatment. Serum cystatin C was 0.67 ± 0.11 mg/L compared to normal controls 0.69 ± 0.09. mg/L. In these same individuals serum creatinine was severely reduced (0.27 ± 0.12 mg/dL) versus normals (0.75 ± 0.15 mg/dL, $p < 0.01$). In one DMD subject in renal failure, cystatin C was elevated.

Discussion: This study demonstrates the potential value of cystatin C as a biomarker for monitoring renal function in DMD. Its applicability extends to other neuromuscular diseases.

Keywords

Duchenne muscular dystrophy; Cystatin C; serum creatinine; biomarker; renal function

Introduction

The glomerular filtration rate (GFR) is a universally recognized marker for kidney failure[1, 2]. For clinical studies, creatinine clearance, based on either serum or plasma creatinine, is accepted as a useful estimate of GFR (eGFR). Normal values are related to age, gender, race, weight and height of the subject[3–5]. For muscle disease patients, the use of creatinine to monitor eGFR has limitations because of its origin from skeletal muscle. Creatinine is a biosynthetic product of creatine phosphate, a molecule of key importance for energy production in muscle. Creatine is converted to creatinine and transported from muscle through the circulation to the kidneys. In patients with muscular dystrophy, reduced muscle mass lowers serum and plasma creatinine levels creating difficulties for interpretation of

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eGFR6,7. For wheelchair-dependent muscular dystrophy patients, the problem is compounded when calculations require both 24-hour urine and height measurements. In this population, incomplete urine collections are frequent, and heights are inaccurate because of limb contractures and scoliosis. Alternative approaches for assessing GFR using inulin8, radioisotopes9 and iohexol can be used for the DMD population, but such methods introduce added expense and inconvenience, delay in turnaround time for results, and possible allergic reactions from reagents used for testing. Our recent clinical trial testing aminoglycosides in DMD to promote mutation suppression of premature termination codons, based on pre-clinical data10, brought into focus the need for an accurate and validated method for continuous monitoring of GFR.

Cystatin C, a known cysteine protease inhibitor, offers the potential for use as a surrogate measure for GFR in adults and children11,12. It is a non-glycosylated, 13.3 kDa basic protein which is produced by all nucleated cells, and it is not affected by circadian rhythm, gender, or lean tissue mass13,14. We designed this study to explore cystatin C levels for use in clinical studies for DMD patients, especially those in need of ongoing monitoring of pharmacologic agents with possible nephrotoxicity. We compared serum creatinine, which is known to be reduced by loss of muscle mass7, with cystatin C levels in DMD subjects. Results were further compared with normal children and young adults and in others with proven renal failure. We purposely included DMD patients on prednisone and deflazacort, because there is controversy regarding the influence of corticosteroids on serum cystatin C levels15-17. Corticosteroids are ubiquitously used to treat DMD, and any current or future clinical trials will have patients on these drugs18,19,20. The findings in this study will be of value throughout the neuromuscular community including other forms of muscular dystrophy, spinal muscular atrophy, and acquired muscle disease with reduced muscle mass.

Materials and Methods

Patient population

Thirty-five DMD study subjects, age 5 and older, with proven mutations of the dystrophin gene, were enrolled from the Muscular Dystrophy Clinics at Nationwide Children's Hospital (NCH) in Columbus, Ohio, the University of Utah and the Cleveland Clinic. Ambulatory status and corticosteroid regimen at the time of the blood draw were recorded. DMD subjects had no prior history of renal disease, no known concomitant disease, and were not participating in any experimental treatment. Twenty-nine normal control subjects were recruited for the study. None had a family history of muscular dystrophy. Cystatin C comparisons were extended to Renal Failure Clinic patients who had elevated serum creatinine levels because of abnormal kidney function of diverse etiology.

The study was begun after approval of the institutional review boards at all participating sites. Consent forms were signed by subjects ≥18 years of age, or parent or legal representatives of participants ≤ 17 years old. An assent form was signed by participants 9 to 17 years of age.

Protocol

All subjects participating in this study were enrolled in an outpatient setting and were actively engaged in their usual activities of daily living. Blood for creatinine and cystatin C was collected in distinct serum-separator tubes. Cystatin C was measured at ARUP Laboratories (Salt Lake City, UT), using an automated and rapid particle-enhanced nephelometric immunoassay (PENIA) as described previously21. Serum creatinine was measured at Nationwide Children's Hospital using the VITROS ® MicroSlide™ method according to the manufacturer's specifications (Ortho-Clinical Diagnostics, Inc).

Statistics

Statistical analyses were based on differences between the groups using a paired t test comparing cystatin C in DMD and normals, between DMD subjects on corticosteroids or untreated, and ambulatory versus wheelchair-dependent.

Results

To ensure relevance to a broad range of DMD patients, we included ambulatory (n = 26) and non-ambulatory (n = 9) subjects ranging in age from 5 to 24 years of age (mean 10.8 ± 5.4 years), and subjects could be taking either prednisone (n = 11) or deflazacort (n = 8). Tables 1 and 2 show comparative data between groups. The serum creatinine levels in DMD were significantly different compared to a normal population of boys of the same age (0.27 ± 0.12 mg/dL versus 0.75 ± 0.15 mg/dL, $p < 0.01$), indicative of reduced muscle mass. The value of serum cystatin C levels as a biomarker that ignores muscle mass is illustrated by the virtually identical levels in DMD and normal volunteers (DMD 0.67 ± 0.11mg/L versus normal 0.69 ± 0.09 mg/L). Serum creatinine is further reduced in wheelchair-dependent DMD subjects (0.23 ± 0.07 mg/dL), while serum cystatin C remains unchanged from ambulatory DMD patients (0.67 ± 0.11 mg/L). Corticosteroids did not alter serum cystatin C in subjects who were taking this drug (DMD corticosteroids 0.69 ± 0.11 mg/L versus non-corticosteroids 0.63 ± 0.10 mg/L, $p = 0.09$).

The relationship of serum cystatin C and creatinine levels in renal failure patients (n = 10; age 14.3 ± 5.1 years old) proved useful (cystatin C = 4.0 ± 3.5 mg/L versus creatinine 5.38 ± 4.97 mg/dL), especially in considering compromised renal function in DMD. Severe renal insufficiency was observed in one patient with terminal congestive heart failure (age 15.6 years; serum creatinine = 2.4 mg/dL and cystatin C = 3.4 mg/L). Extrapolating from the renal failure cohort, a cystatin C level of this magnitude would be associated with a serum creatinine two-fold higher than observed in the DMD patient.

Discussion

Experimental treatment protocols are evolving for DMD and other inherited (e.g., spinal muscular atrophy) and acquired (amyotrophic lateral sclerosis) neuromuscular disorders that may require frequent monitoring for renal toxicity. In DMD, examples include agents designed to suppress premature termination codons or skip exons, or repair or replace mutant genes. These studies must follow FDA safety guidelines that mandate vigilant monitoring of all organ systems. In DMD and other neuromuscular disorders where muscle mass is reduced, standard clinical chemistry measures of serum or plasma creatinine do not accurately reflect alterations in renal function. Attempts to use alternative methods of study, like iohexol infusions, require four to six hour outpatient visits and extended wait times for results that can interrupt dosing schedules. The need for a more practical approach is essential for clinical trials. Cystatin C has been demonstrated to be of value for monitoring renal failure in a variety of conditions[12,22,23]. One prior study of cystatin C in DMD has been published, but the information is difficult to access (printed in Japanese without an abstract or translation)[24].

Serum cystatin C levels are particularly applicable to DMD for clinical trials for several reasons. This cysteine protease inhibitor demonstrates a steady state in the circulation, so that it can be tested any time of day. Levels initially decline in infancy but stabilize and reach a plateau after 1 to 1.5 years of age [13,25-29]. This age factor will have minimal impact for most DMD clinical trials. Cystatin C levels in children are also independent of gender and height[12,26,28,30]. Prior studies have suggested that body composition does not

influence cystatin C,13،14،29 but no study has systematically examined this issue in the muscular dystrophy population.

In certain circumstances, screening for endocrinopathies may be appropriate. Ketonuria in diabetes and hyperthyroidism reduce cystatin C levels, and hypothyroidism raises cystatin C levels31،32. A particular advantage in the DMD population is that neither oral prednisone nor deflazacort influenced cystatin C in our DMD patient population or in treatment of the nephrotic syndrome16

For immune-mediated neuromuscular disorders, cystatin C appears to be a useful monitoring tool for renal complications based on findings that cyclosporine, tacrolimus and mycophenolate mofetil do not affect the levels33. Use of cystatin C to monitor renal function during the treatment of malignancies, however, can result in falsely elevated levels. This includes drugs such as methotrexate and cyclophosphamide, because rapid breakdown of cells can release cystatin C into the serum34.

In summary, the findings in this study support the use of cystatin C as a biomarker for monitoring renal function in DMD. Cystatin C levels in DMD patients and controls were not different despite significant differences in serum creatinine levels. Hydration, a particular problem in the non-ambulatory wheelchair-dependent patient, did not influence levels of cystatin C35. Corticosteroid regimens, commonly used to treat DMD were also free of influence on serum cystatin C levels. Patients with DMD and renal failure are rare, and we had the opportunity to perform cystatin C levels on a single case example in whom we found the levels to be very high. This case demonstrates proof of principle that cystatin C is elevated in overt renal failure (in fact this patient died within 48 hours of blood draw). On the other hand, in the setting of future clinical trials, additional data will be required to establish the range of cystatin C that accompanies early compromise of renal function when serum creatinine levels for the DMD patient reach or just exceed the upper limits of “normal”. There is clearly a sense of urgency to identify novel agents for treatment of DMD, and the findings in this report will have applicability for clinical trials where monitoring renal disease is appropriate.

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Abbreviations

DMD	Duchenne muscular dystrophy
eGFR	estimated glomerular filtration rate

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Table 1

Serum creatinine and cystatin C levels in Duchenne muscular dystrophy (DMD) patients, normal control population and patients with renal disease

Cohort	Age (years)	Serum creatinine (mg/dL)	Cystatin C (mg/L)
DMD (n=35)	10.8 ± 5.4	0.27 ± 0.12	0.67 ± 0.11
DMD with renal failure patient (n=1)	15.6	2.41	3.4
Normal population (n=29)	12.8 ± 3.4	0.75 ± 0.15	0.69 ± 0.09
Renal disease patients (n=10)	14.3 ± 5.1	5.38 ± 4.97	4 ± 3.45

Serum creatinine (DMD vs Normal, $p < 0.01$); cystatin C (DMD vs Normal, p = ns)

Table 2

Comparison of serum creatinine and cystatin C levels in DMD patients

Cohort	Serum creatinine (mg/dL)	Cystatin C (mg/L)
DMD patients on corticosteroids (n=19)	0.31 ± 0.12	0.69 ± 0.11
DMD patients untreated (n=16)	0.22 ± 0.09	0.63 ± 0.10
Ambulatory DMD patients (n=26)	0.29 ± 0.13	0.67 ± 0.11
Wheelchair DMD patients (n=9)	0.23 ± 0.07	0.68 ± 0.14

Serum creatinine (DMD corticosteroids vs untreated, $p < 0.02$; Ambulatory DMD vs Wheelchair DMD. p = ns); Cystatin C (DMD corticosteroids vs DMD untreated, p = ns; Ambulatory DMD vs wheelchair DMD, p = ns)