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Renal function in children and adolescents with Duchenne muscular dystrophy

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Abstract

Improved life expectancy and the need for robust tools to monitor renal safety of emerging new therapies have fueled the interest in renal function in Duchenne muscular dystrophy (DMD) patients. We aimed to establish a methodology to accurately assess their renal function. Twenty DMD patients (5–22 years) were included in this prospective study. After obtaining medical history, all patients underwent a clinical examination, 24-hour ambulatory blood pressure monitoring, ultrasound of the kidneys, direct GFR measurement (^{51}Cr-EDTA, mGFR), complete blood and urine analysis. Seventeen of 20 patients were treated with corticosteroids and 5/20 with angiotensin converting enzyme inhibitor (lisinopril). No patient suffered from urinary tract infections or other renal diseases. Hypertension (systolic or diastolic blood pressure >P95) was found in 9/20 patients (8/9 patients were on steroid treatment) and a non-dipping blood pressure profile in 13/20 subjects (10/13 patients were on steroid treatment). Urinary protein to creatinine ratio was elevated in 17/18 patients, whereas 24-hour urine protein excretion was normal in all subjects. Median interquartile range (IQR) mGFR was 130.4 (29.1) mL/min/1.73 m^2. Hyperfiltration (mGFR >150 mL/min/1.73 m^2) was found in 5/20 patients. Inverse correlation between mGFR and age was observed ($R^2 = 0.45$, $p = 0.001$). Serum creatinine based estimated GFR (eGFR) equations overestimated mGFR up to 300%. eGFR based on cystatin C Filler equation was closest to the mGFR (median eGFR (IQR) of 129.5 (39.7) mL/min/1.73 m^2). Our study demonstrates a high prevalence of hyperfiltration and hypertension in children and adolescents with DMD. Because the majority of hypertensive patients were under corticosteroid treatment, the iatrogenic cause of hypertension cannot be excluded. Serum or urine creatinine measurements are of no value to evaluate renal function in DMD patients due to the reduced skeletal muscle mass.

Keywords: Duchenne muscular dystrophy; Kidney function; GFR; Hyperfiltration; Blood pressure

1. Introduction

Duchenne muscular dystrophy (DMD, MIM 310200) is an X-linked recessive muscle disorder affecting around 1 in 3500 to 6000 newborn boys [1]. An absent or reduced expression of the dystrophin protein, caused by mutations (mainly deletions) in the dystrophin gene, results in progressive muscle degeneration [2]. Without treatment patients rarely survive beyond their teens as the disease also causes cardiorespiratory failure. In recent decades, life expectancy of patients with DMD has increased, due to improved respiratory, cardiac and orthopedic treatment and potentially also due to long-term corticosteroid treatment [3–6]. Emerging therapies, targeting dystrophin restoration, muscle growth or pathophysiological events downstream from the dystrophin deficiency have moved into clinical development. Among these, RNA modulating approaches such as antisense mediated exon skipping and nonsense codon suppression aim to restore the production of partially functional or full length dystrophin protein [7]. A recent phase 1-2a study of local intramuscular administration of the antisense oligonucleotide PRO051 showed a modest improvement in the 6-minute walk test after 12 weeks of extended treatment, but variable proteinuria was an adverse event in all patients [8]. However, in general, little is known about renal function in DMD patients. A Japanese cause-of-death analysis reported death caused by renal failure in 14% of

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286 DMD patients [9]. The same group demonstrated increased plasma levels of cystatin C (CysC) in more than 30% of these patients over the age of 30 [10].

Improved life expectancy and the need for robust tools to monitor renal safety of emerging new therapies have fueled the interest to evaluate renal function in DMD patients. Methodologically, estimating glomerular filtration rate (GFR) and urinary excretion of proteins and other electrolytes in this patient population are seriously hampered by the uselessness of creatinine, due to reduced skeletal muscle mass. Therefore, we evaluated other accurate and validated creatinine-independent methods for monitoring GFR and urinary excretions. In this regard, CysC, which is independent of muscle mass and hydration, was suggested as a valuable alternative for calculating estimated GFR (eGFR) in these patients [11,12].

In this study we aimed to establish a methodology for studying renal function in patients with DMD and, using this methodology, to describe their renal function in detail.

2. Patients and methods

2.1. Patients: medical history and clinical examination

Twenty DMD patients, aged between 5 and 22 year, with proven mutations in the dystrophin gene attending the neuromuscular reference center at the University Hospitals Leuven, Belgium, were enrolled in this cross-sectional prospective study. Medical history, personal or family history of urinary tract infections or other renal diseases, cardiac shortening fraction, corticosteroid regimen, treatment with angiotensin converting enzyme inhibitors (ACEi), age of diagnosis and ambulatory status were recorded. During a general clinical examination, length, weight and body mass index (BMI) were determined. Twelve non-ambulatory patients were weighed with a hoist and their height was predicted from their ulnar length, measured with a Harpenden anthropometer in a sitting position, based on the formula of Gauld et al. [13].

2.2. Blood and urine analyses

The blood tests included complete blood count, electrolytes, total serum protein, creatine kinase, 1,25-dihydroxy vitamin D, serum creatinine (Scr), urea and CysC. The enzymatic assay of Roche was used to determine Scr. CysC was measured using nephelometry (BN II Nephelometer). A 24-hour urine specimen was used to measure total protein, alfa-1 microglobulin, creatinine, electrolytes and glucose.

2.3. Ambulatory blood pressure monitoring (ABPM)

An oscillometric device (Mobil-O-Graph NG) was attached to the non-dominant arm with an appropriate cuff. Measurements were performed every 15 minutes during daytime and every 30 minutes during the night and lasted for 24 hours. Except for one participant, treatment with ACEi lisinopril was temporarily stopped 7 days before ABPM. We used the reference values of Wühl et al. [14] defining hypertension as a blood pressure (BP) >95th percentile. We defined a non-dipping BP profile as a nocturnal decrease <10% of daytime BP [15].

2.4. Renal ultrasound

Ultrasound of the urinary bladder and kidneys was performed using a Philips iU22 ultrasound system. Bipolar diameters were measured and compared with the reference values according to age [16] and length [17]. We defined nephromegaly as a kidney length > mean + 2 standard deviations for length. We also considered kidney length according to reference values for age.

2.5. Measured GFR (mGFR)

After administering a single bolus injection of ^{51}Cr-EDTA, eight blood samples were taken at 15, 30, 45, 60, 120, 180, 240 and 300 minutes after injection. Data were bi-exponentially fitted followed by correction for body surface area (BSA), according to the formula of Du Bois and Du Bois [18]. Hyperfiltration was defined as mGFR >150 mL/min/1.73 m^2. This value is based on reference values of Pottel et al. [19]: a median GFR of 107.3 mL/min/1.73 m^2 with a standard deviation of 21.5 mL/min/1.73 m^2 leads to a 97.5th percentile of 150 mL/min/1.73 m^2.

2.6. Estimated GFR (eGFR)

Estimated GFR values were calculated using four Scr-based equations (Schwartz [20], Flanders Metadata [19], simple height-independent [21], Q(height) [22]), three CysC-based equations (Larsson [23], Filler [24], Zappitelli [25]) and two Scr/CysC-based equations (Zappitelli [25], Bouvet [26]). Only formulas based on enzymatic Scr and/or nephelometry were included. The different formulas are shown in Table 1.

2.7. Statistics

SPSS 20 and GraphPad Prism 6.0 (La Jolla, CA, USA) were used for statistical analyses. The median and interquartile range (IQR) were calculated for all the variables. Associations between categorical variables were evaluated using the Pearson Chi-Square test. For continuous variables, Spearman's rank test was used. P-values are considered significant at the 5% significance level but should be considered as explorative, therefore there is no correction for multiple testing. There was no a-priori power analysis (or sample size calculation) as there was no pre-set hypothesis for this study. Consequently, recruitment of patients in the DMD population for this invasive direct mGFR measurement was limited to 20 for ethical reasons.

2.8. Ethical approval

The study was approved by the Institutional Ethical Board of UZ Leuven. Consent forms were signed by parents of participants ≤17 years of age or subjects ≥18 years old. Assent forms were signed by participants 5–17 years old.

3. Results

The clinical characteristics of the patients are presented in Table 2.The median age was 15.5 years. Sixteen of the 20 patients were treated with daily corticosteroids (deflazacort or