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Original Article

Isocitrate Dehydrogenase 1 and 2 Mutations in Pediatric Neuroblastoma Patients

Pediatrik Nöroblastom Hastalarında İzositrat Dehidrogenaz 1 ve 2 Mutasyonları

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ABSTRACT

Objective: Neuroblastoma is one of the common tumors of childhood. The demonstration of new factors such as isocitrate dehydrogenase 1 (IDH1) and isocitrate dehydrogenase 2 (IDH2) mutations will be important in the diagnosis and treatment. IDH1 and IDH2 mutations have been found in many types of cancer, such as malignant gliomas, acute myeloid leukemias, chondrosarcoma, and thyroid carcinoma. This study aimed to investigate the presence of IDH1 or IDH2 mutations in patients with neuroblastoma and to determine whether these mutations were different in terms of age, clinical findings, and response to treatment.

Methods: Biopsy specimens of 25 patients with pediatric neuroblastoma patients were evaluated for IDH mutations. The clinical and laboratory features of the patients with/without mutation were retrospectively analyzed from a hospital database.

Results: A total of 25 patients for whom genetic analysis could be performed were included in the study (60% male, n=15). The mean age was 32.2±25.9 months (3 days-96 months). IDH1 mutation was detected in 8 (32%) and IDH2 mutations in 5 (20%) patients. These mutations showed no statistically significant relationship with age, tumor localization, laboratory results, stage, and prognosis. However, in the case of IDH mutation, patients were diagnosed at the advanced stage.

Conclusions: This study demonstrated the relationship between neuroblastoma and IDH mutation for the first time. Because to the fact that the mutation is very heterogeneous, it would be appropriate to conduct a larger series of patients in terms of the impact of the clinical significance of each mutation on the diagnosis and prognosis.

Keywords: Neuroblastoma, isocitrate dehydrogenase mutation, pediatrics

ÖZ

Amaç: Nöroblastom çocukluk çağının sık görülen tümörlerinden biridir. İzositrat dehidrogenaz 1 (IDH1) ve izositrat dehidrogenaz 2 (IDH2) mutasyonları gibi yeni faktörlerin gösterilmesi tanı ve tedavide önemli olacaktır. IDH1 ve IDH2 mutasyonları malign gliomlar, akut miyeloid lösemiler, kondrosarkom ve tiroid karsinomu gibi birçok kanser türünde bulunmuştur. Bu çalışmanın amacı nöroblastom tanılı hastalarda IDH1 veya IDH2 mutasyonlarının varlığını araştırmak ve bu mutasyonların yaş, klinik bulgular ve tedaviye yanıt açısından farklı olup olmadığını belirlemektir.

Yöntemler: Yirmi beş pediatrik nöroblastom hastasının biyopsi örnekleri IDH mutasyonları açısından değerlendirildi. Mutasyonu olan ve olmayan hastaların klinik ve laboratuvar özellikleri hastane veri tabanından retrospektif olarak analiz edildi.

Bulgular: Genetik analiz yapılabilen toplam 25 hasta çalışmaya dahil edildi (%60 erkek, n=15). Ortalama yaş 32,2±25,9 aydır (3 gün-96 ay). Hastaların 8'inde (%32) IDH1 mutasyonu ve 5'inde (%20) IDH2 mutasyonu saptandı. Bu mutasyonlar yaş, tümör lokalizasyonu, laboratuvar sonuçları, evre ve prognoz ile istatistiksel olarak anlamlı bir ilişki göstermemiştir. Ancak IDH mutasyonu durumunda hastalar ileri evrede tanı almışlardır.

Sonuçlar: Bu çalışma, nöroblastom ve IDH mutasyonu arasındaki ilişkiyi gösteren ilk çalışmadır. Mutasyonların oldukça heterojen olması nedeniyle, her bir mutasyonun klinik öneminin tanı ve prognoz üzerindeki etkisi açısından daha geniş bir hasta serisi ile çalışmanın yapılması uygun olacaktır.

Anahtar kelimeler: Nöroblastom, izositrat dehidrogenaz mutasyonu, pediatri

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INTRODUCTION

Neuroblastoma is a common extracranial tumor of childhood and originates from primordial neural crest cells found in the adrenal medulla or sympathetic ganglia. The biological behavior of this tumor varies[1,2]. The demonstration of new factors predicting the biological behavior will be important in the diagnosis and treatment of neuroblastoma. These candidate factors include isocitrate dehydrogenase 1 (IDH1) and isocitrate dehydrogenase 2 (IDH2) mutations. The important metabolic function of IDH1 and IDH2 is its role in the conversion of IDH to alpha ketoglutarate in the krebs cycle[3]. IDH activity in mammalian cells increases in response to oxidative damage. Both enzymes can produce nicotinamide adenine dinucleotide phosphate; therefore, thus the cell is protected against oxidative damage[4]. IDH1 and IDH2 mutations have been found in many cancer types such as acute myeloid leukemias (AML), intrahepatic cholangiocarcinoma, chondrosarcoma, thyroid carcinoma and malignant glioma. Mutant IDH proteins acquire a neomorphic enzyme activity that causes the production of D-2-hydroxyglutarate (D2HG). In the physiological conditions, the accumulation of D2HG in the cell is restricted due to the D2HG dehydrogenase enzyme. However, due to the neomorphic activity of the mutant IDH, the intracellular level of D2HG increased to supraphysiological levels. As an oncometabolite, D2HG inhibits cellular differentiation by inhibiting alfaketoglutarate-dependent dehydrogenases in the demethylation of histone and deoxyribo nucleic acids. The inhibition of normal cell differentiation causes pathological self-reproduction and malignant transformation of stem cell-like progenitor cells[5,6]. The aim of this study was to investigate the presence of IDH1 and 2 mutations in patients with neuroblastoma and to determine whether these mutations are different in terms of age, clinical findings, and response to treatment.

MATERIALS and METHODS

Patients

In this study, 25 patients with neuroblastoma who had biopsy specimens, were followed-up and treated between 2005-2018 years in the oncology department were included. Informed consent was obtained from the parents of the patients following institutional guidelines for the study sample collection as well as permission for its use in research. All patients were diagnosed according to the American Society of Clinical Oncology diagnostic criteria. Definitive diagnosis requires one of the following conditions: Unequivocal histologic diagnosis from tumor tissue by light microscopy in the setting of increased urine catecholamines or evidence of metastases to the bone marrow on bone marrow aspiration and biopsy in the setting of increased urine catecholamines[7]. Clinical information was collected from the medical records. The birth dates, age of diagnosis, gender, and consanguinity marriage status of the cases who were followed-up and treated were recorded. All patients were questioned for clinical findings of neuroblastoma; abdominal pain, abdominal distension, fatigue, weight loss, anemia, infection, hemorrhage, skin ecchymosis, or skin nodules were recorded.

Assay Results

Ferritin level was measured by the chemiluminometric method using a Siemens Advia Centaur XP device; lactate dehydrogenase (LDH) level was measured with the Beckman Coulter AU5800 device in the central laboratory of our hospital. Also neuron specific enolase (NSE) level in the Synlab Laboratory with a Bosch brand device; vanil mandelic acid (VMA) level in the Deltalab Laboratory by SPLC device was measured. Abdominal and cervical ultrasonographies were performed a radiology specialist with a 7.5-MHz linear probe and a Toshiba Alpro 500 device at our hospital. The abdomen, thorax, and central nervous system tomography images taken with the Somotom Emotion device were examined. The final reports of the biopsy specimens that were previously taken and were evaluated by the specialists in the pathology department in our hospital were examined. The scintigraphy of the patients was performed using a General Electric Millenium brand device and a gamma camera with I123 MIBG at our hospital. The activity involvement was evaluated. The stages of the diagnosis of the patients were determined using the International Neuroblastoma Staging System[8]. The prognosis and survival rates after treatment were evaluated. Biopsy specimens were examined for IDH mutation with molecular genetics methods.

DNA Isolation

DNA isolation was performed using the QIAamp DNA FFPE Tissue Kit (Qiagen Inc) from the samples and adhered to the manufacturer's protocol. For the study, the 4th exon containing the IDH1 gene codon 132 and the 4th exon containing the IDH2 gene codon 172 were selected. For the *IDH2* gene, the entire 4th exon was designed, and the *IDH1* gene was primed after the 54th codon. Although the focal point was 132 codon for the *IDH1* gene and 172 for the *IDH2* gene, all of the amplified regions were analyzed. The amplification of the regions to be studied was obtained using the polymerase chain reaction (PCR) of the designed primers. The reaction

was monitored by 2% agarose gel electrophoresis. Two amplicons obtained for each sample were mixed in such a way that each region was represented at an approximately equal rate, considering the reaction efficiency. The generated PCR pool was purified using the NucleoFast® 96 PCR kit (MACHEREY-NAGEL GmbH). The purified sample was quantified by a spectrophotometer (Nanodrop N1000, Thermo Inc.). The amount of DNA was standardized to be 0.2 ng/iu. The standardized sample was made ready for the next generation of sequencing using illumina's NexteraXT sample preparation kit. The alignment of the readings was performed using Miseq Reporter (illumina Inc.) Inc. Analysis of the aligned BAM files was performed using the IGV 2.3 (Broad Institute) software. The threshold value was set to 0.03 so that low-rate somatic mutations could be visualized during IGV analysis.

This study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by University of Health Sciences Turkey, Ankara Children's Health and Diseases Hematology Oncology Training and Research Clinical Research Ethics Committee with approval number 2018-163 (date: 12.11.2018).

Statistical Analysis

In the presentation of descriptive statistic, the data obtained by measurement were expressed as mean ± standard deviation and (minimum-maximum) and categorical data as number (percentage). Cross-table analyses and chi-square tests were used to compare the qualitative characteristics of the groups. The Shapiro-Wilks test was used to determine the normal distribution of numerical measurements in groups. Two groups were compared with t-test in the independent groups and Mann-Whitney U test for those who did not show normal distribution. The IBM SPSS Statistics version 22 package program was used for all statistical analysis. Significance level $p<0.05$ was taken.

RESULTS

Clinical Features

A total of 25 patients for whom genetic analysis could be performed were included in the study (60% male, n=15). The mean age was 32.2±25.9 months (3 days-96 months). Six (30%) patients had consanguinity marriage between their parents (n=20). Primary tumor localization was the abdomen in 24 (96%) patients and the cervical region in 1 (4%) patient. Seven (28%) patients had bone marrow involvement, and 7 (28%) had bone metastasis. The number of patients with orbital involvement was 2 (8%). The mean NSE value was 114.2±115.2 ng/mL (11.6-370), VMA 44.2±44.5 mg/grcre (5.1-153); ferritin 227.6±459.4 ng/mL (3-2013), and mean LDH was 854.2±876.7 IU/L (175-3112). Because of pathological examination, 21 (84%) patients were diagnosed with neuroblastoma and 4 (16%) patients were diagnosed as ganglioneuroblastoma. Seven (28%) patients had stage I, 6 (24%) patients had stage II, 4 (16%) patients had stage III, and 8 (32%) patients had stage IV. Three of the cases (12%) were separated from our hospital to continue their treatment at another center. It was observed that 14 (63.6%) cases had remission and 8 (36.4%) cases died during follow-up and treatment (n=22).

Mutations of Isocitrate Dehyrogenases 1 and 2

IDH1 or IDH2 mutation was detected in 11 (44%) patients. When mutations were evaluated separately, 8 (32%) patients had IDH1 and 5 (20%) had IDH2 mutations. Two (8%) patients had mutations in the *IDH1* and *IDH2* genes together.

Clinical Features According to Isocitrate Dehyrogenase 1/2 Mutations

The clinical characteristics of the study patients stratified by their IDH mutation profile are listed in Table 1. In the univariate analysis, there were no statistical differences in age, gender, consanguinity marriage rates, primary tumor locations, bone marrow and bone metastasis, orbital involvement, mean NSE, VMA, LDH and ferritin values, pathological diagnosis, stages, treatment responses and overall survival rates between patients with or without IDH mutations.

IDH mutations were detected in 7 (46.7%) males and 4 (40%) females (p=0.742). While 3 (27.3%) patients with IDH mutations were diagnosed during the neonatal period, no patient without mutation was diagnosed with neuroblastoma in that period (p=0.060). The data of the consanguinity marriage status between the parents of 20 cases could reach. Although not statistically significant, the rate of consanguineous marriage was higher in the presence of mutations (n=11) than in the absence of mutations (n=9), (44.4% vs. 18.2%, p=0.202). In the case of IDH mutation, the primary tumor localization was abdominal in all patients, whereas this rate was 92.2% in patients without mutation (p=0.366). Bone marrow involvement and bone metastasis were higher in IDH mutation status, albeit nonsignificant statistically (36.4% vs. 21.4%, p=0.409, for both). The orbital involvement was not observed among patients with an IDH mutation (p=0.191). Ten (90.9%) patients with IDH mutation had neuroblastoma and 1 (9.1%) ganglioneuroblastoma, whereas 11 (78.6%) patients without IDH mutation were

diagnosed with neuroblastoma and 3 (21.4%) were ganglioneuroblastoma (p=0.404). In case of mutation, the proportion of patients evaluated as stage V was higher than those without mutation, although not statistically significant (36.4% vs. 28.6%, p=0.850). The remission rate was slightly lower numerically in the case of mutations (60% vs. 66.7%, n=22, p=0.746). When mutations were evaluated separately, the remission rate was lower in IDH1 mutations compared with those without mutation (42.9% vs. 73.3%), again not statistically significant (p=0.166). The 5-year overall and event-free survival rate for all patients was 68%. When evaluated according to mutation status, this rate was slightly lower in patients with IDH mutations compared with patients without mutations, albeit nonsignificant statistically (63.6% vs. 71.4%, p=0.446). The mutation specific clinical data of the patients are summarized in Table 2.

Case 1 with IDH1 and IDH2 mutations together was 6 years and 10 months of age, and she was referred to our hospital due to a mass in the abdominal ultrasound due to recurrent urinary tract infection. The patient who has been evaluated as stage I, is in remission and continues the follow-up. Another patient with IDH1 and IDH2 mutations together was 2 years old boy who had 5 different IDH1 mutations and 2 different IDH2 mutation; he was evaluated as stage IV and he died within two months after the diagnosis. In cases 6 and 7, the same mutation was detected, and V69I mutant protein was produced. A 42-months-old boy patient was evaluated as stage IV. The patient left our hospital at 68th month of treatment. The case 7 case was a 16-days-old girl. The patient was evaluated as stage I and died from a surgical complication related to bleeding while being operated for total excision. Three different mutations were detected in the *IDH2* gene in case 9. This patient was a 3-day-old boy, and he was evaluated as stage I. He has been on the follow-up for more than 10 years after complete excision.

DISCUSSION

In this study, IDH1 and IDH2 mutations were examined in pediatric patients with neuroblastoma. It was investigated whether there was a difference in terms of epidemiological, laboratory, and clinical findings between the groups with and without mutations. Although the IDH1 mutation was detected in 8 (32%) and IDH2 mutations in 5 (20%) patients, these mutations showed no statistically significant relationship with age, tumor localization, laboratory results, stage and prognosis. However, in the case of IDH mutation, patients were diagnosed at the advanced stage.

Nineteen different mutations were detected in our study. Four of the mutations are previously known mutation; however, fifteen of them have been recently described. The previously known mutation frequencies are between 0.1% and 0.001%[9-12].

Postzygotic mosaic mutations occur after conception of the human zygote during the developmental cycle of the fetus. The individual has a mosaic or diversity of mutated and non-mutated cells. The earlier the mutation, the more widespread it is, or, if it occurs at a relatively late stage, it may be limited to a tissue. Although these mutations have been associated with various diseases in the past, their role in neuroblastoma is unclear[13,14]. However, in our study, mutations were examined only in the biopsy materials and tumoral tissue samples of the patients. No additional laboratory tests or other tissue sampling were performed during the study period. Therefore, it is impossible to comment on the presence or absence of mosaicism in patients. However, it would be valuable and meaningful to draw attention to mosaicism in neuroblastoma genetics in a future prospective study.

Male/female ratio was 1.5 in this study. In a previous study, the ratio was found to be 1.2, similar to our ratio[15]. However, there is no statistically significant difference between sex in terms of IDH1 and IDH2 mutations. This shows that IDH1 and 2 mutations have no sex-related predisposition. Additionally, in cancer-related IDH studies in the literature, there was no sex predisposition in these enzyme mutations[16]. The mean age was 32.2±25.9 months in our study. In a previous study, the average age at the diagnosis of neuroblastoma was 22 months[17]. There was no significant difference between age at diagnosis and mutation status. However, mutations may cause age-specific clinical findings when evaluated individually. In our study, it is not possible to show which mutation is related to age due to the small number of cases. The comparison of similar mutations with each other in a larger series will be more accurate.

In a previous study, IDH1 mutation was found to be 3-16% in patients with grade 4 primary glioblastoma and 73-88% in grade 4 secondary glioblastoma[18]. In another study, the IDH1 mutation was found to be 0-8% and IDH2 mutation was found in 0-15% of patients with AML[19]. Also, in a study performed in patients with AML, the IDH1 mutation was found to be 6-16% and the IDH2 mutation was 8-19%[20]. Although IDH2 mutations are relatively more common in AML, IDH1 mutations are more common than IDH2 mutations in other types of cancers[21]. In our study, IDH1 mutation is more common in accordance with the literature.

Table 1. The clinical features of patients.

No of	Age of		Consang	IDH mutations						Primary tumor
Patient	Diagnosis	Gender	Marriage	Gene	Exon	Codon	Position	cDNA	Protein	Localization
1	82	F	No	*IDH 1*	4	63	1	c.187G>A	A63T	Abdomen
				IDH 2	4	132	2	c.395G>A		
2	24	M	Yes	*IDH 1*	4	77	1	c.229A>G	T77A	Abdomen
				IDH 1	4	89	1	c.265A>G	K89E	
				IDH 1	4	101	1	c.301A>G	N101D	
				IDH 1	4	121	2	c.362T>C	V121A	
				IDH 1	4	133	2	c.398A<G	H133R	
				IDH 2	4	136	2	c.407A>G	N136S	
				IDH 2	4	158	2	c.473C>A	P158Q	
3	1	M	No	*IDH 1*	4	137	1	c.409G>A	D137N	Abdomen
4	34	M	Yes	*IDH 1*	4	134	2	c.401C>T	A134V	Abdomen
5	24	F	No	*IDH 1*	4	134	1	c.400G>A	A134T	Abdomen
6	42	M	No	*IDH 1*	4	69	1	c.205G>A	V69I	Abdomen
7	0.5	F	-	*IDH 1*	4	69	1	c.205G>A	V69I	Abdomen
8	60	F	Yes	*IDH 1*	4	125	1	c.373G>A	V1125I	Abdomen
9	0.1	M	No	*IDH 2*	4	159	1	c.475C>T	R159C	Abdomen
				IDH 2	5	197	2	c.590C>T	T197I	
				IDH 2	5	198	2	c.593C>T	P198L	
10	3	M	Yes	*IDH 2*	4	155	2	c.464A>G	K155R	Abdomen
11	56	M	-	*IDH 2*	4	137	1	c.409G>A	G137R	Abdomen
12	57	M	No	-	-	-	-	-	-	Abdomen
13	13	M	No	-	-	-	-	-	-	Abdomen
14	54	M	-	-	-	-	-	-	-	Abdomen
15	36	F	Yes	-	-	-	-	-	-	Cervical
16	36	F	No	-	-	-	-	-	-	Abdomen
17	36	F	No	-	-	-	-	-	-	Abdomen
18	11	M	No	-	-	-	-	-	-	Abdomen
19	22	F	No	-	-	-	-	-	-	Abdomen
20	6	M	-	-	-	-	-	-	-	Abdomen
21	60	M	-	-	-	-	-	-	-	Abdomen
22	18	M	-	-	-	-	-	-	-	Abdomen
23	24	F	Yes	-	-	-	-	-	-	Abdomen
24	12	M	No	-	-	-	-	-	-	Abdomen
25	96	F	No	-	-	-	-	-	-	Abdomen

"F" represents female and "M" represents male. Age at diagnosis is given in months. IDH: Isocitrate dehydrogenase, NSE: Neuron-specific enolase, VMA: Vanil mandelic acid, LDH: Lactate dehydrogenase, NBL: Neuroblastoma

Table 1. Continued

Bone marrow	Bone	Orbital	NSE	VMA	Ferritin	LDH	Pathological		Survival
Involvement	Metastasis	Involvement	mg/mL	mg/grcre	ng/mL	IU/L	Subgroup	Stage	Condition
No	No	No	12	5	15	223	ganglionNBL	1	Alive
Yes	Yes	No	-	-	169	2397	NBL	IV	Deceased
Yes	No	No	49.7	80.1	283.1	287	NBL	III	Alive
No	No	No	109	-	37.2	1281	NBL	II	Alive
Yes	Yes	No	90	-	100	1178	NBL	Deceased	Death
Yes	Yes	No	-	-	669	944	NBL	IV	-
No	No	No	31	5	-	-	NBL	Deceased	Death
No	Yes	No	132		11	235	NBL	Deceased	Death
No	No	No	-	-	-	-	NBL	I	Alive
No	No	No	-	-	237	345	NBL	II	Alive
No	No	No	42	153	92	-	NBL	II	Alive
No	No	No	11	5	11	235	ganglioNBL	I	Alive
No	No	No	13	11	4	175	NBL	I	Alive
Yes	No	No	-	-	580	3112	NBL	Deceased	Death
No	No	No	16	35.5	3	187	ganglioNBL	II	-
Yes	Yes	Yes	370	97.4	95.2	896	ganglioNBL	Deceased	Death
No	Yes	Yes	122.9	80	25	341	NBL	IV	Alive
No	No	No	-	-	65.2	838	NBL	II	Alive
No	No	No	24	13	9	248	NBL	I	Alive
No	No	No	50.1	59.5	29	246	NBL	I	Alive
Yes	Yes	No	180	18	-	-	NBL	Deceased	Death
No	No	No	230	42.8	-	-	NBL	Deceased	Death
No	No	No	370	-	2013	2395	NBL	III	Alive
No	No	No	-	-	3	667	NBL	II	Alive
No	No	No	200	11	-	-	NBL	III	-

Table 2. Clinical and laboratory features of 25 patients with neuroblastoma and comparison of patients with or without IDH mutation.

	All 25 patients	With IDH mutation	Without IDH mutation	
	25 (100%)	11 (44%)	14 (56%)	
Patient characteristics				p-value
Age: month (min-max)	32.2 (0.1-96)	29.6 (0.1-82)	34.3 (6-96)	0.223
Gender				
Male n (%)	15 (60%)	7 (46.7%)	8 (53.5%)	0.742
Female n (%)	10 (40%)	4 (40%)	6 (60%)	
Consang. marriage (n=20) n (%)	6 (30%)	4 (44.4%)	2 (18.2%)	0.202
Tumor localization n (%)				
Abdomen	24 (96%)	11 (100%)	13 (92.9%)	0.366
Cervical	1 (4%)	0 (0%)	1 (7.1%)	
Bone marrow invol. n (%)	7 (28%)	4 (36.4%)	3 (21.4%)	0.409
Bonee metastasis n (%)	7 (28%)	4 (36.4%)	3 (21.4%)	0.409
Orbital involvement n (%)	2 (8%)	0 (0%)	2 (14.3%)	0.191
NSE ng/mL mean (min-max)	114.2 (11.6-370.0)	31.2 (12.8-49.7)	86.9 (11.6-370)	0.665
VMA mg/mL mean (min-max)	44.2 (5.1-153.0)	42.7 (5.4-80.1)	43.3 (5.8-97.4)	0.900
Ferritin ng/mL mean (min-max)	227.6 (3.0-2013.0)	149.2 (15.3-283.1)	25.5 (3.4-95.2)	0.639
Pathological subgroup n (%)				
NBL	21 (84%)	10 (90.9%)	11 (78.6%)	0.404
GanglioNBL	4 (16%)	1 (9.1%)	3 (21.4%)	
Stage n (%)				
I	7 (28%)	3 (27.3%)	4 (28.6%)	0.850
II	6 (24%)	3 (27.3%)	3 (21.4%)	
III	4 (16%)	1 (9.1%)	3 (21.4%)	
IV	8 (32%)	4 (36.4%)	4 (28.6%)	
Survival condition (n=22) n (%)				
Alive	14 (63.6%)	6 (60%)	8 (66.7%)	0.746
Death	8 (36.4%)	4 (40%)	4 (33.3%)	
5 years OS and EFS (%)	68%	63.6%	71.4%	

IDH: Isocitrate dehydrogenase, NSE: Neuron-specific enolase, VMA: Vanil mandelic acid, LDH: Lactate dehydrogenase, NBL: Neuroblastoma, OS: Overall survival, EFS: Event-free survival, Min-max: Minimum-maximum

In two of the cases, mutations were detected in both *IDH1* and *2* genes. One patient had a mutation in the *IDH1* and *2* genes, while in the other patient, 5 different mutations were detected in the *IDH1* gene and 2 different mutations in the *IDH2* gene. The case with a high number of mutations was diagnosed at an earlier age and evaluated as stage IV. We may speculate that, as the number of mutations increases, the tumor may be more aggressive, and the disease may be more common at an earlier age. The same mutations were observed in the same region in the *IDH1* gene in two cases, and the same mutant proteins were produced. When the stages of these patients were compared, it was seen that the stages of the patients were 1 and 4. While the patient with stage IV died, the stage I patient was diagnosed during the neonatal period because of a mass in the antenatal ultrasonography. The importance of early diagnosis is noteworthy, even if an aggressive mutation is encountered here. In the case of IDH mutation, the proportion of patients evaluated as stage V was higher than those without mutation in our study. The values were not statistically significant, but it was noteworthy that in the group with IDH1 mutation, the proportion of advanced stage disease was higher. In the presence of the IDH1 mutation, this mutation can be thought to cause cancerogenesis and lead to a more advanced stage of the disease. However, to achieve healthier results, mutation -specific evaluations should be performed.

The rate of 5-year overall survival was 82% in patients with neuroblastoma[22]. In our study, the 5-year overall and event-free survival rate of all patients was 68%. This rate was slightly lower in patients with IDH mutations compared to patients without mutations, albeit nonsignificant statistically. This may be because the stages of patients with IDH1 mutations were more advanced. In a previous study, patients with low-grade glioblastoma had a longer survival time in the presence of IDH mutation; therefore, IDH mutations may be a good prognostic factor for the glioblastoma[23]. In another study, D2HG caused by mutant IDH1 and 2 caused cancer onset; however, it inhibits proliferation and cell survival in cancer cells with high fat mass and obesity-associated levels[24]. In our cases, the IDH1 mutation may have an increased effect on mortality because of the more proliferative behavior of the tumor on the MYC pathway.

Determination of the gene expression in mutated and non-mutated tumors of specific tumor types may provide an idea about the mechanism of the mutagenesis of IDH mutation[25]. Such studies may clarify the functional role of mutations and provide information to guide neuroblastoma management. Determining whether the status of IDH for any type of tumors involving these mutations is an independent prognostic factor may be a guide to the clinical management of a lethal group of cancer. IDH1 and 2 genetic mutations can be used as determinants for the subset of peripheral neuronal tumors[26]. It may also be useful when there is insufficient material for histopathological analysis. Determination of D2HG levels associated with IDH1 and 2 mutations, especially high in serum or urine, may simplify the diagnosis and management of patients with neuroblastoma[27]. Additionally, even in the absence of a diagnosis of neuroblastoma, examination of D2HG levels at the cell or tissue level in individuals with IDH1 or IDH2 mutations can be quite striking for a possible carcinogenesis.

CONCLUSION

The functional significance of IDH mutations in pediatric patients with neuroblastoma is unknown. The evaluation of the characteristics of these mutations will provide an understanding of neuroblastoma biology and will contribute to the diagnosis and treatment of neuroblastoma. The IDH mutations were not found to be prognostic in any parameter in this study; however, it may be worth investigating in a larger cohort.

Ethics

Ethics Committee Approval: The study protocol was approved by University of Health Sciences Turkey, Ankara Children's Health and Diseases Hematology Oncology Training and Research Clinical Research Ethics Committee with approval number 2018-163 (date: 12.11.2018).

Informed Consent: Informed consent was obtained from the parents of the patients following institutional guidelines for the study sample collection as well as permission for its use in research.

Peer-review: Externally and internally peer-reviewed.

Author Contributions

Surgical and Medical Practices: G.S., S.Y., C.B., N.Y., A.F., S.T., B.K.B., E.C., N.E., S.A.T., A.U.E., Concept: E.L., G.S., S.Y., C.B., A.F., S.T., A.U.E., Design: E.L., G.S., S.Y., C.B., A.F., S.T., A.U.E., Data Collection and/or Processing: E.L., G.S., S.Y., C.B., N.Y., A.F., S.T., B.K.B., E.C., N.E., S.A.T., A.U.E., Analysis and/or Interpretation: E.L., G.S., N.Y., S.T., B.K.B., E.C., N.E., S.A.T., Literature Search: E.L., G.S., S.Y., B.K.B., Writing: E.L., G.S., B.K.B.

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