

Carrier Woman of Duchenne Muscular Dystrophy Mimicking Inflammatory Myositis

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Carrier woman of Duchenne muscular dystrophy (DMD) can mimic the inflammatory myositis in presenting symptoms. Two diseases should be differentiated by the clinical history, muscle biopsy and genetic study. There are few reports in which both histochemical and genetic study showed the possible link of overlapping inflammatory pathophysiology with dystrophinopathy. We report a 40-yr-old woman who presented with subacute proximal muscle weakness and high serum level of creatine kinase. She had a history of Graves' disease and fluctuation of serum liver aminotransferase without definite cause. MRI, EMG and NCV were compatible with proximal muscle myopathy. Muscle biopsy on vastus lateralis showed suspicious perifascicular atrophy and infiltration of mono-macrophage lineage cells complicating the diagnosis. Dystrophin staining showed heterogeneous diverse findings from normal to interrupted mosaic pattern. Multiple ligation probe amplification and X chromosome inactivation test confirmed DMD gene deletion mutation in exon 44 and highly skewed X inactivation.

Key Words: Muscular Diseases; Muscular Dystrophy, Duchenne; Carriers State

INTRODUCTION

Duchenne muscular dystrophy (DMD) is a severe human X linked recessive disorder of muscle characterized by progressive skeletal muscle wasting. The disease is caused by mutations in the dystrophin gene located on Xp21.2, which encodes a cytoskeletal protein in muscle. The incidence of DMD is about 1 in 3,500 male births and approximately one-third of the DMD patients originate through new mutations, while the rest are inherited through carrier mothers or arise from germ line mosaicism (1). The disease generally affects male as it is X linked recessive but female can manifest symptoms as heterozygous carriers of the disease (2). Late onset manifestation of carrier woman could confuse the diagnosis for myopathy as it has often indeterminate clinical features of inflammatory myopathy. Furthermore, overlapping inflammatory features on biopsy specimen could complicate the diagnosis. Recently we encountered a 40 yr old woman who presented with subacute proximal muscle weakness and had more than ten times of the upper normal limit of serum creatinine kinase (CK) level mimicking inflammatory myositis, but later she was revealed as a manifesting DMD carrier by gene analysis and X chromosome inactivation (XCI) study. Herein we describe the case and review the literatures.

CASE DESCRIPTION

A 40-yr-old housewife woman was admitted on January 25, 2010 to the rheumatism center because of 6-month history of increasing proximal muscle weakness which was exacerbated rapidly one month earlier.

She had been an athlete during her high school period and had been in usual state of health until 3 yr earlier, when she developed weight loss and fatigue with diagnosis of Graves' disease with thyrotoxicosis. At that time, her serum liver enzyme, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level increased to 184 IU/mL, 94 IU/mL respectively without serologic evidence of viral hepatitis, which endocrinologist considered as a feature of thyrotoxicosis. After one and a half year long methimazole treatment, remission of hyperthyroidism was achieved. But her liver enzyme continued to fluctuate within two times of upper normal range during the follow up. Eighteen month before admission, because she reported fatigue and weakness despite of euthyroid status, she was referred to gastroenterologist for the evaluation of persistent mild liver enzyme elevation. Her viral marker for hepatitis B and C were negative and ultrasonography showed innumerable small gall stone and small liver parenchymal calcification. She was

instructed to be followed up regularly at gastroenterology department. One year before admission, weight loss and myalgia were developed with elevation of liver aminotransferase again up to 187 IU/mL of AST. She was undertaken ultrasonography guided liver biopsy which revealed minimal porto-periportal activity and minimal portal fibrosis. Anti-nuclear antibody (ANA) showed titer of 1:20 with speckled pattern. Anti-smooth muscle antibody and anti-LKM-1 antibody was negative. Prednisone and azathiopurine were prescribed by gastroenterologist for her long lasting elevation of liver enzyme under the impression of autoimmune hepatitis without definite cause. Six month before admission, recurrent myalgia, muscle weakness, and anorexia developed with fluctuation of liver aminotransferase serum levels despite of treatment with azathiopurine and low dose prednisone. Three month earlier, proximal muscle weakness became evident and she felt difficulty in climbing upstairs and worsening even thereafter.

On initial examination, she showed chronic ill-looking appearance and vital signs were within normal range. She could walk but complained of difficulty in climbing upstairs or raising arms. There was no specific skin lesion on her face or body. During the interview, we found that her two sons had been diagnosed as DMD in childhood and were bed-ridden status since long before. Her two brothers and parents were in usual health status, which suggests de novo mutation. Her serum CK level was 2,436 IU/mL, aldolase 11.9 IU/mL, LDH 312 IU/L. Serum TSH and free T4 were normal. Antinuclear antibody, anti-Jo-1 antibody, and anti-PM-SCL antibody were all negative. Chest radiography showed normal finding. Fat suppression T2 weighted MR image in the thigh level showed multiple atrophy in thigh muscles such as gluteus maximus, tensor fasciae latae, vastus lateralis and hamstring muscles. When contrast media admin-

istered, there was no gadolinium enhancement. Transthoracic 2D echocardiography showed normal chamber size and good systolic function without evidence of pulmonary hypertension. Muscle biopsy of right vastus lateralis revealed myopathic change showing small group muscle atrophy with a few regenerating fibers and infiltration of inflammatory cell as well as suspicious area of perifascicular atrophy mimicking dermatomyositis. Additional special staining showed positive staining for leukocyte common antigen (LCA), CD 68 but rarity for CD 4, CD 8, which meant the main inflammatory cells are macrophage-lineages. Dystrophin staining was not negative, but showed diverse staining pattern from normal to interrupted sarcolemmal activity in some area (Fig. 1). EMG-NCV study revealed short duration, small amplitude, increased insertional activity, positive sharp wave and fibrillation compatible with myopathy. Based on progressive features of myopathy with inflammatory evidence, high-dose prednisone was tried at dosage of 1.0 mg/kg/day. After 2 weeks of corticosteroid treatment, good response was observed as decreasing serum CK level to near normal and improving weakness from grade 4 minus to grade 4 plus by Medical Research Council's grading system. But initial steroid response was not lasted longer and her muscle weakness and fluctuating serum CK level was repeated on follow up despite continuation of oral prednisone. After 6 month of referral to our center, intravenous immunoglobulin and oral cyclosporine was tried but previous improvement of muscle enzyme and weakness were not observed any more.

To identify the underlying genetic defect, we performed molecular genetic testing for the *DMD* gene. After we had obtained informed consent, blood sample was collected from the patient and genomic DNA was isolated. We performed multiple ligation probe amplification (MLPA) analysis using the test kits SALSA

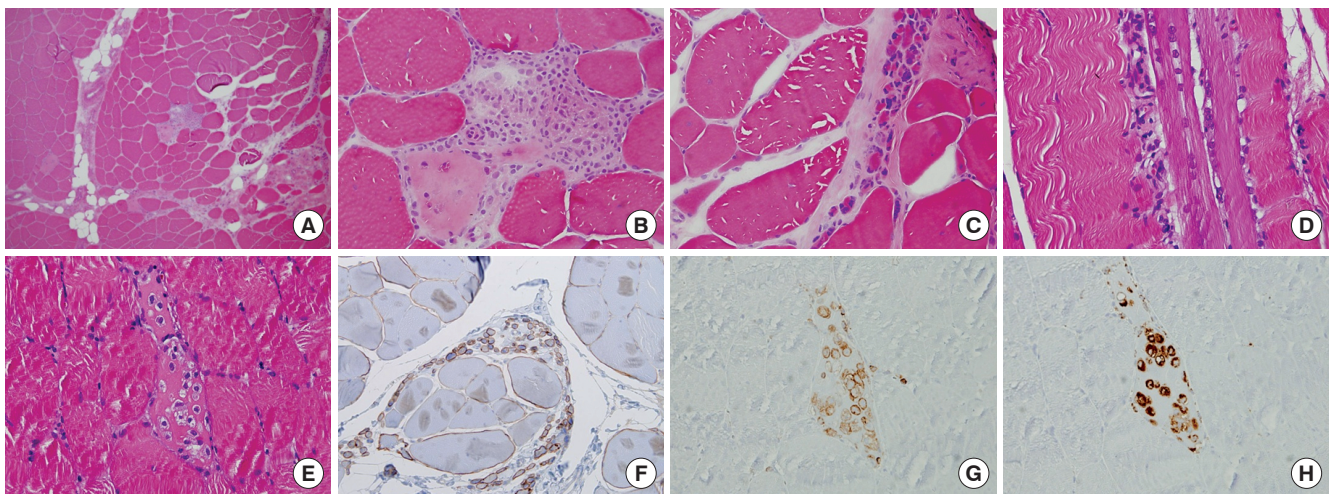


Fig. 1. Muscle biopsy specimen of right vastus lateralis muscle. It shows variable sized muscle fiber, fatty infiltration, perimysial fibrosis, focal inflammatory infiltrates with the suspicious area of perifascicular atrophy in low power (A) and high power view (B). Perimysial atrophy mimicking dermatomyositis are also shown (C). Also regenerating fibers with inflammatory infiltration was shown (D, E). Dystrophin staining showed variable different staining pattern from normal to interrupted mosaic pattern (F). Inflammatory cells are mainly macrophage-lineages on LCA (G), CD 68 (H) special staining with rarity of CD 4 and CD 8 positive cells (not shown).

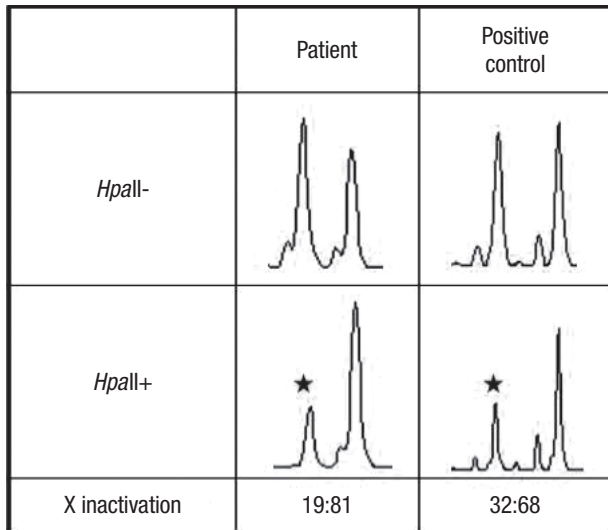


Fig. 2. X chromosome inactivation (XCI) analysis in this patient. After digestion with *HpaII*, a PCR product is obtained from the inactive X chromosome. Highly skewed X chromosome inactivation is found with ratio of 19:81 compared to positive control.

P034 DMD and P035 DMD from MRC-Holland (Amsterdam, Netherland) and revealed a heterozygote deletion mutation involving exon 44 in this patient (3).

The XCI pattern was determined by PCR analysis of a polymorphic CAG repeat in the *HUMARA* (4). Methylation of sites located close to this short tandem repeat correlates with XCI. After digestion with the methylation sensitive enzyme *HpaII*, a PCR product is obtained only from the inactive X chromosome. The PCR product was analyzed by GeneScan Software (Applied Biosystems, Foster City, CA, USA). XCI was calculated as the ratio between the intensities of the PCR products of the two alleles with the smallest allele given first. The presence of skewed XCI was considered if the ratio was $\geq 35:65$, and considered extremely skewed if the ratio was $\geq 20:80$ (5). The heterozygous allele of the X chromosome in non-digested sample was shown with a CAG repeat polymorphism (Fig. 2). After DNA was digested with *HpaII*, the patient showed one distinct peak and one faint peak, indicating that these had a highly skewed XCI and the ratio was 19:81.

DISCUSSION

Differential diagnosis for adulthood onset myopathy without definite infective or pharmacologic etiology in woman usually include inflammatory myositis such as polymyositis or dermatomyositis but, though rarely encountered in general population, late onset genetic disorder of muscular dystrophy also should be sought by means of special staining of muscle cells as well as genetic study as shown in this case.

There are few reports in which both histochemical and genetic study showed the possible link of overlapping inflammatory pathophysiology with dystrophinopathy in a normal karyo-

type woman. This case showed that late onset of myopathic symptoms in manifesting carrier of DMD with normal karyotype could complicate the diagnosis by overlapping inflammatory features on muscle biopsy specimen if physician is unaware of the family history of DMD. In contrast to our case of normal karyotype, in Korea, there are two case reports of manifesting carrier of DMD in Turner syndrome (6, 7). One of them, 6 yr old girl, was confirmed by MLPA method but without histochemical finding and the other, 12 yr old girl, was confirmed by negative dystrophin staining on histochemical study but multiplex PCR was negative for DMD detection. Relatively early onset of manifestation in these two cases is related to their karyotype of Turner syndrome because of X-recessive nature of DMD. One retrospective study for DMD in Korea identified three carrier girls (8-10 yr of age) among 93 patients diagnosed with Duchenne or Becker muscular dystrophy from 1989 to 2008, but histochemical findings or karyotype of carriers were not reported (8).

DMD, which is characterized by compromised sarcolemmal integrity from defective dystrophin-associated protein complex (DAPC), bound in skeletal myocyte membrane, cause progressive myopathic weakness, gait disturbance and grossly elevated serum creatine kinase (CK) as a result of degenerating fibers. In male DMD patients, severe myopathic features develop in early and most of the patients are wheelchair bound by 10 to 15 yr of age (9). Women, as a heterozygous carrier of DMD gene in one of their two X chromosome, usually do not show severe myopathic features because of possible compensation by normal X chromosome with inactivation of defected DMD gene in diseased X chromosome, which occur randomly (10). But sometimes, this compensation could be reversed by nonrandom inactivation of X chromosome enough to cause some degree of myopathic features. Non-random skewed X chromosome inactivation is one of possible mechanism which inactivate preferentially normal X chromosome (11). Other mechanisms of manifesting carrier include X chromosome rearrangement involving DMD locus and complete or partial absence of X chromosome (Turner syndrome).

Unlikely to male DMD patients, manifesting carrier woman showed variable disease activity and could be undetected. Incidence of manifestation carrier among DMD carrier women could be hardly assessed because of both variable severity of disease among carriers and different definitions for manifesting carrier. About 2.5%-10% of female carriers are classified as manifesting carriers for showing some degree of muscle weakness, and frequently having enlarged calves (9, 12). One cohort study in Netherlands reported that DMD carrier women showed symptomatic muscle weakness in 19%, myalgia in 5%, dilated cardiomyopathy in 8%, EKG abnormality in 47%, echocardiographic abnormality including cardiac chambers dilatation in 36%. Mean age at onset of symptoms was 33.6 yr while severe phenotypes

in girls were reported with rather young age (2, 13). Elevation of serum creatine kinase was common in as much as 50% of carrier. Though manifestation of DMD is not uncommon in carrier woman, the exact prevalence of carrier status among general population and the frequency of severe manifestation are not yet known in Korea.

Our patient is also noticeable in the onset of symptoms in her late thirties and elevation of liver aminotransferase could not be regarded as a manifesting features initially, which leads to misdiagnosis of autoimmune hepatitis before referral to us. In one retrospective study in Korea, 37% of Duchenne/Becker dystrophy patients presented elevation of liver enzyme before the diagnosis in retrospective study in Korea (8).

Muscle biopsy findings of manifesting carrier were investigated in previous reports in views of dystrophin staining (14). While the absence of dystrophin staining in muscle specimen is diagnostic in male DMD patients, specimen from manifesting carrier showed variable findings from normal to regional absence or mosaic pattern of sarcolemmal staining with anti-dystrophin which meant different presentation of abnormal dystrophin gene by myocyte within same patient (10). Mosaic pattern of dystrophin staining could be the result of compensated dystrophin production from multinucleated skeletal myocyte. Muscles of patients with DMD patients also exhibit inflammatory changes, which is same for manifesting carriers. Class I MHC antigens are known to be strongly expressed in skeletal myocytes of DMD patients and mononuclear cell infiltration with possible role of inflammatory cytokines to induce myocyte degeneration are observed as well as atrophy (15, 16). Infiltration by macrophages and T-cells as well as varying degree of degeneration could mimic inflammatory myopathy. In the presenting case, finding of dominant macrophage infiltration with rare CD 4 and CD 8 T cells could be caused by prior corticosteroid therapy, which might obscure inflammatory T cell from biopsy specimen.

Because of tremendous size (2.2 megabases), complexity (8 promoters), and diversity of mutations within DMD gene, DNA testing for DMD presents a challenge for clinical laboratories. DMD gene contains 79 exons and its protein product, dystrophin, has 3,685 aminoacids with molecular weight of 427 kDa (17). While multiplex polymerase chain reaction (PCR) could detect 98% of mutations in affected male, multiplex PCR is not useful for detecting female carriers in whom deletions are masked by amplification of the normal X chromosome. In that reason, we used multiple ligation probe amplification (MLPA) for deletion/duplication analysis of DMD gene and found deletion mutation in exon 44 area (3). Furthermore we performed non random X chromosome inactivation, skewed XCI, study to ascertain the diagnosis of manifesting carrier and found highly skewed XCI ratio over 20:80, which correlated with severity of our patient.

Exact pathophysiological mechanism of muscle degeneration

is not yet determined in DMD and several possible mechanisms are mechanical membrane fragility, abnormal calcium homeostasis with activating protease and mitochondrial overloading, abnormal gene regulation, and inflammation induced by immune cells and cytokines (18). If it is the same for carrier woman, in case of manifesting carrier of DMD with skewed X inactivation, symptomatic myopathic features could be originated dependent to individual threshold for compensation of muscle degeneration. So the changing extent of imbalance between muscle degeneration and regeneration could result in both asymptomatic carrier with high serum CK level in one patients and manifesting carrier with myopathic feature in another (18).

Inflammatory myositis such as polymyositis or dermatomyositis is caused by immunological pathologic mechanism without defect in cytoskeleton membrane protein but the diagnostic criteria could be met in the case of manifesting carrier of DMD without careful consideration for muscular dystrophy even though there must be differentiation of these two disease entity (19). Most important point would be a different long-term treatment strategy in relation to immunosuppressive agent and prognosis. In pediatric DMD patients, studies have shown that prednisone improves the strength and function. It is hypothesized that prednisone has a stabilizing effect on membranes and perhaps an anti-inflammatory effect could be beneficial for delay in degeneration process. Though there is few report regarding to corticosteroid use in manifesting carrier, our patient initially responded to corticosteroid therapy, which was not lasted longer. In pediatric patients, cyclosporine also was reported to improve clinical function in DMD children. Other emerging therapies under evaluation include synthetic cyclosporine analogue as cyclophilin inhibitor, calpain inhibitor, oxandrolone as anabolic steroid, pentoxifylline, and β -agonist such as albuterol. In regarding to overlapping inflammatory features of DMD, tumor necrosis factor- α (TNF- α) blocking agents are suggested based on the studies from mdx mouse, animal model for DMD, but not yet investigated in patients (20).

In conclusion, we report a 40-yr-old woman of myopathic feature mimicking inflammatory myositis as a manifesting carrier of DMD with highly skewed-XCI pattern by molecular genetic analysis. Physicians should take caution in diagnosing inflammatory myositis based on clinical criteria because muscular dystrophy of adulthood could present with overlapping inflammatory features on biopsy. Careful pattern recognition for dystrophin staining with genetic study is necessary to make correct diagnosis.

REFERENCES

1. Roberts RG. *Dystrophin, its gene, and the dystrophinopathies*. *Adv Genet* 1995; 33: 177-231.
2. Hoogerwaard EM, Bakker E, Ippel PF, Oosterwijk JC, Majoor-Krakauer

- DF, Leschot NJ, Van Essen AJ, Brunner HG, van der Wouw PA, Wilde AA, de Visser M. *Signs and symptoms of Duchenne muscular dystrophy and Becker muscular dystrophy among carriers in The Netherlands: a cohort study. Lancet* 1999; 353: 2116-9.
3. Gatta V, Scarciolla O, Gaspari AR, Palka C, De Angelis MV, Di Muzio A, Guanciali-Franchi P, Calabrese G, Uncini A, Stuppia L. *Identification of deletions and duplications of the DMD gene in affected males and carrier females by multiple ligation probe amplification (MLPA). Hum Genet* 2005; 117: 92-8.
 4. Allen RC, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW. *Methylation of HpaII and HhaI sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. Am J Hum Genet* 1992; 51: 1229-39.
 5. Xinhua Bao, Shengling Jiang, Fuying Song, Hong Pan, Meirong Li, Wu XR. *X chromosome inactivation in Rett Syndrome and its correlations with MECP2 mutations and phenotype. J Child Neurol* 2008; 23: 22-5.
 6. Lee KA, Han SH, Choi JR, Chung JS, Choi YC. *Becker muscular dystrophy with r(X) carrying an out-of-frame DMD deletion. Pediatr Neurol* 2008; 39: 129-32.
 7. Yang MS, Lee DK, Oh KY, Choi KS, Lee KH. *Duchenne muscular dystrophy in a girl with Turner syndrome. J Korean Acad Rehabil Med* 2005; 29: 537-40.
 8. Seo CD, Lee YJ, Lee EH, Jeong MH, Yum MS, Ko JM, Yoo HW, Ko TS. *The clinical features, immunostaining and genetic study in Duchenne/Becker muscular dystrophy. J Korean Child Neurol Soc* 2009; 17: 40-9.
 9. Emery AE. *The muscular dystrophies. Lancet* 2002; 359: 687-95.
 10. Bonilla E, Schmidt B, Samitt CE, Miranda AF, Hays AP, de Oliveira AB, Chang HW, Servidei S, Ricci E, Younger DS, Dimauro S. *Normal and dystrophin-deficient muscle fibers in carriers of the gene for Duchenne muscular dystrophy. Am J Pathol* 1988; 133: 440-5.
 11. Yoshioka M, Yorifuji T, Mituyoshi I. *Skewed X inactivation in manifesting carriers of Duchenne muscular dystrophy. Clin Genet* 1998; 53: 102-7.
 12. Norman A, Harper P. *A survey of manifesting carriers of Duchenne and Becker muscular dystrophy in Wales. Clin Genet* 1989; 36: 31-7.
 13. Hoogerwaard EM, van der Wouw PA, Wilde AA, Bakker E, Ippel PF, Oosterwijk JC, Majoor-Krakauer DF, van Essen AJ, Leschot NJ, de Visser M. *Cardiac involvement in carriers of Duchenne and Becker muscular dystrophy. Neuromuscul Disord* 1999; 9: 347-51.
 14. Hoogerwaard EM, Ginjaar IB, Bakker E, de Visser M. *Dystrophin analysis in carriers of Duchenne and Becker muscular dystrophy. Neurology* 2005; 65: 1984-6.
 15. McDouall RM, Dunn MJ, Dubowitz V. *Nature of the mononuclear infiltrate and the mechanism of muscle damage in juvenile dermatomyositis and Duchenne muscular dystrophy. J Neurol Sci* 1990; 99: 199-217.
 16. Nirmalanathan N, Holton JL, Hanna MG. *Is it really myositis? A consideration of the differential diagnosis. Curr Opin Rheumatol* 2004; 16: 684-91.
 17. Muntoni F, Torelli S, Ferlini A. *Dystrophin and mutations: one gene, several proteins, multiple phenotypes. Lancet Neurol* 2003; 2: 731-40.
 18. Deconinck N, Dan B. *Pathophysiology of duchenne muscular dystrophy: current hypotheses. Pediatr Neurol* 2007; 36: 1-7.
 19. Dalakas MC. *Polymyositis, dermatomyositis and inclusion-body myositis. N Engl J Med* 1991; 325: 1487-98.
 20. Radley HG, De Luca A, Lynch GS, Grounds MD. *Duchenne muscular dystrophy: focus on pharmaceutical and nutritional interventions. Int J Biochem Cell Biol* 2007; 39: 469-77.