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Utility of Hypotonia Diagnostic Investigations: A 12-year Single Center Study

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ABSTRACT

Introduction: Hypotonia is a common presentation that child neurologists encounter daily. The hypotonic neonate represents a diagnostic challenge as a lesion at any level in the neuro-axis may cause hypotonia. In this paper, we study the diagnostic yield of investigations commonly used as part of a hypotonia work-up.

Methods: A 12-year retrospective cohort study was conducted at a tertiary care center in Saudi Arabia from 2007 to 2018. Final diagnoses, clinical presentations, laboratory tests, imaging and genetic studies were reviewed from the patient's electronic health records.

Results: 164 patients were identified as fitting the inclusion criteria of the study. 50% had central hypotonia, 18% peripheral hypotonia and 32% mixed hypotonia. Molecular testing was performed for 82% (74) of patients. 65 Microarray studies were done; 27% abnormal and 9% diagnostic. 55 gene panels were done; 58% abnormal and 30% diagnostic. 53 single-gene tests were done; 57% abnormal and 40% diagnostic. 61 whole exome sequences were done; 72% positive and 59% diagnostic. 126 MRIs were reviewed; 56% abnormal and 33% contributed to the diagnosis.

Conclusion: Molecular genetic testing is our recommended next step in the diagnosis of patients with hypotonia after careful phenotyping. Neuroimaging is helpful to guide further costly workup of patients with hypotonia.

1. Introduction

Hypotonia or low muscle tone is defined as decreased resistance to passive movement, which may or may not be associated with decreased muscle strength. Recognition of hypotonia in the newborn may be straightforward, but determining the cause can be challenging, even to an expert neurologist.

Muscle tone is controlled by the peripheral fusimotor system with input from the central nervous system (CNS). The afferent fibers detect muscle spindle stretch and subsequently direct the motor unit system to cause muscle contraction. Failure of any component of the motor unit, from the anterior horn cell, motor neuron, neuromuscular junction, or muscle itself, will result in hypotonia [1].

Historical features and examination findings make a major contribution to determining the final diagnosis. However, we still encounter lots of children with no apparent etiology for their hypotonia, and we might end up with a vast array of diagnostic workups. Those investigations might pose a clinical challenge and cost. Available modalities include various forms of neuroimaging; electrophysiological tests, including electroencephalography (EEG), nerve conduction studies (NCS), electromyography (EMG), and repetitive nerve stimulation (RNS); muscle and nerve biopsy; and other laboratory studies, such as serum creatine kinase (CK), metabolic studies, and genetic studies [2].

Few published studies have addressed the utility of diagnostic workups and the outcome of infants presenting with generalized hypotonia. In a study by Dubowitz et al., a definitive diagnosis was established in 67.4% of cases, 40% of which were diagnosed on purely clinical grounds, whereas, in 60% of cases, additional investigations were necessary [3,4]. In another study of 50 hypotonic neonates, the investigations contributed to or helped in the diagnosis as follows:

Abbreviations: MRI, Magnetic resonance imaging; CH, Central Hypotonia; MH, Mixed Hypotonia; PH, Peripheral Hypotonia; VLCFA, Very-Long-Chain Fatty Acids; CNS, Central Nervous System; EEG, Electroencephalography; NCS, Nerve Conduction Studies; EMG, Electromyography; RNS, Repetitive Nerve Stimulation; CK, Creatine Kinase; aCGH, Microarray-based Comparative Genomic Hybridization; WES, Whole-Exome Sequencing

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neuroimaging 38%; EEG 30%; muscle biopsy 14%; nerve biopsy 8% [5].

Since we live in a genomic medicine era, we are observing a change in practice, with the utilization of biochemical and molecular genetics tests more than other invasive approaches like muscle and nerve biopsies. We aim in our study to investigate the diagnostic yield of clinical history, examination, metabolic tests, neuroimaging, and genetic testing in the diagnosis of patients with hypotonia and to determine the diagnostic profile of hypotonic children in Saudi Arabia. We also aim to characterize brain MRI abnormalities in patients with hypotonia.

2. Methodology

The patients were identified through a hospital database search of electronic health records. The diagnostic code for hypotonia was used to enroll patients from outpatient visits, inpatient consultations and imaging requests. Our investigators used a structured data collection form to collect data. They retrospectively reviewed patients from 2007 till 2018; the study was conducted in a tertiary care center, King Fahad Medical City (KFMC), which receives referrals from all regions of Saudi Arabia.

Patients with a documented examination or preliminary diagnosis with appendicular or central hypotonia were included in the study. Either neurology or genetic assessment records were required for patient enrollment; charts without proper history and neurological examination documentation were excluded from the study.

Upon review of patient charts, we classified hypotonia into central, peripheral, or mixed hypotonia. Central hypotonia was defined as any patient with axial or appendicular hypotonia, preserved or exaggerated deep tendon reflexes, preserved muscle power, abnormal neuroimaging, and symptoms suggestive of CNS involvements, cognitive developmental delay, seizure etc. Peripheral hypotonia was defined as any patient with axial or appendicular hypotonia, hyporeflexia, presence of weakness, normal brain neuroimaging, normal cognition, and presence of other signs suggestive of peripheral nervous system disease (e.g., fasciculation). Mixed hypotonia is defined as any patient with appendicular or axial hypotonia with a combination of CNS and peripheral nervous system involvement.

The clinical variables obtained included demographic data, the presence of an affected family member, consanguinity, decreased fetal movements, abnormal antenatal ultrasound, polyhydramnios, delivery by cesarean section, gestational age (full-term or preterm), birth weight (Low Birth Wight < 1500 Gram), respiratory distress requiring resuscitation and intubation, the occurrence of neonatal seizures and the presence of other Chronic Disease.

The physical examination features that were obtained included the presence of dysmorphic features, presence of spontaneous limb movement, deep tendon reflexes, head circumference, appendicular, and axial tone.

All investigations relevant to the diagnosis were reviewed, including electrophysiologic studies, neuroimaging studies (brain MRI), selected laboratory tests and biopsies (skin, muscle, and nerve biopsies). Electrophysiologic tests included EMG, NCS, and EEG. A single neuroradiologist reviewed all neuroimaging. The laboratory tests included lactate, ammonia, CK, tandem MS, VLCFA, urine organic Acids, serum amino acids, liver function test, renal profile, and thyroid function test. All genetic and molecular studies were included, which includes karyotype, aCGH, single-gene testing, gene panels, whole-exome sequencing (WES). Expert geneticists and neurologists assessed the patients and the molecular diagnostic investigations were requested based on the clinical phenotype rather than a preset orders.

All positive investigations were categorized according to their contribution and guidance toward the final diagnosis, as follows: (1) diagnostic if the result was pivotal in the final diagnosis; (2) contributive if the result contributed to the final diagnosis by suggesting a pathophysiologic mechanism (e.g., fiber type grouping on muscle biopsy).

Hypotonia was classified into three groups: central, mixed, and peripheral hypotonia. The metric data was described in terms of Mean ± SD, and the intergroup comparison among the three study groups was measured by Ronal Fisher's F-test for one-way Analysis of Variance. The non-metric data were described in terms of frequency and percentages, for which the intergroup group variance was measured by Chi-square test. All inferences were drawn at 95% confidence interval, with the help of MS-Excel 10 and SPSS 25.0 software. The study was approved by our hospital international review board.

3. Results

Among the 1592 patients' charts that were screened, 164 patients met the inclusion criteria of the study, and among those, 129 patients had good quality MRIs that were reviewed.

50% of our cohort were males, and 50% were females. Central hypotonia accounted for 50%, peripheral hypotonia 18%, and mixed hypotonia 32% of patients. 55% of hypotonic patients presented between 12 and 60 months of age; 60% of this age group had central hypotonia. 35% of patients presented between 1 and 12 months, 43% of whom had central hypotonia and 44% had peripheral hypotonia. 6% of patients presented after five years of age and only 4% of the whole cohort presented with neonatal hypotonia, 50% of whom had peripheral hypotonia. The age of presentation of hypotonic patients correlated significantly with the hypotonia type (*P*-value 0.017).

Out of 164 patients, 88% (146) had abnormal history and examination, 53% had central hypotonia, 19% had peripheral hypotonia, and 28% mixed hypotonia. Consanguinity was found in 70% of patients. 22% of patients with a positive history of consanguinity had a family history of hypotonia, and overall positive family history of hypotonia was reported in 33.1%. 25 antenatal ultrasounds were documented, 24% of which reported abnormal findings, and all had central hypotonia. Polyhydramnios was found in 40% (10/25) of the documented charts. Global developmental delay was present in 78% (128) of patients (central 55%, mixed 36%, peripheral 9%, *P*-value < 0.01). Seizure disorder was present in 22% of the patients (central 67%, mixed 28%, peripheral 22%, P-value 0.17).

A low birth weight (below 1500 g) was found in 15.3% (20/131) of patients. History of NICU admission was found in 41.1% (60/146) of patients and occurrence of neonatal seizure was found in 11% (13/120), while neonatal fever was present in 9% (3) of patients.

Appendicular hypotonia was present in 87% (central 53%, mixed 28%, peripheral 19%, *p*-value 0.01), axial hypotonia in 85% (central 51%, mixed 31%, peripheral 19%, *p*-value 0.7), muscle weakness in 44% (central 23%, mixed 36%, peripheral 41%, *P*-value 0.001) and absent reflexes (central 0%, mixed 62%, peripheral 38%, p-value 0.001). Head circumference of 129 patients was documented. 8.5% (1/129) were found to have macrocephaly, 43% (56/129) had microcephaly, and 48% were normal. Central hypotonia was the predominant hypotonia type among patients with microcephaly, accounting for 48%, followed by mixed hypotonia 36% and then peripheral hypotonia 16%. The presence of dysmorphic features was high; up to 46.4% (64/138) of our cohort (central 63%, mixed hypotonia 25%, peripheral hypotonia 13%, *P*-value 0.06). Muscle weakness was found in 44% of patients; 41% of those had peripheral hypotonia. Table 1 summarizes the clinical variables of hypotonia patients.

Molecular genetic testing was performed for 82% of patients, which included aCGHs, gene panels, and whole exome sequencing. A total of 74 karyotypes were carried out, of which 4% were abnormal, and 1% was diagnostic.

65 aCGH studies were performed, of which 27% reported as abnormal. 9% were considered diagnostic, and 67% of abnormal aCGHs were found in patients with central hypotonia (Table 4). Five Methylation Tests for Prader-Willi Syndrome was done, three of them were

Table 1
Clinical variables of hypotonic patients cohorts.

	N = 164 100%	Central 83 50%	Mixed 52 31%	Peripheral 29 17%	P-value
Age (month)					
0 month	6(3.70%)	33%	17%	50%	0.017
1–12	57(34.80%)	44%	33%	23%	
12–60	90 (54.9%)	60%	30%	10%	
≥ 60	11 (6.7%)	18%	46%	36%	
Gender					
Female	82 (50.0%)	51%	34%	15%	0.554
Male	82 (50.0%)	50%	29%	21%	
Head circumference					
Microcephalic	56 (43.4%)	48%	36%	16%	0.81
Macrocephalic	11 (8.5%)	55%	27%	18%	
Abnormal fetal movement	9 (30.0%)	45%	22%	33%	0.206
Polyhydramnios	10 (40.0%)	80%	10%	10%	0.134
Low birth Wight (< 1500 g)	20 (15.3%)	60%	30%	10%	0.619
NICU/intubation/ resuscitation	60 (41.1%)	55%	25%	20%	0.445
Neonatal seizure	13 (10.8%)	61%	31%	8%	0.538
Consanguinity	110 (69.6%)	51%	29%	20%	0.242
Positive family Hx	50 (33.1%)	40%	34%	26%	0.094
Dysmorphism	64 (46.4%)	62%	25%	13%	0.067
Appendicular hypotonia	143 (88.8%)	53%	28%	19%	0.015
Axial hypotonia	140 (86.4%)	50%	31%	19%	0.797
DTR					
Normal	61 (38.1%)	85%	7%	8%	< 0.001
Decreased / absent	61 (38.1%)	0%	62%	38%	
Brisk	38 (23.8%)	76%	21%	3%	
Global Developmental Delay (GDD)	128(78%)	55%	36%	9%	< 0.00001
Weakness	44 (44.4%)	23%	36%	41%	< 0.001
Seizure	36(22%)	67%	28%	5%	0.17

abnormal. 55 gene panels were prepared, of which 58% were abnormal, with 30% diagnostic. Interestingly, 50% of the abnormal panels were associated with peripheral hypotonia. 53 single-gene tests were carried out, with 57% showing abnormal results, but only 40% were diagnostic. 19 SMN gene deletions was performed, 11 of them were abnormal. 61 whole exome sequences were performed, of which 72% were positive, and 59% were deemed diagnostic. 44% of the abnormal WESs were associated with central hypotonia, 36% with mixed hypotonia, and 19% with peripheral hypotonia. All single gene variations associated with hypotonia are reported in Table 3.

Regarding metabolic investigations, 108 CK tests were done; 19% were abnormal, 7% of them contributed to the diagnosis, and 42% were associated with peripheral hypotonia (p-value 0.027). 117 Tandom MS tests were carried out, of which 6% were abnormal, and only 1% contributed to the diagnosis. A total of 59 VLCFA were completed, of which 8% were abnormal, and 6% contributed to the diagnosis. 101 urine samples were tested for organic acid; 10% were abnormal, 1% was diagnostic, and 2% contributed to the diagnosis. Thirty-four other specific metabolic tests were done, with 35% proving abnormal, and 3% contributing to the diagnosis.

Neurophysiological investigations were also reviewed. 45 Electroencephalograms (EEG) were performed. 68% (31) EEGs were reported as abnormal, 61% of those patients had central hypotonia. In addition to that, 33 nerve conduction and electromyogram (NCS/EMG) studies were done, 45% (15) of them resulted in abnormal examination. Total of nine muscle biopsy result were reviewed, all of them were abnormal, but only one contributed to the final diagnosis.

126 MRIs were reviewed by a certified neuroradiologist, who found that 56% were abnormal, and 48% (42/88) contributed to the diagnosis. We found a strong correlation between hypotonia type and abnormal brain MRI: among 88 patients with abnormal brain MRIs, 57% had central hypotonia, followed by 31 with mixed hypotonia and 10% with peripheral hypotonia (P-value 0.001). Table 2 summarizes the diagnostic yield of hypotonia investigations that were reviewed.

4. Discussion

Hypotonia is one of the commonest reasons for consultation with pediatric neurologists. Most of the studies investigating hypotonia were conducted before genetic workup became readily available, so few studies addressed the diagnostic yield of a hypotonia workup that included molecular genetics testing. Our study is one of the largest cohorts that addressed that question and the only one, as far as we know, that specifically studied the Arab population.

In comparison to the previous studies, our study demonstrates no significant changes in the proportion of central and peripheral hypotonia. Our results confirm the large predominance of central causes of hypotonia over peripheral causes, in a nearly 3:1 ratio. Our finding of central hypotonia is lower than other studies, which varied from 67% to 85% [6,7]. The presence of the following was higher in patients with central hypotonia, although the number was not high enough to reach statistically significant value: abnormal head circumference, low birth weight, NICU admission, neonatal seizure, and dysmorphic features.

Vincent et al. reported mixed hypotonia as 'undetermined type' of hypotonia, which accounted for 16% of cases. Mixed hypotonia in our study is significantly higher (31%) compared to other studies. This could be attributed to under-reporting or the fact that this was not categorized into a separate entity in the previous studies [8]. We speculate that the high percentage we found could be due to the unique

Table 2
Diagnostic yield of hypotonia investigation.

Investigation modality	#of patients	%Abnormal test	%Diagnostic test	%Contributing test	Test versus hypotonia type p-value
MRI	157	56%	–	26%	0.001
CK	108	19%	–	7%	0.027
Tandem MS[a]	117	6%	–	1%	0.349
VLCFA	59	8%	–	6%	0.547
Urine for organic acid	101	10%	1%	2%	0.767
Karyotype	74	4%	1%	–	0.645
Microarray	65	28%	9%	2%	0.783
Specific Gene Panel	55	58%	30%	–	0.138
Whole Exome Sequencing	61	72%	59%	–	0.049
Single gene	53	57%	40%	–	0.803
Muscle Biopsy	9	100%	–	11%	–
EEG	45	68%	–	–	–
NCS/EMG	33	45%	–	–	–

[a] Tandem mass spectrometry (Tandem MS) was used in the initial screening and diagnosis of IEMs in high risk neonatal and pediatric populations. Tandem MS evaluate Amino Acids & Urea Cycle Disorders, Organic Acid Disorders & Fatty Acid Oxidation Disorders.

Table 3
Single gene disorders in hypotonic patients.

#	Patient phenotype	Gene	Variant	Classification	Zygosity	Disease mode of inheritance	Associated disease phenotype
1	Hypotonia, progressive thoracolumbar scoliosis, muscle atrophy, no respiratory involvement	*IGHMBP2*	Chr11;NM_002180.2:c.2474del(p.(Pro825fs))	Pathogenic	Homozygous	AR	Charcot-Marie-Tooth Disease Type 2S (OMIM# 616155)
2	Global developmental delay (GDD), Hypotonia, dysmorphic features, classic molar tooth sign on MRI brain	*CC2D2A*	Chr4;NM_001080522.2;c.3364C > T(p.(Pro1122Ser))	Pathogenic	Homozygous	AR	Overlapping syndromes, COACH syndrome and Joubert syndrome (OMIM# 612013)
3	Bilateral hearing loss and retinitis pigmentosa	*MYO7A*	Chr11;NM_000260.3:c.5001_5002del (p.(Tyr1668fs))	Pathogenic	Homozygous	AR	AR USHER type 1B (OMIM#276900)
4	Developmental delay, Failure to thrive, abnormally high propanayl carnitine	*PCCA*	Chr13;NM_000282.4;c.425G > A;(p.G142A)	Pathogenic	Homozygous	AR	Propionic Acidemia (OMIM# 606054)
5	Spastic tetraplegia, GDD normal brin MRI	*ALS2*	Chr2: NM_020919.42q33.1;c.305A > G;(p.His102Arg)	Pathogenic	Homozygous	AR	Infantile onset ascending spastic paralysis (OMIM#607225)
6	GDD (mainly cognitive)	*PURA*	Chr5;NM_005859.4:c.305_308del (p.(Leu102fs))	Pathogenic	Heterozygous, denovo	AD	AD mental retardation 31 (OMIM#600473)
7	Hypotonia, GDD, hepatosplenomegaly, Sphingomyelinase enzyme activity is reduced, MRI diffuse hypomyelination	*SMPD1*	Chr11;NM_000543.5;c.1267C > T; (p.His423Tyr)	Pathogenic	Homozygous	AR	Niemann-Pick disease Type A
8	GDD, swallowing dysfunction, seizure, unremarkable brain MRI	*PLCB1*	Chr20: NM_015192.4c.550C > T; (p.Arg184)	Pathogenic	Homozygous	AR	Early infantile epileptic encephalopathy 12 (OMIM# 613722)
9	Hypotonia, weakness, normal cognition, swallowing difficulty, high CK, brain MRI showed delayed myelination	*LAMA2*	Chr6;NM_000426.3:c.1762del (p.(Ala588fs)). Chr6;NM_000426.3:c.4686G > A (p.(Trp1563))	Pathogenic	Compound heterozygous	AR	Merosin-deficient muscular dystrophy (OMIM#607855)
10	Hypertrophic cardiomyopathy, hypotonia, GDD, swallowing dysfunction	*GAA*	Chr17; NM_000152.5;c.896 T > C; (p.L299P)	Pathogenic	Homozygous	AR	Pompe Disease (GSD 2) (OMIM# 232300)
11	Hypotonia, Left Ventricular hypertrophy, high CK, brain MRI showed white matter changes	*LAMA2*	chr6;NM_000426.3: c.3924 + 2 T > C	Pathogenic	Homozygous	AR	Merosin-deficient muscular dystrophy type A (OMIM#607855)
12	Proximal myopathy, peripheral hypotonia, Gower's sign is positive, cognitively normal	*COL6A2*	Chr21;NM_001849.3:c.1053 + 1G > T	Pathogenic	Heterozygous	AD, AR	Bethlem myopathy 1 (OMIM# 158810)
13	Developmental regression, hypotonia ataxia, spasticity	*PLA2G6*	chr22; NM_003560.2: c.2070_2072del (p.Val691del)	Pathogenic	Homozygous	AR	Infantile neuroaxonal dystrophy 1 (OMIM#256600)
14	Microcephaly, hypotonia, seizure disorder, GDD, failure to thrive, brain MRI showed genesis of corpus collosum and delayed demyelination	*AMT*	chr3; NM_000481.3:c.982dub (p.(Ala328fs))	Pathogenic	Homozygous	AR	Glycine encephalopathy (OMIM#605899)
15	Epilepsy, GDD, Central Hypotonia, Spastic diplegia	*ADAT3*	Chr19;NM_138422.2:c.430G > A (p.(Val144Met))	Pathogenic	Homozygous	AR	AR mental retardation 36 (OMIM#615286)
16	GDD, spasticity, MRI brain showed Molar Tooth sign	*AHI1*	Chr;NM_017651.4:c.1328 T > A; (p.(Val443Asp))	Pathogenic	Homozygous	AR	Joubert syndrome type 3 (OMIM#608629)
17	GDD, progressive leukodystrophy, severe Hydrocephalus, cerebellar atrophy and cyst	*LAMA1*	chr18; NM_00559.3:c.6779C > A (p.(Ser2260Tyr))	Likely pathogenic	Homozygous	AR	Poretti-Boltshauser syndrome (OMIM# 615960)
18	Myopathic face, peripheral hypotonia, respiratory failure, Cryptorchidism, DM type 1	*MTM1*	ChrX;NM_000252.2: c.679G > A (p.Val227Met)	Pathogenic	Hemizygous	XR	Myotubular myopathy (OMIM#310400)
19	Hepatosplenomegaly, jaundice, hypotonia, failure to thrive	*GALT*	Chr9; NM_000155.4;c.983G > A; (p.R328H)	Pathogenic	Homozygous	AR	Classic galactosemia (OMIM# 230400)
20	Severe failure to thrive, hypotonia, chronic pancreatitis, brain MRI is unremarkable	*CTRC*	Chr1; NM_007272.2:c.649G > A (p.(Gly217Ser))	Pathogenic	Heterozygous	AD	AD chronic pancreatitis (OMIM#167800)
21	Peripheral hypotonia, normal cognition, motor delay, contracture, high CK	*LAMA2*	Chr6;NM_000426.3;c.1762delG; (p.Ala588Leufs*11)	Pathogenic	Homozygous	AR	Merosin-deficient muscular dystrophy type A (OMIM#607855)
22	Hypotonia, weakness, brain MRI showed Dandy-Walker continuum with hydrocephalus	*FKRP*	Chr19;NM_024301.5;c.1364C > A; (p.Ala455Asp)	Pathogenic	Homozygous	AR	Muscular dystrophy-dystroglycanopathy type 5A (MDDG) (OMIM#613153)
23	GDD, brain MRI showed cerebellar atrophy, hypothyroidism, hypotonia,microcephaly,	*PMM2*	Chr16; NM_000303.2:c.43G > A (p.(Gly15Arg))	Pathogenic	Homozygous	AR	Congenital disorder of glycosylation type 1A (OMIM#212065)

(*continued on next page*)

Table 3 (*continued*)

#	Patient phenotype	Gene	Variant	Classification	Zygosity	Disease mode of inheritance	Associated disease phenotype
	carbohydrate deficient transferrin showed abnormal result						
24	Subcutaneous nodules, failure to thrive, progressive impaired vision, impaired swallowing, hoarseness, joint deformity	*ASAH1*	Chr8;NM_004315.5:c.338 T > G; (p.Val113Gly)	Likely pathogenic	Homozygous	AR	Farber lipogranulomatosis (OMIM# 228000)
25	GDD, Visual impairment, Bilateral Sensory neural hearing deficit, brain MRI showed cerebellar atrophy, clava hypertrophy, reduced signal intensity in basal ganglia	*PLA2G6*	Chr22;NM_003560.4;c.2370 T > G (p.Tyr790Ter)	Pathogenic	Homozygous	AR	Infantile neuroaxonal dystrophy 1 (OMIM#256600)
26	Developmental regression, hypotonia, no seizure disorder, brain MRI suggestive of mitochondrial cytopathy	*SURF1*	Chr9; NM_003172.4;c.870delT (p.(Phe290 Leu fs*55)	Pathogenic	Homozygous	AR	Leigh disease (OMIM#256000)
27	Developmental regression, hypotonia, ataxia, MRI showed cerebellar atrophy, clava hypertrophy	*PLA2G6*	Chr22; NM_003560:c.1771C > T (p.(Arg591Trp))	Pathogenic	Homozygous	AR	Infantile neuroaxonal dystrophy 1 (OMIM#256600)
28	Developmental regression, seizure disorder, nystagmus, MRI showed cerebellar atrophy, clava hypertrophy	*PLA2G6*	Chr22;NM_003560; c.2070_2072delTGT (p.(Val691del)). Chr22; NM_003560;c.1771C > T (p.(Arg591Trp))	Pathogenic	Compound Heterozygous	AR	Infantile neuroaxonal dystrophy 1 (OMIM#256600)
29	Distal arthrogryposis, bilateral developmental hip dysplasia, ptosis, hypotonia, developmental delay	*ECEL1*	Chr2; NM_004826.3;c.1470G > A (p.(Trp490*))	Pathogenic	Homozygous	AR	Distal arthrogryposis 5D (OMIM#605896)
30	GDD, hypotonia, liver impairment, brain MRI showed molar tooth sign	*CC2D2A*	Chr4; NM_001080522.2:c.3364C > T (p.(Pro1122Ser))	Pathogenic	Homozygous	AR	COACH syndrome (OMIM#216360)
31	GDD, intractable seizures, epileptic encephalopathy	*FRRS1L*	Chr9; NM NM_014334.3; c.961C > T (p.(Gln321Ter))	Pathogenic	Homozygous	AR	Early infantile epileptic encephalopathy 37 (OMIM#616981)
32	Developmental regression, spasticity, sensory neuropathy, brain MRI showed diffuse supra-tentorial cortical atrophy	*UBTF*	Chr17; NM_014233.4; c.628G > A (p.(Glu210Lys))	Pathogenic	Heterozygous	AD	Neurodegenerative childhood-onset brain atrophy
33	Microcephaly, developmental delay, infantile spasm, stereotyped hand movements, brain MRI showed subtle periventricular white matter changes	*PGAP1*	Chr2; NM_024989.4; c.508A > G (p.(Ile170Val)). Chr2; NM_024989.4; c.148-1G > T	Pathogenic	compound heterozygous	AR	Mental retardation type 42 (OMIM#615802)
34	GDD, hypotonia, dysmorphic features, joint laxity, MRI delayed myelination	*FBXL4*	Chr6; NM_001278716.2; c.1698A > G (p.(Ile566Met))	Pathogenic	Homozygous	AR	Mitochondrial DNA depletion syndrome 13 (OMIM#615471)
35	Cardiac disease (left ventricular hypertrophy), spasticity, obesity, GDD, hypotonia, nystagmus	*ALMS1*	Chr2; NM_015120.4:c.8164C > T (p.(Arg2722*))	Pathogenic	Homozygous	AR	Alstrom syndrome (OMIM#203800)
36	GDD, hypotonia, microcephaly, dysmorphic features, and normal brain MRI	*UNC80*	Chr2; NM032504.1:c.3793C > T (p.(Arg1265*))	Pathogenic	Homozygous	AR	Hypotonia, infantile, with psychomotor retardation and characteristic facies 2 (OMIM# 616801)

Table 4
Copy number variations in hypotonic patients.

#	Dysmorphism	Consanguinity	Abnormal karyotype	Abnormal CGH Array	Human Genome Assembly	Abnormality	Pathogenic Vs.VUS	WES
1	+	+		+	Hg19	Arr[hg19] 2q11.21(18916842-21,465,659)x1	Pathogenic Deletion	NA
2	–	–	–	+	Hg19	Arr[hg19] 15q11.2(22770421-23,290,819)x1	VUS	NA
3	–	–	–	+	Hg19	Arr [hg19] 4q21.21q21.23(82359656-84155605px1	VUS	NA
4	–	–	+	+		Arr[18]x3	Pathogenic Trisomy 18	NA
5	–	–	–	+	Hg19	Arr[hg19] 3p12.3 (78847640-79,145,358)x1 pat	VUS	–
6	+	+	–	+	Hg19	Arr[hg19] 6q27(168981939-169,229,402)x3 par, 6q27 (169230654-16,997,930)x4 pat	Benign Duplication and Triplication	+
7	+	+	–	+	Hg19	Arr[hg19] 10q22.3q23.2(81617260-89,027,024)x1	Pathogenic Deletion	NA
8	+	+	–	+	Hg19	Arr[hg19] 16p12.1(21787504-22,431,357)x3	VUS	–
9	–	+	–	+	Hg19	Arr[hg19] 11q23.1(111629692-112,385,875)x1	Like Pathogenic	–
10	–	–	+	+	Hg19	Arr[hg19] 8pterp23.2(158048-5,473,064)x1, 8q21.2pter(86778228-146,295,771)x3	Likely Pathogenic Deletion, Pathogenic Duplication	NA
11	–	+	–	+	Hg19	Arr[hg19] 9q34.11(131190616-131,917,003)x1	VUS	NA
12	–	–	–	+	Hg19	Arr[]hg19] Xp11.23(46,950,670-47,045,430)x2	VUS	+
13	–	+	–	+	Hg19	Arr[hg19] qpqwpqq.w (120,468,424-121,343,783)x3	VUS	+

WES, Whole –exome Sequencing; VUS, Variant of unknown significance; CGH array, Array comparative genomic hybridization

genetic etiologies of our population, which might have mixed central and peripheral features.

The occurrence of peripheral hypotonia was significantly lower in our study, at only 17% compared to other studies, which can be attributed to the fact that comparable studies investigated specific patient categories, e.g., neonatal hypotonia or patients admitted to ICUs. In our cohort, we tried to minimize the selection bias that had been reported by previous studies, by including patients of all age groups from multiple hospital areas (ED, ICU, Wards, and clinics) and further classified patients into central hypotonia, peripheral and mixed hypotonia [5,9].

The age of the presentation was significantly correlated with hypotonia type in our study. Half of the patients aged younger than one month had peripheral hypotonia. Moreover, almost 54% of patients aged between 1 and 60 months of age had central hypotonia. After 60 months of age, the percentage of central hypotonia reduces significantly to 20%, while mixed and peripheral hypotonia become more predominant. The high rate of consanguinity in our population did not help guide the diagnostic workup of hypotonia patients, as it was not significantly associated with an abnormal physical examination or abnormal molecular testing. The presence of a family history of hypotonia and the investigation results of previously affected siblings or family relatives were more helpful to guide the hypotonia investigations.

The importance of history and physical examination in hypotonia diagnosis is well established in the literature [3][10,11]. However, only a few studies elaborated on the history and examination that were obtained [8,10]. In our study, patients with central hypotonia (53%) had more significant abnormal history compared to patients with peripheral hypotonia (19%) and mixed hypotonia (28%), which is also reported by Laguna et al. [8] NICU admission was observed in 50% of central hypotonia, while 60% of peripheral hypotonia patients were associated with abnormal fetal movement compared to 30% of central hypotonia patients.

Dysmorphism was noticed in all patient groups; however, it was noticed more in central hypotonia patients, although it did not reach statistical significance. This might support the notion that having a dysmorphic face might indicate a dysmorphic brain. Richer and colleagues observed this finding, as they reported facial dysmorphism in 42% of central hypotonia and 29% of peripheral hypotonia [5].

Despite the unique nature of our cohort, with a very high rate of consanguinity and high rate of autosomal recessive conditions, we were surprised to find that having a positive family history of hypotonia did not increase the diagnostic yield of the condition. This could be attributed to the lack of definitive diagnosis in the first affected child.

In our study, we found that muscle weakness was more associated with peripheral hypotonia and mixed hypotonia, as mentioned above, which support the emphasis in the literature on the importance of physical examination in hypotonic patients.

Deep tendon reflex, muscular weakness, and appendicular hypotonia were significantly correlated with the clinical type of hypotonia. On the other hand, axial hypotonia was not. Peripheral hypotonia was associated with absent reflexes, absence of antigravity movement, and appendicular hypotonia, as reported by other studies, but mixed hypotonia was associated with absent reflexes, appendicular hypotonia, and axial hypotonia, with or without weakness [8].

Our study demonstrates that the diagnostic yield of metabolic workup has not changed compared to previous studies. Metabolic investigations were not diagnostic in cases of hypotonia, but contributed to reaching the final diagnosis in 3–6% of the cases. Creatine kinase was the only biochemical test that correlated with the type of hypotonia, and it showed significantly high readings with peripheral hypotonia compared to central hypotonia: this finding is supported by other studies [5,6]. Although abnormal neuroimaging were significantly correlating with the type of hypotonia, which is commonly abnormal with central hypotonia, but it contributed to reaching a final diagnosis in 26% of our cohort, which is similar to what has been reported by Birdi et al. which is 33% of the cases [6].

We found that muscle biopsy has a low diagnostic value; however, our result could be biased due to the fact that we had only a small number of biopsied patients, and this could be due to a change in the practice toward molecular genetic testing with fewer invasive procedures.

Our study demonstrates a significant paradigm shift toward the use of genetic studies. We reviewed all molecular tests that were performed in relation to hypotonia patients. Chromosomal karyotyping was not helpful in the diagnosis as the diagnostic yield was only 1%. Array CGHs were diagnostic in 10% of patients, however, no significant association was found with the presence of dysmorphic features, this would be best addressed in a prospective study design with particular focus on dysmorphology including subtle changes. Gene panels and single-gene testing had higher diagnostic yields; 30% and 39%, respectively. Whole-exome sequencing had the highest diagnostic yield of all molecular testing, which helped to reach the final diagnosis in 59% of cases. These findings were noticed in other studies, such as Laguna et al. [8], who stated that DNA-based diagnostic tests were used to save time and money in the journey of hypotonia diagnosis [12,13].

Limitations of this study include limited documentation of some perinatal history, as it was obtained from the neurology clinic charts without an official perinatal report, and we were unable to accurately analyze the severity of the functional motor impairment; this offers a future opportunity for prospective cohort studies.

In conclusion, diagnosing a hypotonic patient still poses a diagnostic challenge despite the advancement of investigations. History and physical examination still play a major role in classifying hypotonia type. Neuroimaging is helpful in guiding further costly workup, e.g., molecular testing such as whole-exome sequencing. Molecular genetic testing, in the form of single-gene testing, gene panels, and whole-exome sequencing, whenever feasible, is our recommended next step in the diagnosis of patients with hypotonia after careful phenotyping. Whole exome sequencing has evolved to be more cost-effective and time saving for the diagnosis of hypotonia patients. Although metabolic diseases are important as treatable causes of hypotonia, the diagnostic yield of metabolic testing outside the neonatal period and among stable hypotonic patients is low, making molecular testing a better option to diagnose metabolic disease in children, especially with border-line metabolic investigation abnormalities.

Declaration of Competing Interest

None.

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