World Journal of Medical Sciences 10 (4): 503-507, 2014
ISSN 1817-3055

DOI: 10.5829/idosi.wjms.2014.10.4.953

Congenital Muscular Dystrophy: Hospital Based Study in Egyptian Pediatric Patients

[1]G. Nakhla, [2]L. Selim, [1]Lubna O. Abdel-Salam,
[3]Amr Abdel Rahim and [1]Somia A.M. Soliman

[1]Department of Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt
[2]Department of Pediatrics, Faculty of Medicine, Cairo University, Cairo, Egypt
[3]Department of Surgery, Faculty of Medicine, Cairo University, Cairo, Egypt

Abstract: Congenital muscular dystrophies are a group of heterogenous inherited autosomal recessive disorders characterized by muscle weakness, hypotonia and delayed developmental milestones. They are classified into two groups, one with CNS affection and the other classic form with no affection of the intellect. The classic form is subdivided into merosin positive and merosin deficient groups. This article describes the clinical picture, electromyography, neuroimaging and muscle biopsy findings of six Egyptian pediatric patients with the clinical presentation of congenital muscular dystrophy, three males and three females. There were 4 cases of congenital merosin negative dystrophies and 2 congenital merosin positive dystrophies. The main clinical findings are muscle weakness and hypotonia, brain MRI demyelinating changes in 2 merosin negative cases. Serum Creatinine Kinase ranged from 109-5064 IU. Electromyography revealed myopathic picture in all cases. Each patient undergoes muscle biopsy. Each specimen was stained with H&E, Masson's trichrome and PAS. Immunohistochemistry was done to classify congenital muscular dystrophies, using merosin antibodies.

Key words: Congenital Muscular Dystrophy · Merosin · Muscle Disorders

INTRODUCTION

Congenital muscular dystrophies (CMDs) are clinically and genetically heterogeneous neuromuscular disorders with onset at birth or in infancy and in which the muscle biopsy is compatible with the presence of a dystrophic myopathy [1].

Congenital muscular dystrophies can be divided according to the structural involvement of the central nervous system (CNS) into two categories; one form is Fukuyama CMD, Walker-Warburg syndrome and muscle-eye-brain disease which are associated with structural abnormalities of the brain, severe mental retardation and seizures and the classic form of CMD which has no apparent clinical involvement of the CNS and the patient has normal intellect [2]. The classic form of CMD is further subdivided into merosin-positive or merosin-negative CMD. The merosin-negative CMD form is thought to be the most severe with inability to walk unaided and can be associated with white matter abnormalities as seen with brain imaging [2].

The aim of this work was to describe the clinical, neuro-imaging and immunohistochemical features in a group of pediatric patient who presented clinically to the Cairo University Children Hospital with congenital myopathy.

MATERIALS AND METHODS

- An open muscle biopsy from the Quadriceps femoris muscle was performed under local anesthesia in 6 patients. All biopsies were taken after an oral informed consent from the parents of the diseased children and a 1–2 cm long specimen was collected from all patients and immediately transported in a sterile container to the pathology laboratory for sectioning and preparation of slides.
- The muscle portion was cut in a transverse section then frozen in liquidnitrogen for one minute, then the cork was attached to the chuck of the cryostat microtome and frozen sections were performed with 10 mm thickness.

Corresponidng Author: Amr Abdel Rahim Abouzeid, Department of Surgery, Faculty of Medicine, Cairo University, Cairo, Egypt E-mail: amrorahim@kasralainy.edu.eg.

- Cryosections were obtained on glass slides then stained with haematoxylin/eosin, Masson's trichrome (To assess degree of endomysial&perimysial fibrosis) and periodic acid-Schiff (PAS) (Detection of glycogen) as a routine.
- Additional sections were prepared from the frozen section on charged glass slides.

Staining Procedure:

- Allow frozen sections to equilibrate to 25° C.
- Apply a 50 µL of appropriately diluted primary antibodies. Incubate for 1 hour at 37° C.
- Wash sections 3x10 minutes in phosphate buffered saline (PBS).
- Apply a 50µL of appropriately diluted biotinylated secondary antibody which binds to primary antibodies; it is polyvalent and universal. Incubate for 45 minutes at 25° C.
- Wash sections 3x10 minutes in PBS.
- Apply streptavidin-peroxidase reagent. Incubate for 45 minutes at 37° C.
- Apply DAB substrate solution (Freshly made just before use: 0.05% DAB 0.1% - H_2O_2 in PBS) to the sections.
- Dehydrate, clear and mount labeled sections for permanent preparations.

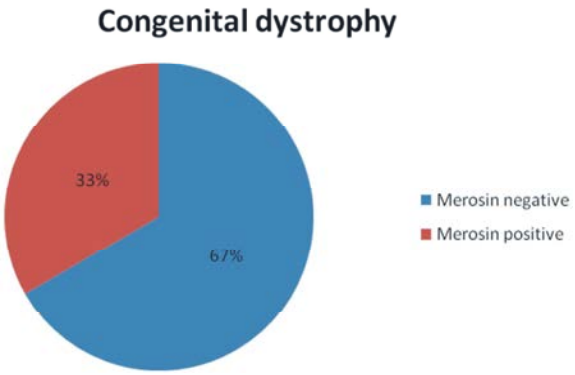
Primary Antibodies Used Are:

- Spectrin: it is a mouse monoclonal antibody (NCL-SPEC1, novacastra, laboratories, New Castle, Uk), which react with human spectrin protein. It was used diluted 1:100 in PBS. Normal muscle fibersshowed a continuous rim of labeling at the periphery of muscle fibers.
- Merosin (Laminin Alpha 2 Chain): it is a mouse monoclonal antibody (NCL-MEROSIN, novacastra, laboratories, New Castle, Uk) which react with human merosin protein. It was used diluted 1:100 in PBS. Normal muscle fibers showed continuous labeling of the extracellular matrix outside muscle fiber membranes.

RESULTS

This study included 6 patients (3 males and 3 females) suspected to have congenital muscular dystrophy attending pediatric clinics in Cairo University Children Hospital.

Congenital Muscular Dystrophies Comprised: merosin deficient CMD (4) cases (Showing no sarcolemmal staining of merosin antibodies) and merosin positive CMD (2) cases (Showing dystrophic picture with complete sarcolemmal staining of merosin antibodies) Graph 1.



Graph 1. Frequency of congenital muscular dystrophy

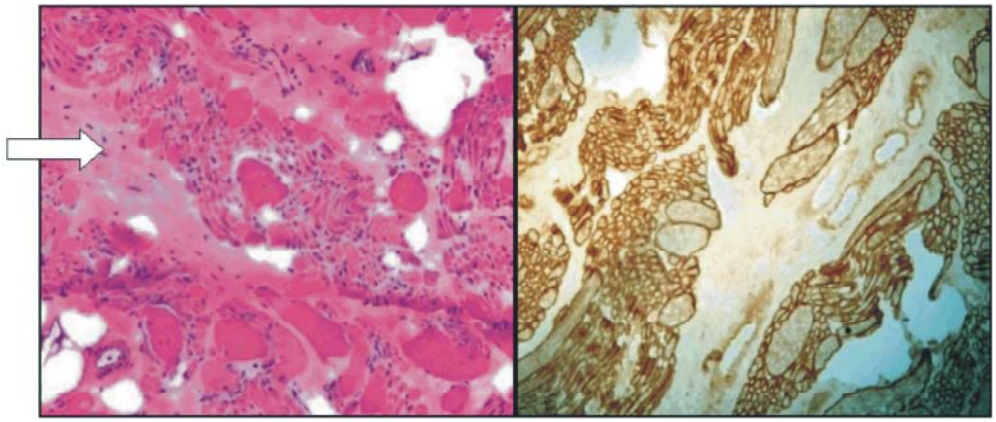


Fig. 1: A case of congenital (Merosin positive) muscular dystrophy. Variations of muscle fiber size, many small atrophic fibers (Arrow), connective tissue & fat replacement (H&E stained frozen section original x200). Normal sarcolemmalimmunostaining for Merosin, (Immunoperoxidase frozen scetionoriginal x200),

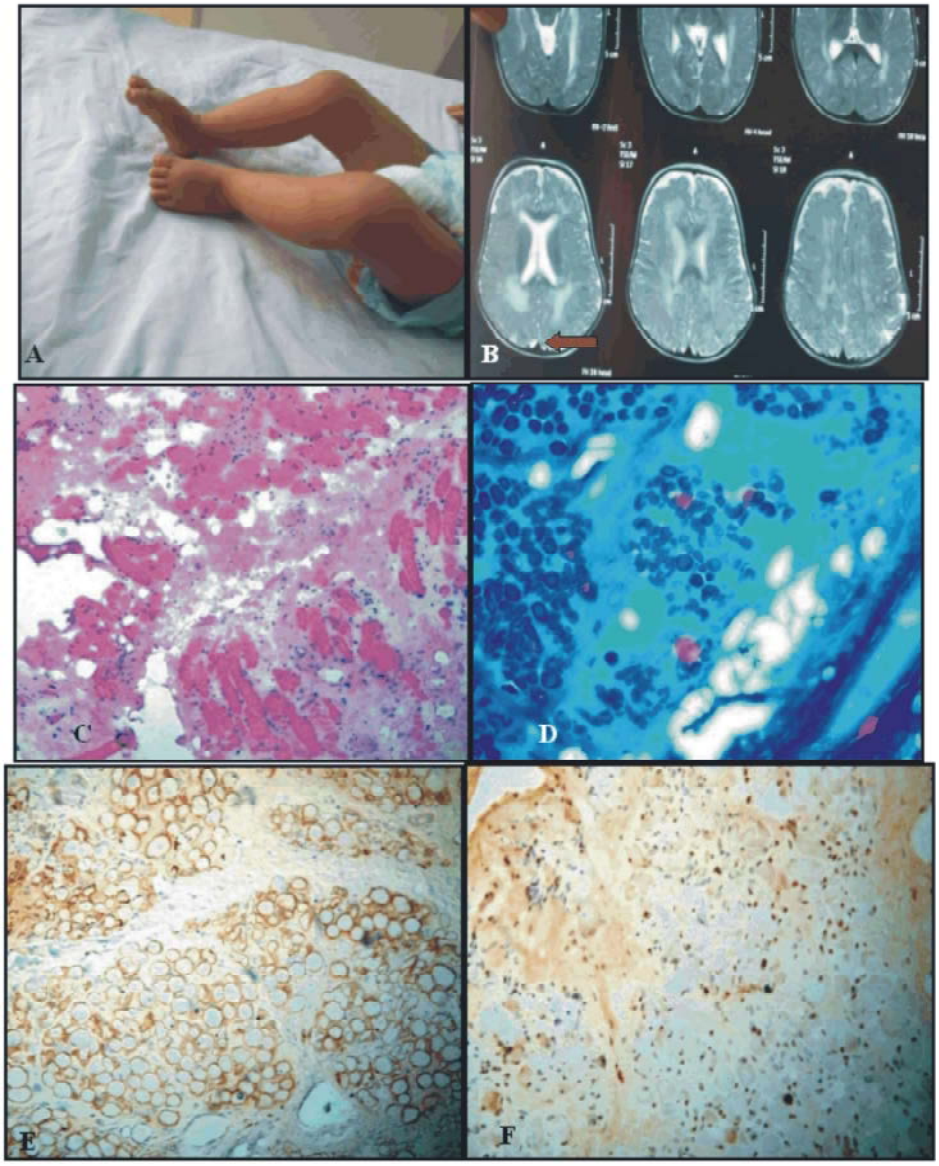


Fig. 2: A case of congenital (Merosin deficient) muscular dystrophy. (A) Child with knee contracture & calf pseudo hypertrophy, (B) MRI demyelinating changes (Arrow), (C) variations in fiber size, marked endomysial&perimysial fibrosis, mild fat replacement (H&E stained frozen section original x200), (D) Marked endomysial&perimysial fibrosis (Masson's trichrome staining original x200), (E) Normal sarcolemmal staining for spectrin (immunoperoxidase original x200), (F) Negative immunostaining of the sarcolemma for merosin (immunoperoxidase original x200).

Their ages of presentation ranged from 1 to 3 years (Mean ± SD: 1.33 ± 0.82).

The clinical presentation in all patients were hypotonia and delayed motor milestones, mentality was normal in all patients, positive family history was found in 5 (83.3%) out of 6 cases.

Serum creatine Kinase ranged from 109 – 5064 IU (Mean ± SD: 1823.00± 1754.99).

Electromyography performed in all patients showed a myopathic pattern.

Brain MRI study showed white matter demyelinating changes in 2 patients out of 4 merosin negative patients. All cases showed variations in fiber size, connective tissue & fat replacement, one case of merosin positive CMD showed many groups of markedly atrophic small fibers Figure 1.

DISCUSSION

Congenital muscular dystrophies (CMDs) describes a heterogenous group of inherited muscle disorders, characterized by a combination of early onset hypotonia, weakness, contractures, normal or elevated CK level, myopathic changes on Electromyography and usually associated with a dystrophic muscle biopsy [3].

All patients were of classic form (no apparent clinical involvement of the CNS) [4]. CMD with CNS manifestation were totally lacking in the present study as these comprise muscle eye brain disease, almost exclusively reported in Finland [5], the Walker-Warburg syndrome which is also a rare disease but spread worldwide [6] and Fukuyama CMD which is associated with major brain abnormalities and is almost exclusively observed in Japan [7].

The 6 patients were further subgrouped according to the presence or absence of merosin on immunohistochemistry according to Tome *et al*. [6] merosin positive CMD (33%) were less common than merosin deficient (67%).

This finding did not agree with the findings of Philpot *et al*. [8] who reported that merosin was present in (54.16%) cases and markedly deficient in (45.8%) and also our merosin deficient were higher than that reported by Tome *et al*. [6] Who reported that merosin deficient CMD accounts for about 30% of classical CMDs. As in the present series merosin positive (MP) CMD were the forms of CMD that showed dystrophic features and were not deficient in merosin, but could not be further classified as other immunohistochemical or molecular studies are not available.

However results of the present study were in concordance with the results of Talim *et al.* [9] who studied 76 patients from 70 families based on the immunohistochemical or linkage analysis, 39 were merosin deficient and 37 merosin positive.

The mean age in congenital muscular dystrophy group is 1.3 years, this agrees with Bertini *et al*. [1]. Also agrees with Laila Abdel Moteleb Selim *et al*. [10] who found the mean age is 1.2 years (14 months)in congenital deficient cases.

Results of the present study were also in concordance with Talim *et al.* [9] who studied 76 patients from 70 families based on the immunohistochemical or linkage analysis and found the age of presentation was younger than 2 years of age.

In our study 2 (50%) out of 4 congenital merosin deficient muscular dystrophy cases showed MRI demyelinating changes, Hui *et al.* [2] studied congenital muscular dystrophy cases and stated that most of the merosin negative cases show severe weakness and white matter abnormalities in brain imaging.

In our study the mean CK value in Congenital muscular dystrophy group was 1823 IU/L, This result matches with Egyptian study by Laila Abdel Moteleb Selim *et al*. [10] who found mean CK level in congenital muscular cases 1533 IU/L.

Results of the present study were also in concordance with the results of Talim *et al.* [9] who studied 76 patients and found the average values of Ck level was, 2021 IU/L.

All CMD cases showed variations of fiber size, connective tissue and fat replacement, except one case of merosin positive CMD showed many groups of small atrophic muscle fibers (Similar to those seen in spinal muscular atrophy) surrounded by connective tissue. This was stated by Mendell *et al*. [11] that muscle biopsy in CMD cases shows variability in fiber size, endomysial&perimysial connective tissue proliferation and varying degrees of necrosis and regeneration. Fiber atrophy is common; the prescence of endomysial connective tissue around atrophic fibers can help distinguish these fibers from the group atrophy seen in spinal muscular atrophy.

REFERENCES

1. Bertini, E., A. D'Amico, F. Gualandi and S. Petrini, 2011. Congenital muscular dystrophies: a brief review. SeminPediatrNeurol, 18(4): 277-88.
2. Hui, C.M., L. Kwong, S.Y. Lam and K.T. Loo, 2004. Merosin-deficient congenital muscular dystrophy in two siblings. Hong Kong Med J., 10(6): 423-6.
3. Muntoni, F. and T. Voit, 2004. The congenitalmuscular dystrophies in 2004: A century of exciting progress. Neuromuscul Disord, 14(10): 635-49.
4. Jones, K.J. and K.N. North, 1997. Recent advances in diagnosis of the childhood muscular dystrophies. J Paediatr Child Health, 33: 195-201.
5. Lenard, H.G., 1991.Congenital muscular dystrophies--problems of classification. Acta Paediatr Jpn, 33(2): 256-60.
6. Tome, F.M.S., T. Evangelista, A. Leclerc, Y. Sunada, E. Manole, B. Estournet, A. Barois, K.P. Campbell and M. Fardeau, 1994. Congenital muscular dystrophy with merosin deficiency. C R AcadSci, Paris, Life Sc., 317: 351-7.

7. Okada, M., G. Kawahara, S. Noguchi, K. Sugie, K. Murayama, I. Nonaka, Y.K. Hyashi and I. Nishino, 2007. Primary collagen VI deficiency is the second most common congenital muscular dystrophy in Japan. Neurology, 69: 1035-42.
8. Philpot, J., C. Sewry, J. Pennock and V. Dubowitz, 1995. Clinical phenotype in congenital muscular dystrophy: correlation with expression of merosin in skeletal muscle. NeuromusculDisord, 5(4): 301-5.
9. Talim, B., G. Kale, H. Topaloglu, Z. Akcoren, M. Caglar, S. Gagus and M. Elkay, 2000. Clinical and histopathologicalstudy of merosin-deficient and merosin-positive congenital muscular dystrophy. Pediatr Dev Pathol., 3(2): 168-76.
10. Laila Abdel motelebSelim, Dina Ahmed Mehaney, Fayza Abdel Hamid Hassan, Sawsan Abdel Hady Hassan, ImanGamaleldin, RandaSabry and Enrico Bertini, 2013. Merosin deficient congenital muscular dystrophy: Clinical, neuroimaging and immunohistochemical study of 8 Egyptian pediatric patients. Journal of Genetic Engineering and Biotechnology, 11(1): 61-68.
11. Mendell, J.R., DR Boué and PT. Martin, 2006. The congenital muscular dystrophies: recent advances and molecular insights. Pediatr Dev Pathol., 9(6): 427-43.