N-CAM expression: The study of muscle disease in a tertiary center of Thailand

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Research article

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Abstract

Objectives To evaluate the correlation between N-CAM expression and diagnosis of common muscle diseases in the Thai population.

Methods The expression of N-CAM was interpreted in 75 muscle biopsy specimens diagnosed with myopathies in a 3-year retrospective study. We used anti-CD56 (MRQ-42) rabbit monoclonal primary antibody (1:200, Cell Marque, MilliporeSigma, Rocklin, CA, USA) in our immunohistochemical study.

Results Of our 75 patients, 41 cases were female and 34 were male. The mean age of the patients was 35-year-old, with the range from 3 months to 83 years. 35 (46.67%) of the specimens were N-CAM-positive, and 40 (53.33%) were N-CAM-negative. There were 35 (46.67%) cases diagnosed with nonspecific myopathy, 17 (22.67%) with inflammatory myopathy, 9 (12.00%) with neurogenic dystrophy, 7 (9.33%) with muscular dystrophy, 5 (6.67%) with non-diagnostic myopathy, and 2 (2.67%) with mitochondrial myopathy.

Chi-squared testing was used to analyze the N-CAM expression data and the diagnosis. There is a statistically significant correlation between inflammatory myopathy and N-CAM positivity (p-value <0.001, OR 14.250, 95% CI 2.960-68.606), with 15 out of 17 cases being N-CAM-positive. N-CAM was also positive in muscular dystrophy, neurogenic myopathy, and nonspecific myopathy but they were not statistically significant. We did not find N-CAM expression in our 2 mitochondrial myopathy cases, nor in the 5 non-diagnostic myopathy cases.

Conclusion N-CAM expression can be a complementary tool for diagnostic evaluation in muscle diseases with regeneration or denervation of muscle fibers. We recommend further study in a larger group.

1. Introduction

Muscle disease or myopathies are neuromuscular disorders in which the primary symptom is muscle weakness due to dysfunction of muscle fibers. The condition can be acquired, familial, or congenital disorders of the skeletal muscle. Common muscle diseases include muscular dystrophy, congenital myopathy, mitochondrial myopathy, inflammatory myopathy, and neurogenic myopathy.[1] The diagnosis of muscle disease requires all modalities; clinical examination, serologic markers, electrodiagnostic test, pathologic findings from the muscle biopsy specimen, and molecular genetic study are of aid in making a final diagnosis. Among these techniques, a muscle biopsy is a useful tool for the diagnostic evaluation of muscle disease, but the pathological findings gained from biopsies can vary from obvious to subtle myopathic changes that may be difficult to identify.[2] Immunohistochemistry (IHC) is an ancillary tool complementing in the diagnosis of muscle diseases.[3]

Neural cell adhesion molecule (N-CAM) is an integral membrane glycoprotein theorized to play many distinct roles in the process of myogenesis, synaptogenesis, and synaptic maintenance.[4–7] In embryonic muscle, N-CAM is transiently present on the surface of myotubes and myoblasts, as well as intramuscular nerves. It is lost as the myotubes develop and the nerves become myelinated.[8,9]

In normal adult muscle, N-CAM is not expressed on muscle fiber except in the portions which comprise the neuromuscular junction, where it is present on the surface of the muscle fiber and motor nerve, as well as the portion of perisynaptic Schwann cells capping nerve terminals.[6] Furthermore, there is evidence that N-CAM is expressed in satellite cells.[10,11]

It is demonstrated that for abnormal muscle, N-CAM is expressed in regenerating and denervated muscle fibers, and research has been done to investigate its potential as a complementary tool in the diagnosis of muscle disease. Many previous studies found that N-CAM is also presented in degenerated muscle fibers in myopathy[12] and showed a possible association between these muscle fibers in mitochondrial myopathies, inflammatory myopathies, and muscular dystrophies.[12–14] The presence of regenerating or denervated muscle fibers, combined with clinical information, can be used in the diagnosis of muscle disease or myopathy.[4,7,11,15,16]

Our present study aims to establish the pattern of N-CAM expression in common muscle diseases found in the Thai population, including muscular dystrophy, neurogenic myopathy, inflammatory myopathy, mitochondrial myopathy, and nonspecific myopathy. We hope to investigate the potential of N-CAM expression in muscle diseases as a complementary tool for diagnostic evaluation.

2. Materials And Methods

2.1 Muscle specimens

A retrospective study was performed on all muscle biopsy specimens from 2017–2019 in Ramathibodi Hospital. They included 75 clinical myopathy cases who had a muscle biopsy. The diagnosis criteria were based on both clinical correlation and histologic findings. Clinical features and patient characteristics including age, sex, serum creatine phosphokinase level, and specimen site were obtained from the patients' medical records.

2.2 Histologic evaluation

Muscles from biceps brachii or quadriceps femoris in each case were collected as 2 cm-long specimens and divided into three parts. The first part was prepared fresh with the snap-frozen section technique. The second part was prepared in 10% neutral buffered formalin fixation. The third part was fixed in 3% glutaraldehyde for electron microscopic study. Only the first part, prepared with the snap-frozen section technique, was used in our study.

The series of routine histochemical stains included hematoxylin and eosin (H&E), enzymatic histochemistry, and immunohistochemistry. The muscle biopsies were evaluated and correlated with clinical information to diagnosis. (Fig. 1)

In our study, we used only biopsied muscle from snap frozen section to explore the N-CAM expression. The diagnosis of muscle diseases from clinical and histopathology findings was categorized as inflammatory myopathy, neurogenic myopathy, muscular dystrophy, mitochondrial myopathy, nonspecific myopathy, and non-diagnostic specimen.

2.3 Immunohistochemical study

The muscle biopsy specimens were snap-frozen in isopentane and liquid nitrogen. Fresh muscle specimens were rapidly frozen in isopentane (-150 °C) and cooled in liquid nitrogen (-80 °C). Cryostat sections (10 μm) were cut and dried on glass slides at room temperature. No fixation or pretreatment was performed before the IHC analysis. Samples were incubated for 40 minutes with primary antibodies diluted in Bond Primary Antibody Diluent (Leica). Visualization with DAB and subsequent counterstaining with Mayer's hematoxylin were performed using the Bond Polymer Refine Detection System (Leica). Sections were then dehydrated, cleared, and mounted.

The CD56(Leu-19) antigen is similar and may even be identical to N-CAM, as research shows that anti-CD56 and anti-N-CAM antibodies have identical immunoreactive patterns, and they both immunoprecipitate a 140–220 kDa glycoprotein.[16] The anti-CD56 (MRQ-42) rabbit monoclonal primary antibody (1:200, Cell Marque, MilliporeSigma, Rocklin, CA, USA) was optimized for use as a fully automated IHC assay on the BenchMark ULTRA (Ventana Medical Systems Inc., Tucson, AZ). Briefly, the primary antibody was applied for 1 hour at 36 °C; amplification was done for 12 minutes amplifier/12 minutes multimer (OptiView Amplification Kit (Ventana Medical Systems Inc.), and counterstained for 16 minutes with hematoxylin II and post-counterstained with bluing reagent for 4 minutes. Palatine tonsil tissues were used as the internal control.

2.4 Interpretation

The N-CAM positivity was shown by membrane staining in regenerating fibers. Generally regenerating fibers were easily identified by enlarged nuclei with a bluish stain sarcoplasm on H&E staining, as shown in Fig. 2A. The bluish stain is due to the increased concentration of RNA within the cell.[2] Some regenerating fibers looked like normal fiber and were challenging to identify but showed positive N-CAM staining. The N-CAM immunohistochemical results were divided into three groups. The strongly positive had intensely diffuse membrane staining, as shown in Fig. 2B. The weakly positive fibers had faint membrane staining, as shown in Fig. 2C. The negatively stained fibers show a complete loss of membrane staining, as shown in Fig. 2D.

2.5 Statistical analysis

The relationship between N-CAM expression and common muscle diseases were evaluated using the Chi-squared test. Odds ratios (ORs) were estimated in myopathies which correlated with N-CAM expression.

The p-values less than 0.05 were considered statistically significant and ORs more than 1.0 indicated an increased risk among the compared muscle diseases, whereas ORs less than 1.0 indicated a decrease in risk in each muscle disease. All data were analyzed by using SPSS (version 25.0.0.0).

3. Results

3.1 Patient characteristic

Of 75 patients, 41 (54.66%) cases were female and 34 (45.33%) cases were male. The mean age was 35 years. The specimens were collected from quadriceps femoris in 59 (78.67%) cases and collected from

biceps brachii in 16 (21.33%) cases. (Fig. 3).

3.2 Common muscle diseases

The most common diagnosis was nonspecific myopathy with 35 (46.66%) cases, and the second most common diagnosis was inflammatory myopathy with 17 (22.66%) cases. There were 9 (12.00%) neurogenic myopathy cases, 7 (9.33%) muscular dystrophy cases, 5 (6.66%) non-diagnostic myopathy cases, and 2 (2.66%) cases of mitochondrial myopathy. (Fig. 4)

3.3 Correlation between muscle diseases and N-CAM expression

The result of N-CAM expression showed positive staining in 35 cases and negative staining in 40 cases.

The inflammatory myopathy showed positive N-CAM in 15 out of 17 cases with statistical significance (p-value < 0.001, OR 14.250, with 95% CI 2.960-68.606). N-CAM was also positive in muscular dystrophy, neurogenic myopathy, and nonspecific myopathy but was not statistically significant in p-value. No positive N-CAM was found in mitochondrial myopathy and non-diagnostic myopathy. (Tables 1 and 2)

Table 1
Categorization of muscle disease and its correlation with N-CAM expression

N-CAM expression	Categorization of muscle diseases (n = 75)					
	Muscular dystrophy n. (%)	Neurogenic myopathy n. (%)	Inflammatory myopathy n. (%)	Mitochondrial myopathy n. (%)	Nonspecific myopathy n. (%)	Non-diagnostic specimen n. (%)
Positive	6 (85.71%)	4 (44.44%)	15 (88.24%)	0 (0.00%)	10 (28.57%)	0 (0.00%)
Negative	1 (14.29%)	5 (55.56%)	2 (11.76%)	2 (100.00%)	25 71.43%)	5 (100.00%)

Table 2
Categorization of muscle disease and N-CAM expression

N-CAM expression	Categorization of muscle disease (n = 35)					
	Muscular dystrophy	Neurogenic myopathy	Inflammatory myopathy	Mitochondrial myopathy	Nonspecific myopathy	Non-diagnostic specimen
Present	6 (85.71%)	4 (44.44%)	15 (88.24%)	-	10 (28.57%)	-
p-value	0.030	0.887	< 0.001	-	0.003	-
ORs	8.069	0.903	14.250	-	0.240	-
(95% CI)	(0.920 – 0.732)	(0.223–3.666)	(2.960-68.606)		(0.091–0.635)	

We also correlated the positive group with the presence of regenerating fibers in histology. All positive cases showed regenerating fibers in the muscle biopsy. The strongly positive N-CAM mostly appeared in inflammatory myopathy and muscular dystrophy. While the weakly positive N-CAM appeared in neurogenic myopathy and nonspecific myopathy. (Table 3)

Table 3
Categorization of muscle disease and characteristics of N-CAM expression

Characteristics of N-CAM expression	Categorization of muscle disease (n = 35)					
	Muscular dystrophy n. (%)	Neurogenic myopathy n. (%)	Inflammatory myopathy n. (%)	Mitochondrial myopathy n. (%)	Nonspecific myopathy n. (%)	Non-diagnostic specimen n. (%)
Staining grade	1 (16.67%)	4 (100.00%)	4 (26.67%)	-	8 (80.00%)	-
Weak	5 (83.33%)	0 (0.00%)	11 (73.33%)	-	2 (20.00%)	-
Strong	0 (0.00%)	0 (0.00%)	0 (0.00%)	-	0 (0.00%)	-
Degenerated fibers	6 (100.00%)	4 (100.00%)	15 (100.00%)	-	10 (100.00%)	-
Absent						
Present						

4. Discussion

N-CAM accumulates in regenerating and denervated fibers but is not necessarily expressed in muscle diseases.[8] In a study on the expression of N-CAM in mitochondrial myopathy by Heuss et al. in 1995, it was discovered that some muscle fibers are N-CAM-positive but do not appear as ragged red fibers or as cytochrome C oxidase deficient, while all the ragged red fibers or cytochrome C oxidase deficient fibers were N-CAM-positive. This may suggest that the expression of N-CAM precede the manifestation of morphological or

enzyme defects, or the technique used to identify N-CAM expression is more sensitive than those used to identify ragged red fibers and cytochrome C oxidase deficient fibers.[12]

Double-staining with anti-vimentin and anti-N-CAM can be used to identify denervated muscle fibers, which show as vimentin-negative and N-CAM-positive, from regenerating muscle fibers, which show as vimentin-positive and N-CAM-positive.[13]

There are many isoforms of N-CAM, and the isoform expressed can be used to identify the type of fiber by the usage of polysialylation-specific antibodies. Non-activated cells like satellite cells and denervated fibers express only non-sialylated isoforms of N-CAM while regenerating fibers express N-CAM and their sialylated isoforms on the cell membrane and cytoplasm.[10]

N-CAM-positive muscle fiber has been included as one of the suggestive muscle biopsy findings for dermatomyositis according to the 239th European Neuromuscular Centre (ENMC) international workshop classification in 2018.[17]

In our study, inflammatory myopathy was the only muscle disease that had a strong association with N-CAM expression in muscle fibers (15 out of 17 cases, p-value < 0.01) with an odds ratio of 14.250 (95% CI 2.960-68.606). This may be because pathogenesis in inflammatory myopathy is either antibody/immune complex-mediated or cytotoxic T-cell mediated, resulting in necrosis and regeneration of muscle fibers.[18] Regenerating fiber which appears as a morphologically normal fiber on hematoxylin and eosin stain and other histochemistry can be highlighted by immunohistochemistry against N-CAM.

We also found that only inflammatory myopathy and muscular dystrophy groups had strong-intensity immunohistochemistry against N-CAM (73% and 83%, respectively). This pattern of staining could be found in regenerating fibers of muscular dystrophy, even though the number of positive N-CAM in muscular dystrophy was statistically insignificant (6 out of 7 cases, p-value = 0.03).

Our study only had 2 mitochondrial myopathy cases, all cases showing negative immunohistochemistry for N-CAM. These results are limited for evaluation due to the small sample size of the specimen. The small sample size and the unknown underlying pathological mechanism of the disease may be reasons for the discrepancy between our study and a previous study by Heuss et al. in 1995.[12]

5. Conclusion

Within our study, inflammatory myopathy showed a statistically significant correlation with positive N-CAM expression, and it alongside muscular dystrophy are the groups that showed strongly positive N-CAM. Weakly positive N-CAM expression was found in neurogenic myopathy and nonspecific myopathy.

All of the cases with positive N-CAM had regenerating muscle fibers on muscle biopsy. N-CAM helps to narrow down the differential diagnosis of myopathies, especially in inflammatory myopathies and muscular dystrophy which can reveal strongly positive N-CAM staining.

The authors conclude that N-CAM expression can be a useful complementary tool in diagnosing muscle diseases with regeneration or denervation of muscle fibers. We recommend further study in a larger group.

Abbreviations

N-CAM: Neural cell adhesion molecule

IHC: immunohistochemistry

H&E: hematoxylin and eosin

OR: Odds ratio

ENMC: European Neuromuscular Centre

DM: Dermatomyositis

Declarations

Ethics approval and consent to participate

The protocol for this study was approved by the Ethical Clearance Committee on Human Rights Related to Research Involving Human Subjects of the Faculty of Medicine Ramathibodi Hospital, Mahidol University (MURA) (COA no. 2019/721). All procedures performed in this study that involved human participants were in accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standard. Written informed consent was obtained from all individual participants included in this study.

Consent for publication

Not applicable

Availability of data and materials

All data are presented in this paper and there are no additional supporting files. The hematoxylin and eosin-stained and immunohistochemical slides are stored at the Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University.

Competing interests

Not applicable

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Authors' contributions

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Contributions

The authors made substantial contributions to the conception, design of the work, the acquisition, analysis, interpretation of data, drafted the work or substantively revised it, and approved the submitted version.

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Figures

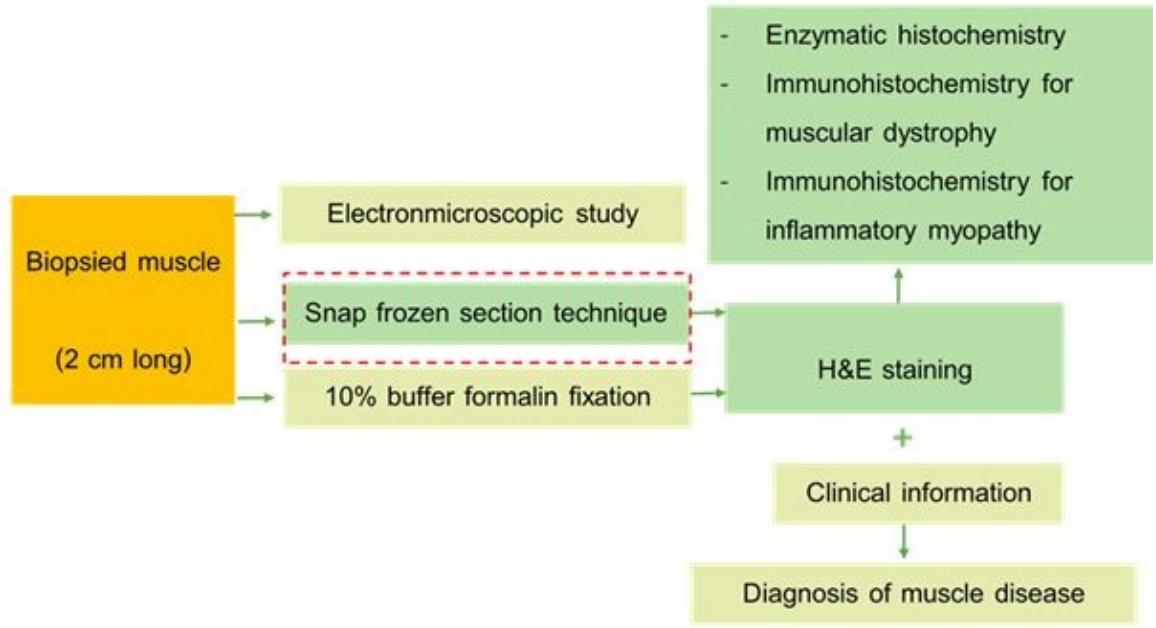

Figure 1

Illustrative flow chart of muscle biopsy in Ramathibodi Hospital

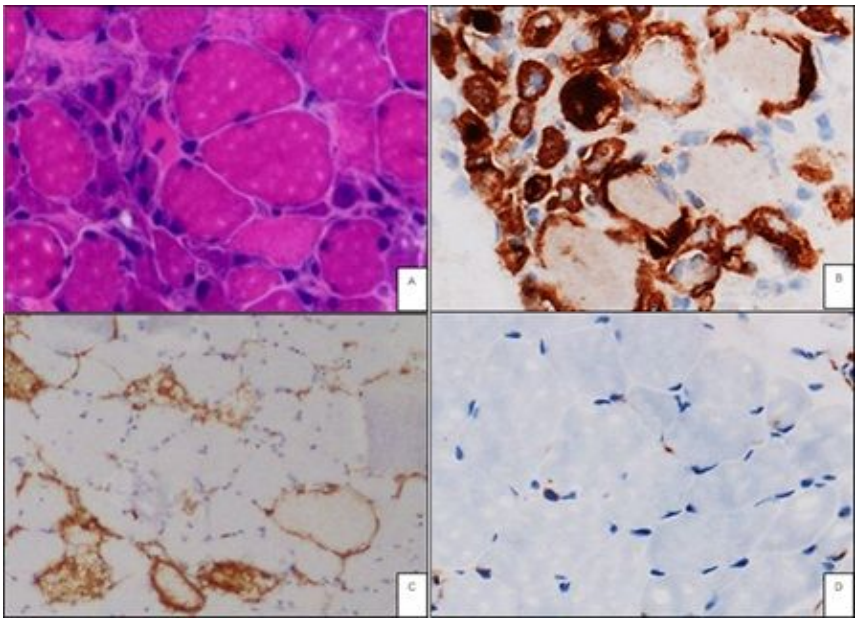

Figure 2

N-CAM interpretation. A, H&E shows regenerating muscle fibers. B, N-CAM was strongly expressed on the sarcolemma of all regenerating fibers. C, Scattered regenerating fibers (N-CAM weakly positive). D, No regenerating fiber (N-CAM negative).

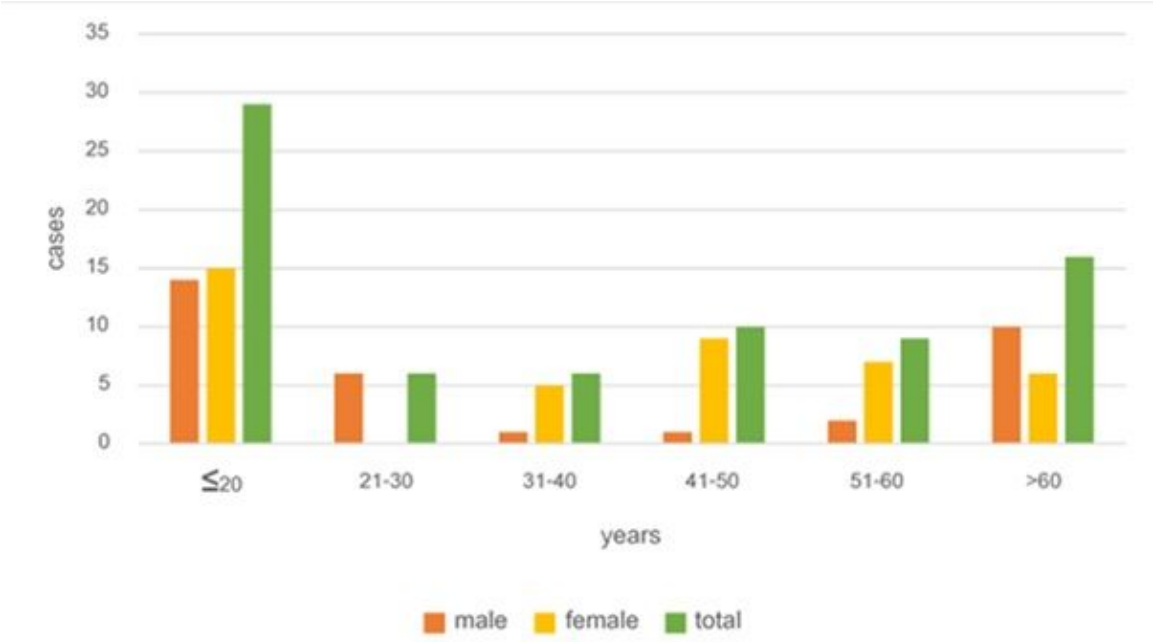

Figure 3

Demographic data of the patient, patient characteristics

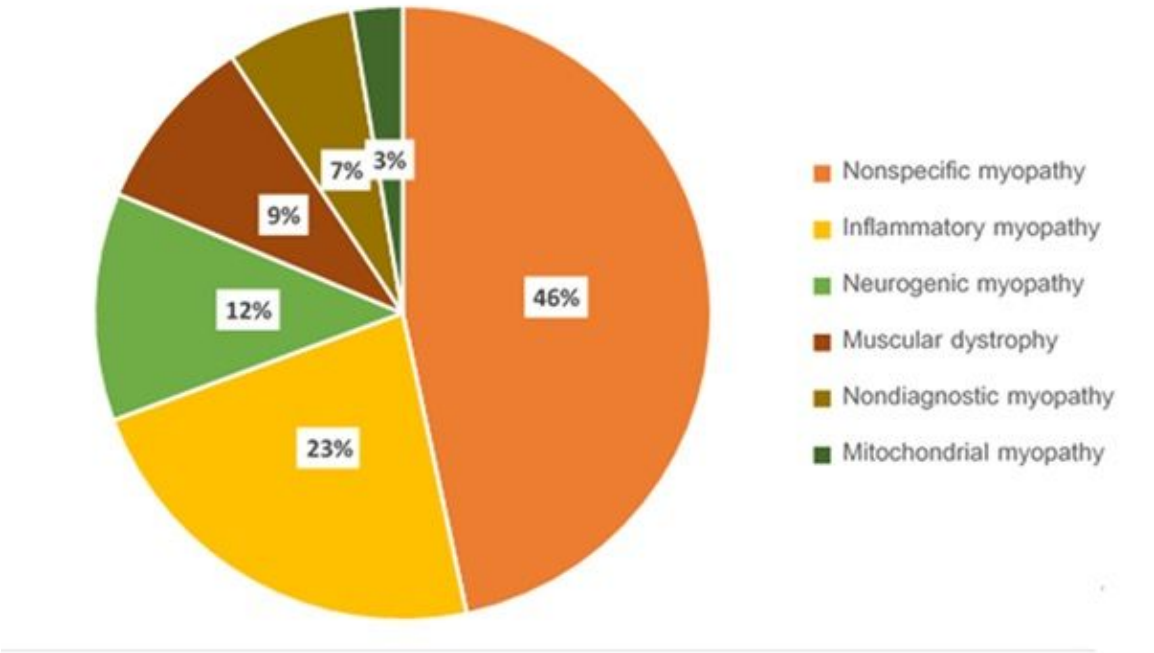

Figure 4

Demographic data of the patient, categorization of common muscle diseases (Percentages rounded)