JOURNAL OF
CONTEMPORARY MEDICINE

DOI:10.16899/jcm.1296330
J Contemp Med 2023;13(4):652-656

Original Article / Orijinal Araştırma

Changes in the Ubiquitination System in Children with Cerebral Palsy

Serebral Palsili Çocuklarda Ubiqütinasyon Sisteminde Değişiklikler

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Abstract

Aim: We aimed to investigate the levels of Ubiquitin Carboxy Terminal Hydrolase-L1 enzyme (UHC-L1), Transactive Response DNA Binding Protein-43 (TDP-43) and Cullin-3 in peripheral blood associated with ubiquitination processes in children with cerebral palsy (CP).

Material and Method: We included 50 children with CP in the first patient group. In the control group, there were 30 healthy children who were matched with the patient groups in terms of age and gender. We also recorded risk factors for CP, CP type, botox application, orthosis use, maternal age at birth, and additional problems. Patients aged 6-10 years, diagnosed with CP, without genetic, metabolic disease or mental retardation history were included in this study.

Results: There were 32 female and 18 male patients in the CP group, while there were 19 female and 11 male volunteers in the control group. Maternal age was significantly higher in the CP group (p=0.002). In our study, as a result of the comparison between the control group and the CP group in terms of UCH-L1, TDP-43 and Cullin 3 levels; the levels of UCH-L1 (p=0.048), TDP-43 (p=0.028) and Cullin 3 (p=0.042) in the CP group were found to be statistically significantly lower than the levels of the control group.

Conclusion: The low serum concentrations of UCHL-L1, Cullin 3 and TDP-43 molecules in the CP group and the statistically positive correlation of these molecules with each other may help to understand the neuronal pathophysiology after disruption of the ubiquitination system.

Keywords: Ubiquitin Carboxy Terminal Hydrolase-L1 enzyme, Transactive Response DNA Binding Protein-43, Cullin-3, cerebral palsy

Öz

Amaç: Serebral palsili (SP) çocuklarda ubikitinasyon süreçleri ile ilişkili periferik kanda Ubiquitin Karboksi Terminal Hidrolaz-L1 enzimi (UHC-L1), Transaktif Yanıt DNA Bağlayıcı Protein-43 (TDP-43) ve Cullin-3 düzeylerini araştırmayı amaçladık. .

Gereç ve Yöntem: Birinci grubuna SP'li 50 hasta dahil edildi. Kontrol grubunda yaş ve cinsiyet açısından hasta grupları ile eşleşen 30 sağlıklı çocuk vardı. SP, SP tipi, botoks uygulaması, ortez kullanımı, annenin doğum yaşı ve ek sorunlar için risk faktörlerini kayddildi. Bu çalışmaya 6-10 yaş arası, SP tanısı almış, genetik, metabolik hastalık veya mental retardasyon öyküsü olmayan hastalar dahil edildi.

Bulgular: SP grubunda 32 kadın ve 18 erkek hasta bulunurken, kontrol grubunda 19 kadın ve 11 erkek gönüllü vardı. Anne yaşı SP grubunda anlamlı olarak yüksekti (p=0,002). Çalışmamızda kontrol grubu ile SP grubunun UCH-L1, TDP-43 ve Cullin 3 düzeyleri açısından karşılaştırılması sonucunda; SP grubunda UCH-L1 (p=0,048), TDP-43 (p=0,028) ve Cullin 3 (p=0,042) düzeyleri kontrol grubuna göre istatistiksel olarak anlamlı düşük bulundu.

Sonuç: SP grubundaki UCHL-L1, Cullin 3 ve TDP-43 moleküllerinin düşük serum konsantrasyonları ve bu moleküllerin birbirleriyle istatistiksel olarak pozitif korelasyonu, ubiquitination sisteminin bozulmasından sonra nöronal patofizyolojinin anlaşılmasına yardımcı olabilir.

Anahtar Kelimeler: Ubiquitin Karboksi Terminal Hidrolaz-L1 enzimi, Transaktif Yanıt DNA Bağlayıcı Protein-43, Cullin-3, serebral palsi

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Received *(Geliş Tarihi)*: 12.05.2023 **Accepted *(Kabul Tarihi)*:** 07.06.2023

INTRODUCTION

The ubiquitin-proteasome system is an important proteolytic pathway in eukaryotic cells. The ubiquitin-proteasome system is involved in numerous important processes required for cellular homeostasis, such as cell cycle regulation, apoptosis, receptor signaling, endocytosis, and others.[1,2] Cell growth and proliferation; It is controlled by ubiquitin-mediated degradation of tumor suppressors, protooncogenes and signal transduction components.[3,4] As a natural consequence of these, disorders in one or more of the ubiquitin-proteasome system components are considered to be one of the important causes of human diseases.[5,6]

Cerebral Palsy (CP) is the most common pediatric neurological disorder that occurs in the developing fetal or infant brain and is characterized by limitation due to developmental movement and posture disorders. CP is a lifelong neurodevelopmental disease that begins in early childhood.[7,8] In clinical and preclinical studies, neurodegenerative diseases have higher oxidative stress marker levels in the brain and peripheral tissues; antioxidant defense marker levels were shown to be lower. This indicates that oxidative stress is an important factor potentially playing a role in the pathogenesis of many different diseases affecting the brain such as mitochondrial disorders, cerebral ischemia and epilepsy.[9,10] It has been proven that oxidative stress markers increase and antioxidants decrease in CP, and as a result, the oxidative/antioxidant balance shifts to the oxidative side in children with cerebral palsy.[11,12] We think that disruptions in ubiquitination processes, which are caused by oxidative stress and ischemia, may also occur in children with cerebral palsy. In order to investigate this hypothesis, we aimed to investigate the levels of Ubiquitin Carboxy Terminal Hydrolase-L1 enzyme (UHC-L1), Transactive Response DNA Binding Protein-43 (TDP-43) and Cullin-3 in peripheral blood associated with ubiquitination processes in children with cerebral palsy.

MATERIAL AND METHOD

This study was planned retrospectively. The study was carried out with the permission of Hitit University Medical Faculty Clinical Researchs Ethics Committee (Date: 05.07.2022, No: 2022/17). The sample was selected from children between the ages of 6-10 who applied to our physical therapy and rehabilitation outpatient clinic. Accordingly, we included 50 children with CP in the first patient group. In the control group, there were 30 healthy children who were matched with the patient groups in terms of age and gender. We also recorded risk factors for CP (premature, prolonged delivery, etc.), CP type, botox application, orthosis use, maternal age at birth, and additional problems. Informed consent was obtained from parents and children for voluntary participation in the study. Patients aged 6-10 years, diagnosed with CP, without genetic, metabolic disease or mental retardation history were included in this study.

Blood Collection

The blood samples of the children with cerebral palsy and the control group were taken into 8 mL capacity clot activator tubes by the blood collection nurse in our polyclinic between 08.00 and 10.00 after 12 hours of fasting. The blood, which was kept in tubes with clot activator at room temperature for half an hour, was centrifuged at 4,000 g for 10 minutes and then 4 mL of serum was obtained. Serum separated into Eppendorfs were stored at -70°C until analysis.

Biochemical Measurements

Measurement serum levels of Cullin-3, UCHL1 and TDP-43 were determined with enzyme linked immunosorbent assay method (SUNRED Biotechnology CO. LTD China; catalog number is 201-12-3552 for Cullin-3, catalog number is 201-12-2329 for UCHL1, and catalog number is 201-12-0334 for TDP-43, according to the manufacturer's instructions. These kits use a double-antibodys and wich ELISA to assay the level of Cullin-3, UCHL1 and TDP-43 in samples. Briefly, samples were added to wells which were pre-coated with monoclonal antibody and incubated; then, antibodies labeled with biotin were added, and combined with Streptavidin-HRP to form immune complex; then incubation and washing were carried out. Then chromogen solutions were added, and at the effect of stop solution, the color finally became yellow. We measured the optical density of each well under 450 nm wave length within 10 minutes after having added stop solution. According to standard concentrations and corresponding optical density values, we calculated the linear regression equation of the standart curve and we determined Cullin-3, UCHL1 and TDP-43 concentration of samples.

Statistical Methods

Frequency analysis was used for nominal and ordinal parameter description; mean and standard deviation were used for scale parameter description. Chi-Square, Likelihood Ratio and Fischer's Exact test were used for differences between categorical variables. Kolmogorov Smirnov test was used for normality test of scale parameters. Kruskal Wallis and Mann Whitney U tests were used for non-normally distributed variables. Independent Samples t-test and one Way ANOVA were used for normally distributed variables. Spearman's eho correlation analysis was used for relationship analysis. Multinominal logistic regression was used for multivariate analysis. All analysis was performed at SPSS 17.0 for windows, at 95% confidence interval and 0.05 significance level.

RESULTS

In our study, there were 32 female and 18 male patients in the CP group, while there were 19 female and 11 male volunteers in the control group. There was no significant difference between the groups in terms of age and gender (p=0.110, p=0.102). More spastic diplegic type (42%) pattern was observed in the CP group. Maternal age was significantly higher in the CP group (p=0.002). In our study, as a result of

the comparison between the control group and the CP group in terms of UCH-L1 levels; The levels of UCH-L1 (p=0.048), TDP-43 (p=0.028) and Cullin 3 (p=0.042) in the CP group were found to be statistically significantly lower than the levels of the control group (**Table 1**). In the correlation results, a positive correlation was observed between UCH-L1 and TDP-43 and Cullin-3 (**Table 2**).

Table 1. Baseline and research parameter differences between groups

	CP (n=50)	Control (n=30)	p value
Age, mean ± SD	8.00±1.66	8.32±1.36	0.110[a]
Gender, n (%)			
Female	32 (64.0)	19 (63.3)	0.102[b]
Male	18 (36.0)	11 (36.7)	
CP Type, n (%)			
Spastic tetraplegic	15 (30.0)		
Spastic diplegic	21 (42.0)		
Hypotonic	5 (10.0)		
Ataxic	4 (8.0)		
Diskinetic	5 (10.0)		
Risk factor, n (%)			
Premature	38 (76.0)		
Prolonged birth	8 (16.0)		
Postnatal throid	4(8.0)		
Mental retardation, n (%)	12 (24.0)		
Hearing disorder, n (%)	9 (18.0)		
Vision defect, n (%)	20 (40.0)		
Epilepsy, n (%)	28 (56.0)		
Device usage, n (%)			
None	10 (20.0)		
SOLID AFO	33 (66.0)		
KAFO	4 (8.0)		
PAFO	3 (6.0)		
Maternal age	31.11±4.89	26.54±4.39	0.002[c]
Cullin-3	6.17±4.17	6.33±5.20	0.042[a]
UCH-L1	4.28±2.99	5.56±4.93	0.048[a]
TDP-43	1077.38±769.39	1085.59±623.26	0.028[a]

a. Kruskal Wallis Test, b. Chi-Square Test, c. One Way ANOVA, d Independent Samples t-test, SD: Standard Deviation, CP: Cerebral palsy, AFO: Ankle foot orthosis, KAFO: Knee-ankle-foot orthosis, UCH-L1: Ubiquitin Carboxy Terminal Hydrolase-L1 enzyme, TDP-43: Transactive Response DNA Binding Protein-43

Table 2: Spearman's rho correlation analysis between UCH-L1, Cullin-3 and TDP-43 for CP group

UCH-L1	CP (n=50)
Cullin 3	0.426*
TDP 43	0.463*

*p<0.05, UCH-L1: Ubiquitin Carboxy Terminal Hydrolase-L1 enzyme, TDP-43: Transactive Response DNA Binding Protein-43, CP: Cerebral palsy

DISCUSSION

In our study, as a result of the comparison of the control group and the CP group in terms of UCH-L1, TDP-43 and Cullin 3 levels; It was found that the levels of all three molecules in the CP group were statistically significantly lower than in the control group.

Ubiquitination, the covalent attachment of ubiquitin to a target protein, regulates most cellular processes and is involved in several neurological disorders.[13,14] The process of ubiquitination is reversible and the reverse process is called deubiquitination which is accomplished by deubiquitinating enzymes.[15] The UCH-L1 molecule is highly and specifically expressed in neurons and plays a role in the ubiquination process of proteins to be degraded via the proteasomal pathway, and plays a role in clearing oxidized or misfolded proteins that occur in normal and pathological processes.[14,16] In the study conducted by Linrui et al.[17] in Parkinson's patients, they found a low level of UCH-L1 in the patient group and associated it with cognitive dysfunction. UCH-L1 concentration has been reported to be elevated in a number of neurological diseases including aneurysmal subarachnoid hemorrhage, traumatic brain injury, stroke and neonatal hypoxic-ischemic encephalopathy.[18-23] In the studies performed, it was observed that the levels of UCH-L1 in the cerebrospinal fluid and plasma after the seizure were increased in patients with epileptic disorders, and when the patients with recurrent seizures were compared with the patients with a single seizure, the UCH-L1 levels were higher in the group of patients with recurrent seizures than in the other group.[24,25] There is only one study in the literature measuring the level of UCH-L1 in patients with CP. In the clinical efficacy study of scalp acupuncture in 45 patients with spastic CP, researchers concluded that scalp acupuncture can effectively treat spastic CP, improve cerebral hemodynamics and gross motor function, reduce muscle tension and spasticity, and improve daily living ability. In this study, they found the UHC-L1 level to be significantly higher in the treatment group compared to the control group.[26] Similar to this study, in our study, the UCH-L1 level in patients with CP was found to be significantly lower than in the healthy group. This result may show us that there is a disruption in the ubiqunitation system in SP.

TDP-43 is a 43 kDa heterogeneous nuclear ribonuclear protein (hnRNP) composed of 414 amino acids.[27] TDP-43 is synthesized in the cytoplasm and shuttled into the nucleus where it primarily resides to perform its physiological functions.[28] Pathological forms of TDP-43 were first identified in 2006 when Amyotrophic Lateral Sclerosis and Frontotemporal Lobar Degeneration patients were found to have tau-negative, ubiquitin-positive cytoplasmic inclusion bodies. The pathogenic mechanisms in these brains ultimately result in TDP-43 depletion from the nucleus, TDP-43 mislocalization into the cytoplasm, and the formation of insoluble aggregates that contain TDP-43 with multiple posttranslational modifications including ubiquitination, phosphorylation, and truncation. These TDP-43 inclusion bodies found in neurons, neuronal cell processes, and glia are now characteristic of the pathology in the most common forms of Amyotrophic Lateral Sclerosis and Frontotemporal Lobar Degeneration.[29,30] In the study of Meneses et al30, they found that TDP-43 pathology is a risk factor for the development of Alzheimer's type dementia and that TDP-43 pathology increases the rate of hippocampal atrophy

in AD. Aging is considered a risk factor for developing TDP-43 pathology in neurologically normal individuals. From 286 consecutive autopsy brains, Uchino and coworkers[31] reported that 40% of control elderly individuals (78.5 ± 9.7 years) with minimal senile plaques had TDP-43 pathology. TDP-43-positive individuals were reported to be significantly older than those without TDP-43 pathology from a study investigating TDP-43 in the anterior temporal pole cortex. [32] TDP-43-positive dystrophic neurites have been found in patients with the parkinsonism-dementia complex on Guam. [33] In the literature, we could not find any study conducted in patients with CP with TDP-43. In our results, lower TDP-43 levels in the patient group compared to the health group can be considered as an indicator of the deterioration in the ubiquitination mechanism.

Cullins are proteins that play a role in post-translational modification of proteins, including ubiquitination that confer substrate specificity to multimeric complex of E3 ligases acting as scaffold proteins. So far, seven members of the cullin family of proteins have been identified. Cullin 3 has begun to emerge as a protein involved in the etiopathology of multiple diseases.[34,35] The deregulation of Cullin 3 activity could be a mechanism involved in pathologies mainly associated with oxidative stress and cell cycle deregulation. Therefore, strategies oriented against Cullin 3 activity comprise a high therapeutic potential for regulation of cellular processes related to the development of several pathologies.[35]

Limitation

The biggest limitation of this study is that we looked at the levels of UCH-L1, TDP-43 and Cullin 3 molecules in the cerebrospinal fluid. The reason is that we cannot compare our results with other comparison results, as there is no other study including all parameters.

CONCLUSION

The results obtained from our study, the difference in the molecular levels of the patients with CP and the control group show that the neuronal structure is impaired in CP. The low serum concentrations of UCHL-L1, Cullin 3 and TDP-43 molecules in the CP group and the statistically positive correlation of these molecules with each other may help to understand the neuronal pathophysiology after disruption of the ubiquitination system. We believe that these results will contribute to the literature and will be a good reference for future studies. In order for these parts to be used in CP pathogenesis and treatment approaches, arrangements with more patient groups are needed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Hitit University Medical Faculty Clinical Researchs Ethics Committee (Date: 05.07.2022, No: 2022/17).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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