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Role of biomarkers of inflammation and MRI technique for the early detection of cystinosis-associated myopathy

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Abstract

Background Cystinosis is an autosomal recessive lysosomal storage disorder caused by cystine crystals accumulation within lysosomes resulting in multi-organ dysfunction. Infantile nephropathic cystinosis is the most common phenotype of the disease. Cystinosis distal myopathy was first described in 1994 with a 24% prevalence in cystinosis adult patients.

Methods We prospectively evaluated the clinical, biochemical, and radiological data of 22 nephropathic cystinosis pediatric patients from 19 unrelated families (17 males and 5 females, their ages 105.7 ± 41.5 months) recruited at the cystinosis clinic at Cairo University Children's Hospital. Biochemical assessment included several inflammatory biomarkers, such as CRP, ESR, chitotriosidase, and galectin-3. We further performed conventional MRI for the muscles of both upper and lower limbs for potential detection of early myopathic involvement. We compared these findings with 44 CKD children as pathological controls and 22 healthy pediatric controls.

Results Clinically, no evidence of neuromuscular involvement was detected in our cystinosis pediatric cohort. Chitotriosidase was elevated significantly in cystinosis patients compared to CKD controls. Regarding MRI, morphologically, there were no significant differences between the muscles of cystinosis patients and healthy controls.

Conclusion A significantly higher chitotriosidase activity in cystinosis patients seems to better represent the overall disease burden and cannot be linked to neuromuscular involvement. MRI findings in muscles of the cystinosis cohort are not striking and indicate no early changes at a younger age. Follow-up and further studies for higher age groups are required to accurately elucidate the MRI's role in assessing cystinosis myopathy.

Keywords Nephropathic cystinosis, Cysteamine, Myopathy, Inflammatory markers, Chitotriosidase, Galectin-3, MRI muscles

Background

Cystinosis is the most common cause of hereditary renal Fanconi syndrome in children. It is a rare lysosomal storage disease with an autosomal recessive inheritance that results from pathogenic variants affecting the *CTNS* gene encoding (cystinosin), the lysosomal cystine transporter, leads to intra-lysosomal cystine accumulation and crystallization within all body cells and organs with subsequent organ damage [1].

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Cystinosis is a monogenic disease; however, three clinical phenotypes are present based on age of onset and renal involvement. Infantile nephropathic phenotype is the most severe and most frequent represents (95%) of the disease [2]. Being a systemic disease, cystine crystals, the pathologic landmark, accumulate in all body cells and result in major extra-renal affection including retinal, endocrinal, and neuromuscular complications [3].

Examination of corneal cystine crystals is a specific and simple test for diagnosis. However, leucocyte cystine assay is essential for children less than 20 months of age with free ophthalmological examination [4]. Genetic detection of the *CTNS* gene is a well-established method that detects over 95% of variants causing the disease [5].

Management of cystinosis includes treating renal Fanconi symptoms and extra-renal complications and administering cysteamine, a cystine-depleting agent [6].

Cystinosis myopathy is classified as distal vacuolar myopathy. It was first described in 1988 affecting adult nephropathic cystinosis patients causing progressive distal muscle wasting and weakness resembling neurogenic atrophy [7]. Weakness and wasting commonly involve the hand muscles. Distal muscles of the lower limbs and proximal muscles are further involved as the disease progresses [8].

Cystinosis neuromuscular spectrum was expanded to include early proximal myopathy with symptomatic motor and sensory axonal neuropathy. Furthermore, asymptomatic neuromuscular involvement may develop early in the pediatric age group. Neurophysiological studies are needed for early detection of such complications before the overt clinical manifestations. Prompt diagnosis with early initiation of cysteamine-specific therapy with adequate doses may delay the occurrence of neuromuscular involvement [9].

Inflammation mediated by macrophage activation plays an important role in the cystinosis pathogenesis and macrophage activation biomarkers have a potential role for the therapeutic monitoring of cystinosis [10]. Chitotriosidase enzyme from activated macrophages is a human chitinase. This enzyme is elevated in several lysosomal storage disorders and is currently established as a promising biomarker for clinical and therapeutic monitoring in cystinosis patients [11, 12]. Another important biomarker is galectin-3, which interacts with the pro-inflammatory cytokine MCP-1 stimulating macrophages and is significantly elevated in cystinosis patients [13].

MRI is one of the best imaging techniques for soft tissue abnormalities. Conventional MRI allows morphological evaluation of muscle through qualitative analysis regarding the presence of volume or signal intensity changes. MRI allows accurate phenotypic characterization of muscle affection through detection of the degree of muscle compromise during early stages and follow-up [14].

Herein, we aim to describe the characteristics of the neuromuscular involvement in Egyptian children with nephropathic cystinosis. We further tried to identify its predisposing factors through proper clinical assessment, biochemical evaluation of important inflammatory biomarkers, and MRI evaluation of upper and lower limb muscles.

Methods

Patients

We prospectively evaluate the clinical, biochemical, and radiological data for 88 children, in outpatient clinics of the Department of Pediatrics, Cairo University Children's Hospital, classified into 3 groups: group (A) included 22 patients with clinical, biochemically, and/or genetically proved cystinosis; group (B) 44 pathological control patients previously known chronic kidney disease (CKD) other than cystinosis, matching the age and renal stage of cystinosis patients; and finally group (C) 22 healthy pediatric volunteers with matching age. The study design and inclusion of patients are illustrated in a flow chart (Fig. 1).

The study has been performed in accordance with the Helsinki Declaration Ethics Code, 1964 for experiments including humans with its later amendments and was approved by the research ethics committee at the Faculty of Medicine, Cairo University (Approval code #MD-88–2020). Informed written consents from guardians of all participants were obtained after elucidating the aim of the work and methodology.

Demographic and clinical data of cystinosis and other CKD children were obtained from patients' follow-up medical records. CKD staging in the study population was classified according to eGFR values calculated by the modified Schwartz formula [15].

Neuromuscular manifestations with any subsequent impairment of function were reported. A full neuromuscular evaluation was performed for all patients, including assessments of muscle tone and power using the Medical Research Council (MRC) muscle power scale, deep reflexes of both upper and lower limbs, proximal and distal reflexes using the National Institute of Neurological Disorders and Stroke (NINDS) scale, as well as sensory examination, coordination, and gait assessment. Other possible complications that may result from neuromuscular affection were also reported, such as swallowing dysfunction and restrictive lung disease.

Biochemical assay

Biochemical evaluation (for participants of group A&B) includes kidney function tests, electrolytes, and inflammatory biomarkers: CRP, ESR, chitotriosidase enzyme

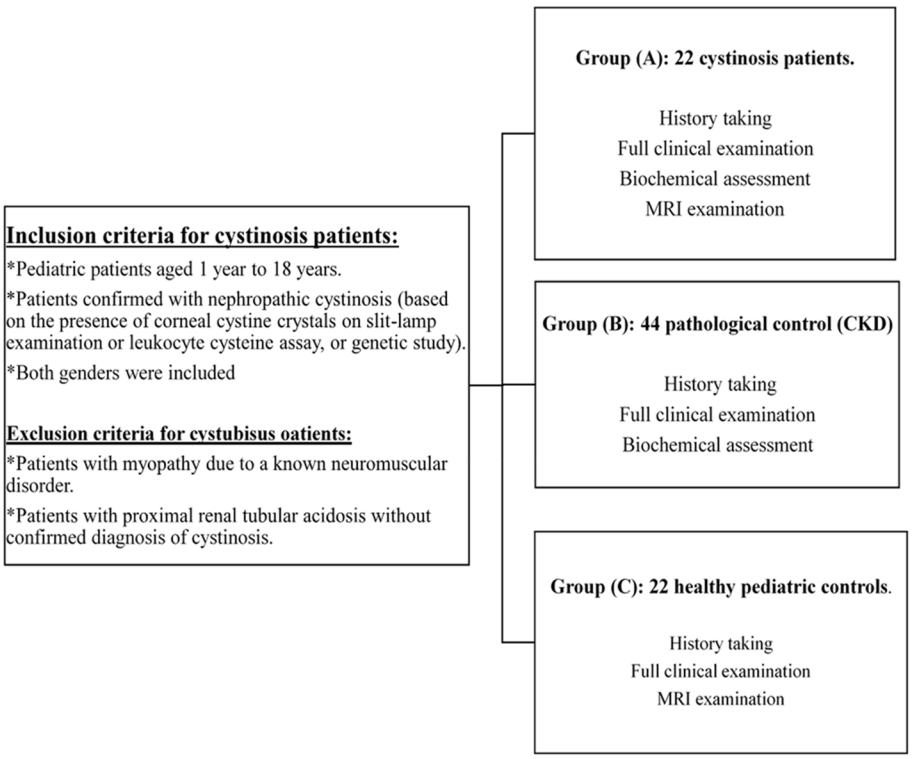


Fig. 1 Flow chart of study design and inclusion of patients

activity, and human galectin-3 (GAL-3). The activity of plasma chitotriosidase enzyme was checked through the fluorogenic substrate 4-methylumbelliferyl-β-DN,N′,N″-triacetylchitotrioside (4-MU-C3) as previously described [16] In short, ten microliter of plasma was mixed with 100 µL of 0.022 mmol/L 4-MU-C3 (Sigma) in Citrate/Phosphate buffer, pH 5.2, 0.1/0.2 mol/L and incubated at 37 °C for 15 min. The reaction was stopped using 2 mL of 0.5 mol/L Carbonate/Bicarbonate buffer, pH 10.7. Quantification of the resulting fluorescence using a calibration curve for the substrate product 4-methylumbelliferone on the spectrofluorometer (FP6200, Jasco, Tokyo, Japan). Results were reported in nmol/ml plasma/h. Galectin-3 in serum was assayed by a commercial ELISA kit (ELK2790, ELK, Wuhan, China) according to the manufacturer's protocol.

MRI of upper and lower limb muscles

MRI radiological evaluation was conducted for participants of groups A & C. MR imaging of shoulders, arms, hands, thighs, and calf muscles was performed. MR studies were performed without IV contrast injection. Sedation was required for children less than 6 years and uncooperative older patients. Only seven patients needed sedation using oral chloral hydrate as a sedative and hypnotic drug.

Conventional magnetic resonance imaging was performed for both cases and control in random order. The radiologist was blinded to their clinical and laboratory data to avoid bias and to enhance the reliability of the findings.

The following muscles were evaluated:

Upper limb: proximal and distal muscles: deltoid, biceps, intrinsic muscles of the hands (thenar and hypothenar) (Fig. 2).

Lower limbs: posterior compartment of thighs and leg (biceps femoris, semitendinosus, and semimembranosus) and (soleus, gastrocnemius, and peroneal muscles) (Fig. 3).

Image sequences included axial FSE T1 without fat suppression and axial STIR WI with fat suppression. The MRI sequence parameters used in our study include the following:

*T1: [out of phase] TE 2.4, TR6.7 and [in phase] TE 2.8, TR 6.7.
*T2: [STIR] TE 72, TR 1300.
*slice thickness: 4–5 mm.

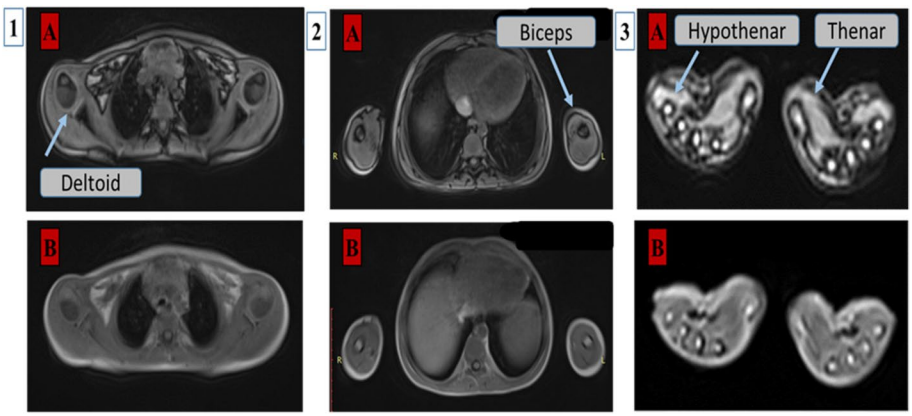


Fig. 2 Axial T1WI of upper limb muscles in cystinosis patient: **1** deltoid, **2** biceps, **3** intrinsic muscles of the hand (thenar and hypothenar). **A** Out of phase. **B** In phase

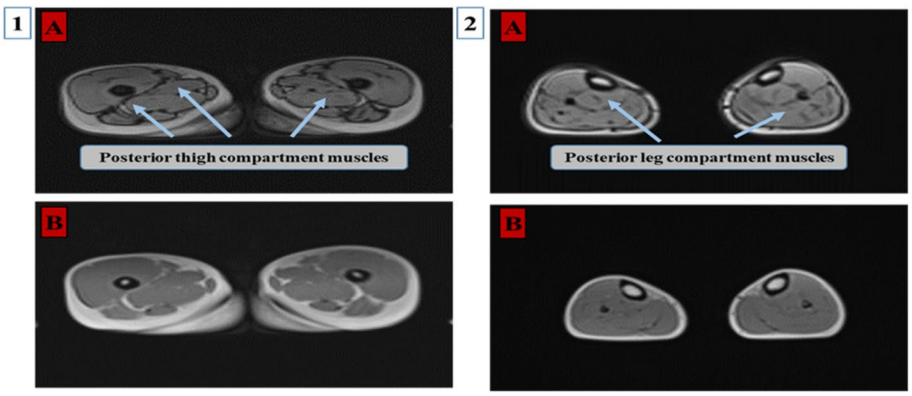


Fig. 3 Axial T1WI of lower limb muscles in cystinosis patient: **1** muscles of posterior thigh compartment: (biceps femoris, semitendinosus, and semimembranous). **2** Muscles of posterior leg compartment: (soleus, gastrocnemius, and peroneal muscles). **A** Out of phase. **B** In phase

*FOV: adjusted according to patient size and imaged body part. For example, the axial image to include both shoulders FOV 357 X 409.

All imaging studies were performed using a 1.5 Tesla MRI machine (Magnetom Aera, Siemens Healthiness, Germany) with a 32-channel phased-array torso coil.

MR data were transferred to and analyzed at an imaging workstation (Syngo MMWP, Siemens Healthineers, Erlangen, Germany). Conventional MR sequences (T1WI and STIR WI) were analyzed for visual assessment of muscle volume and signal intensity.

Statistical methods

Data was analyzed using IBM© SPSS© Statistics version 23 (IBM© Corp., Armonk, NY) and JMP® version 13.2.1 (SAS© Institute Inc., Cary, NC). Continuous numerical variables were presented as mean and standard deviation (SD) representing normally distributed data and median and interquartile for non-normally distributed data. Intergroup differences were compared by the independent-samples *t*-test, Mann–Whitney *U* test, and Kruskal–Wallis test. Categorical data was presented as numbers and percentages and differences were compared using Fisher's exact test. Ordinal data was compared using the chi-squared test. A two-sided p value < 0.05 was considered statistically significant.

Results

Demographic and clinical features

Our study included 22 patients from 19 unrelated families with known nephropathic cystinosis. Their ages ranged from 41 to 164 months at recruitment, with an average age of 105.7 months. Seventeen children were males, and 5 were females. All the 22 patients (100%) were offsprings of consanguineous parents, 15 patients (68.2%) had previous one or more affected siblings, and 5 families (22.7%) suffered from sibling deaths due to cystinosis.

The median age of onset of symptoms was 6 months (range 3–12 months), while the median age of diagnosis was 42 months (range 3–120 months). One patient was diagnosed pre-symptomatically during screening after sibling affection. The median eGFR for cystinosis children was 59.4 mL/min/1.73 m^2 (range 7.8–165). The need for hemodialysis as RRT in cystinosis patients started at an age ranging from 78 to 132 months with a mean age of 103.4 months. Three patients required regular hemodialysis, and six patients had undergone kidney transplantation (following previous hemodialysis). All the studied cystinosis patients were receiving oral cysteamine therapy with a dose ranging from 620 to 1120 mg/m^2/day and for extended durations (median 51, range 36–88 months). Specific cysteamine therapy was started once patients were diagnosed and dosage was increased gradually until a maximum dose was reached and was continued whenever available. Table 1 summarizes the main demographic and clinical features of the recruited cystinosis and CKD children.

Neuromuscular affection

Regarding clinical patterns of neuromuscular affection in cystinosis patients, no clinical evidence of neuromuscular involvement was found, neurological examination revealed normal muscle bulk, tone and normal muscle power with normal reflexes, normal gait, and normal bulbar muscle. In CKD controls, none presented with any

Table 1 Demographic and clinical data of recruited cystinosis ($n=22$) and CKD ($n=44$) children

Demographic and clinical data			Group (A) Cystinosis cases No. = 22	Group (B) Pathological control No. = 44	Test value	*P* value
1. Demographic data						
Gender		Female	5 (22.7%)	14 (31.8%)	NA	NA
		Male	17 (77.3%)	30 (68.2%)	NA	NA
Age (months)		Mean ± SD	105.73 ± 41.57	108.52 ± 37.39	−0.276•	0.784
		Range	41–164	37–177		
Consanguinity (degree)		Yes	22 (100.0%)	17 (38.6%)	22.846*	0.000
		No	0 (0.0%)	27 (61.4%)		
Same condition in the family		Yes	15 (68.2%)	12 (27.3%)	10.154*	0.001
		No	7 (31.8%)	32 (72.7%)		
Other sibling deaths		Yes	5 (22.7%)	3 (6.8%)		
		No	17 (77.27%)	41 (93.18%)		
2. Growth data						
Weight (kg)		Mean ± SD	19.41 ± 8.65	28.47 ± 10.20	−3.571•	0.001
		Range	9–38	13–60		
Height (cm)		Mean ± SD	107.93 ± 18.89	123.77 ± 17.22	−3.411•	0.001
		Range	80–145	92–162		
BMI (kg/m^2)		Mean ± SD	15.75 ± 2.57	18.14 ± 2.87	−3.298•	0.002
		Range	11.36–23	13–29.76		
HC (cm)		Mean ± SD	50.86 ± 2.21	51.32 ± 1.79	−0.899•	0.372
		Range	46–55	46–54		
3. CKD staging						
GFR (ml/min/1.73 m^2)		Mean ± SD	59.38 ± 39.34	68.15 ± 38.16	−0.871•	0.387
		Range	7.8–165	8–153.7		
CKD stage		Stage 1	6 (27.3%)	14 (31.8%)	7.827	0.098
		Stage 2	3 (13.6%)	15 (34.1%)		
		Stage 3	7 (31.8%)	4 (9.1%)		
		Stage 4	3 (13.6%)	3 (6.8%)		
		Stage 5	3 (13.6%)	8 (18.2%)		
Mode of RRT	No RRT	Conservative	13 (59.1%)	38 (86.4%)	6.212*	0.013
	RRT	Hemodialysis	3 (13.6%)	6 (13.6%)	0.000*	1.000
		Transplantation	6 (27.3%)	0 (0.0%)	13.200*	0.000

•: Independent t-test; *:Chi-square test

neuromuscular symptoms; however, one patient (2.3%) presented with clinical features consistent with muscle affection in the form of decreased muscle power grade IV with normal muscle bulk, muscle tone, and normal sensations. Muscle power could not be assessed in one cystinosis patient (4.5%) and 2 CKD control patients (4.5%) due to their young age and inability to obey orders.

Inflammatory biomarkers

Regarding inflammatory markers, chitotriosidase enzyme activity shows significantly high results in cystinosis patients with a median 57.2 (IQR 21–116.4) nmol/ml/h compared to the CKD control group 23.4 (15–32.6) nmol/ml/h, $P=0.002$. Fourteen cystinosis patients (63.6%) had chitotriosidase levels above the reference range, while only 10 CKD controls were above the reference range (22.7%), $P=0.0014$. The area under the ROC curve (AUC) for chitotriosidase to differentiate cystinosis from other CKD patients was 0.736 (95% CI 0.694–0.791). At the cut-off value of 45.4 nmol/ml/h, the sensitivity of chitotriosidase as a predictor for cystinosis was (63.64%), the specificity was (86.36%), the predictive positive value was 70% and the predictive negative value was 82.6%, thus the biomarker is rather sensitive for predicting cystinosis among CKD pediatric patients (Fig. 4).

ESR (1st hour) was higher in cystinosis patients 29(15–37) mm/h compared to CKD controls 15(10–30) mm/h, $P=0.06$. Moreover, 14 cystinosis patients (63.6%) had high ESR, while only 4 CKD patients (9.1%) were above the reference range, $P<0.0001$.

Regarding CRP, no difference between cystinosis patients and CKD patients was observed. As for galectin-3, only one cystinosis patient (4.5%), and 3 CKD patients (6.8%) had high galectin-3 levels, with no significant difference, $P=0.203$ (Table 2).

Regarding the correlation between inflammatory markers and different clinical and biochemical parameters. There was a negative significant correlation between ESR (1st) and sodium level with (P value $=0.007$, $r=-0.556$). Otherwise, no significant correlations exist between inflammatory markers and the other studied variables (Table 3).

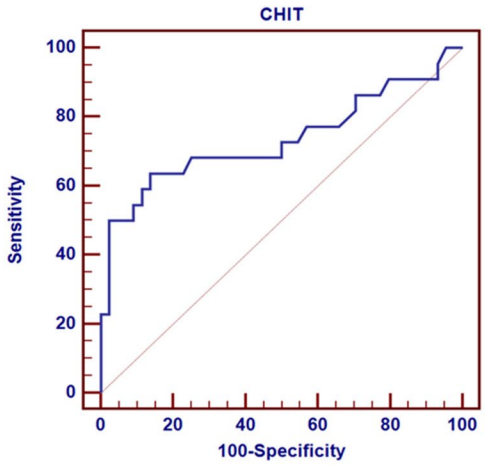
MRI findings in upper and lower limb muscles

MRI was performed to detect early myopathic changes in cystinosis patients compared to the healthy pediatric cohort. In conventional MRI technique; Fatty infiltration of muscles is demonstrated as increased T1-weighted images (T1WI) signal intensity, while muscle edema appears on T2-weighted and STIR sequences as hyperintensity.

Muscles in T1WI images appeared normal anatomically with no changes in the muscle volume, (no atrophy or hypertrophy) and no fatty infiltration. In the STIR or T2-weighted images we detected no muscle edema.

Discussion

In this study, we conducted a case–control study for the detection of the clinical value of inflammatory biomarkers and macrophage activation markers together with conventional MRI of limb muscles as potential additional



Parameter	AUC	Cut-off Point	Sensitivity	Specificity	PPV	NPV
Chitotriosidase	0.736	>45.4	63.64	86.36	70.0	82.6

AUC=Area under curve, PVP=Predictive value for positive, PVN= Predictive value for Negative

Fig. 4 ROC curve illustrating validity of chitotriosidase as predictor for cystinosis

Table 2 Routine labs and inflammatory biomarkers in recruited cystinosis ($n=22$) and CKD ($n=44$) children

Variable (normal value)		Group (A) Cystinosis cases No. = 22	Group (B) Pathological control No. = 44	Test value	*P* value
1. Routine labs					
Serum creatinine (0.3–0.7 mg/dl)	Median (IQR)	0.95 (0.7–1.4)	0.85 (0.6–1.2)	−0.635‡	0.526
	Range	0.4–6.4	0.4–6.2		
Urea (6–24 mg/dl)	Median (IQR)	42.5 (22–70)	30 (20–44)	−1.055‡	0.291
	Range	11–290	10–270		
Serum sodium (135–150 mmol/L)	Mean ± SD	136.86 ± 5.55	141.23 ± 2.70	−2.372•	0.021
	Range	122–147	136–149		
Serum potassium (3.5–5.2 mmol/L)	Mean ± SD	3.89 ± 1.10	4.42 ± 0.79	−2.241•	0.029
	Range	2.1–7	2.5–7		
Calcium (8.8–10.5 mg/dl)	Mean ± SD	9.70 ± 0.86	9.51 ± 0.83	0.829•	0.410
	Range	7.9–10.9	7.6–11.2		
Serum phosphorous (4.5–6.5 mg/dl)	Mean ± SD	4.61 ± 1.48	4.85 ± 1.19	−0.709•	0.481
	Range	2.1–7.9	2.6–8.2		
Serum magnesium 1.6–2.6 mg/dl)	Mean ± SD	2.32 ± 0.62	2.21 ± 0.41	0.869•	0.388
	Range	1.3–3.9	1.2–3.5		
Alkaline phosphatase (180–550 U/L)	Median (IQR)	328.5 (266–424)	256 (187–336.5)	−2.347‡	0.019
	Range	160–927	100–1800		
LDH (90–190 U/L)	Mean ± SD	318.05 ± 120.27	254.77 ± 73.18	2.653•	0.010
	Range	158–654	123–510		
CPK (1–171 U/L)	Median (IQR)	105 (75.5–118)	89 (64–110)	−1.197‡	0.231
	Range	35–409	10–335		
Average PH	Mean ± SD	7.34 ± 0.05	7.36 ± 0.06	−0.732•	0.467
	Range	7.23–7.43	7.2–7.45		
Average serum bicarbonate (HCO3)	Mean ± SD	21.09 ± 3.50	19.73 ± 2.92	1.677•	0.098
	Range	15.3–29	13–25		
2. Inflammatory biomarkers					
CRP	Median (IQR)	6 (6–6)	6 (6–6)	−1.447	0.148
	Range	6–6	6–48		
ESR (1st)	Median (IQR)	29 (15–37)	15 (10–30)	−1.878	0.060
	Range	3–80	0–115		
CHIT	Median (IQR)	57.2 (21–116.4)	23.4 (15–32.6)	−3.108	0.002
	Range	1.4–365	0.3–166.6		
Gal-3	Median (IQR)	4.85 (4.16–5.63)	5.28 (4.6–6.45)	−1.272	0.203
	Range	3.77–23.37	3.57–22.19		

•: Independent t-test; ‡: Mann Whitney test

monitoring factors for neuromuscular involvement in nephropathic cystinosis children.

Neuromuscular affection is a long-term complication in adult nephropathic cystinosis patients with a major effect on their quality of life. Skeletal muscle involvement was first reported in 1988 [17]. It was first detected in an adult cystinosis patient 20 years old presented with worsening dysphagia and weakness in both upper and lower limbs muscles and a muscle biopsy postmortem showed the pathogenic cystine crystals within muscle perimysium and endomysium [18], that commonly presents as a progressive distal myopathy ranging from mild weakness and atrophy affecting intrinsic muscles of the hand that proceed proximally causing proximal muscle weakness and contractures [19]. Cystinosis distal myopathy may be asymptomatic with absent clinical weakness which has been previously described as an early sign of cystinosis myopathy during the first three decades of life [7].

The current study was conducted on 22 cystinosis patients (from 19 unrelated families) with confirmed nephropathic cystinosis, 17 males (77.3%) and 5 females (22.7%) with mean age (105.73 ± 41.57) months. Patients

Table 3 Correlation between inflammatory markers and different clinical and biochemical parameters

	ESR (1st)		Chitotriosidase		Galectin 3	
	r	*P* value	*r*	*P* value	*r*	*P* value
1. Age of study population						
Age (months)	−0.175	0.436	0.055	0.809	−0.047	0.836
Age of onset of symptom	0.092	0.684	0.045	0.844	0.228	0.307
Age of diagnosis (months)	−0.402	0.063	−0.089	0.692	0.265	0.233
Lag between onset and Dx	−0.390	0.073	−0.079	0.726	0.246	0.270
2. Growth percentiles						
Weight (kg)	−0.282	0.203	−0.122	0.588	−0.142	0.530
Height (cm)	−0.313	0.157	−0.109	0.629	−0.138	0.539
BMI	−0.091	0.686	0.029	0.899	−0.066	0.770
3. Chronic renal disease						
eGFR	−0.328	0.136	−0.352	0.109	−0.313	0.155
Age of onset of RRT	0.509	0.162	0.350	0.356	−0.133	0.732
4. Different laboratory parameters						
Na	**−.556**	**0.007**	−0.214	0.339	0.201	0.370
K	−0.187	0.404	−0.020	0.930	−0.257	0.249
Urea	0.405	0.061	0.223	0.318	−0.338	0.124
Serum Creatinine	0.288	0.194	0.337	0.125	−0.199	0.375
Ca	−0.136	0.545	0.051	0.820	−0.015	0.946
PO4	0.382	0.079	0.209	0.350	−0.170	0.451
Mg	0.365	0.095	0.408	0.060	−0.186	0.407
ALP	−0.385	0.077	−0.030	0.895	0.212	0.344
PH	0.181	0.421	0.044	0.845	−0.083	0.714
PCO2	−0.007	0.975	−0.152	0.499	0.236	0.291
HCO3	−0.033	0.884	−0.088	0.698	0.145	0.519
CPK	0.189	0.399	−0.145	0.519	0.107	0.634
LDH	0.233	0.297	−0.169	0.451	−0.093	0.680
5. Specific cysteamine therapy						
Age of start of therapy	−0.402	0.063	−0.089	0.692	0.265	0.233
Duration of therapy	0.084	0.712	0.162	0.472	−0.267	0.230

were followed at the cystinosis clinic, at Cairo University Children's Hospital. No clinical evidence of neuromuscular involvement was found in our cystinosis pediatric patients, neurological examination revealed normal muscle bulk, tone, and power with normal reflexes, normal gait, and normal bulbar muscles.

There was a statistically significant higher median level of chitotriosidase enzyme in cystinosis patients compared to the CKD control group. There was no significant difference in the median values of ESR, CRP, and Galectin-3 levels between cystinosis patients and the CKD control group.

Among the evaluated biomarkers of inflammation, plasma chitotriosidase enzyme activity showed promise for use as an additional clinical and therapeutic monitor of cystinosis patients. Another inflammatory marker galectin-3 (Gal-3), a profibrotic mediator, is attributed to fibrosis in animal models, kidney and correlates inversely with the reduction of GFR in humans, but it is unknown if galectin-3 could expect the degree of kidney disease [20].

MRI is highly valuable in the assessment of soft tissue abnormalities**.** Conventional magnetic resonance imaging is a highly efficient modality for accurate assessment of the degree of diffuse symmetrical inflammatory myopathy, MRI is highly sensitive in edema detection that correlates with, and sometimes precedes, clinical findings [21]. In this study, conventional MR sequence for the qualitative analysis of upper and lower limb muscles regarding the presence of volume or signal intensity changes revealed no differences between the cystinosis patients and the healthy cohort. This goes hand in hand with the clinical examination findings, which may signify that it is still too early to notice such differences in the recruited age group.

Fatty infiltration of muscles is demonstrated as elevated signal intensity on T1-weighted images (T1WI) that reflect muscle damage and assess the volume of muscle for either hypertrophy or atrophy. Replacement of muscle fibers with connective tissue, endomysial fibrosis, is a good indicator of muscle function loss than fatty infiltration, however no accurate imaging technique to assess muscle fibrosis. Edema is seen as hyperintensity on T2-weighted images (T2WI) and STIR sequences, which reflect ongoing muscle damage [22]. In our study, morphologically, there were no significant differences between the muscles of cystinosis patients and healthy controls. In T1WI, muscles appeared normal anatomically with no changes in the muscle volume (atrophy or hypertrophy) and no fatty infiltration, while in STIR or T2-weighted images, no muscle edema was detected.

To our knowledge, there was no previous study that used MRI to evaluate muscle affection in patients with nephropathic cystinosis.

Previous studies used MRI to evaluate other lysosomal storage diseases such as Pompe disease. In Pompe disease, MRI reveals fatty infiltration of the subscapularis, abdominal wall and posterior paravertebral muscles [23], adductor magnus, and hamstrings, with relative sparing of the lower leg muscles. An interesting finding is the striking involvement of the tongue muscles known as the "bright tongue sign" [24]. The severity of infiltration correlates with the severity of disease, and muscle function and can predict prognosis. MRI appearances of skeletal muscle can also be used to assess progress upon commencement of treatment [25].

Conclusion

In conclusion, our data point out that the chitotriosidase enzyme is a clinically significant biomarker for nephropathic cystinosis; however, its usefulness in the evaluation of cystinosis myopathy progression is yet to be confirmed.

Regarding muscle MRI in cystinosis patients, normal results for conventional MRI do not exclude the possibility of early changes in muscles that need more advanced MRI techniques for accurate evaluation of muscles.

Limitations and recommendations

Further studies are required with more advanced MRI techniques to assess muscle MRI changes in a larger sample size and a wider age spectrum, possibly as a multi-center collaboration for the early detection and characterization of neuromuscular involvement patterns in nephropathic cystinosis patients.

Advanced MRI techniques, such as diffusion-weighted imaging (DWI) and muscle T2 mapping, should be considered to detect subtle muscle changes that may precede overt myopathy. DWI can reveal changes in water diffusion within muscles, indicating early pathological changes. T2 mapping provides quantitative data on muscle edema and inflammation. Incorporate DWI and T2 mapping in the MRI protocol to detect early myopathic changes.

Also, the inclusion of muscle quantitative MRI, such as fat fraction quantification using the Dixon technique or magnetic resonance spectroscopy (MRS), would provide more detailed insights into muscle composition and early myopathic changes. The Dixon technique separates fat and water signals, allowing precise measurement of fat infiltration in muscles. MRS provides metabolic information that can detect biochemical changes before structural changes become apparent.

Future research is needed with follow-up muscle MRI for early detection of any findings with detailed analysis of the potential clinical implications of these MRI findings and how these imaging results could influence patient management and treatment decisions, such as potential changes in patient management and early intervention strategies.

Abbreviations

4-MU-C3	4-Methylumbelliferyl-triacyl chitotrioside
AR	Autosomal recessive
CHIT	Chitotriosidase
CKD	Chronic kidney disease
CPK	Creatinine phosphokinase
CRP	C-reactive protein
CTNS	Cystinosis
DWI	Diffusion-weighted imaging
EDTA	Ethylene diamine tetra acetic acid
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
GAL-3	Galectin-3
GFR	Glomerular filtration rate
HS	Highly significant
IQR	Interquartile range
LDH	Lactate dehydrogenase
MCP-1	Monocyte chemoattractant protein-1
MRC	Medical Research Council
MRI	Magnetic resonance imaging
NINDS	National Institute of Neurological Disorders and Stroke
NS	Non-significant
RRT	Renal replacement therapy
S	Significant
STIR	Short inversion time inversion recovery
T1WI	T1-weighted images

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Authors' contributions

All authors listed have contributed sufficiently to the project. RH contributed to the design of the work and the idea of the study. R.S. collected the clinical data, analyzed them, and drafted the manuscript. M.A. contributed to the idea of study and helped in laboratory work. SE performed muscle MRI for the patients and analyzed the data. NA generated the idea. RH, MA, S.E., and NA conceived and designed the evaluation and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data used during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The study was conducted in accordance with the declaration of Helsinki for studies including human participants and was approved by the institutional research ethics committees at the Faculty of Medicine, Cairo University (Approval code #MD-88–2020). Written informed consent was obtained from the parents. Participant data has been anonymized.

Consent for publication
Written informed consent was obtained from the parents. Participant data have been anonymized.

Competing interests
The authors declare that they have no competing interests.

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