Impact of Age at Starting Cysteamine Therapy on Serum Chitotriosidase in Cystinotic Iraqi Children

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Abstract

Cystinosis is a rare autosomal recessive lysosomal storage disease with high morbidity and mortality. It is caused by mutations in the CTNS gene that encodes the cystine transporter, cystinosin, which leads to lysosomal cystine accumulation**.** It is the major cause of inherited Fanconi syndrome and should be suspected in young children with failure to thrive and signs of renal proximal tubular damage. The diagnosis can be missed in infants, because not all signs of renal Fanconi syndrome are present during the first months of life. Elevated white blood cell cystine content is the corner stone for the diagnosis. Since chitotriosidase (CHIT1 or chitinase-1) is mainly produced by activated macrophages both in normal and inflammatory conditions. Which suggests that cystinosis should be included in the differential diagnosis of disorders with increased plasma chitotriosidase activity. This study is aimed to investigate the impact of cystinosis on the renal function in relation to age at detection and initiation of treatment course,besides estimating serum chitotriosidase level,as a screening marker and therapeutic monitor for cystinosis disease in Iraqi children with cystinosis.

The present study is a case-control study included a samples of 30 children with nephropathic cystinosis, compared to 25 healthy control children from those attending at The Genetic Rare Diseases Center/AL-Emamain AL-Kadhimain teaching hospital, Baghdad-Iraq.

Our results report patients who started taking the medication (cysteamine) before two years of age were presented with significantly lower levels of cystine and chitotriosidase in addition to better renal function (higher GFR) (P-value = 0.0001) .In other words, earlier treatment led to better disease control and less deterioration of renal function.

In conclusion serum chitotriosidase activity estimation might aid in monitoring therapeutic benefit of cysteamine therapy and the prognosis of the disease when WBC cystine assessment is not available.

Keywords: *Cystinosis, Cysteamine, Chitotriosidase.*

Introduction

Cystinosis is a rare autosomal recessive lysosomal storage disease with high morbidity and mortality. It is caused by mutations in the CTNS gene that encodes the cystine transporter, cystinosin, which leads to lysosomal cystine accumulation[(1)].Three clinical forms of cystinosis can be distinguished depending on the age at presentation and the degree of disease severity,(1) Infantile Nephropathic Form: Also known as renal Fanconi syndrome, the most frequent and most severe form of the disease. Patients are generally present before the age of 12 months with polyuria, polydipsia and failure to thrive, caused by generalized proximal tubular damage.(2) Late-Onset or Juvenile Nephropathic Form: Also known as Intermediate Cystinosis, Adolescent Form, which is characterized by the same symptoms

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as infantile cystinosis, but with a disease onset mostly after the first decade of life and a slower rate of disease progression[2]. (3) Ocular Non-nephropathic Form of Cystinosis: Also known as Non-nephropathic adult Form, Benign Non-nephropathic Cystinosis, which is characterized by photophobiadue to corneal cystine crystals deposits, while other organs remain spared[3].

Early symptoms of classical nephropathic cystinosis include renal tubular Fanconi syndrome, rickets, impaired growth, hypothyroidism and photophobia[4]**.** However, cystine accumulation continued in non-renal organs, including the muscle, brain, bone marrow, liver, spleen, lymph nodes, cornea, conjunctiva, thyroid, pancreas, testes and intestines[5]. Consequently, the clinical course of cystinosis changed from that of a largely renal disease to that of a multisystemic disorder with significant non-renal involvement, including a distal vacuolar myopathy, decreased pulmonary function, swallowing impairment, deterioration of the central nervous system (CNS), endocrinopathies, vascular calcifications, retinal damage and other ophthalmic complications[6]. Definitive diagnosis is based upon a high indexof suspicion because of the clinical presentation including: polyuria, thirst, failure to thrive, growth retardation, vomiting, periods of dehydration, constipation, developmental delay and rickets in some patients. Biochemically, the patients were presented with hypokalemia, hypophosphatemia, metabolic acidosis, low serum uric acid, low carnitine, and, sometimes, hyponatremia. Proteinuria can reach grams per day and consists of LMW proteins, albumin and high molecular weight proteins[7] supported by slit lamp examination of the corneas showing crystals, which are generally present by 16 months of age. The detection of elevated intracellular cystine content is the cornerstone for the diagnosis.[8]

Nationwide birth prevalence data concerning cystinosis are only reported in few populations. However, the prevalence of cystinosis in Iraq about is 163 patients according to data collected from The Genetic Rare Diseases Center/AL-Emamain AL-Kadhimain Teaching Hospital, Baghdad-Iraq. The incidence of cystinosis is estimated as 1 in 100,000 –200,000 live births per year[9].

Cystine depleting therapy with cysteamine orally is the only specific targeted therapy available for managing cystinosis and needs to be combined with cysteamine eye drops for corneal disease involvement[10].

The control of nephropathic cystinosis is complex owing to its severity and multisystemic nature and the requirement of treatment with several drugs with a very strict dosage schedule. Early diagnosis, prompt cysteamine administration and treatment adherence influence morbidity and prognosis[11].

Subjects and Method:

The present study is a case-control study included 30 patients were diagnosed to have cystinosis from those attending at The Genetic Rare Diseases Center/AL-Emamain AL-Kadhimain teaching hospital, Baghdad-Iraq. This study was approved by the Ethics Committee of the College of pharmacy/University of Baghdad. All participants were informed about the aim and the proposed benefits of the study before obtained their agreements. Apparently healthy individuals (25) were included to serve as a control group.

Cystinotic children included in the study were thoseaged less than 10 years, and diagnosed to have cystinosis according to clinical symptoms of disease and eye examination; demonstrating corneal cystine crystals. Whereas the exclusion criteria were:

- Patients that have other inborn error of metabolism.
- Patients that have any endocrinopathy (DM, thyroid disorder, congenital adrenal hyperplasia).
- Patients that have other renal disorders (nephrotic syndrome, CKD, Fanconi syndrome/not related to cystinosis).

Data Collection: The patient's information sheet included all the recorded details: anthropometric measures, medical and social history that are related to the clinical diagnosis of cystinosis patients, as summarized in table (1). The questionnaire was posed to patient parents who were diagnosed with cystinosis at the time of a regular renal care follow-up visit to the hospital.

Table 1: Patients & Control Demographics and Disease Characteristic

Characteristic	Patient group (Mean± SD)	Control group (Mean± SD)	P-value
Age_(months)	65.20 ± 34.27	62.32 ± 35.45	.728
Gender (M/F)	(17/13)	(13/12)	.729
Weight (Kg)	11.20 ± 3.00	22.28 ± 10.71	.0001*
Height (cm)	87.27 ± 11.88	108.56 ± 18.98	.0001*
BMI kg/m^2	14.68 ± 2.36	17.71 ± 2.96	.0001*
Cystine (nmol/mg protein)	2.98 ±1.80	0.20 ± 0.06	.0001*
S. Creatinine (µmol/L)	179.76 ± 177.53	38.86 ± 14.16	.0001*
Chitotriosidase(nmol/hr/ml)	308.30 ± 134.789	115.96 ± 32.44	.0001*
GFR (mL/min/1.73 m^2)	44.09 ± 37.64	110.13 ± 12.16	.0001*
S.Calcium (mmol/L)	1.80 ± 0.55	2.15 ± 0.20	.004*
Age at diagnosis (months)	25.60 ± 20.88	-	-
Duration of disease(months)	39.53 ± 33.24	-	-
Dose of Cysteamine (mg/kg/day)	56.67±7.581	-	-
Cysteamine Frequency	3.9 ± .712	-	-
Treatment duration(month)	19.93 ± 28.89	-	-
Age when treatment started (month)	45.27 ± 31.04	-	-

N for patients=30, N for Control=25, SD=Standard deviations, GFR=Glomerular Filtration Rate, Normal S.Cr range is 26 to 62 µmoles/ liter, Normal cystine level (Mixed leukocytes test) is less than 0.2 nm/mg protein, Normal S. Calcium range is 2.2-2.6 mmol/L, Normal BMI range is 18.5 to 24.9, Normal Chitotriosidase level is < 78.5 nmol/hr/ml.

Sample Collection and Preparation: From each participant (patient and control subjects), venous blood samples were collected after at least 6 hours from taking treatment (**Cysteamine**). Six milliliters was withdrawn, about (2 ml) was transferred to a tube containing Lithium Heparin and stored at (+2 to +8 °C, for several hours) to be taken it to the laboratory for separation of white blood cell (WBC) to be used for measuring cystine content by applying high performance liquid chromatography (HPLC) [(12)].

The other part (4 ml) of the blood sample was transferred to a plane tube and centrifuged at (3000 rpm) for 5 minutes to obtain serum, which is used for the measurement of creatinine and calcium level. The remaining aliquot of serum was kept in eppendorff tubes and frozen at (–20°C) for later analysis of serum chitotriosidase concentration. Also, urine specimens were collected using urine cups from each participant for measuring glucose in urine. Laboratory assay procedures were blinded for assays (regarding the sample was for a control or a patient) of the participants. Samples were assayed in a random order. Because, the lack of blinding could have introduced bias into the assessment of subjective outcomes such as health-related quality of life and adverse events. Additionally, it's the first time to measure cystine level in Iraq for those patients.

Statistical analysis: The analyses were conducted using the Statistical Package for the Social Science (SPSS, version 22, IBM, New York, USA).Descriptive statistics (means, standards deviations, frequencies and percentages) of the participants (both patient and control group) were calculated. Because the variables were not normally distributed, we used non-parametric tests including Mann-Whitney (between 2 groups) tests to measure the difference in multiple measures according to participating groups (patient vs control) and different age groups. Spearman correlation was used to measure the relationships among different measures in patient group. A p-value of less than 0.05 was considered to be significant statistically.

Results

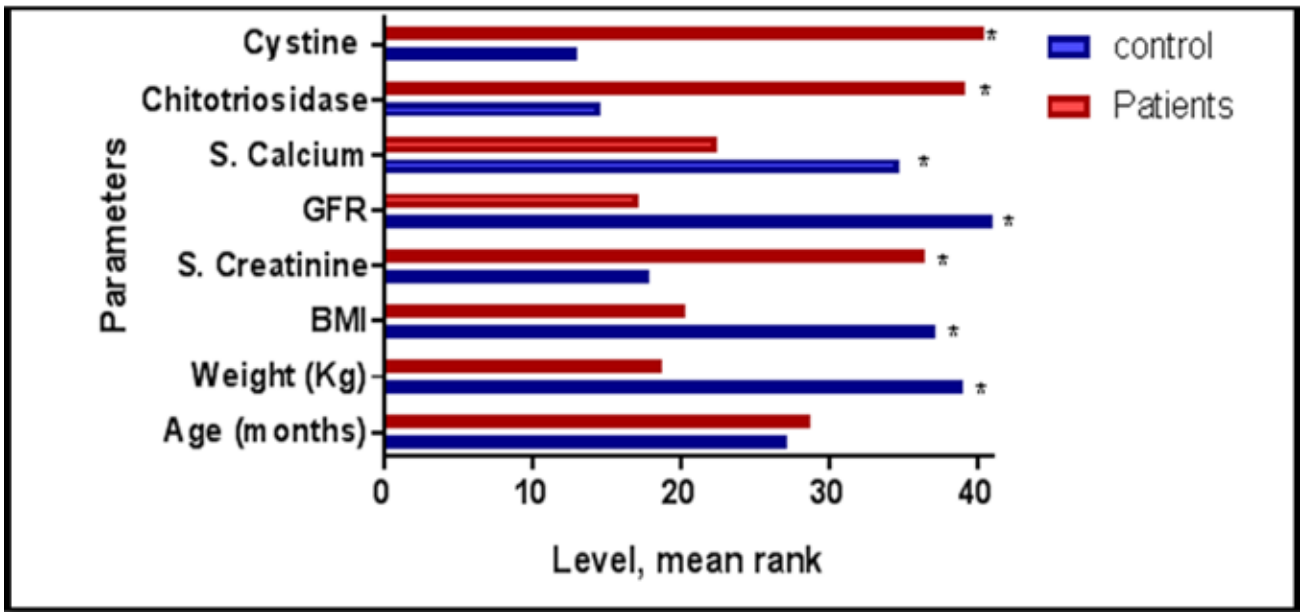
Patients & Control Demographics and Disease Characteristics: The participating patients aged

between 1.5 and 10 years (18 and 120 months) with average age of (5.4 years)(± SD 2.86)/(65.20 months) (± SD 34.27). The control group had a comparable age to patients group with average age of (5.2 years)(± SD 2.96)/(62.32 months) (±SD 35.45) and ranged between one and 10 years old (12 and 120 months). Control group included 13 male participants (52.0%) and 12 (48.0%) female. The thirty cystinotic patients, included (17 male) and (13 female) representing 56.7% and 43.3% respectively (**Table -1**).

Considering BMI, the patients BMI level is below normal, as compared to the control group (P-value <0.05). Meanwhile, lower weight, height, GFR and serum calcium measures were recognized compared to control group. On the other hand, the control children had significantly lower levels of serum creatinine, cystine and chitotriosidase levels compared to patients group (**Table -1 & Figure-1**).

The children were about two years old in average when they were diagnosed with the disease (cystinosis). The patients also had abnormally higher levels of cystine, creatinine and Chitotriosidase. In contrast, they had abnormal lower levels of serum calcium and GFR (glomerular filtration rate). In average, the treatment started after 20 months of the disease diagnosis (**Table -1**). Additionally, the average disease duration in patients group was about 40 months, while the average duration of the treatment was half this period (about 20 months). In other words, the patients had an average of 20 months without treatment due to unavailability of the medication (cysteamine) additionally the medication is too expensive.

The average doses as prescribed by physician were 56.67 mg/kg/day (±SD 7.581) ranging between 40mg/kg/day for newly diagnosis patient to 60mg/kg/day for the patients that had been diagnosed some time ago, the newly diagnostic patients were started at (40 mg/kg/day) to be increased over the course of 4 to 6 weeks until reaching a dose of (60 mg/kg/day), whereas the average doses frequency was 4.00 (±SD 0.695) (range 3-5 times daily).



*Significant (P-value <0.05) difference between mean rank

Figure 1: Comparison between different parameters of control and patients

Difference in Disease Measures According to Age at Starting Medication (Cysteamine): Patients who started taking the medication (cysteamine) before years two of age were presented with significantly lower levels of cystine and chitotriosidase in addition to better renal function (higher GFR values) (P-value = 0.0001) (Table-2). In other words, earlier treatment led to better disease control and less deterioration of renal function.

Table 2: Difference in the Disease Measures According to Age for Starting Medication (Cysteamine)

Disease parameter	Treatment starting age (month)	N	Mean Rank	P-value
Chitotriosidase ŋmol/hr/ml))	0-24	13	7.54	.0001*
	>24	17	21.59	
	Total	30		
Cystine (ŋmol/mg protein)	0-24	13	7.92	.0001*
	>24	17	21.29	
	Total	30		
GFR (ml/min/1.73 m^2)	0-24	13	23.54	.0001*
	>24	17	9.35	
	Total	30		

*Significant (P-vale< 0.05) according to Mann-Whitney test

Discussion

The Relationship between Age at Starting Cysteamine Therapy and Kidney Functional Outcomes in Nephropathic Cystinosis: Nephropathic cystinosis is a systemic disease that results in kidney failure at the end of the first decade of life when untreated [(13)] or even when treatment is initiated after 5 years of age with immediate release cysteamine therapy. In spite of cysteamine is not ordinarily able to reverse Fanconi syndrome but in some isolated cases of prenatal diagnosis who starting of cysteamine therapy through the first weeks of life averted the occurrence of the renal tubular disorder[(14)]. The importance of early treatment was reported by Gahl among others, by estimating that for every month of treatment prior to 3 years of age, finding that 14 months' worth of later renal function were preserved[(15)].

As shown in table (3) the patients were categorized into two groups: A-Children started cysteamine therapy before 2 years of age (n=13, 43.3%), B- Children started cysteamine therapy after 2 years of age (n=17, 56.7%).

GFR (glomerular filtration rate) was significantly increased in cysteamine treated patients before 2 years of age with average 82.53 (±28.46) in comparison with that started the treatment after 2 years of age had average 18.46 (±12.42). Additionally, the average WBC cystine level (a marker of disease control) in patients started the treatment before 2 years of age was 1.35 (±0.57) nmol ½ cystine/mg protein (therapeutic goal of cysteamine therapy must have average WBC cystine level < 2 nmol ½ cystine/mg protein)[(16)], while average WBC cystine level in patients started the treatment after 2 years of age 4.05 (±1.48) nmol ½ cystine/mg protein.

Besides that, the level of serum chitotiosidase is significantly lower than the patients who started the treatment after 2 years of age with average 176.92 (±48.24)(nmol/hr/ml), whereas those started therapy after 2 years 383.3 (±105.67) (nmol/hr/ml). Wherefore when the treatment applied before 2 years of age and with an average WBC cystine level < 2 nmol ½ cystine/ mg protein there was preservation for kidney function and limiting the renal deterioration, while after the age of two years, the glomerular filtration rate (GFR) was lowered as indicated by the increased serum creatinine which evolves to advanced CKD and hence,the serum levels of cystine and chitotiosidase were significantly increased. Data analysis of 30 child with nephropathic cystinosis shows that early treatment with cysteamine has a positive effect on the onset of renal and extrarenal complications.

A study by Cochat et al cystinotic patients reached ESRD at a mean age of 9.8 years, even though 83% had received treatment, probably due to the late introduction of cysteamine therapy (7.3 years) [(17)], while in the present study the average age at starting cysteamine treatment was 3.9 (ranged from 1–10) years and the patients reached ESRD at average age of less than 9.8 years,approximately 6.8 years in patients who started the treatment after 2 years of age and non- compliance to the treatment.

In conclusion serum chitotriosidase activity estimation might aid in monitoring therapeutic benefit

of cysteamine and the prognosis of the disease when WBC cystine assessment is not available, despite that chitotriosidase enzyme is not specific for this disease. However, it is believe a useful clinical screening test and a promising therapeutic monitor since a link between cystine accumulation and plasma chitotriosidase activity comes from the observation that plasma chitotriosidase activity and leukocyte cystine content ran in parallel and were both affected by the dosing regimen.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq.

Conflict of Interest: Non

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