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
Diagnostic yield of hypotonia investigations. Table 2

| 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*             | 117           | 6%              | –                 | 1%                  | 0.349                            |
| VLCFA                  | 59            | 8%              | –                 | 6%                  | 0.547                            |
| Urine for organic acid | 101           | 10%             | 1%                | 2%                  | 0.767                            |
| Karyotype              | 74            | 4%              | –                 | –                   | 0.645                            |
| Microarray             | 65            | 28%             | 9%                | 2%                  | 0.735                            |
| Specific Gene Panel    | 55            | 58%             | –                 | –                   | 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%             | –                 | –                   | –                                 |

* 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 | Chr4; NM_000260.3:c.5001_5002del (p.(Tyr1668fs)) | Pathogenic | Homozygous | AR | Protopian Audacia (OMIM# 60954) |
| 4 | Developmental delay, Failure to thrive, abnormally high propanoyl carnitine | PCCA | Chr13; NM_000282.4:c.425G > A; (p.G142A) | Pathogenic | Homozygous | AR | Propionic Acidemia (OMIM# 606054) |
| 5 | Spastic tetraplegia, GDD | ALS2 | Chr2; NM_020919.4;2q33.1: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, AD | AR | Mental retardation 31 (OMIM# 604673) |
| 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_015192.4;2c.550C > T; (p.Arg184) | Pathogenic | Homozygous | AR | Early infantile epileptic encephalopathy 12 (OMIM# 617272) |
| 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_000260.3;c.4686G > A (p.(Trp1563)) | Pathogenic | Compound | heterozygous | AR | Merosin-deficient muscular dystrophy (OMIM# 607855) |
| 10 | Hypertrophic cardiomyopathy, hypotonia, swallowing dysfunction | GAA | Chr17; NM_001525.2:c.896 T > C; (p.L299P) | Pathogenic | Homozygous | AR | Pompe Disease (GSD 2) (OMIM# 232200) |
| 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 | COLA2, AHI1, PLA2G6, AMT, LAMA2 | Chr21; NM_01849.4;3:c.1053 + 1G > T; (p.Val691del) | Pathogenic | Homozygous | AR | Bethlem myopathy 1 (OMIM# 158810) |
| 13 | Developmental regression, hypotonia ataxia, spasticity | AMT | chr3; NM_000481.3;c.982dub (p.(Ala328fs)) | Pathogenic | Homozygous | AR | Glycine encephalopathy (OMIM# 608997) |
| 14 | Microcephaly, hypotonia, seizure disorder, GDD, failure to thrive, brain MRI showed genesis of corpus callosum and delayed demyelination | ADAT3 | Chr19; NM_138422.2:c.430G > A; (p.(Val144Met)) | Pathogenic | Homozygous | AR | AR mental retardation 36 (OMIM# 615280) |
| 15 | Epilepsy, GDD, Central Hypotonia, Spastic diplegia | AHI3 | Chr16; NM_017651.4;c.1328 T > A; (p.(Val443Asp)) | Pathogenic | Homozygous | AR | Joubert syndrome type 3 (OMIM# 608629) |
| 16 | GDD, spasticity, MRI brain showed Molar Tooth sign | LAMA1 | chr18; NM_000559.3;c.679C > A; (p.(Ser226Tyr)) | Likely pathogenic | Homozygous | AR | Poretti-Bolshauer syndrome (OMIM# 615969) |
| 17 | GDD, progressive leukodystrophy, severe Hydrocephalus, cerebellar atrophy and cystic Myopathic face, peripheral hypotonia, respiratory failure, Cryptorchidism, 21q11 deletion | MTM1 | ChrX; NM_000252.2:c.679G > A; (p.(Va.L227Met)) | Pathogenic | Homozygous | XR | Myotubular myopathy (OMIM# 310400) |
| 18 | Myopathy, hypotonia, failure to thrive, brain MRI showed delayed myelination | GALT | Chr9; NM_000155.4:c.983G > A; (p.(R328H)) | Pathogenic | Homozygous | AR | Classic galactosmia (OMIM# 239400) |
| 19 | Severe failure to thrive, hypotonia, chronic pancreatitis, brain MRI is unremarkable | CTRC | Chr1; NM_007272.2;c.649G > A; (p.(Gly217Ser)) | Pathogenic | Homozygous | AD | AD-chronic pancreatitis (OMIM# 167800) |
| 20 | Peripheral hypotonia, normal cognition, motor delay, contracture, high CK | LAMA2 | Chr6; NM_000260.3;c.1762delG; (p.(Ala588Leufs*11| Pathogenic | Homozygous | AR | Merosin-deficient muscular dystrophy type A (OMIM# 607855) |
| 21 | Hypotonia, weakness, brain MRI showed Dandy-Walker continuum with hydrocephalus | FKRP | Chr19; NM_024301.5;c.1364C > A; (p.(A.G15Arg)) | Pathogenic | Homozygous | AR | Congenital disorder of glycosylation 1A (OMIM# 212063) |

(continued on next page)
| #   | Patient phenotype                                                                 | Gene       | Variant                                                                 | Classification  | Zygosity | Disease mode of inheritance | Associated disease phenotype                                                                 |
|-----|----------------------------------------------------------------------------------|------------|-------------------------------------------------------------------------|-----------------|----------|----------------------------|------------------------------------------------------------------------------------------------|
| 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.879delT (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.(Val69Idel)). Chr22; NM_003560:c.1771C > T (p.(Arg591Trp)) | Pathogenic      | Homozygous | AR                         | Infantile neuroaxonal dystrophy 1 (OMIM# 256600)                                          |
| 29  | Distal arthrogryposis, bilateral developmental hip dysplasia, proso, hypotonia, developmental delay | ECEL1      | Chr2; NM_004026:3:c.1470G > A (p.(Trp490*))                            | Pathogenic      | Homozygous | AR                         | Distal arthrogryposis 3D (OMIM# 655960)                                                     |
| 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                              | FRARS1L    | Chr9; NM_0014334:3; 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 supratentorial cortical atrophy | UBTF       | Chr17; NM_014231:4; c.628G > A (p.(Glu218Lys))                         | Pathogenic      | Heterozygous | ADD                        | Neurodegenerative childhood-onset brain atrophy (OMIM# 126582)                            |
| 33  | Microcephaly, developmental delay, infantile spasm, stereotyped hand movements, brain MRI showed subtle periventricular white matter changes | PGAP1      | Chr2; NM_0024989:4; c.508A > G (p.(Ile170Val)). Chr2; NM_0024989:4; c.148-1G > T | Pathogenic      | compound heterozygous | AR                         | Mental retardation type 42 (OMIM# 655802)                                                   |
| 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.(Arg272*))                            | Pathogenic      | Homozygous | AR                         | Alström syndrome (OMIM# 203800)                                                            |
| 36  | GDD, hypotonia, microcephaly, dysmorphic features, and normal brain MRI          | UNC20      | Chr2; NM_003250:4; 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.

| Abnormality | Pathogenic VUS | Abnormal CGH | Abnormal WES | Pathogenic Deletion | Abnormal | Consanguinity | Abnormal | Pathogenic Duplication and Truncation | Abnormal | Pathogenic Deletion | Abnormality |
|-------------|----------------|--------------|--------------|---------------------|----------|---------------|----------|--------------------------------------|----------|---------------------|--------------|
| # | Dysmorphism | Centisomy | Abnormal | Abnormal | Abnormal | Abnormal | Abnormal | Abnormal | Abnormal | Abnormal | Abnormal | Abnormal |
| 1 | + | + | + | + | + | + | + | + | + | + | + | + |
| 2 | + | + | + | + | + | + | + | + | + | + | + | + |
| 3 | + | + | + | + | + | + | + | + | + | + | + | + |
| 4 | + | + | + | + | + | + | + | + | + | + | + | + |
| 5 | + | + | + | + | + | + | + | + | + | + | + | + |
| 6 | + | + | + | + | + | + | + | + | + | + | + | + |
| 7 | + | + | + | + | + | + | + | + | + | + | + | + |
| 8 | + | + | + | + | + | + | + | + | + | + | + | + |
| 9 | + | + | + | + | + | + | + | + | + | + | + | + |
| 10 | + | + | + | + | + | + | + | + | + | + | + | + |
| 11 | + | + | + | + | + | + | + | + | + | + | + | + |
| 12 | + | + | + | + | + | + | + | + | + | + | + | + |
| 13 | + | + | + | + | + | + | + | + | + | + | + | + |

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

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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