Radiological and Genetic Evaluation in Hypotonic Infants

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ABSTRACT

Objective: To investigate the importance and diagnostic yield of genetic and radiological evaluations in children with hypotonia.
Study Design: Comparative observational study.
Place and Duration of the Study: Department of Pediatrics Neurology, Namik Kemal University, Tekirdag, Turkey, between 2019 and 2022.
Methodology: Patients' medical histories, laboratory results, radiological examinations, and genetic tests, if any, were obtained retrospectively from the patients' clinic files. Children with hypotonia detected since the infantile period and who were on regular follow-up were included in the study. Patients who lost the follow-up were excluded.
Results: Out of one hundred and seventy patients, 61.8% (n=105) were boys and 38.2% (n=65) were girls. The admission age of the patients ranged from 1 to 121 months; the mean age at presentation was 13.52±17.35 months. Hypotonia was central in 85.3% (n=145), peripheral in 12.4% (n=21), and mixed in 2.3% (n=4). Cerebral palsy was the predominant, non-genetic clinical cause of hypotonia (n=66, 39%). Brain magnetic resonance imaging (MRI) was normal in 48.2% (n=82). The most common MRI abnormality was periventricular leukomalacia in 15.9% (n=27). Sixty-five (38.2%) patients were diagnosed genetically. More than half of the patients with a genetic diagnosis were diagnosed by whole exome sequencing (WES).
Conclusion: Brain MRI is the first choice for the patients with central hypotonia. Patients who cannot be diagnosed with clinical findings and brain MRI should undergo WES. This is helpful for the long-term prognosis and management.

Key Words: *Hypotonia, Whole exome sequencing, Magnetic resonance, Spinal muscular atrophy, Cerebral palsy.*

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INTRODUCTION

Hypotonia refers to a decreased muscle tone in arms, trunk, or cranial muscles.[1-3] Hypotonia may be associated with many disorders originating from neuromuscular, genetic, central nervous system, soft tissue or inherited metabolic disorders. But sometimes, the aetiology cannot be found. Although it is difficult to define the underlying aetiology in hypotonic infants, it is necessary to know the aetiology for prognosis prediction and treatment, if any.

Although there are many genetic, radiological, and biochemical tests that can be done to determine the aetiology of hypotonia, the diagnosis of hypotonia in an infant can only be made by a physical examination. History-taking and physical examination should be supported by the laboratory tests and neuroimaging methods. However, diversity may require a genetic diagnosis.

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The aim of this study was to investigate the importance and diagnostic yield of genetic and radiological evaluations in children with hypotonia.

METHODOLOGY

Children referred to the pediatric neurology outpatient clinic at Namik Kemal University, Tekirdag, Turkey, between 2019 and 2022 with the diagnosis of hypotonia were restrospectively reviewed. Patients whose hypotonicity was detected since the infantile period and whose follow-up was continued regularly were included in the study. Patients without follow-up were excluded.

The patients' examination findings, medical histories, laboratory results, radiological reports and genetic tests, if any, were obtained retrospectively from the patient files.

All of the patients were evaluated by a paediatric neurologist at the first admission and were divided into three main groups as central (n=145), peripheral (n=21), and mixed type (n=4), according to the examination findings.

The laboratory tests performed on patients for aetiological evaluation are; plasma electrolytes, liver and kidney function tests, creatine kinase, thyroid function tests, urine and plasma amino acids, Tandem MS, very long chain fatty acids (VLCFA), urine organic acids, lactate, and ammonia. Electromyography and

nerve conduction studies were performed in patients with peripheral and mixed type hypotonia, and brain MRI was performed in all patients.

All patients were evaluated by a medical geneticist, and in the light of clinical findings, starting with the basic tests such as karyotype to advanced genetic analyses such as array CGH and whole exome sequencing were planned, respectively.

Magnetic resonance imaging (MRI) of the patients were evaluated. The frequency and variety of pathologies were evaluated by listing the detected radiological pathologies.

An approval was taken from the local ethics committee (Approval number: 2022.173.09.20, Namik Kemal University, Tekirdag, Turkey).

NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) programme was used for the statistical analysis. The descriptive statistical methods (mean, standard deviation, median, first quartile, third quartile, frequency, percentage, minimum, maximum) were used while evaluating the study data. Pearson Chi-square test, Fisher's exact test, and Fisher-Freeman-Halton exact test were used to compare qualitative data. Statistical significance was accepted as $p<0.05$.

RESULTS

A comparison of descriptive characteristics, perigestational history, laboratory studies and final diagnosis by types of hypotonia and presence of genetic diagnosis are shown in Table I.

The study was conducted with a total of 170 patients, 38.2% (n=65) females and 61.8% (n=105) males who were referred to the paediatric neurology outpatient clinic with the diagnosis of hypotonia between 2019 and 2022. The admission age of the patients participating in the study ranged from 1 to 121 months; the mean age at presentation was 13.52±17.35 months. When the age groups at admission were examined, 66.5% (n=113) of the patients was aged under 1 year, 31.2% (n=53) was between 1 and 5 years, and 2.4% (n=4) was over the age of 5 years.

While 85.3% (n=145) of the patients had central hypotonia; 12.4% (n=21) had peripheral and 2.3% (n=4) were mixed type. Only four patients had mixed hypotonia. Three out of four patients were diagnosed with Andersen disease, rigid spine congenital muscular dystrophy, and pontocerebellar hypoplasia type 8, but one patient still does not have a final diagnosis.

Mixed type hypotonia was not included in the statistical analysis because it was considered to be too small in number and would cause confusion in the evaluation of central and peripheral hypotonia.

While neuroradiological tests were normal in 48.2% (n=82) of the patients; 51.8% (n=88) were found to be abnormal. When the abnormal MRI results of the patients were examined, the most common pathologies were periventricular leukomalacia in 15.9% (n=27), total cerebral atrophy in 7.6% (n=13), hydrocephalus in 7.1% (n=12), and gliotic lesions in 5.9% (n=10).

EMG was performed in 17 of the patients, and an abnormality was detected in only eight (4.7%) of them. No patient underwent muscle or nerve biopsy.

It was observed that at least one genetic analysis was performed in 63.5% (n=108) of the patients. Ten patients had no pathology in genetic tests (karyotype analysis, array CGH, and WES analysis). An analysis of genetic test results is summarised in Table II.

While 81.8% (n=139) of the patients participating in the study had a final diagnosis, 1.8% (n=3) did not; 16.5% (n=28) of them had on-going genetic examinations. Finally, 38.2% (n=65) had genetic diagnoses and 43.5% (n=74) had clinical diagnoses, while 18.3% (n=31) did not have a final diagnosis. Among the genetic mutation types, 73.8% (n=48) were monogenic, 10.8% (n=7) were microdeletion, and 15.4% (n=10) were chromosomal.

The list of patients with a non-genetic diagnosis is given in Table III, and the most common diagnosis was cerebral palsy.

As the diagnostic tests were evaluated; 48.9% (n=68) of the patients were diagnosed by clinical and routine laboratory tests, 1.4% (n=2) by metabolic tests, 5.8% (n=8) by karyotype analysis, 9.4% (n=13) by array CGH, 5.8% (n=8) by single gene analysis, 25.9% (n=36) by WES, and 2.9% (n=4) of them were diagnosed by MRI scan.

When clinical findings were compared according to the hypotonia type, it was found that central type hypotonia was predominantly seen in patients with microcephaly, preterm birth, neonatal convulsion, and a history of pregnancy complications ($p<0.05$). In the study, brain MRI was performed in all patients, including patients who were suspected with peripheral hypotonia. MRI abnormalities were found to be significantly more common in patients with central hypotonia as expected ($p<0.05$).

DISCUSSION

Hypotonia constitutes a patient group frequently encountered by paediatricians and paediatric neurologists. Although it is common, the process of diagnosis is not always easy. While previous studies on this subject mostly evaluated patients based on clinical findings, this study differed as it evaluated patients clinically, radiologically, and genetically.

In this study, the cases of central hypotonia were more common than peripheral hypotonia. The rate of central hypotonia varies between 50% and 87% in other studies and is in line with this study.[4-7] There were only four mixed hypotonia patients in this study. These patients were considered as an undetermined group and hence, were not included in the statistical analysis.

It was observed that most of the patients admitted to the paediatric neurology outpatient clinic before the age of one year, and patients with central hypotonia were diagnosed earlier than the peripheral ones. A presence of striking symptoms such as seizures and cognitive accompanied with central hypotonia may be a reason for the early referral to a neurologist.

Table I: Comparison of descriptive characteristics, perigestational history, laboratory studies, and final diagnosis in terms of types of hypotonia and presence of genetic diagnosis.

		Hypotonia Type		p	Genetic Diagnosis		p
		Central (n=145)	Peripheral (n=21)		Present (n=105)	Absent (n=65)	
Gender	Female	50 (34.5)	12 (57.1)	[a]0.045*	41 (39)	24 (36.9)	[a]0.782
	Male	95 (65.5)	9 (42.9)		64 (61)	41 (63.1)	
Age	<1 year	17 (11.7)	1 (4.8)	[b]0.579	13 (12.4)	6 (9.2)	[b]0.185
	1-5 years	114 (78.6)	17 (81)		85 (81)	49 (75.4)	
	>5 years	14 (9.7)	3 (14.3)		7 (6.7)	10 (15.4)	
Initial application age	<1 year	104 (71.7)	7 (33.3)	[b]0.002**	84 (80)	29 (44.6)	[b]0.001**
	1-5 years	38 (26.2)	13 (61.9)		20 (19)	33 (50.8)	
	>5 years	3 (2.1)	1 (4.8)		1 (1)	3 (4.6)	
Age of diagnosis (n=139)	<1 year	91 (77.1)	5 (27.8)	[b]0.001**	69 (93.2)	29 (44.6)	[b]0.001**
	1-5 years	24 (20.3)	12 (66.7)		5 (6.8)	32 (49.2)	
	>5 years	3 (2.5)	1 (5.6)		0 (0)	4 (6.2)	
Head circumference	<-2SD	60 (41.4)	1 (4.8)	[b]0.001**	36 (34.3)	29 (44.6)	[b]0.425
	(-2SD) - (+2SD)	79 (54.5)	18 (85.7)		64 (61.0)	33 (50.8)	
	>2SD	6 (4.1)	2 (9,5)		5 (4.8)	3 (4.6)	
Dysmorphic features	Var	59 (40.7)	10 (47.6)	[a]0.547	31 (29.5)	38 (58.5)	[a]0.001**
	Yok	86 (59.3)	11 (52.4)		74 (70.5)	27 (41.5)	
Delivery type	Vaginal	39 (26.9)	10 (47.6)	[a]0.052	26 (24.8)	24 (36.9)	[a]0.091
	C/S	106 (73.1)	11 (52.4)		79 (75.2)	41 (63.1)	
Gestational age	<37 weeks	69 (47.6)	2 (9.5)	[a]0.001**	53 (50.5)	20 (30.8)	[a]0.012*
	37-42 weeks	76 (52.4)	19 (90.5)		52 (49.5)	45 (69.2)	
Birth weight (grams)	<1500	35 (24.1)	0 (0)	[b]0.001**	30 (28.6)	5 (7.7)	[b]0.001**
	1500-2500	35 (24.1)	0 (0)		27 (25.7)	9 (13.8)	
	2500-4000	72 (49.7)	21 (100)		45 (42.9)	51 (78.5)	
	>4000	3 (2.1)	0 (0)		3 (2.9)	0 (0)	
Gestational and labour complications	Present	78 (53.8)	3 (14.3)	[a]0.001**	61 (58.1)	23 (35.4)	[a]0.004**
	Absent	67 (46.2)	18 (85.7)		44 (41.9)	42 (64.6)	
NICU admission	Present	96 (66.2)	8 (38.1)	[a]0.013*	76 (72.4)	32 (49.2)	[a]0.002**
	Absent	49 (33.8)	13 (61.9)		29 (27.6)	33 (50.8)	
Neonatal convulsion	Present	32 (22.1)	0 (0)	[c]0.015*	28 (26.7)	4 (6.2)	[a]0.001**
	Absent	113 (77.9)	21 (100)		77 (73.3)	61 (93.8)	
Parental consanguinity	Present	28 (19.3)	9 (42.9)	[c]0.024*	20 (19)	19 (29.2)	[a]0.125
	Absent	117 (80.7)	12 (57.1)		85 (81)	46 (70.8)	
Creatin Kinase	Normal	137 (94.5)	19 (90.5)	[c]0.617	99 (94.3)	60 (92.3)	[c]0.750
	High	8 (5.5)	2 (9.5)		6 (5.7)	5 (7.7)	
Vitamin B12	Normal	107 (73.8)	17 (81)	[b]0.317	79 (75.2)	48 (73.8)	[b]0.896
	Low	3 (2.1)	1 (4.8)		2 (1.9)	2 (3.1)	
	High	35 (24.1)	3 (14.3)		24 (22.9)	15 (23.1)	
MRI	Normal	64 (44.1)	15 (71.4)	[a]0.019*	35 (33.3)	47 (72.3)	[b]0.001**
	Pathologic	81 (55.9)	6 (28.6)		70 (66.7)	18 (27.7)	
EMG	Normal	3 (2.1)	5 (23.8)	[b]0.001**	4 (3.8)	5 (7.7)	[b]0.005**
	Pathologic	1 (0.7)	7 (33.3)		1 (1)	7 (10.8)	
	Non applied	141 (97.2)	9 (42.9)		100 (95.2)	53 (81.5)	
Metabolic screening	Normal	98 (67.6)	14 (66.7)	[b]0.914	89 (84.8)	26 (40)	[b]0.001**
	Pathologic	5 (3.4)	1 (4.8)		2 (1.9)	4 (6.2)	
	Non applied	42 (29)	6 (28.6)		14 (13.3)	35 (53.8)	
Genetic screening	Present	83 (57,2)	21 (100)	[a]0.001**	43 (41)	65 (100)	[a]0.001**
	Absent	62 (42,8)	0 (0)		62 (59)	0 (0)	
Karyotype	Normal	72 (49.7)	14 (66.7)	[b]0.574	38 (36.2)	51 (78.5)	[b]0.001**
	Pathologic	8 (5.5)	0 (0)		0 (0)	8 (12.3)	
	Non applied	62 (42.8)	7 (33.3)		63 (60)	6 (9.2)	
	Invest. cont.	3 (2.1)	0 (0)		4 (3.8)	0 (0)	
Array CGH	Normal	51 (35.2)	12 (57.1)	[b]0.143	27 (25.7)	37 (56.9)	[b]0.001**
	Pathologic	10 (6.9)	2 (9.5)		0 (0)	13 (20,0)	
	Non applied	82 (56.6)	7 (33.3)		75 (71,4)	15 (23,1)	
	Invest. cont.	2 (1.4)	0 (0)		3 (2,9)	0 (0)	
Single gene	Normal	16 (11)	9 (42.9)	[b]0.001**	16 (15.2)	11 (16.9)	[b]0.001**
	Patologic	2 (1.4)	6 (28.6)		0 (0)	8 (12,3)	
	Non applied	127 (87.6)	6 (28.6)		89 (84.8)	46 (70.8)	
WES	Normal	8 (5.5)	2 (9.5)	[b]0.008**	10 (9.5)	0 (0)	[b]0.001**
	Pathologic	25 (17.2)	10 (47.6)		0 (0)	36 (55.4)	
	Non applied	104 (71.7)	8 (38.1)		86 (81.9)	29 (44.6)	
	Invest. cont.	8 (5.5)	1 (4.8)		9 (8.6)	0 (0)	
Final diagnosis	Present	118 (81.4)	18 (85.7)	[b]0.333			
	Absent	2 (1.4)	1 (4.8)				
	Investigation continues	25 (17.2)	2 (9.5)				
Final diagnosis type	Genetical diagnosis	45 (31)	18 (85.7)	[b]0.001**			
	Clinical diagnosis	73 (50.3)	0 (0)				
	No final diagnosis	27 (18.6)	3 (14.3)				
Genetical diagnosis	Absent	100 (69)	3 (14.3)	[a]0.001**			
	Present	45 (31)	18 (85.7)				
Genetical mutation (n=65)	Monogenic	30 (66.7)	16 (88.9)	[b]0.074			
	Microdeletion	5 (11.1)	2 (11.1)				

*[a]Pearson Chi-square test; [b]Fisher Freeman Halton Test. **p<0.01, *p<0.05.*

Table II: List of monogenic disorders, chromosomal diseases and microdeletion syndromes, respectively.

Monogenic disorders				
Gene	**Zygosity**	**Variant**	**Inheritance**	**Associated disease**
COL5A1	Heterozygous	Nm_001278074.1:c.3852+2T>C	AD	Ehler Danlos Syndrome type 1 OMIM:130000,
GCDH	Homozygous	Nm_000159.3:c.1204C>T	AR	Glutaric acidemia type 1 OMIM:231670
GJB2	Heterozygous	Nm_004004.6:c.35delG	AD	Deafness, Autosomal Dominant 3a OMIM:601544
SYNE2	Heterozygous	Nm_182914.3:c.5284G>T	AD	Emery-Dreifuss Muscular Dystrophy type 5 OMIM:612999
Xq28	Hemizygous	Arr(grch38)Xq28(153438774_154121747)x2	X linked recessive	MECP2 Duplication Syndrome OMIM:300260
SET	Heterozygous	Nm_003011.4:c.1A>T	AD	Intellectual developmental disorder, autosomal dominant 58 OMIM:618106
SELENON	Heterozygous	Nm_020451.3:c.1421A>C	AR/AD	Rigid Spine Syndrome 1 OMIM:602771
ZEB2	Heterozygous	Nm_014795.4:c.1046del	AD	Mowat Wilson Syndrome OMIM:235730
BCAP31	Hemizygous	Nm_001139441.1:c.627delT	X linked recessive	Deafness, dystonia, and cerebral hypomyelination OMIM:300475
AMT	Homozygous	Nm_000481.3:c.878-1G>A	AR	Nonketotic Hyperglicinemia OMIM:605899
KMT2A	Heterozygous	Nm_001197104.1:c.4575G>A	AD	Wiedemann- Steiner sydnrome OMIM:605130
AARS1	Homozygous	Nm_181798.1:c.704A>G	AD	Charcot-Marie-Tooth Disease tip2n OMIM:613287
GAA	Heterozygous	Nm_002529.4:c.2303C>T	AR	Pompe Disease OMIM:232300
POMC	Homozygous	Nm_033453.4:c.304C>T	AR	Obesity, adrenal insufficiency, and red hair due to POMC deficiency OMIM:609734
ERCC2	Homozygous	Nm_000400.4:c.2164C>T	AR	Trichothiodystrophy 1, photosensitive OMIM:601675
CLCN7	Heterozygous	Nm_001114331.3:c.1259dupG	AD	Osteopetrosis Autozomal Dominant 2 OMIM:166600
FGFR3	Heterozygous	Nm_001163213.1:c.1144G>A	AD	Achondroplasia OMIM:100800
HARS1	Heterozygous	Nm_001258040.3:c.112C>T	AD	Charcot-Marie-Tooth disease Tip2w OMIM:616625
GNE	Heterozygous	Nm_001128227.3:c.2179G>A	AD	Sialuria OMIM:269621
ARID1A	Heterozygous	Nm_006015,6:c.2345A>C	AD	Coffin Siris Syndrome 2 OMIM:614607
DOCK6	Compound Heterozygous	Nm_020812.4:c3517C>T Nm_020812.4:c.6004G>T	AR	Adams Oliver Syndrome 2 OMIM:614219
MORC2	Heterozygous	Nm_014941.3:c.2621C>T	AD	Charcot-Marie-Tooth Disease Tip 2Z OMIM:616688
SLC17A8	Heterozygous	Nm_139319.3:c.842A>G	AD	Deafness, Autosomal Dominant 25 OMIM:605583
SLC17A8	Heterozygous	Nm_139319.3:c.842A>G	AD	Deafness, Autosomal Dominant 25 OMIM:605583
CEP290	Homozygous	Nm_025114.4:c.3176delT	AR	Leber congenital amaurosis 10 OMIM:611755
ARID1B	Heterozygous	C.6700_6701delC>T	AD	Coffin Siris Syndrome 1 OMIM:135900
ARID1B	Heterozygous	Nm_001374820.1:c.2316dupC	AD	Coffin Siris Syndrome 1 OMIM:135900
ZNF462	Heterozygous	Nm_021224.6:c.3163G>A	AD	Weiss-Kruszka Syndrome OMIM:618619
KCNMA1	Heterozygous	Nm_001161352.2:c.2116A>T	AD	Liang-Wang Syndrome OMIM:618729
SPARC	Heterozygous	Nm_003118.4:c.57+1G>T	AR	Osteogenesis Imperfecta type 17 OMIM:616507
MECP2	Heterozygous	Nm_001110792.2:c.799C>T	X linked dominant	Rett Syndrome OMIM:312750
THOC6	Homozygous	Nm_024339.5:c.299G>A	AR	Beaulieu-Boycott-Innes Syndrome OMIM:613680
GRIA2	Heterozygous	Nm_000826.6:c.1382A>T	AD	Neurodevelopmental disorder with language impairment and behavioural abnormalities OMIM:618917
CCDC78	Heterozygous	Nm_001031737.3:c.43_49dupTCTCGGC	AD	Centronuclear Myopathy type 4 OMIM:614807
RYR1	Heterozygous	Nm_000540.2:c.9796A>C	AD	King-Denborough Syndrome OMIM:619542
RAB3GAP2	Homozygous	Nm_012414.4:c.1277G>A	AR	Warburg Mikro Syndrome OMIM:614225
ARID1A	Heterozygous	Nm_006015.6:c2718C>G	AD	Coffin Siris Syndrome 2 OMIM:614607
NSD1	Heterozygous	Nm_022455:c.6165_c.6170delCTTCAA	AD	Sotos Syndrome OMIM:117550
SEMA3A	Homozygous	Nm_006080,3:c.1406G>A	AD	Hypogonadotropic Hypogonadism 16 OD OMIM:614897
GBE1	Homozygous	Arr(grch38)3p12.2(81536218_81700597)x1	AR	Andersen Disease OMIM:232500
Xq28	Hemizygous	Arr(grch37)Xq28(152697283_153387918)x2	X linked recessive	MECP2 Duplication Syndrome OMIM:300260
SMN1	Homozygous	Del E7-E8	AR	Spinal muscular atrophy type 1 OMIM:253300 (7 patients)

Continued...

Chromosomal Abnormalities	
Karyotype	**Associated Disease**
46 XY t(4;20)(q12;q13.3)	t(4;20)
46 XY der(8)t(3q;8q)	Derivative chromosome 8
46 XX der(14;21)(q10;q10) +21	Down Syndrome, Robertsonian translocation (OMIM:190685)
47 XY +21	Down Syndrome (OMIM:190685) (4 patients)
Microdeletion Syndromes	
Variants	**Associated Disease**
arr(GRCh37)15q11.2q13.1(23648792_28544359)x1	Angelman Syndrome (OMIM:105830)
22q11.2 deletion	Di George Syndrome (OMIM:188400)
arr(GRCh37)2q24.3q31.3(165429235_181417009)x1	2q31.2 Microdeletion Syndrome (OMIM: 612345)
arr(GRCh38)11p15.5p12(203788_38506056)x2 hmz(0.3)	Beckwith Wiedemann Syndrome (OMIM:130650)
15q11.2 delesyonu	Angelman Syndrome (OMIM:105830)
arr(GRCh37)9p24.3(1591811_1759811)x1, arr cgh(hg 19)22q13.31-q13.33(44,554,083-51,224,252)x1	Chromosome 9q24.3 Deletion Syndrome (OMIM:154230) Phelan-Mcdermid Syndrome (OMIM:606232)

AR: Autosomal recessive, AD: Autosomal dominant.

Table III: List of patients who had clinical diagnosis.

Cerebral palsy (due to preterm birth)	44
Cerebral palsy (due to HIE)	12
Cerebral palsy (due to neonatal hypoglycemia)	2
Benign hypotonia	5
Biotidinase deficiency	1
CHARGE sequence	1
FRYNS syndrome	1
Ketoglutaric aciduria	1
Congenital CMV infection	1
Congenital syphilis infection	1
Cortical developmental malformation: Cortical dysplasia	1
Cortical developmental malformation: Cortical dysplasia	1
Cortical developmental malformation: Lissencephaly	1
Cortical developmental malformation: Schizencephaly	1
Lowe syndrome	1
Pontocerebellar hypoplasia type 8	1
Occipital encephalocele	1
Central nervous system infection	1
Undiagnosed	31
Total	105

A complete gestational and birth history, and physical examination lead the specialist to distinguish the central and peripheral hypotonia. Preterm birth, neonatal convulsions, maternal gestational problems, and microcephaly are the symptoms that clearly point to the central nervous system problems. As expected, these findings are significantly higher in central hypotonia. The previous studies have emphasised the importance of clinical findings in diagnosis of hypotonia as these studies have reported the rate of patients diagnosed with only clinical findings to be approximately 50%.[1,5,8,9] The clinical diagnosis of approximately 50% of central hypotonia emphasises the importance of basic physical examination and medical history.

Brain MRI is the most supportive method after medical history and physical examination. Cortical developmental abnormalities, gliotic changes secondary to hypoxic ischemic encephalopathy (HIE), calcifications due to TORCH infections, and genetic diseases such as Aicardi-Goutierrez syndrome and Adams Oliver syndrome can be seen on MRI and lead to the diagnosis. In the literature, it has been shown that the most helpful test for diagnosis is brain MRI which is similar to the finding in this study.[3] In this study, 51.8% of the patients were diagnosed by evaluating the clinical findings and brain MRI findings together. The most common MRI finding is periventricular leukomalacia and is almost typical for neurological sequelae of preterm birth. It was thought that in a patient with central hypotonia, the first test to be requested after medical history and physical examination should be a brain MRI. In a patient with peripheral hypotonia, brain MRI does not need to be one of the initial tests.

In patients with central hypotonia, karyotyping and array-based Comparative Genomic Hybridization (CGH) are the first applied chromosomal studies. These tests can diagnose the common causes of syndromic central hypotonia, such as Down syndrome and Prader Willi syndrome, as well as rarer microdeletion syndromes. Sixty-five of the patients (38%) had a genetic diagnosis. Monogenic mutations were found in 48 cases, chromosomal changes in 10, and microdeletion syndromes in seven of them. Total of four patients were diagnosed with mixed hypotonia and two of them were genetic conditions of Andersen disease and rigid spine muscular dystrophy.

It was observed that 85.7% of the patients with peripheral hypotonia were genetically diagnosed, but this rate was much lower in central hypotonia. In a study of 144 patients by Laulgel *et al*., peripheral hypotonia was observed in 22 patients and genetic diagnosis was made except for three of these patients.[3] This rate is similar to the present result. The reason for this difference is that most of the central causes are non-genetic diagnoses such as hypoxic ischemic encephalopathy, intraventricular haemorrhage due to preterm birth.

Haliloglu reported that hypotonia and respiratory distress in the neonatal period may be the first signs of hypotonic infants.[10] Patients diagnosed with hypotonia in the neonatal period may have been exposed to hypoxia and misdiagnosed as HIE due to the inability to complete the ascent movements in labour and lack of respiratory effort. For this reason, it has been emphasised that studying a genetic panel with next generation sequencing (NGS) in these patients is the most practical and diagnostic test.[10,11] In this

study, a patient was thought to have mixed type hypotonia and diagnosed as pontocerebellar hypoplasia type 8 with brain MRI findings. He is still hospitalised in the paediatric intensive care unit due to the respiratory failure and non-suction, and genetic test results are awaited.

Patients who cannot be diagnosed by karyotyping and array CGH, targeted clinical gene panel, and whole exome sequencing tests according to the type of hypotonia are recently adapted. Clinical exome sequencing is more cost-effective, and WES, with its broader spectrum, reduces the time to diagnose and treatment-free time in curable diseases. In this study, 36 (21.2%) patients were diagnosed by WES. The importance of WES in the diagnosis of hypotonia is indisputable, since patients diagnosed by WES constitute more than half of those with genetic diagnosis. In the study of Waldrop *et al*.,[12] patients diagnosed with hypotonia but without a definitive diagnosis were enrolled and 39% of patients were genetically diagnosed after WES. The rate of diagnosis by WES in Waldrops'study is slightly higher than this study.[12] Although WES seems to be an expensive test for the developing countries, it is a time saver considering the short time available for diagnosis of such patients.[13-15]

SMA is the prototype disease for hypotonia. An early diagnosis and initiation of treatment are of great importance.[16,17] In this study, SMA type 1 was detected in seven patients, and all of them were diagnosed after physical examination and by screening the SMN 1 and 2 genes with a single gene analysis. This shows that SMA disease's clinical characteristics are well-known by clinicians. The authors think that with the initiation of SMA screening in newborn period in Turkey, the patients will be diagnosed and treated earlier.

There are three main limitations in this study. First, since the diseases mentioned in the study were very rare, the patients were analysed in three main groups. Secondly, the mixed type of hypotonic patients was very rare and therefore, cannot be included in the statistical analysis due to the insufficient number. The final limitation is that the age of the patients included in the study constitutes a heterogeneous group.

CONCLUSION

Although hypotonic infant evaluation can sometimes be very complicated, most patients are diagnosed by medical history and physical examination. Brain MRI is the first choice as an adjunctive test in patients where central hypotonia is suspected. For patients who cannot be diagnosed with clinical findings and brain MRI, WES can be a good option for diagnosis. It is also helpful in the immediate and long-term management plan, and the counselling of parents.

ETHICAL APPROVAL:
The study was approved by Namik Kemal University Clinical Research Ethics Committee (Approval No. 2022.173.09.20).

PATIENTS' CONSENT:
The study was conducted in accordance with the principles of the Declaration of Helsinki.

COMPETING INTEREST:
The authors declared no competing interest.

AUTHORS' CONTRIBUTION:
SG: Substantial contribution to the concept and drafting of the manuscript.
GG: Interpretation of the data and drafting of the work.
HT: Drafting and revising the final manuscript.
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REFERENCES

1. Paro-Panjan D, Neubauer D. Congenital hypotonia: is there an algorithm? *J Child Neurol* 2004; **19(6)**:439-42. doi: 10.1177/088307380401900608.
2. Richer LP, Shevell MI, Miller SP. Diagnostic profile of neonatal hypotonia: An 11-year study. *Pediatr Neurol* 2001; **25(1)**:32-7. doi: 10.1016/s0887-8994(01)00277-6.
3. Laugel V, Cossee M, Matis J, De Saint-Martin A, Echaniz-Laguna A, Mandel JL, *et al*. Diagnostic approach to neonatal hypotonia: Retrospective study on 144 neonates. *Eur J Pediatr* 2008; **167(5)**:517-23. doi: 10.1007/s00431-007-0539-3.
4. Mesquita M, Ratola A, Tiago J, Basto L. Neonatal hypotonia: Is it a diagnostic challenge? *Rev Neurol* 2018; **67(8)**: 287-92. doi: 10.33588/rn.6708.2018141.
5. AlBanji MH, AlSaad AN, AlAnazi RF, Aleisa ZA, Alam DS, Alhashim AH. Utility of hypotonia diagnostic ınvestigations: A 12-year single center study. *Mol Genet Metab Rep* 2020; **25**:100665. doi: 10.1016/j.ymgmr.2020.100665.
6. Birdi K, Prasad AN, Prasad C, Chodirker B, Chudley AE. The floppy infant: Retrospective analysis of clinical experience (1990–2000) in a tertiary care facility. *J Child Neurol* 2005; **20(10)**:803-8. doi: 10.1177/08830738050200100040.
7. Dua T, Das M, Kabra M, Bhatia M, Sarkar C, Arora S, *et al*. Spectrum of floppy children in Indian scenario. *Indian Pediatr* 2001; **38(11)**:1236-43.
8. Johnston HM. The floppy weak infant revisited. *Brain Dev* 2003; **25(3)**:155-8. doı: 10.1016/s0387-7604(02)00168-7.
9. Ozaydın E, Turgut U, Gündüz M, Güven A, Kose G. Hipotonik infantların ayirici tanisinda algoritma faydali mıdır? 53 Olgunun Değerlendirilmesi. *Turkiye Cocuk Hastalikları Dergisi*; **7(3)**:128-33. doı: 10.12956/tjpd.2013.3.05.
10. Haliloglu G. Neonatal presentations of neuromuscular disorders. *Eur J Paediatr Neurol* 2022; **38**:A6-A11. doi: 10.1016/j.ejpn.2022.04.003.
11. François-Heude MC, Walther-Louvier U, Espil-Taris C, Beze-Beyrie P, Rivier F, Baudou E, *et al*. Evaluating next-generation sequencing in neuromuscular diseases with neonatal respiratory distress. *Eur J Paediatr Neurol* 2021; **31(7)**: 78-87. doi: 10.1016/j.ejpn.2021.01.011.
12. Waldrop MA, Pastore M, Schrader R, Sites E, Bartholomew D, Tsao CY, *et al*. Diagnostic utility of whole exome

sequencing in the neuromuscular clinic. *Neuropediatrics* 2019; **50(2)**:96-102. doi: 10.1055/s-0039-1677734.

13. Aaltio J, Hyttinen V, Kortelainen M, Frederix GWJ, Lönnqvist T, Suomalainen A, *et al*. Cost-effectiveness of whole-exome sequencing in progressive neurological disorders of children. *Eur J Paediatr Neurol* 2022; **36**:30-6. doi: 10.1016/j.ejpn.2021.11.006.
14. Floriach-Robert M, Cabello A, Simon De Las Heras R, Mateos Beato F. Neonatal hypotonia of muscular origin: Analysis of 50 cases. *Neurologia* 2001; **16(6)**:245-53.
15. Miller VS, Delgado M, Iannaccone ST. Neonatal hypotonia, *Semin. Neurol* 1993; **13(1)**:73-83. doi: 10.1055/s-2008-1041110.
16. Nicolau S, Waldrop MA, Connolly AM, Mendell JR. Spinal muscular atrophy. *Semin Pediatr Neurol* 2021; **37**:100878. doi: 10.1016/j.spen.2021.100878.
17. Butterfield RJ. Spinal muscular atrophy treatments, newborn screening, and the creation of a neurogenetics urgency. *Semin Pediatr Neurol* 2021; **38**:100899. doi: 10.1016/j.spen.2021.100899.