Special Pathologic Features of Adolescent Idiopathic Scoliosis: Could There be a New Type of Muscular Dystrophinopathy?

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Research article

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Abstract

Background: Adolescent idiopathic scoliosis (AIS) is characterized by vertebral rotation and lateral curvature of the spine and affects 2-4% of the population throughout the world and the cause of the disease is still controversial. Recent researches suggest that there is an internal correlation between certain neuromuscular diseases and AIS. This study aims to characterize the paraspinal muscles of AIS patients, and to further explore the its etiology.

Methods: Eighteen AIS patients treated with posterior scoliosis correction surgery were included and had biopsies taken from the paraspinal muscle at the apex vertebra region. Serial sections with conventional H&E staining and histochemical staining were obtained, and immunohistochemical examinations were employed to detect Dystrophin-1, -2, -3, Myosin, MHC-1, CD4, CD8, CD20, and CD68 or CD163 antibodies. Biopsy samples were grouped according to the subjects' Cobb angle and Nash-Moe's classification respectively, and the corresponding pathological changes were compared between groups.

Results: The immunohistochemical staining results showed significant differences in the expression pattern of Dystrophin-2 (P=0.023) and Dystrophin-total (P=0.018) between the mild and severe scoliosis groups, with the expression of Dystrophin more abnormal or absent in the severe scoliosis group. There were also significant differences in the expression pattern of Dystrophin-2 (P=0.035) between the Nash-Moe classification subgroups. The expression of Dystrophin-3 was absent to various extent in all patients. Besides, we observed an infiltration of CD4+ and CD8+ cells in the paraspinal muscles and tendons. In all patients, the expression of MHC-1 on myolemma was present in some muscle fibers.

Conclusions: The expression of dystrophin protein was significantly reduced and was correlated with the severity of scoliosis, suggesting that dystrophin protein dysfunctions contribute to the development of scoliosis. Meanwhile, the inflammatory changes of AIS mainly manifested in T cell activation and infiltration, and there seemed to be certain correlations between the expression of MHC-1 and the abnormal expression of dystrophin protein. Further studies along these results may open up new ideas for the diagnosis of scoliosis and the treatment for paraspinal myopathy.

1. Background

Adolescent idiopathic scoliosis (AIS) is a disease characterized by vertebral rotation and lateral curvature of the spine, which affects the development of children during their adolescence(1). It is a very common type of spinal deformity with a prevalence of 2–4% worldwide among children younger than 16 years old(2). But the cause of the disease has not yet been clarified.

As early as the mid-1970s, the proportion and size of type 1 and type 2 skeletal muscle fibers in AIS were measured by several researchers(3, 4) and paraspinal muscle imbalance was reported to be one of the key features of AIS. In the 1980s, ultrastructural study of multifidus muscle in progressive idiopathic scoliosis showed structural changes of the sarcolemma and at the myotendinous junction(5, 6). According to the previous literature, AIS is often accompanied by certain but unspecified pathological changes in paraspinal nerves or muscles(7). In 2015, Luciano et al.(8) reported cases of AIS patients who didn't show the typical symptoms of decreased limb muscle strength and of limited respiratory muscle function; however, the results of the paraspinal muscle biopsies of two patients with AIS indicated a high likelihood of core myopathy, but the patients did not develop core myopathy eventually. A hypothetical explanation is that this sort of AIS patients may be a special manifestation of the core myopathy. On the other hand, some neuromuscular diseases, such as Duchenne muscular dystrophy can also affect the paraspinal muscles in the late stage and cause scoliosis(9). Therefore, whether there is an internal correlation between certain neuromuscular diseases and AIS is still a problem that calls for further etiological studies, and paraspinal myopathy is one of the key potential factors for the initial pathogenesis of idiopathic scoliosis.

Based on the hypothesis that the onset and clinical progression of AIS may be associated with certain neuromuscular diseases, we used pathological methods to study the paraspinal muscle changes in AIS patients, and introduced immunohistochemical antibody markers that are commonly used in the diagnosis of neuromuscular diseases through routine morphology, which enabled further analysis of and discussions on the relationship between AIS and neuromuscular diseases.

2. Methods

2.1 Subjects

A total of 18 AIS patients were included, all of whom received posterior scoliosis correction surgery. The subjects were followed up in our hospital from November 2018 to August 2019, and their average age was 16.11 ± 3.77 years old (Table 1). All subjects had main thoracic curves with a Cobb angle of above 40° by the standing posteroanterior radiographs, and they had normal BMI and no related complications. Patients diagnosed of neuromuscular diseases by the neurology department were excluded from the study. This study received approval from the institutional reviewing board. All patients voluntarily provided informed consent and were protected by law.

2.2 Clinical group and classification

Based on the Cobb Angle, the patients in this study were divided into two groups: severe scoliosis group and mild scoliosis group. According to the Nash-Moe classification standards, the patients in this study were also divided into three groups: Nash-Moe type Ⅰ, Nash-Moe type Ⅱ, and Nash-Moe type Ⅲ.

2.3 Pathological sampling and specimen preparation

During the operation, the surgeon performed paraspinal muscle biopsy of the multifidus muscle at the apex vertebra region. The biopsy tissue was wrapped in a semi-humid saline gauze and immediately transferred to the laboratory, where it was drained with blotting paper and was consecutively embedded in tragacanth and OCT compound (Tissue-Tek) and finally frozen in isopentane pre-cooled in liquid nitrogen. Cryostat sections were obtained with 7 to 10 μm thickening. Conventional H&E staining, histochemical staining (NADH-TR, Gomori trichrome, PAS, oil red O), and EnVision two-step immunohistochemical staining were performed under standard techniques that used the following primary antibodies: Dystrophin-1 (Anti-dystrophin rod domain, Leica Biosystems, USA), Dystrophin-2 (Anti-dystrophin C-terminal, Leica Biosystems, USA), Dystrophin-3 (Anti-dystrophin N-terminal, Leica Biosystems, USA), Myosin (Zsbio, China), MHC-1 (Zsbio, China), CD4 (Zsbio, China), CD8 (Zsbio, China), CD20 (Zsbio, China), and CD6 (Zsbio, China)8 or CD163 (Zsbio, China) antibodies respectively(10).

2.4 Interpretation of pathological results

The following items were observed and evaluated respectively: 1) degrees of muscle atrophy, hypertrophy, fatty infiltration, and muscle degeneration; 2) the presence of whorled fibers or internal nuclei; 3) the proportion and distribution of muscle fiber types; 4) the expression of Dystrophin and MHC-1 proteins, 5) the interstitial condition of muscle tissues, and 6) the expression and distribution of inflammatory cells in tendons and muscles. Two pathologists independently analyzed each subject, and there were no inconsistent judgments.

2.5 Statistical analysis

The pathological changes of patients in different Cobb Angle groups and Nash-Moe classification groups were analyzed with χ^2 test, and the correlation between clinical classification and the pathological changes was also examined. All statistical analyses were performed using SPSS 24.0 (IBM Corporation, Armonk, New York, USA). And the statistical significance was defined as a P value less than 0.05 in a two-sided hypothesis test.

3. Results

3.1 Clinical data of the enrolled patients

The subjects included three males and 15 females, with an average age of 16 years old. The average Cobb curve angle of the thoracic segment was 51.04° and the median Cobb angle in scoliosis was 55.9°. Thus, we adopted 55° as the grouping criteria for the degree of deformity among the patients. There were ten patients with severe scoliosis (Cobb angle > 55°) and eight with mild scoliosis (Cobb angle < 55°). In the Nash-Moe type classification, ten patients were classified as type Ⅰ, seven as type Ⅱ, and three as type Ⅲ. (Table 1)

Table 1
Clinical features of the AIS patients

Patient	age	Age of onset	Nash-Moe Classification	Cobb Curve Angle		Lance Classification			King classification
				thoracic vertebra	lumbar vertebra	type	Lumbar correct	Sagittal correct	
1	12	12	□	51.4	N/A	1	C	-	□
2	16	16	□	58.5	37.5	3	C	N	□
3	18	18	□	73.6	N/A	1	C	+	□
4	22	22	□	25.8	N/A	1	C	N	□
5	15	15	□	28.3	N/A	1	C	N	□
6	16	16	□	29.9	N/A	1	C	-	□
7	20	20	□	76.5	N/A	1	C	+	□
8	27	27	□	62.5	41.6	3	C	+	□
9	16	16	□	33.3	N/A	1	B	-	□
10	16	16	□	55.1	48.4	3	C	+	□
11	15	15	□	56.8	58.2	3	C	-	□
12	13	13	□	57.5	N/A	5	C	N	□
13	15	15	□	65.6	N/A	1	C	N	□
14	12	12	□	58.1	N/A	1	C	N	□
15	15	15	□	44.8	48.5	3	C	N	□
16	14	14	□	24.7	31.9	5	C	N	□
17	16	16	□	45.4	N/A	1	C	N	□
18	12	12	□	70.9	N/A	1	C	-	□

3.2 The histological changes of paraspinal muscles and comparisons between the scoliosis groups (Table 2)

Muscle atrophy was found in all the enrolled patients, and the proportion of moderate and severe atrophy accounted for 94.4% of the subjects. The percentage of severe atrophy was 70.83%, suggesting that severe paraspinal atrophy was prevalent.

Compensatory hypertrophy of paraspinal muscle was identified in 88.89% of the enrolled patients, and in 33.33% of the patients, the paraspinal muscle was accompanied by several whorled fibersin. Meanwhile, internal nuclei was observed in muscle fibers in 88.89% of the patients (Figure. 1A). The paraspinal muscles of all patients were subject to varying degrees of muscle fiber degeneration, with 55.56% of the patients presented edema and 22.22% presented extensive edema. Besides, 27.8% of the patients had minor myofiber destruction and myolysis in the biopsy tissue, indicating that myogenic lesions are more predominant than single neurogenic muscular atrophy in pathological changes.

Moth-eaten fibers were observed in the NADH-TR staining of muscle biopsy in 70% of the patients with severe scoliosis and 25% of the patients with mild scoliosis (Figure.1B). Although there was no statistically significant difference between the two groups ($P = 0.053$), it shows a trend that the severer the scoliosis is, the more moth-eaten fibers may appear.

As for Myosin staining, type 2 fibers were positive in the muscles of AIS patients, but the staining was uneven to various degrees. There was a small amount of uneven staining in 80% of the severe scoliosis group and 50% of the mild scoliosis group, though

there was no statistically significant difference between the two groups (P = 0.327). In addition, myosin staining suggested that there were slightly more type 2 muscle fibers than type 1 in the biopsy muscle tissues (Fig. 1C).

Dystrophin protein immunostaining showed that Dystrophin-2 (C-terminal epitope) and Dystrophin-total were both absent in most of the atrophic muscle fibers and some of the non-atrophic muscle fibers, and the differences between the two groups were statistically significant. In the severe scoliosis group, the expression of anti-atrophic protein was more abnormal or absent. There were significant differences in the expression patterns of both Dystrophin-2(P = 0.023) and Dystrophin-total(P = 0.018) between the two groups (Fig. 2), and the expression of dystrophin was subject to increased abnormities or absence in the severe scoliosis group. As for Dystrophin-1 (rod domain epitope, P = 0.054) and Dystrophin-3 (N-terminal epitope, P = 0.411), the sarcolemma of dystrophic and degenerated myofibers faded in all the cases involved (Fig. 3), even though there was no significant difference between the mild and severe scoliosis groups.

Table 2
Pathological features of muscle biopsy in AIS patients in the severe and mild scoliosis groups

Pathological feature or staining pattern		Total scale	Severe scoliosis (Cobb > 55°, n = 10)	Mild scoliosis (Cobb < 55°, n = 8)	p
Atrophy Degree	Mild-Moderate	1/18	0	12.5%	0.247
	Moderate-Severe	16/18	90%	87.5%	
	Severe	1/18	10%	0	
Atrophy Pattern	Big group	4/18	10%	37.5%	0.159
	Small group	14/18	90%	62.5%	
Percentage of Atrophy		18/18	72.50 ± 23.24	68.75 ± 20.83	0.075
Degeneration of myofibers	None	13/18	70%	75%	0.813
	Present	5/18	30%	25%	
Edema	None	8/18	40%	50%	0.796
	Few	6/18	40%	25%	
	Most	4/18	20%	25%	
Whorled fibers	None	12/18	80%	50%	0.178
	Present	6/18	20%	50%	
Hypertrophic fibers	None	2/18	0	25%	0.059
	Present	16/18	100%	75%	
Internal nuclei	None	2/18	10%	12.5%	0.867
	Present	16/18	90%	87.5%	
Moth-eaten in NADH-TR	None	9/18	30%	75%	0.053
	Present	9/18	70%	25%	
Myosin staining pattern	None	1/18	0	12.5%	0.327
	Several	5/18	20%	37.5%	
	Small part	12/18	80%	50%	
Dystrophin-1 staining pattern	Dizzy lineation	8/18	30%	62.5%	0.054
	Light- colored lineation	4/18	40%	0	
	Discontinuous lineation	6/18	30%	37.5%	
Dystrophin-2 staining pattern	Dizzy lineation	10/18	30%	87.5%	0.023
	Light- colored lineation	3/18	30%	0	
	Discontinuous lineation	5/18	40%	12.5%	
Dystrophin-3 staining pattern	Dizzy lineation	1/18	0	12.5%	0.411
	Light- colored lineation	8/18	50%	37.5%	

Pathological feature or staining pattern		Total scale	Severe scoliosis (Cobb > 55°, n = 10)	Mild scoliosis (Cobb < 55°, n = 8)	p
	Discontinuous lineation	9/18	50%	50%	
Dystrophin-total staining pattern	Dizzy lineation	10/18	30%	87.5%	0.018
	Light- colored lineation	4/18	40%	0	
	Discontinuous lineation	4/18	30%	12.5%	

3.3 Inflammatory cell infiltration of paraspinal muscle biopsy of AIS and comparisons between the scoliosis groups (Table 3)

MHC-1 immunostaining showed weakly positive results of some sarcolemma of dystrophic and degenerated myofibers in almost all AIS cases. The infiltrating inflammation cells were dominantly CD4 + T cells and CD8 + T cells (Fig. 4), and the inflammation cells distributed around small vessels or scattered among the stroma in muscle bundles. Muscle tissues and the tendons of paraspinal muscle were both affected and the level of inflammation was almost the same. Few CD20 + B cells were observed to infiltrate in muscle bundles or tendons. The presence of CD68/CD163 + cells indicates that the monocyte-macrophage system may play a role in the progression of AIS (Fig. 5).

Table 3
Pathological features of inflammation in muscle biopsies of AIS patients in severe and mild scoliosis groups

Pathological feature or staining pattern		Total scale	Severe scoliosis (Cobb > 55°, n = 10)	Mild scoliosis (Cobb < 55°, n = 8)	p
MHC-1 staining		18/18	38 ± 24.74	33.13 ± 30.81	0.241
MHC-1 staining pattern	Normal fiber	9/18	50%	50%	0.956
	Dystrophic fiber	4/18	20%	25%	
	Degenerated fiber	5/18	30%	25%	
CD4 in muscle	None/Several	13/18	70%	75%	0.813
	Numerous	5/18	30%	25%	
CD4 in tendon*	None/Several	7/17	60%	14.29%	0.050
	Numerous	10/17	40%	85.71%	
CD8 in muscle	None/Several	17/18	90%	100%	0.269
	Numerous	1/18	10%	0	
CD8 in tendon	None/Several	17/18	90%	100%	0.427
	Numerous	1/18	10%	0	
CD68/CD163 in muscle	None	6/18	20%	50%	0.178
	Several	12/18	80%	50%	
CD68/CD163 in tendon*	None	8/17	60%	28.57%	0.196
	Several	9/17	40%	71.43%	
CD20 in muscle	None	17/18	100%	87.5%	0.193
	Several	1/18	0	12.5%	
CD20 in tendon*	None	16/17	100%	85.71%	0.172
	Several	1/17	0	14.29%	
*Patient 6 has no tendon samples.					

3.4 Pathological features of muscle biopsy and inflammatory cell infiltration of AIS patients in different Nash-Moe subgroups (Tables 4 and 5)

Most of the pathological changes in the muscle biopsies of AIS patients did not show any statistically significant differences among different Nash-Moe subgroups, and the only exception was the expression of dystrophin protein. Immunostaining showed significantly different expression patterns of Dystrophin-2 among the subgroups, and the higher the group type was, the more abnormal or absent the Dystrophin-2 expression was (P = 0.035). However, although the expression of Dystrophin-1 showed statistically significant differences among the three Nash-Moe classification groups (P = 0.034), it was not related to the severity of the disease, with the Nash-Moe patients showing more Dystrophin-1 light staining and more discontinuous muscle fibers in muscle biopsies compared with the other two groups.

Table 4
Pathological features of muscle biopsy in AIS patients in different Nash-Moe subgroups

Pathological features or staining pattern		Total scale	Nash-Moe Type I (n = 8)	Nash-Moe Type II (n = 7)	Nash-Moe Type III (n = 3)	p
Atrophy Degree	Mild-Moderate	1/18	12.5%	0	0	0.241
	Moderate-Severe	16/18	87.5%	100%	66.67%	
	Severe	1/18	0	0	33.33%	
Atrophy Pattern	Big group	4/18	25.00%	28.57%	0	0.428
	Small group	14/18	75.00%	71.43%	100.00%	
Percentage of Atrophy		18/18	76.88 ± 16.68	77.14 ± 19.97	40 ± 10	0.346
Degeneration of myofibers	None	13/18	75%	71.43%	66.67%	0.962
	Present	5/18	25%	28.57%	33.33%	
Edema	None	8/18	25%	57.14%	66.67%	0.360
	Few	6/18	50%	28.57%	0	
	Most	4/18	25%	14.29%	33.33%	
Whorled fibers	None	12/18	50%	71.43%	100%	0.178
	Present	6/18	50%	28.57%	0	
Hypertrophic fibers	None	2/18	12.5%	14.29%	0	0.674
	Present	16/18	87.5%	85.71%	100.00%	
Internal nuclei	None	2/18	12.5%	0	33.33%	0258
	Present	16/18	87.5%	100.00%	66.67%	
Moth-eaten in NADH-TR	None	9/18	62.5%	42.86%	33.33%	0.610
	Present	9/18	37.5%	57.14%	66.67%	
Myosin staining pattern	None	1/18	12.5%	0	0	0.530
	Several	5/18	12.5%	42.86%	33.33%	
	Small part	12/18	75%	57.14%	66.67%	
Dystrophin-1 staining pattern	Dizzy lineation	8/18	62.5%	14.29%	66.67%	0.034
	Light- colored lineation	4/18	0	57.14%	0	
	Discontinuous lineation	6/18	37.5%	28.57%	33.33%	
Dystrophin-2 staining pattern	Dizzy lineation	10/18	87.5%	50.00%	0	0.035
	Light- colored lineation	3/18	0	25.00%	33.33%	
	Discontinuous lineation	5/18	12.5%	25.00%	66.67%	
Dystrophin-3 staining pattern	Dizzy lineation	1/18	12.5%	0	0	0.687
	Light- colored lineation	8/18	37.5%	57.14%	33.33%	

Pathological features or staining pattern		Total scale	Nash-Moe Type I (n = 8)	Nash-Moe Type II (n = 7)	Nash-Moe Type III (n = 3)	p
	Discontinuous lineation	9/18	50%	42.86%	66.67%	
Dystrophin-total staining pattern	Dizzy lineation	10/18	62.50%	28.57%	100.00%	0.177
	Light- colored lineation	4/18	12.50%	42.85%	0	
	Discontinuous lineation	4/18	25.00%	28.57%	0	

Table 5
Pathological features of AIS in different Nash-Moe subgroups

Pathological feature or staining pattern		Total scale	Nash-Moe Type I (n = 8)	Nash-Moe Type II (n = 7)	Nash-Moe Type III (n = 3)	p
MHC-1 staining		18/18	35.63 ± 27.70	28.57 ± 24.95	53.33 ± 30.55	0.657
MHC-1 staining pattern	Normal fiber	9/18	50%	42.86%	66.67%	0.780
	Dystrophic fiber	4/18	25%	28.57%	0	
	Degenerated fiber	5/18	25%	28.57%	33.33%	
CD4 in muscle	None/Several	13/18	87.5%	57.14%	66.67%	0.394
	Numerous	5/18	12.5%	42.86%	33.33%	
CD4 in tendon*	None/Several	7/17	14.29%	57.14%	66.67%	0.141
	Numerous	10/17	85.71%	42.86%	33.33%	
CD8 in muscle	None/Several	17/18	87.50%	100%	100%	0.428
	Numerous	1/18	12.50%	0	0	
CD8 in tendon	None/Several	17/18	100%	85.71%	100.00%	0.371
	Numerous	1/18	0	14.29%	0	
CD68/CD163 in muscle	None	6/18	50%	14.29%	33.33%	0.322
	Several	12/18	50%	85.71%	66.67%	
CD68/CD163 in tendon*	None	8/17	42.86%	42.86%	66.67%	0.753
	Several	9/17	57.14%	57.14%	33.33%	
CD20 in muscle	None	17/18	100%	85.71	100%	0.371
	Several	1/18	0	14.29%	0	
CD20 in tendon*	None	16/17	100%	85.71%	100%	0.394
	Several	1/17	0	14.29%	0	
*Patient 6 has no tendon samples.						

4. Discussion

Adolescent idiopathic scoliosis (AIS) is a common three-dimensional spine deformity characterized by a lateral spinal curvature of more than 10°(2). Although the etiopathogenesis of scoliosis is still unclear, various factors that may contribute to the etiology of scoliosis have been identified, such as genetic factors, structural factors, and environmental factors. The progression of the patient's side curvature is particularly evident during puberty, and this stage is also the period when the paraspinal muscles grow rapidly(11). Rehabilitation exercises for paraspinal muscles, such as Schroth gymnastics, also have a therapeutic effect on scoliosis (12). Besides, scoliosis most often occurs in the late stages of neuromuscular diseases and may continue to progress with sagittal deformities(13). This research on the correlation between paraspinal muscle lesions and scoliosis is particularly important for understanding the pathogenesis of the disease, for which muscle biopsy is the most effective research method.

4.1 Paraspinal muscle atrophy and degeneration

Both the degree of paraspinal atrophy and the atrophy occurance among the subjects in our study were slightly higher than those reported by Wajchenberg M. (7). We suggest that, in addition to the severity of the disease, the degree of atrophy is also related to multiple factors, such as the patient's Cobb angle and the course of disease. In addition, the location of the biopsy sampling will also affect the proportion of atrophy. In this study, the paraspinal muscle was sampled relatively closer to the tendon, which may account for the higher atrophy rate than reported in previous studies.

The paraspinal muscle fiber structures of our subjects were observed abnormal to varying degrees and in different forms, including changes in rostral muscle fiber, nuclear shift, and abnormality in myosin structure. The rate of nuclear shift in our study was higher than the results of Wajchenberg et al.(7). At the same time, the Myosin staining results suggested that the Type 1 fiber content among the patients generally decreased, which was similar to previous results (14, 15). Some of the patients experienced a serious decrease in the proportion of Type 1 paraspinal muscle fibers, which was lower than that of Type 2 muscle fibers. Type 2 muscle fibers are fast-shrinking fibers and mostly distribute in skeletal muscles of the extremities. In contrast, Type 1 muscle fibers are slow-muscle fibers and typically distribute in the trunk. Mannion et al. (16) contend that the loss of Type 1 muscle fibers may cause the muscles to withstand the long-term rigid contraction, leading to the loss of spinal stability and eventually to risks of scoliosis.

In addition, some patients had paraspinal muscle degeneration to different degrees, such as lysis and destruction, suggesting the existence of more serious myogenic lesions in the skeletal muscles. The NADH-TH staining indicated that most patients had moth-eaten fibers, which has also been reported in existing literature. Moth-eaten fibers were indicated by the flaky and irregular loss of oxidase in staining, and could be present near the center or the edge of muscle fibers. Their presence near the center of muscle fibers could resemble the presentation of core myopathy, and must be differentiated by clinical manifestations and genetics (7, 8). Furthermore, patients with severe scoliosis tended to have more NADH deficiency than patients with mild scoliosis ($P = 0.053$, although there was no statistically significant difference), suggesting the metabolic dysfunction of myofibers. With the increase of Cobb angle, paraspinal muscles are subject to a trend of gradual degeneration and necrosis.

4.2 Abnormal expression of dystrophin in paraspinal muscles of AIS

Dystrophin is a 427kD structural protein and a cytoplasmic protein associated with sarcolemma. It links the actin cytoskeleton to the transmembrane dystrophin-glycoprotein complex(17, 18) and helps maintain membrane integrity and cellular homeostasis(19).

In our study, three antibodies, whose antigens were respectively Rod-domain, N-terminal, and C-terminal dystrophins, were introduced to conduct immunohistochemical staining and the evaluation of paraspinal musculoskeletal membranes in AIS patients. And our results suggested that there was a significant loss of Dystrophin protein in the paraspinal muscles of patients with idiopathic scoliosis. And compared to patients with mild scoliosis, those with severe scoliosis have significantly more total protein loss as revealed by Dystrophin rod-domain and C-terminal immunostaining. With Dystrophin N-terminal immunostaining, all the biopsy samples showed discontinuously linear expression or even loss of expression, especially in atrophic muscle fibers. These results lead to the conclusion that sarcolemma integrity and stability can be damaged by the dysfunction of dystrophin protein in the paraspinal muscles of AIS patients.

As is widely held, Duchenne muscular dystrophy (DMD) gene mutation disables the expression of dystrophin, which is key to the pathogenesis of DMD. As a fatal X-linked recessive disease, DMD is characterized by skeletal, respiratory, and cardiac muscle deteriorations. In terms of the onset gender, most of the patients are boys because of the genetic character of DMD(20, 21). In DMD and Becker muscular dystrophy (BMD) patients, due to mutations in the DMD gene, the synthesis of Dystrophin protein can be reduced or yield abnormal structures, which leads to the destruction of the sarcolemma, cell necrosis and apoptosis, muscle atrophy in the limbs, and severe respiratory muscle and diaphragm muscle weakness; some patients may even have scoliosis due to paraspinal muscle involvement in the later stages(9).

This subjects of this study had no symptoms of weak limb muscles or respiratory and cardiac muscles, and were be excluded if diagnosed with DMD and BMD. However, their paraspinal muscles showed a novel pattern of deficiency in Dystrophin protein, which implies that these AIS patients may represent a special type of muscle dystrophinopathy, just as those patients of BMD and DMD who have decreased or dysfunctional Dystrophin protein. However, unlike DMD or BMD, in this study the pathological changes only existed in the patients' paraspinal muscles, causing asymmetrical traction of the spine and resulting in scoliosis.

As for the dystrophin expression patterns, in DMD, dystrophin is absent except in revertant fibers. According to the "read frame" hypothesis in BMD, truncated dystrophin will lead to reduced intensity of some functional proteins at different epitopes such as the N-terminal and C- terminal(22). For the patients in this study, the AIS dystrophin was subject to reduced immunostaining of dystrophin at rod-domain and C-terminal epitopes and to entirely absent immunostaining at the N-terminal epitope of the bundle of atrophic fibers. This immune expression pattern is similar to the above myodystrophy, but on the other hand, the abnormal expression of anti-myodystrophy in AIS muscle biopsy tissues with atrophic muscle fibers is more obvious, which is different from the expression pattern in DMD or BMD.

4.3 Paraspinal muscle inflammatory cell infiltration

Meanwhile, we also found signs of inflammatory infiltration into the paraspinal muscles in patients with AIS. High expression of MHC-1 was observed, a protein that could release autoantigens to T-lymph receptors on the surface of sensitized CD8 + T cells and activate CD8 + T cells under the action of co-stimulatory factors on the surface of muscle fibers. Activated CD8 + T cells release perforin and granzyme, causing muscle fiber necrosis. This is also the pathogenesis of polymyositis in inflammatory myopathy (23).

In the biopsies of the paraspinal muscles of AIS patients, we also observed the infiltration of CD4 + and CD8 + cells in the paraspinal muscles and tendons, but no CD20 + cells were present. Therefore, the pathological changes are very similar to the toxic effects of T cells. The killing effect of T cells to paraspinal muscle cells may account for the atrophy and necrosis of the paraspinal muscles as well as the unbalanced tractions of the spine that lead to scoliosis. We did not have a clue as to how the toxic effects of T cells were activated in this study. Samaan et al.(24, 25) also observed the infiltration of macrophages into the paraspinal muscles of patients with idiopathic scoliosis, arguing that the immune metabolic response was involved in the pathological process of idiopathic scoliosis. Previous studies have also suggested that neuroinflammatory signals can drive spinal curve formation in zebrafish models of IS(26). Therefore, we speculate that inflammatory cytokines and chemokines mediated inflammatory processes may be involved in the pathological changes of AIS.

Is Dystrophin associated with paraspinal inflammation? It has been reported in the literature that disruptions of dystrophin-glycoprotein complex may lead to membrane damages that result in massive infiltration of immune cells, chronic inflammation, necrosis, and severe muscle degenerations(27). As a non-specific inflammation, the infiltrating immune cells are mostly lymphocytes and mononuclear cells. In addition, whether Dystrophin protein is related to the inflammatory response of paraspinal muscles is also a popular research topic. Alyson et al. reported that certain microRNAs can regulate the expression level of Dystrophin protein through the NK-κB inflammatory pathway(28). This study is among the first attempts to link the degree of inflammation with changes of Dystrophin protein. We argue that the inflammatory response and its pathway can affect the functions of Dystrophin, thus compromising the stability of muscle fiber structures. The pathological outcomes of the paraspinal muscles of our subjects further suggested the existence of this pathway.

5. Limitations

Because of the severe atrophy of the scoliosis and the concave side muscles in some patients, the biopsy areas and locations could be rather limited, which inevitably affect the percentage of atrophy. The paraspinal muscle fibers of AIS presented dystrophin dysfunctions, which were closely related to dystrophin-glycoprotein complex and sarcolemma stability. Further studies need to take into account more relevant proteins and explore the mechanisms of AIS.

6. Conclusion

Paraspinal muscles play an important role in the formation and development of scoliosis. Our study suggests that dystrophin protein may significantly decrease or even disappear in patients with scoliosis and that its amount is associated with the severity of scoliosis. Therefore, dystrophin protein dysfunction may contribute to the occurrence and development of scoliosis. Meanwhile, the inflammatory changes mainly manifested in T cell activation and infiltration, and there seemed to be certain correlations between the expression of MHC-1 in inflammatory cell infiltration and the abnormal expression of anti-myoatrophy protein. With these results, further studies on the treatment for paraspinal muscles may open up new ideas for the diagnosis and treatment of scoliosis.

Abbreviations

AIS: adolescent idiopathic scoliosis; DMD: Duchenne muscular dystrophy; BMD: Becker Muscular Dystrophy

Declarations

Ethics approval and consent to participate

This study was approved by Ethics Committee of Peking University Third Hospital. Informed consent forms for surgery were obtained from all the patients or their legal surrogates. Written consent for the study has been obtained from all participants.

Consent for publication

Written consent was obtained from all participants.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declare no conflict of interest.

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No funding was obtained for this study.

Authors' contributions

Administrative support: MY. Collection and assembly of data: JYL, JXL, DZ. Data analysis and interpretation: JXL. Manuscript writing: JYL, JXL, DZ. Scientific advice: MY, DZ, YZ. Final approval of manuscript: All authors.

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Figures

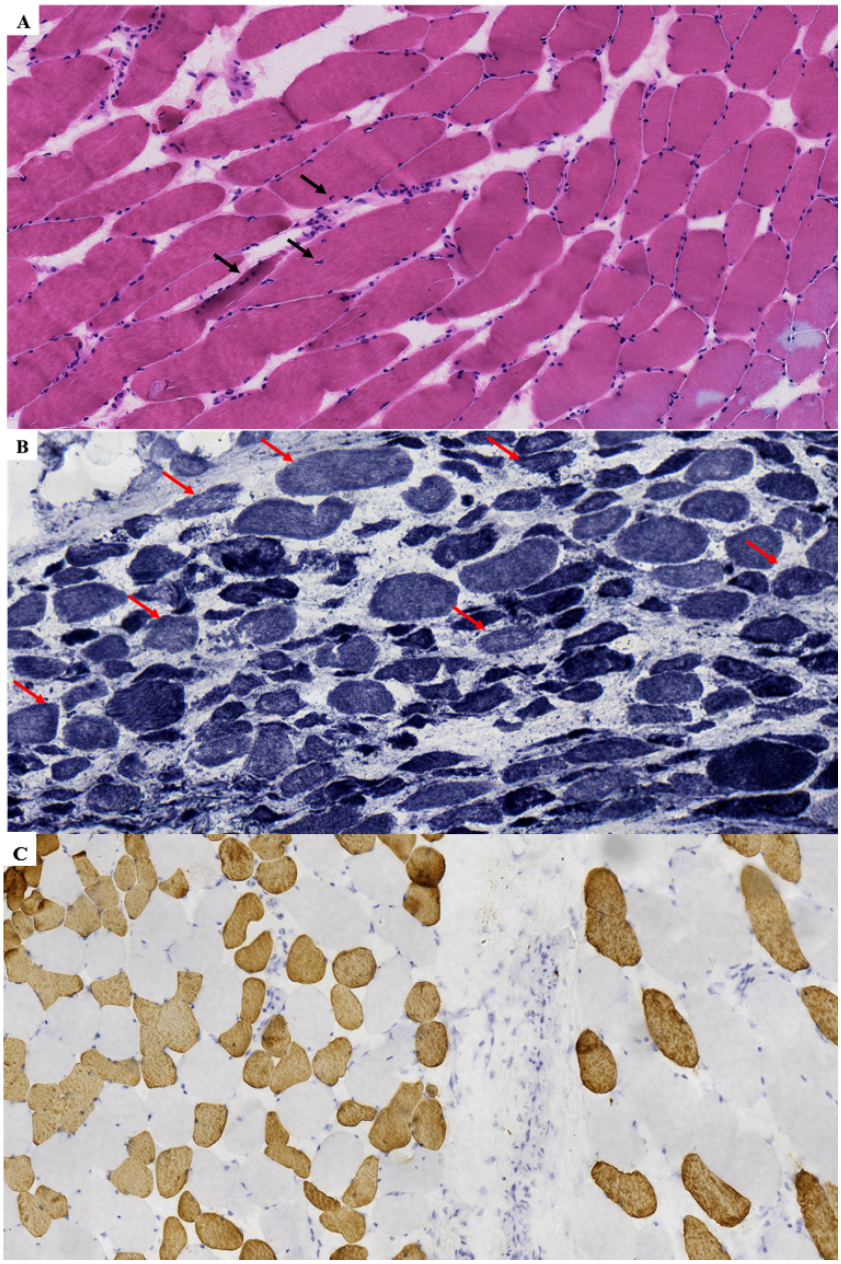

Figure 1

(A) Various degrees of muscle fiber atrophy could be observed in the paraspinal muscle biopsies of the enrolled patients, as indicated by the small bundles. Different proportion of compensative hypertrophy of muscle fibers was present in 88.89% of the patients, and nuclear migration could be observed in the muscle fibers (black arrow) (H&E staining, 40×). (B) Moth-eaten fibers were present in both atrophic and non-atrophic muscle fibers (red arrow) (NADH-TR staining, 20×). (C) Myosin immunohistochemical staining showed positive results, with uneven presence of type 2 muscle fibers, and type 2 fibers were slightly dominant in the biopsies (Myosin immunohistochemical staining, 20×).

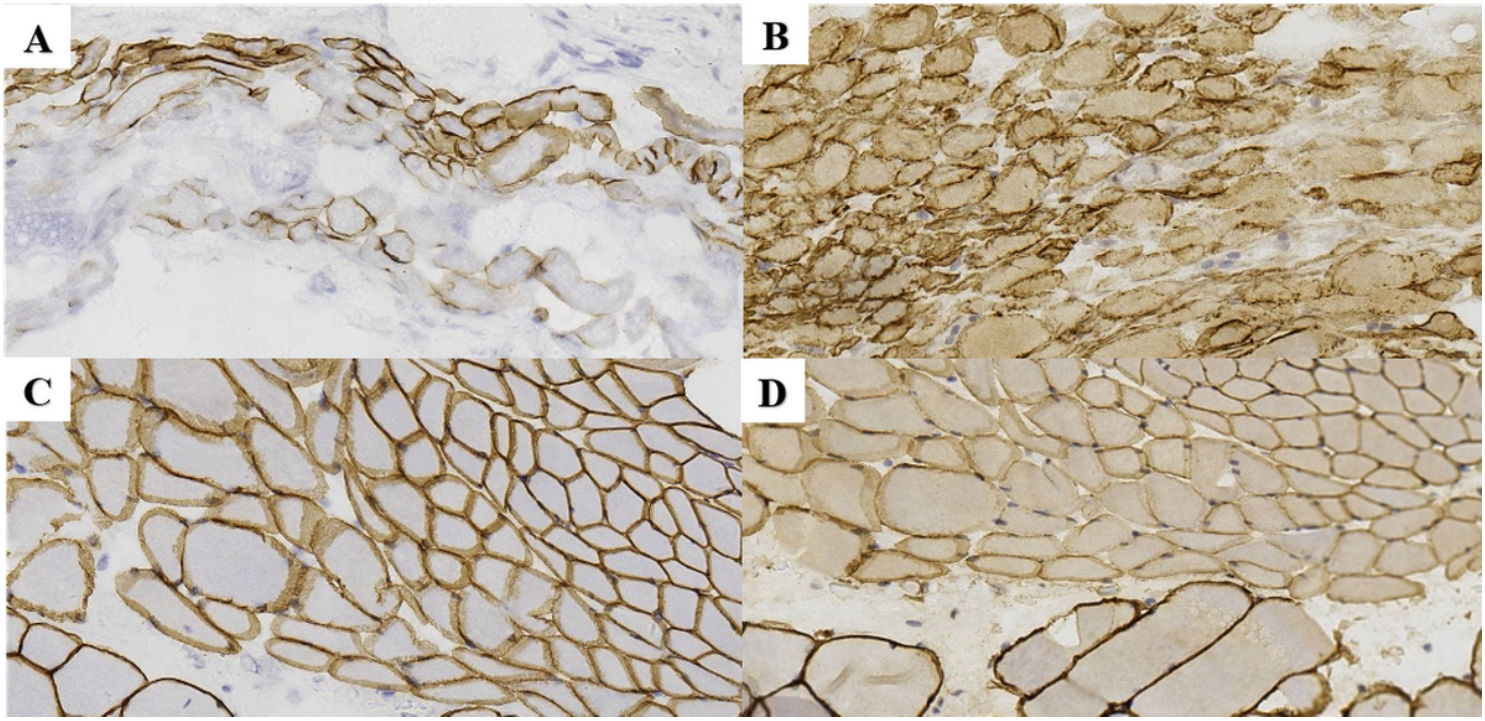

Figure 2

Immunohistochemical staining of paraspinal muscle biopsy in AIS patients. Dystrophin-2 and Dystrophin-total proteins were absent in most of the atrophic muscle fibers and some of the non-atrophic muscle fibers. The expression of dystrophin protein was more abnormal or absent in the severe scoliosis group, and the difference between the two groups was statistically significant. In the severe scoliosis group, the expression of Dystrophin protein was subject to significantly increased abnormities or absence. (A) Severe scoliosis group: Dystrophin-2 was absent in most atrophic muscle fibers and some non-atrophic muscle fibers (Dystrophin-2, 40×). (B) Severe scoliosis group: Dystrophin-total was absent in most atrophic muscle fibers and some non-atrophic muscle fibers (Dystrophin-total, 40×). (C) Mild scoliosis group: Dystrophin-2 was absent in partial atrophic and non-atrophic muscle fibers (Dystrophin-2, 40×). (D) Mild scoliosis group: Dystrophin-total was absent in partial atrophic muscle fibers and non-atrophic muscle fibers (Dystrophin- total, 40×).

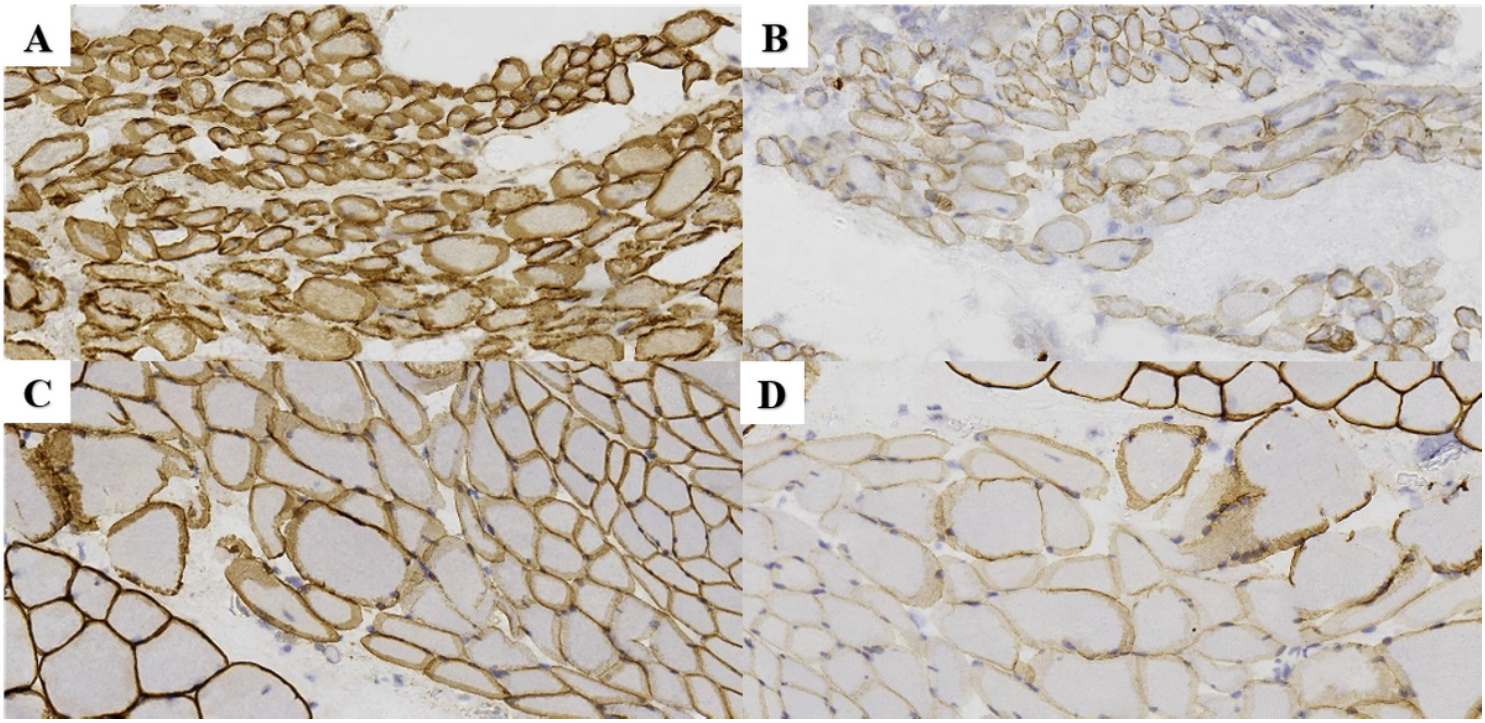

Figure 3

Immunohistochemical staining of Dystrophin-1 (rod-like domain epitopes) and Dystrophin-3 (N-terminal epitopes) in AIS paraspinal muscle biopsy. Dystrophin-1 immunostaining showed dizzy or light-colored lineation in atrophic muscle fibers, with linear discontinuity in a few cases. Dystrophin-3 immunostaining showed that the myolemma of both atrophic and non-atrophic muscle fibers were weakened or even disappeared in all cases. However, there was no statistically significant difference between the mild and severe scoliosis groups. (A) Severe scoliosis group, Dystrophin-1 showed dizzy or light-colored lineation pattern on atrophic myofibers (Dystrophin-1, 40×). (B) Severe scoliosis group, Dystrophin-3 immunostaining showed sarcolemma of atrophic and non-atrophic myofibers faded or even disappeared (Dystrophin-3, 40×). (C) Mild scoliosis group, Dystrophin-1 showed dizzy or light-colored lineation pattern on atrophic myofibers (Dystrophin-1, 40×). (D) Mild scoliosis group, Dystrophin-3 immunostaining showed sarcolemma of atrophic and non-atrophic myofibers faded or even disappeared (Dystrophin-3, 40×).

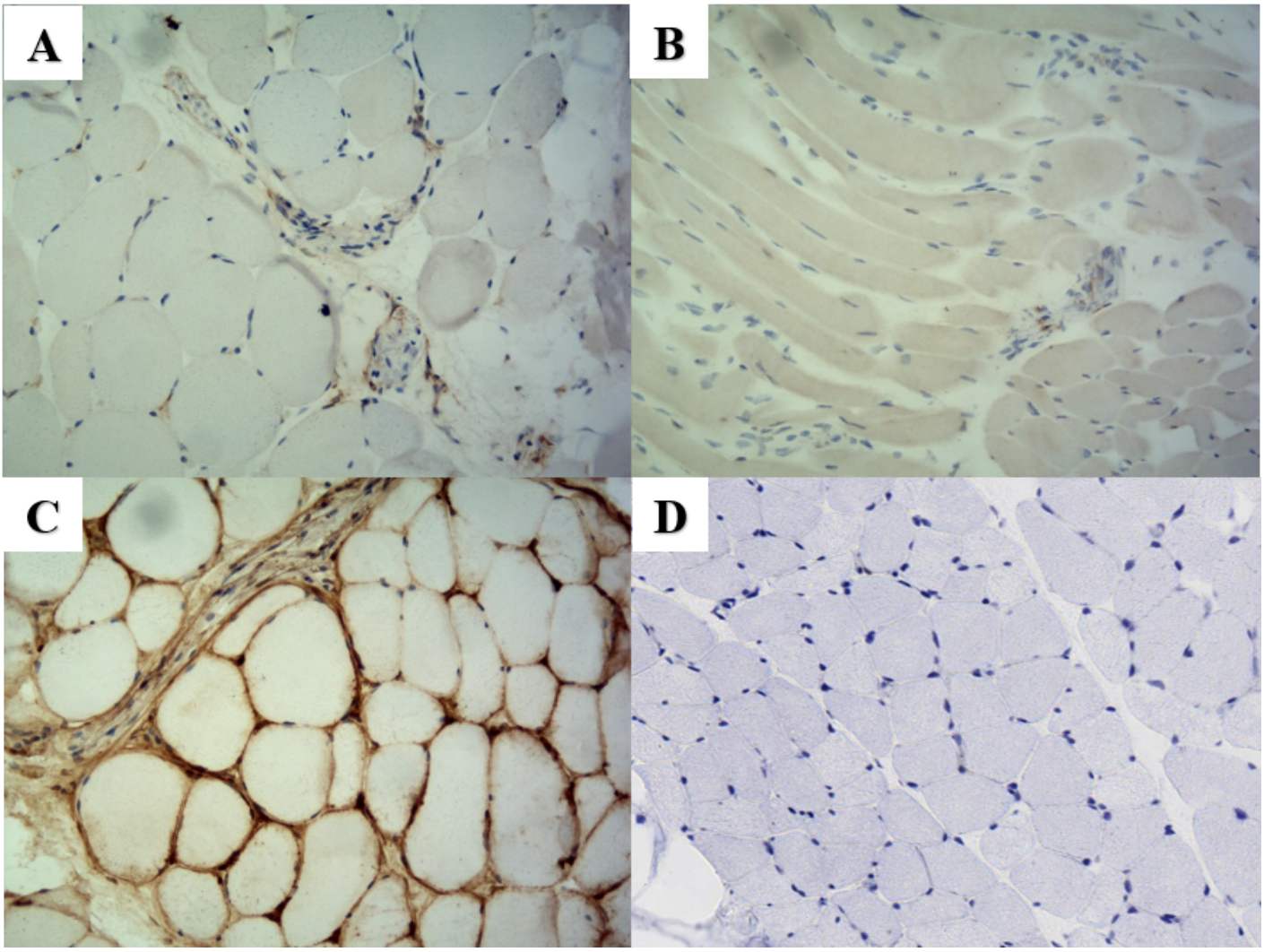

Figure 4

Inflammatory cells in paraspinal muscle biopsies of AIS patients. (A) The infiltrating inflammatory cells were mainly CD4+T cells (CD4, 40×). (B) CD8+T cells could be seen distributing around small vessels or scattered in fasciculus interstitium (CD8, 20×). (C) MHC-1 immunohistochemical staining showed weakly positive results of the myofilm of muscle fibers in almost all cases of AIS (MHC-1, 40×). (D) No CD20+B cell infiltration was observed in the fascicles and tendons (CD20, 20×).

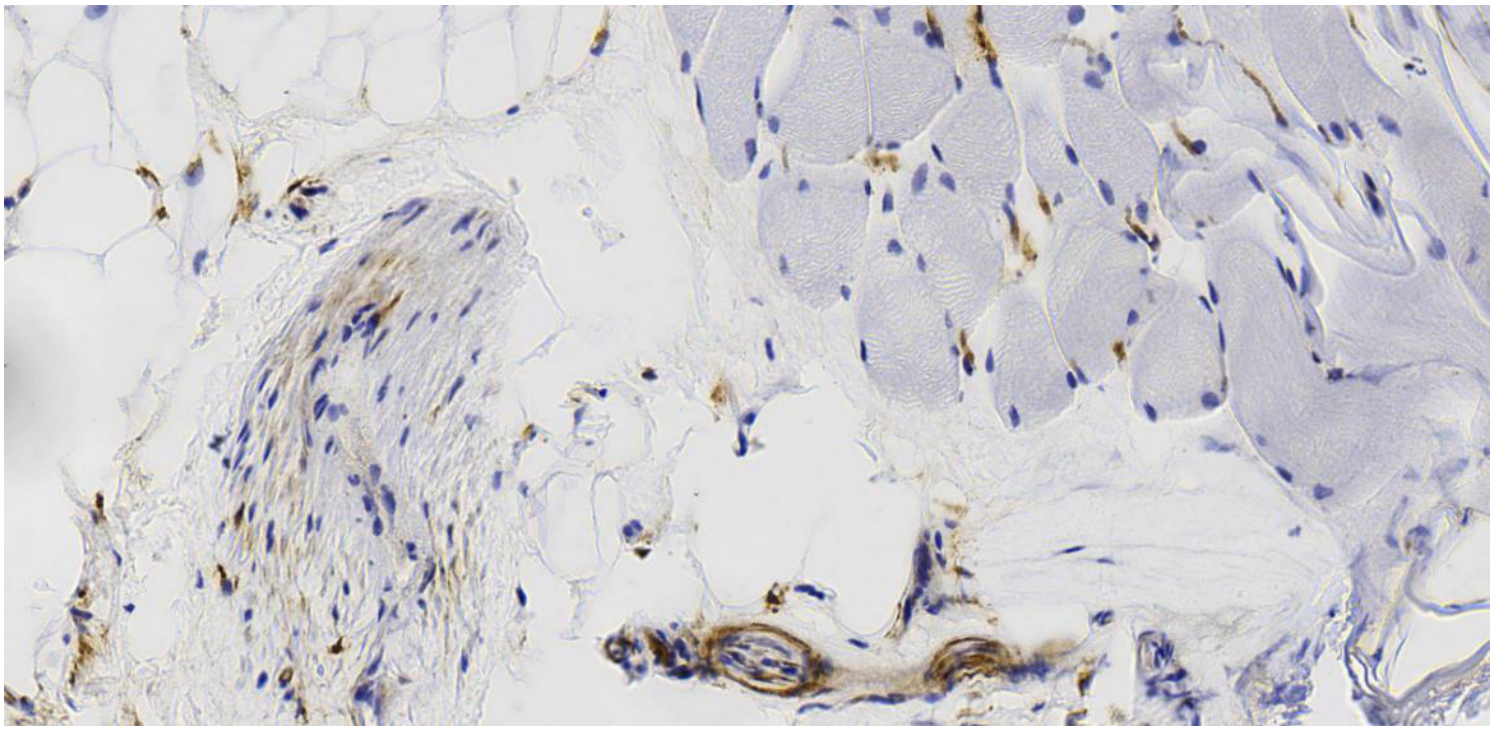

Figure 5

CD163+cells infiltrated in tendons and around small blood vessels in the stroma of muscle bundle, indicating that the monocyte-macrophage system may play a role in the progression of AIS (Immunohistochemistry of CD163 antibody, 40×).

Supplementary Files

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