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ARTICLE

Merosin deficient congenital muscular dystrophy: Clinical, neuroimaging and immunohistochemical study of 8 Egyptian pediatric patients

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KEYWORDS

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Abstract Congenital muscular dystrophies (CMD) are a group of heterogeneous inherited autosomal recessive disorders characterized by muscular weakness, hypotonia and contractures. The Merosin Negative CMD (MNCMD) is considered to be the most severe form and is usually associated with white matter abnormalities as seen with brain imaging. Merosin is also expressed in the nervous system and its deficiency could affect its development. This article describes the clinical picture, muscle biopsy findings and neuroimaging abnormalities of eight Egyptian Pediatric patients with the clinical presentation of merosin negative congenital muscular dystrophy. The leading clinical presentation in almost all patients was severe hypotonia, muscular weakness and failure to achieve motor developmental milestones, only Case 2 walked at 2 years of age. Mentality was normal in most patients with exception of Case 2 in whom scholastic achievement was poor and was associated with behavior abnormality. Serum Creatine kinase ranged from moderate to severe elevation, 536–3563 U/L, Electromyography demonstrated a myopathic pattern in all patients. Brain MRI showed extensive demyelination of the cerebral white matter in 6/8 patients with extension to cerebellar demyelination in Case 5. 5/8 patients underwent muscle biopsy for which immunofluorescence staining for merosin demonstrated complete deficiency of laminin α2 in Case 5 & partial deficiency of laminin α2 in Case 2.

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This report demonstrates the utility of Immunofluorescence staining as a guide to confirm the diagnosis of MDCMD especially when molecular diagnosis is not available.

1. Introduction

Congenital muscular dystrophy (CMD) is a heterogeneous group of disorders, characterized by early onset muscle weakness, hypotonia and contractures [13]. There are several types of CMD with heterogeneous aetiology, one form is the Fukuyama type CMD with severe structural brain anomalies which has been mapped to chromosome 9q31-33. The pure form of CMD can be further subdivided into merosin positive and merosin negative CMD (MNCMD) [3].

Congenital muscular dystrophy (CMD) due to merosin (laminin alpha2 chain) deficiency is an autosomal recessive disorder characterized by severe muscular weakness, hypotonia since birth, absence of overt cerebral or ocular symptoms and muscle pathology consistent with a dystrophic process [1]. It had been suggested to account for 50% of all cases of CMDs [12]. The aim of this work was to describe the clinical, neuroimaging, and immune-histochemical features of eight pediatric patients who presented clinically to the Cairo University Children Hospital (CUCH) with MNCMD.

2. Case reports

2.1. Family 1, Cases 1, 2, 3

Family 1, Case 1, A 7 years old boy, the second child of first degree consanguineous parents, was born preterm by ventouse delivery after an uneventful pregnancy. He was cyanosed at birth and was incubated for 20 days. At birth he was noticed to be floppy. Social smile and mother recognition were present at 2 months of age. He presented to the neuropediatric clinic of the CUCH at the age of 2 years because of failure of achievement of his motor developmental milestones. Neurological examination revealed generalized hypotonia, diminished deep tendon reflexes, muscle power grade 3/6, weak facial musculature as well as generalized muscle wasting. Patient was able to support his head and sit unsupported but no standing nor walking. Speech was normal as well as mentality. His brain Magnetic Resonance Imaging (MRI) revealed extensive demyelination of the white matter Fig. 1. His Electromyographic (EMG) studies revealed myopathic pattern. Nerve Conduction Velocity (NCV) demonstrated features of neuropathic affection. His lab investigations showed marked elevated of Creatine Kinase (CK), 935 (reference interval: 21–232 U/L).

Case 2, The younger brother of Case 1, is a 3 years old male presenting with the same but milder clinical picture. His Ck was moderately increased, 532 U/L. His EMG was myopathic. Brain Computed tomography (CT) showed non-specific increased hypodensity in the white matter of the right parieto-occipital region. Brain MRI done by the age of 5 months and proved to be normal. There are no other affected members in the same family Fig. 2. Case 3, the youngest sister, a 4 months old female presented with generalized hypotonia and marked head lag, her CK was markedly elevated, 2600 U/L. Brain MRI showed extensive demyelination.

2.2. Family 2, Cases 4 & 5

Family 2, Case 4 is a 7 years old male boy, the first son of a first degree consanguineous couple. He presented to the neuropediatric clinic at the CUCH at the age of 20 months with global developmental delay and generalized hypotonia. His prenatal history was uneventful, he was delivered by Cesarean section, with history of cyanosis due to umbilical cord strangulation. Neurological examination revealed a floppy infant with severe generalized hypotonia manifested by marked head lag on traction response, ventral suspension showed the limbs hanging limply and vertical suspension revealed marked shoulder girdle hypotonia. Cranial nerve examination revealed no ptosis or external ophthalmoplegia with normal visual fixation and visual following movements, no difficulty in mastication or swallowing and normal Auditory Brain Stem Evoked Potenial (ABrSEP). Clinical Examination revealed no organomegaly nor cardiomyopathy. His lab investigations showed markedly elevated CK: 1831 U/L. EMG showed a myopathic pattern. Brain MRI done by the age of 2 years revealed an extensive demyelination suggestive of leukodystrophy, particularly at the frontal, parietal and occiptal regions Fig. 3. Based on the MRI findings, a leukodystrophy enzyme assay profile was performed including Aryl Sulfatase, N Acetyl Aspartatic acid, serum Very Long Chain Fatty Acids (VLCFA) and all were in the normal range. Plasma lactate was normal, 1.5 mmol/L (Normal; up to 2.2 mmol/L). Muscle biopsy

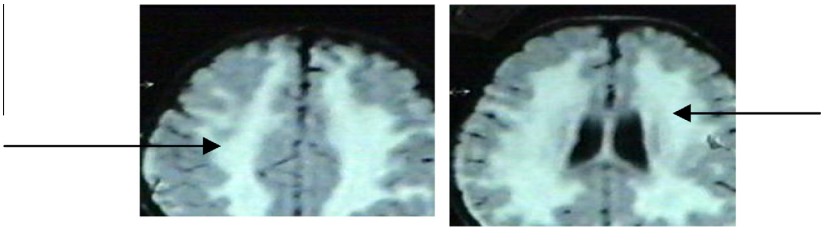

Figure 1 Flair Brain MRI of Case 1 showing bilateral diffuse cerebral demyelination in the deep periventricular white matter (left) and in centrum semiovale (right) (Arrows).

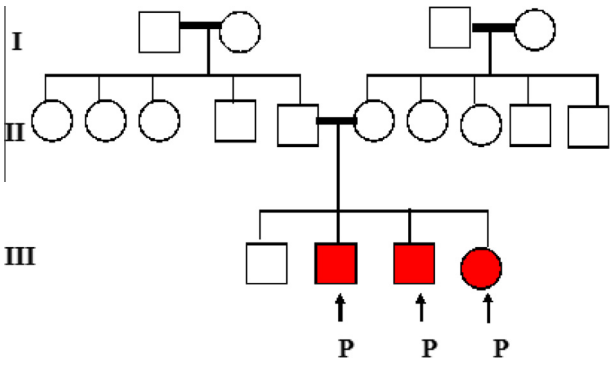

Figure 2 Family pedigree of Cases (1, 2, 3). P: Proband.

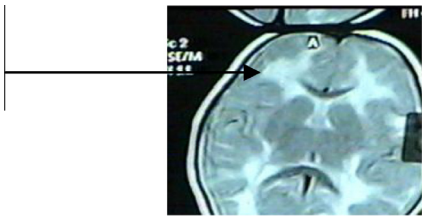

Figure 3 T2W brain MRI of Case 4 showing bilateral symmetric, diffuse, abnormal high signal intensity in the white matter of both cerebral hemispheres involving mainly the deep periventricular white matter (Arrow).

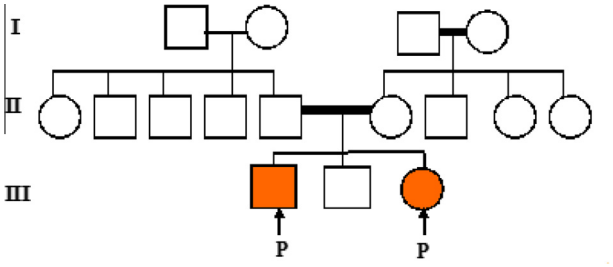

Figure 4 Family pedigree of Cases (4, 5). P: Proband.

analyzed by both light and electron microscopy showed the replacement of muscle fibres by fat and fibrous tissue with the presence of advanced nonspecific atrophic changes.

Case 5, 1.5 years old female, the younger sister of Case 4, presented to the CUPH because of failure of proper achievement of her motor developmental milestones. She was noticed by the mother to be floppy after birth. Neurological Examination revealed floppy infant with severe generalized hypotonia. Cranial nerve examination was normal with no ptosis nor external ophthalmoplegia, normal visual following movement and normal fixation, mastication and swallowing were intact. Normal hearing documented by normal ABrSEP. Abdominal examination did not reveal organomegaly. Cardiac examination and Echocardiography (ECHO) were normal. Her lab investigations showed markedly elevated CK, 1831 U/L. EMG showed myopathic changes and MRI done by the age of 1 year, demonstrated extensive demyelination involving mainly periventricular white matter in both cerebral hemi-

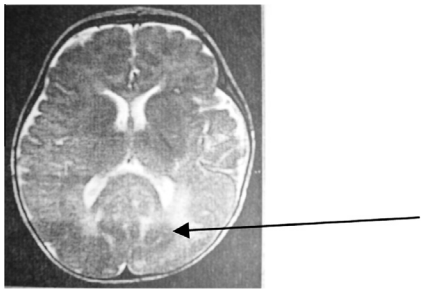

Figure 5 Brain MRI of Case 7 showed irregular high signal intensity involving the white matter of both occipital and peritrigonal regions on the T2WIs(Arrow).

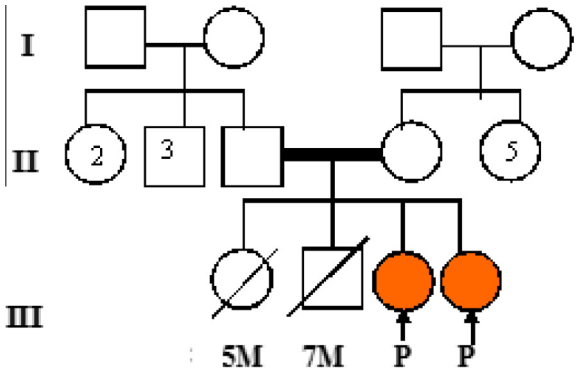

Figure 6 Family pedigree of Cases (6, 7). P: Proband, M: Months.

spheres as well as in cerebellar peduncles. No other affected member in the same family Fig. 4.

2.3. Family 3, Cases 6, 7

Family 3, Case 6, A 6 years old girl, the 3rd child of consanguineous parents. She was born by normal delivery, full term. The condition started by the age of 4 months where the mother noticed that the girl has delayed development, poor suckling and muscle weakness. Clinical examination showed severe hypotonia. EMG by the age of 9 months revealed myopathic pattern. ECHO was normal. CK was markedly elevated 1561 U/L. Plasma lactate was normal. Brain MRI done by the age of 2 years showed bilateral periventricular deep white matter areas of bright T2 and fluid-attenuated inversion recovery (FLAIR) signal intensity suggestive of demyelinating disease. Muscle biopsy done by the age of 1 year, Light microscopic examination showed extensive fat deposition, marked degeneration and necrosis of the muscle fibres. immunofluorescence (IF) staining showed normal dystrophin staining, normal Sarcoglycans staining and negative staining of merosin confirming MDCMD (results are not shown).

Case 7, the younger sister of Case 6, a 3 years old girl. She suffered the same clinical picture but with more progressive coarse. CK was elevated, 707 U/L. Plasma lactate was normal, 0.2 mmol/L. Brain MRI done by the age of 2 years showed irregular high signal intensity involving the white matter of both occipital and peritrigonal regions on the T2 Weighted

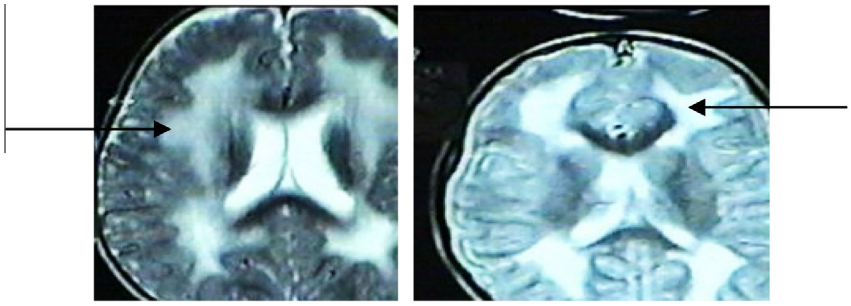

Figure 7 Brain MRI of Case 8 showed extensive demyelination changes of both cerebral hemispheres appearing as high signal intensity on T2WI images (arrows).

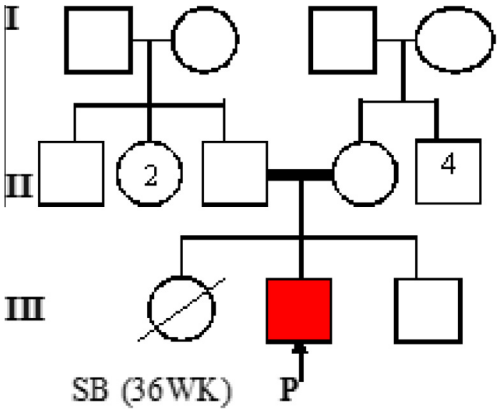

Figure 8 Family pedigree of Case 8. P: Proband, WK: Week, SB: Stillbirth.

Images (WIs) Fig. 5. There are two sibs who died by the age of 5 and 7 months, however the cause of death is not known Fig. 6.

2.4. Family 4, Case 8

Family 4, Case 8, A 5 years old boy, the 2nd child of first degree consanguineous parents, was born by normal vaginal delivery after an uneventful pregnancy. He developed recurrent episodes of chest infections since the age of one month. At the age of 6 months, he was noticed to be hypotonic, with inability to support his head. He presented to the CUCH at the age of one year because of failure to achieve his motor developmental milestones. Physical examination revealed an alert infant, but he was hypotonic with decreased spontaneous movements, he had complete had lag, with facial weakness, weak cry, swallowing and mastication. His body weight was at 5th centile and he has a proximal muscle power of grade 2/6 with generalized muscle wasting. Tendon Achilles was tight and deep tendon reflexes were markedly diminished. Ocular motility and fundus examination were normal, Auditory evoked potential (AEP) demonstrated a bilateral sensoryneural hearing loss with evidence of demyelination from the cochlea to the lower pons. At the age of 4 years, he was able to support his head, sit unsupported, but no walking nor standing with normal intelligence and speech. CK was elevated 536 U/L, Plasma lactate was normal. His metabolic screening was normal as well as enzymatic assay of Aryl Sulphatase and VLCFA.ECHO was normal. Brain MRI showed extensive demyelination changes of both cerebral hemispheres (including deep periventricular white matter, subcortical U fibers and centrum semiovale) in the form of low SI on TI WI and high signal intensity on T2WI & FLAIR images Fig. 7. EMG showed early recruitment, low amplitude, and brief duration of polyphasic motor unit potential, yet, the motor NCV studies were normal. Muscle biopsy showed prominent fatty and fibrous tissue replacement of muscle fibers, and the preserved fibers exhibited variation in diameter. There was no relevant inflammatory cell infiltration and the intermyofibrillary architecture was unremarkable. Electron microscopy showed loss of myofilaments and replacement by structureless material, these findings were consistent with progressive muscular dystrophy. Gomori trichome stain showed no ragged red fibres. There was no family history of neuromuscular disease, however the first baby was stillborn and a younger normal male sib Fig. 8.

3. Materials and methods

An open biopsy from the quadriceps femoris was performed in 5/8 patients under general anesthesia. All biopsies were taken after oral informed consent obtained from the parents of the diseased patients. Muscle biopsy specimens were snap frozen in liquid nitrogen then stored at −80 °C until processed. For the diagnosis of MDCMD, Immunofluorescence (IF) staining was performed on cryosections of all biopsy specimens from the probands along with negative control muscle biopsy specimens. Briefly, 7-mm thick Cryosections were air-dried for 1 h. The sections were then incubated for 1 h at room temperature with the following antibodies: the rat monoclonal anti-merosin (Alexis Co., San Diego, California, USA) diluted 1:1000 and the mouse monoclonal antibody (MAB1922, Chemicon® International, Inc., Temecula, California, USA) diluted 1:2000. The first antibody recognizes the human merosin 300 kDa fragment at the amino-terminal and the second antibody recognizes the merosin 80 kDa fragment towards the carboxy-terminal. Sections washed in 0.1% Tween-20/ phosphate-buffered saline (PBS) and then incubated with anti-rat and anti-mouse biotinylated IgG (Amersham Pharmacia Biotech Inc., Piscataway, USA), respectively, diluted 1:40 for 30 min. After three washes in Tween-20/ PBS, the binding reaction was detected with streptavidin fluorescein (Amersham Pharmacia Biotech Inc., Piscataway, USA) at 1:250 dilution for 30 min. All antibody dilutions were made in PBS (pH 7.4) con-

Table 1 Summary of the demographic and clinical data of the studied patients.

Pedigree/case N	Age at presentation in month	Gender	Consanguinity	Walking	Hypotonia	Ocular motility	Mental subnormality
P1/1	24	M	Positive	Not achieved	Severe	Normal	Normal
P1/2	36	M		Not achieved	Mild	Normal	Poor scholastic achievement, poor memoy
P1/3	4	F		Not achieved	Moderate	Normal	Behaviour abnormalities
P2/4	20	M	Positive	Not achieved	Severe	Normal	Normal
P2/5	18	F		Not achieved	Severe	Normal	NA
P3/6	4	F	Positive	NA	Severe	Normal	NA
P3/7	3	F		NA	Severe	Normal	NA
P4/8	6	M	Positive	not achieved	Severe	Normal	NA

P: pedigree, M: Male, F: Female, NA: Not Available.

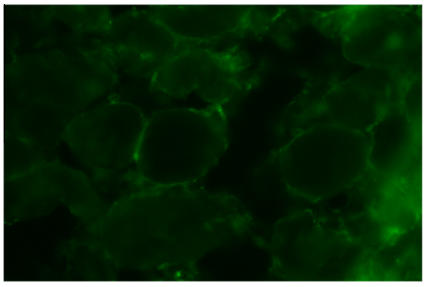

Figure 9 shows negative immunoflurescence staining Merosin 300 kd subunit (Amplified 40X) of Case 5.

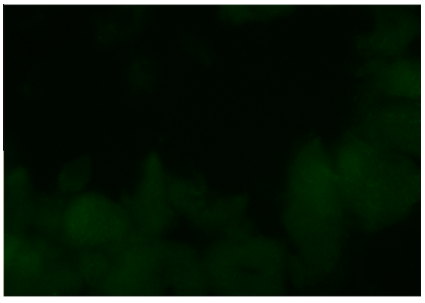

Figure 10 shows negative immunoflurescence staining of Merosin 80 kDa subunit (Amplified 40X) of Case 5.

taining 1% bovine serum albumin (BSA) (Sigma–Aldrich, St. Louis, Mo.). Sections were mounted in glycerol/PBS 1:1 and examined under a Zeiss microscope equipped for epifluorescence.

4. Results

Eight Egyptian patients from 4 different families (4 females & 4 males) had been included in this study. Their ages of presentation ranged from 3 to 36 months (Mean ± SD: 14 ± 11). The leading clinical presentation in almost all patients was severe hypotonia and failure to achieve motor developmental milestones, only Case 2 walked at 2 years of age. Mentality was normal in most patients with exception of Case 2 in whom

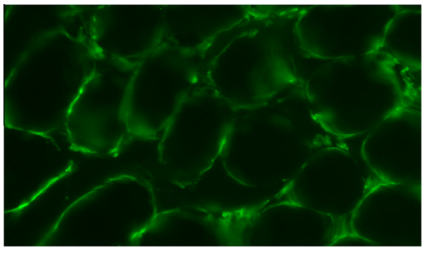

Figure 11 shows positive immunoflurescence staining of Merosin 80 kDa subunit (Amplified 40X) of Case 2.

scholastic achievement was poor and associated with behavior abnormality, positive consanguinity was elicited for all patients (Table 1). Serum Creatine kinase ranged from moderate to severe elevation, 536–3563 IU (Mean ± 2SD: 1533 ± 1033) (Table 2). Electromyography performed in all patients demonstrated a myopathic pattern. Brain MRI showed extensive demyelination of the cerebral white matter in 6/8 patients with extension to cerebellar demyelination in Case 5 (Table 2). 5/8 patients underwent muscle biopsy for which IF staining for merosin was applied. We were able to demonstrate complete deficiency of laminin α2 in Case 5, there was no immunostaining on muscle fibers with the anti-300 kDa antibody (Fig. 9) and the anti-80 kDa antibody (Fig. 10) & partial deficiency of laminin α2 in Case 2, the 80 kDa epitope was preserved (Fig. 11), but the 300 kDa epitope was reduced (Fig. 12). Such results confirmed the diagnosis of MDCMD. Muscle biopsy

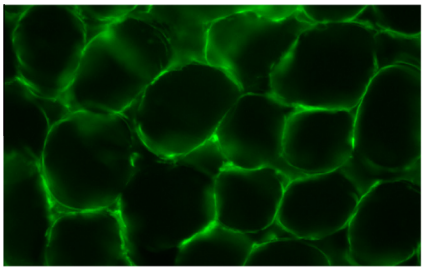

Figure 12 shows reduced immunoflurescence staining of Merosin 300 kDa subunit (Amplified 40X) of Case 2.

Table 2 Data of the laboratory, histochemical and MRI results among the studied patients.

Pedigree/case number	Serum CK reference interval(21–232) U/L	Light microscopy	IF stain for merosin	Brain MRI
P1/1	953	Replacement of muscle fibers by fibrous	ND	Extensive white matterdemyelination
P1/2	532		Partial deficiency	Normal
P1/3	2600	Replacement of muscle fibers by fibrous	ND	Periventricular demelination
P2/4	1831	Replacement of muscle fibres by fibrous	ND	Extensive demyelination involving the periventricular white matter
P2/5	3563		Complete deficiency	Extensive demyelination involving the periventricular white matter in both cerebral hemisphere and cerebellar hemisphere
P3/6	1561		Complete deficiency	Bilateral periventricular deep white matter areas of bright T2 and fluid-attenuated inversion recovery (FLAIR) signal intensity
P3/7	707	Extensive fat deposition & marked degeneration & necrosis of the muscle	ND	Bilateral perventricular deep white matter areas of bright T2w& flair signal intensity irregular high signal intensity involving the white matter of both occipital and peritrigonal region on T2w
P4/8	536	Replacement of muscle fibres by fibrous	ND	Extensive demyelination in both cerebral hemispheres

P: Pedigree, ND: Not done, −: Negative, +: Positive, CK: Creatine kinase.

specimens from patients 1, 4 and 8 showed marked degeneration of muscle fibres and extensive replacement with fatty and fibrous tissue elements interfering with IF staining. Demographic, clinical, neuroimaging, laboratory and IF staining results are summarized in (Tables 1 and 2).

5. Discussion

Congenital muscular dystrophies (CMDs), a heterogeneous group of inherited muscle disorders, characterized by a combination of early onset hypotonia and weakness, contractures, variable progression, normal or elevated CK, and myopathic changes on EMG, usually associated with a dystrophic muscle biopsy [11,18]. This group of conditions is thought to be among the most common of autosomal recessive neuromuscular disorders [17].

There are two categories of CMD depending on the structural involvement of the central nervous system (CNS) [10]. The classic form of CMD has no apparent clinical involvement of the CNS and the patient has normal intellect [10]. MNCMD is a severe form characterized by the absence of laminin α2 chain (formerly named merosin) around muscle fibres [13]. Most patients have normal intelligence but some have been reported to show mental retardation and epilepsy. They form a clinically homogeneous subgroup, in contrast to partial merosin deficiency cases who are less frequent, of variable severity but generally less severe [1].

In 1994, Tome et al. reported the first group of patients with classic CMD that were also deficient in merosin [15]. The merosin-negative cases showed clinical homogeneity with symptoms of severe hypotonia, multiple contractures, delayed developmental milestones and normal mentation accompanied by variable degrees of central hypomyelination as seen with neuroimaging [5,6]. Both complete and partial deficiency of the protein had been described and those with milder symptoms were associated with the partial form [14].

Merosin deficient congenital muscular dystrophy (MDCMD) was clinically suspected in the studied patients as they presented with early onset severe hypotonia, motor-developmental delay with increased CK levels, especially in the early months of life, no independent ambulation due to weakness and contractures and respiratory insufficiency which may need tracheotomy and abnormal cerebral imaging findings [3].

Although merosin-negative CMD cases demonstrate clinical homogeneity, the severity of weakness and CNS manifestation may vary. Patients with MNCMD are likely to develop dilated cardiomyopathy or right bundle branch block [6]. In this report, none of patients had cariac abnormalities. However, In a literature search on 248 reported cases, ECG or ECHO results were abnormal in 35% of the cases [5].

Brain MRI findings, which are found in almost all patients of MDCMD may be a valuable criterion for diagnosis. Characteristic white matter hypodensity was seen on brain MRI with high T2 signal in the periventricular and subcortical white matter [16]. EMG and NCS is performed in all patients with suspected CMD for confirmation of myopathy and exclusion of other diseases. NCS results are normal except in some cases of MDC1A, in which mild neuropathic changes may be seen. EMG changes usually show myopathic pattern and found early in all types of CMD [2]. In this report, all patients

showed normal pattern at NCV and myopathic pattern at EMG.

MNCMD is caused by mutations in the LAMA2 gene on chromosome 6q22–23 [7]. This gene is composed of 65 exons and at least 90 different mutations have been reported (http://www.dmd.nl/). Diagnosis of MDCMD is usually made by the clinical features and immunohistochemical examination of muscle biopsy. Using several antibodies directed against different regions of laminin α2 chain allows a precise distinction between complete and partial deficiency [4]. As there are neither common mutations nor hot spots for mutations, the detection of causative genetic abnormality is laborious, expensive and time consuming [7]. Accordingly, the immunohistochemical staining analysis of the muscle biopsies is a powerful diagnostic tool [1].

In the current study, 7/8 patients presented with severe hypotonia and hyporeflexia and was not able to achieve independent walking due to weakness and contractures, only Case 2 from Family 1, had mild motor delay and achieved walking at the age of 2 years, motor power assessment revealed a good muscle power of grade 4/5. This patient has poor scholastic achievement, poor memory as well as attention deficient hyperactive disorder. These parameters could not be assessed in other patients as their severe motor handicap prevented them from school attendance, however attempts performed by parents for education of these children reports a good response indicating a normal mentality despite extensive demyelinating process relevant to the brain MRI of 7 cases in contrast to mild nonspecific posterior demyelination in the brain MRI of Case 2, F1, so it seems that no correlation exist between the extent of demyelination and the mental affection.

Case 2, Family 1, is mostly suffering from partial merosin deficiency as the patient could achieve complete independent ambulation at the age of 2 years with grade 4/5 muscle power, he has mild mental subnormality and mild brain demyelination. Partial merosin deficiency can be due to LAMA2 defects or to other causes [1]. To our knowledge, all cases with partial merosin deficiency, normal intelligence and white matter changes are due to a primary LAMA2 defect [4]. But a few cases with primary partial merosin deficiency may present a slight mental retardation, epilepsy and/or very mild occipital cortex or cerebellum abnormalities in addition to the characteristic white matter changes [9]. Their precise diagnosis is then difficult in absence of a molecular confirmation as they can be mistaken for other disorders [7].

Results of the present study were in concordance with the results of Talim et al., 2000 who studied 76 patients from 70 families based on the immunohistochemical or linkage analysis, 39 were merosin deficient and 37 merosin positive. Similarly, the age of presentation of the Merosin deficient patients was younger than 2 years of age. Also, the average values of Ck level was high, 2021 IU/L and 26 patients in their studies had white matter hypodensity [13].

In the series of the probands of the present study, brain MRI in patients 1,2,3 showed diffuse extensive white matter signal-changes. In patient 4, there was no abnormal white matter changes on brain MRI at 5 months of age despite of confirming the diagnosis of MDCMD by IF. This finding underscores the age at which MRI is performed, because MRI changes may be hard to see in the early stages of the disease, with incomplete cerebral myelination. These abnormal white matter lesions are generally detectable after six months of age [3]. Also if brain MRI findings in early infancy (usually before four months of age) are normal in a child suspected of having MDCMD, repeated imaging studies after the age of one year should be very useful in evaluating white matter abnormalities.

6. Conclusion

To the best of knowledge, this is the first reported cases of MNCMD in Egypt. In this report, we demonstrated the utility of IF staining as a guide to reach the diagnosis and further molecular testing. As Egypt is a country with high consanguinity, autosomal recessive genetic disorders are very common. Further studies are highly recommended to estimate the prevalence of MDCMD among Egyptian patients. Also, Further molecular genetic analysis is recommended for better assessment of the phenotype/genotype correlation, genetic counseling and prenatal diagnosis.

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