Evaluation of the effect of chenodeoxycholic acid treatment on skeletal system findings in patients with cerebrotendinous xanthomatosis

Serebrotendinöz ksantomatozis hastalarında kenodeoksikolik asit tedavisinin iskelet sistemi bulguları üzerine etkisinin değerlendirilmesi

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Abstract

Aim: The primary purpose of the present study is to evaluate the effect of chenodeoxycholic acid treatment on skeletal system findings in patients with cerebrotendinous xanthomatosis.

Material and Methods: This retrospective study was conducted between June 2013 and December 2018 with seven patients with cerebrotendinous xanthomatosis in Cerrahpasa Medical Faculty Pediatric Nutrition and Metabolism Department. The clinical, epidemiologic, and genotypic features of the patients were reviewed in detail and the following items, especially related with skeletal system involvement, were recorded from medical data: history of a bone fracture, plasma calcium, phosphate, alkaline phosphatase and 25-hydroxy-vitamin D concentrations, bone mineral density values of the posteroanterior lumbar spine (L1-L4), and femoral neck before and after chenodeoxycholic acid treatment.

Results: Regarding the bone mineral metabolism, plasma calcium, phosphate, alkaline phosphatase levels were found in normal ranges in all patients. Plasma 25-hydroxy-vitamin D evaluation at the time of diagnosis showed deficiency in three patients and insufficiency in three patients. Following chenodeoxycholic acid therapy, 25-hydroxy-vitamin D deficiency persisted in only one patient, but insufficiency was observed in four patients. According to the bone mineral density assessments, four patients had Z-scores below the expected range for age both at initial examination and after chenodeoxycholic acid therapy. No significant difference was observed between plasma 25-hydroxy-vitamin D levels and bone mineral density Z-scores before or after treatment.

Conclusion: This study elucidates the necessity of additional medical treatment as a part of chenodeoxycholic acid therapy to improve skeletal system findings in cerebrotendinous xanthomatosis.

Keywords: Bone mineral density, cerebrotendinous xanthomatosis, chenodeoxycholic acid

Öz

Amaç: Bu çalışmanın temel amacı serebrotendinöz ksantomatozis tanılı hastalarda kullanılan kenodeoksikolik asit tedavisinin iskelet sistemi bulguları üzerine olan etkisini tanımlamaktır.

Gereç ve Yöntemler: Bu geriye dönük çalışmaya Haziran 2013 ve Aralık 2018 yılları arasında Cerrahpaşa Tıp Fakültesi Çocuk Metabolizma ve Beslenme Bilim Dalı'nda izlenen serebrotendinoz ksantomatozis tanılı yedi hasta alındı. Hastalara ait klinik, epidemiyolojik ve genotipik özellikler geriye dönük incelendi ve özellikle iskelet sistemini ilgilendiren kemik kırığı öyküsü, kenodeoksikolik asit tedavisi öncesi ve sonrasında plazma kalsiyum, fosfat, alkalen fosfataz, 25-hidroksi-vitamin D düzeyleri, posteroanterior lomber vertebra (L1-L4) ve femur boynu kemik mineral dansitelerine ait bilgiler kaydedildi.

Bulgular: Kemik mineral metabolizmasının değerlendirilmesi açısından ölçülen plazma kalsiyum, fosfat, alkalen fosfataz seviyeleri tüm hastalarda normal sınırlardaydı. Tanı anında yapılan plazma 25-hidroksi-vitamin D değerlendirilmesi; üç hastada eksiklik, üç hastada yetersizlik ile uyumluydu. Kenodeoksikolik asit tedavisi sonrasında 25-hidroksi-vitamin D eksikliği bir hastada devam ederken, dört hastada yetersizlik saptandı. Kemik mineral dansitometri değerlendirmelerinde, dört hastada kemik mineral dansitesi Z-skorlarının başlangıç anında ve tedavi sonunda yaşa göre düşük olarak devam ettiği görüldü. 25-hidroksi-vitamin D ve kemik mineral dansitometrisi Z-skorları arasında tedavi öncesi ve sonrasında istatistiksel olarak anlamlı farklılık izlenmedi.

Çıkarımlar: Bu çalışmada, serebrotendinöz ksantomatozis tanılı hastalarda iskelet sistemi bulgularının iyileştirilmesi amacıyla kenodeoksikolik asit tedavisine ek olarak farklı medikal tedavilerin gerekli olabileceği üzerinde durulmuştur.

Anahtar sözcükler: Kemik mineral dansitesi, kenodeoksikolik asit, serebrotendinöz ksantomatozis

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Introduction

Cerebrotendinous xanthomatosis (CTX) is an autosomal recessively inherited lipid storage disorder caused by mutations of CYP27A1 gene, which encodes for the enzyme sterol 27-hydroxylase. As sterol 27-hydroxlase catalyzes the first step in the process of cholesterol side-chain oxidation, deficient enzyme activity leads to impaired primary bile acid synthesis, increased concentration of bile alcohols, and increased formation of plasma and tissue cholestanol. Progressive accumulation of cholestanol and cholesterol in almost every tissue of the body, particularly in the nervous system, was thought to be responsible for the clinical symptoms of CTX (1).

Patients with CTX can present with diverse clinical manifestations. Besides neurologic manifestations, non-neurologic involvement can be seen in the course of the disease. The first symptoms typically arise from childhood, including chronic infantile diarrhea and juvenile cataracts (2). Signs and symptoms that tend to develop later in adulthood are mostly associated with neurologic involvement. Cognitive decline, ataxia, corticospinal tract abnormalities, speech changes, gait disturbances, psychiatric disturbances, Parkinsonism and seizures are the main neurologic abnormalities in CTX (3). Xanthomas usually appear in the second or third decade. Skeletal system and cardiovascular system involvements can also be observed in CTX (2). Brain imaging of patients reveals supra- and infratentorial atrophy, subcortical and periventricular white matter alterations, brainstem lesions, and cerebellar parenchymal abnormalities involving the dentate nuclei and the surrounding white matter (4).

Cerebrotendinous xanthomatosis is mainly diagnosed based on biochemical testing and molecular genetic analysis in the light of clinical findings and neuroimaging. The biochemical abnormalities, which characterize CTX, include high plasma and tissue cholestanol concentrations, increased concentration of bile, urine and plasma alcohols and virtually absent chenodeoxycholic acid (CDCA) (5). Molecular genetic testing of CYP27A1 gene allows diagnosis confirmation and identification of healthy carriers in CTX (6). Although onset of symptoms generally occurs in childhood, diagnosis of CTX is rarely made before the age of 20 years, which represents a remarkable diagnostic delay. As a result of a comprehensive review of selected international CTX series, a suspicion index for early diagnosis was developed (7).

Chenodeoxycholic acid treatment normalizes biochemical abnormalities by suppressing cholesterol 7α-hydroxylase activity and prevents cholestanol accumulation. However, providing normal cholestanol concentrations do not correlate with improvement in neurologic findings. The timing of CDCA treatment is thought to have a significant role in the outcomes of patients. Improvement or stabilization of neurologic findings could be achieved by starting treatment at earlier ages (8, 9).

Considering the skeletal system involvement, CTX has been reported to be associated with osteoporosis and increased bone fractures (2). However, both the underlying pathogenesis of disrupted bone metabolism and the effects of CDCA treatment on bone mineralization are still not clarified. In this study, we describe the bone densitometry results before and after CDCA treatment and evaluate the role of therapy on skeletal system findings.

Material and Methods

This retrospective study was conducted between June 2013 and December 2018 with seven patients with CTX in Cerrahpasa Medical Faculty, Pediatric Nutrition and Metabolic Department. Patients who were under regular follow-up and in whom the definite diagnosis was made through CYP27A1 gene molecular analysis were included in the study.

The clinical, epidemiologic, and genotypic features of the patients were reviewed in detail and the following items, especially related with skeletal system involvement were recorded from medical data: history of a bone fracture, plasma calcium (Ca), phosphate (P), alkaline phosphatase (ALP) and 25-hydroxy-vitamin D (25-OHD) concentrations before and after CDCA treatment, and bone mineral density (BMD) values of posteroanterior (PA) lumbar spine (L1-L4) and femoral neck before and after CDCA treatment. Initial and final plasma cholestanol levels, age at CDCA therapy initiation, therapy durations, and any other medical treatments that were initiated to improve bone manifestations were also noted according to follow-up records.

Z-scores were preferred to evaluate BMD because our patients included females prior to menopause, males younger than 50 years, and pediatric patients. BMD Z-scores less than or equal to -2.0 were defined as "below the expected range for age," and Z-scores above -2.0 were accepted as being "within the expected range for age." Both PA lumbar spine and femoral neck were used for dual energy X-ray absorptiometry (DXA) determinations in patients who were older than 18 years. However, only analysis of PA lumbar spine was presented in pediatric cases (10). In pediatric cases, Z-score calculations were also corrected according to the pediatric reference data of healthy Turkish children (11). Plasma 25-OHD concen-

Table 1. Demographic information and molecular analysis of the patients with CTX

Pt/Fm	Sex	Age	Consanguinity	CYP27A1 mutation			Cholestanol(µg/mL)[a] (N=0.45–3.75)	Cholestanol(µg/mL)[b] (N:0.45–3.75)	Duration of CDCA treatment
				Allele 1	Allele 2	Allele 3			
1/A	M	13y 4m	(+)	c.808C>T	c.808C>T		22.39	6.12	2y 1m
2/A	M	19y 6m	(+)	c.808C>T	c.808C>T		19.99	5.07	2y 1m
3/B	M	42y 6m	(+)	c.1061A>G	c.1184+1G>A	c.1476+2T>C			2y 4m
4/C	F	31y 11m	(+)	c.446+1G>A	c.446+1G>A				3y 9m
5/D	F	16y 1m	(+)	c.1333C>T	c.1333C>T		27.48	4.99	2y 7m
6/D	M	7y 7m	(+)	c.1333C>T	c.1333C>T		21.89	5.46	2y 7m
7/E	M	25y 4m	(+)	c.256-1G>C	c.256-1G>C		14.35	6.24	12m

Pt: Patient; Fm: Family; +: Present; CTX: Cerebrotendinous xanthomatosis; CDCA: Chenodeoxycholic acid; [a] At the time of diagnosis; [b] After treatment

trations were accepted to be normal if 25-OHD was >30 ng/mL and deficient if below 20 ng/mL. 25-OHD concentrations between 20–30 ng/mL were accepted as vitamin D insufficiency (12).

Patients who had no molecular diagnosis, who were not under a regular follow-up, and patients with missing data were excluded from the study. Our study was designed according to the principles of Ethical Guidelines for Biomedical Research Involving Human Subjects as defined in the Declaration of Helsinki by the World Health Organization (revised in September 2013, www.wma.net). The study protocol was approved by the Ethical Committee of Cerrahpaşa Medical Faculty (104434/05.12.2018). All parents of the patients included in the present study gave informed consent.

Statistical analyses were performed using the Statistical Package for the Social Sciences version 21.0 (SPSS Inc., Chicago, IL, USA). Values are expressed as median and range or mean and standard deviation. Comparisons between two and among groups were performed using the paired sample t-test and a value of $p < 0.05$ was considered to indicate statistical significance.

Results
Seven patients with CTX from five unrelated families were enrolled in the study. Five patients were male and two patients were female. All patients had a family history of consanguinity. The mean age of the patients at the time of diagnosis was 22.3±11.9 (median: 19.53) years. Plasma cholestanol concentrations were obtained in five of seven patients. In these patients, the mean plasma cholestanol concentration was 21.20±4.73 (median: 21.89) µg/mL at the time of diagnosis. The remaining two patients were aged 45 and 35 years, respectively, and CTX was suspected because of their overall neurologic manifestations. Molecular genetic testing of the CYP27A1 gene was performed in all patients and found compatible with the disease. The mutational and epidemiologic features of the patients are summarized in Table 1.

Regarding bone mineral metabolism, plasma Ca, P, and ALP concentrations were found to be in normal ranges in all patients. Plasma 25-OHD evaluation at the time of diagnosis showed deficiency in three patients (patients 3, 5, and 7) and insufficiency in three patients (patients 1, 4, and 6). According to the BMD assessment, four patients were found to have Z-scores below the expected range for age (patients 1, 2, 3, and 4). Two patients had a history of bone fracture causing immobilization in their medical history. In one of these patients, the Z-score was remarkably below the expected range for age (Patient 1), whereas BMD analyses were found as normal in the other patient (Patient 5). The BMD parameters of the patients were in a statistically significant relationship with plasma cholestanol concentrations before CDCA treatment ($p < 0.05$).

Chenodeoxycholic acid treatment was started at a dose of 10 mg/kg/day and no adverse effects or complications were observed during the CDCA treatment in any patients. The mean duration of CDCA

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therapy was 2.3±0.8 (median: 2.27) years. Cholestanol analysis was performed annually after the initiation of CDCA treatment and it was shown that plasma cholestanol concentrations decreased dramatically confirming the biochemical response to therapy. Only one patient received vitamin D therapy at a dosage of 4000 IU/day (Patient 7). Vitamin D treatment was planned to be started to the other patients with osteoporosis and vitamin D deficiency; however, treatment could not be provided because of family issues and the patients' noncompliance. As a result, no other medical treatment was used to improve bone manifestations in the other six patients.

Following the CDCA therapy, 25-OHD deficiency persisted in only one patient (Patient 5), but insufficiency was observed in four patients (patients 1, 2, 4, and 6). One of the two patients with normal plasma 25-OHD concentrations was under vitamin D therapy (Patient 7). According to the BMD assessment, four patients who were found to have Z-scores below the expected range for age at the initial examinations still had low Z-scores. In addition, the final BMD Z-score was found below the expected range for age in Patient 5 who had normal Z-scores at basal investigations and had a history of bone fracture despite regular CDCA treatment. No significant difference was observed between plasma 25-OHD levels and BMD Z-scores before or after treatment. The densitometric and biochemical parameters before and after CDCA treatment are defined in Table 2. The statistical evaluations

of 25-OHD and BMD parameters in patients with CTX before and after CDCA treatment are defined in Table 3.

Discussion

Cerebrotendinous xanthomatosis was first reported to be associated with osteoporosis and increased bone fractures by Berginer et al. (13) depending on radiographs of vertebrae and long bones, the results of BMD scores and bone biopsies, osteoporosis was proven in patients with CTX. Subsequently, the relationship between bone metabolism and CTX has been reported in a few studies based on limited data. In our study, we aimed to evaluate the bone mineral profile and BMD changes under CDCA treatment.

The underlying mechanisms of osteoporosis in CTX have not yet been clarified, but to date, some hypotheses have been suggested in medical literature. Decreased concentration of 25-OHD due to defective bile acid synthesis was suggested to be responsible for disturbed bone metabolism (13). Contrarily, other studies reported normal 25-OHD levels in CTX (14, 15). In addition, in a study with decreased plasma 25-OHD concentrations, a remarkable improvement of 25-OHD values was noted under CDCA treatment. However, no significant correlation has been found between ameliorating BMD Z-scores and changes of plasma 25-OHD concentrations during treatment (16). An alternative hypothesis emphasized that decomposition of bile acids in CTX results in decreased intestinal calcium

Table 2. Densitometric and biochemical parameters in patients with CTX before and after CDCA treatment

	Serum calcium (N=8.40–10.20 mg/dL)		Serum 25-OHD		BMD L1-L4 Z-score		BMD Femoral Neck Z-Score	
	Before	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
1/A	9.80	9.90	20.50	26.40	-4.47	-5.37		
2/A	10.93	10	36.50	20.50	-2.50	-2.50	-1.60	-1.60
3/B	9.11	9.24	17.14	31.20	-3.50	-3.20	-1.80	-1.70
4/C	8.83	9.17	28.20	29.80	-2.60	-2.30	-1	-1.40
5/D	9.70	9.40	4.88	14.91	-1.40	-2.80	-2.10	-2.60
6/D	9.80	9.80	28.80	20.87	-0.08	1.15		
7/E	9.50	9.81	8.5	39.6	-1.50	-1.70	-5.40	-3.70

25-OHD: 25-hydroxyvitamin D; BMD: Bone mineral density; CDCA: Chenodeoxycholic acid; CTX: Cerebrotendinous xanthomatosis

Table 3. Statistical evaluation of 25-OHD and BMD parameters in patients with CTX before and after CDCA treatment

Parameter	Before therapy (Mean±SD)	After therapy (Mean±SD)	Sig. (2-tailed)
Serum 25-OHD	20.60±11.40	26.10±8.20	p=0.376
BMD L1-L4 Z-score	-2.29±1.45	-2.38±1.94	p=0.778
BMD Femoral Neck Z-score	-2.45±1.99	-2.10±1.07	p=0.504

25-OHD: 25-hydroxyvitamin D; BMD: Bone mineral density; CDCA: Chenodeoxycholic acid; CTX: Cerebrotendinous xanthomatosis; SD: Standard deviation

absorption. Impaired intestinal calcium transport was proposed to contribute to the osteoporosis in CTX (15).

The efficacy of CDCA treatment in patients with CTX was first reported in 1984 with the observation of clinical improvement in neurologic findings of 17 symptomatic patients with CTX despite diagnostic delay (17). According to recent studies, the initiation of CDCA treatment in earlier stages of the disease has been thought to have a significant role in the improvement or stabilization of neurologic findings (8, 9). Despite the increasing knowledge about the effect of treatment on neurologic findings, therapy responses of skeletal system findings have not been identified comprehensively. In a study with eight patients with CTX aged between 25 and 45 years, plasma Ca, P, ALP, and 25-OHD concentrations and BMD values were recorded before and after CDCA therapy with duration of 6–12 months. Four patients had pathologic radiography findings compatible with osteopenia. Six patients had lower BMD values in comparison with the control group. Following CDCA therapy, a significant increase in BMD values was detected. However, no Z-score determination was made in this study (15). In another study with 11 patients with CTX aged between 11 and 43 years, measurement of plasma 25-OHD concentrations and BMD values before and after CDCA treatment was recorded. In the initial examinations, five patients had BMD values below the expected range. One of these five patients was in the pediatric population. Four patients had vitamin D deficiency and five patients had vitamin D insufficiency. None of the patients had a history of bone fracture nor were they taking calcium or vitamin D replacement. Following CDCA therapy with duration of approximately 30 months, a significant increase in BMD values and serum 25-OHD concentrations were observed. A negative correlation was found between the age at diagnosis and femoral BMD changes after therapy (r=-0.64, p<0.05). Elevation of 25-OHD concentrations was found to be related with improvement of vitamin D intestinal absorption secondary to bile acid restoration. It was suggested that low bone mass was the main problem in CTX that would respond to CDCA treatment (16).

In our study, both vitamin D deficiency and low bone mass for age were detected in patients with CTX. Chenodeoxycholic acid treatment was not found to be effective on densitometric and biochemical parameters of bone mineral metabolism statistically. Normalization of plasma cholestanol levels after CDCA treatment was accepted as biochemical response of the therapy and was achieved in all patients. According to the mean age at diagnosis and initiation of therapy in our patients, it was shown that CDCA was started during earlier stages of the disease. However, according to our data, it may be suggested that other medical treatments were needed to ameliorate skeletal system findings in addition to CDCA treatment.

The first limitation of our study is the small sample size, which results from the fact that CTX is a rarely seen, inherited metabolic disease. The other limitation is the lack of a control group. CDCA therapy was initiated following the diagnosis in all patients because CTX was accepted to be a treatable disorder. As a result, it could not be possible to compose a control group due to ethical considerations.

Conclusion

Our study confirms the presence of low bone mass and vitamin D deficiency in patients with CTX. Skeletal system manifestations as bone fractures are considered as an important factor that impairs quality of life accompanied by neurologic findings. The effect of CDCA treatment on bone metabolism is still not clarified in detail. Further studies with large sample sizes should elucidate the necessity of additional medical treatment as a part of standard therapy to improve skeletal system findings in CTX.

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