
Pediatric rheumatology

MHC class I and II expression in juvenile dermatomyositis skeletal muscle

A.M.E. Sallum¹, M.H.B. Kiss², C.A.A. Silva³, A. Wakamatsu⁴, S. Sachetti⁵, S. Lotufo⁶, N. Matsumura⁷, S.K.N. Marie⁸

Departments of Pediatrics and Neurology, and Division of Rheumatology of Faculdade de Medicina da Universidade de São Paulo; Department of Pediatrics of Santa Casa de Misericórdia de São Paulo Medical School; Department of Pediatrics of Menino Jesus Hospital and Adolfo Lutz Institute, São Paulo, Brazil.

Abstract

Objective

To assess MHC I and II expressions in muscle fibres of juvenile dermatomyositis (JDM) and compare with the expression in polymyositis (PM), dermatomyositis (DM) and dystrophy.

Patients and methods

Forty-eight JDM patients and 17 controls (8 PM, 5 DM and 4 dystrophy) were studied. The mean age at disease onset was 7.1±3.0 years and the mean duration of weakness before biopsy was 9.4±12.9 months. Routine histochemistry and immunohistochemistry (StreptABComplex/HRP) for MHC I and II (Dakopatts) were performed on serial frozen muscle sections in all patients. Mann-Whitney, Kruskal Wallis, chi-square and Fisher’s exact statistical methods were used.

Results

MHC I expression was positive in 47 (97.9%) JDM cases. This expression was observed independent of time of disease, corticotherapy previous to muscle biopsy and to the grading of inflammation observed in clinical, laboratorial and histological parameters. The expression of MHC I was similar on JDM, PM and DM, and lower in dystrophy. On the other hand, MHC II expression was positive in just 28.2% of JDM cases and was correlated to histological features as inflammatory infiltrate, increased connective tissue and VAS for global degree of abnormality (p<0.05). MHC II expression was similar in DM/PM and lower in JDM and dystrophy, and it was based on the frequency of positive staining rather than to the degree of the MCH II expression.

Conclusions

MHC I expression in muscle fibres is a premature and late marker of JDM patient independent to corticotherapy, and MHC II expression was lower in JDM than in PM and DM.

Key words

Juvenile dermatomyositis, idiopathic inflammatory myopathies, MHC class I, MHC class II, muscle biopsy.
Introduction

Distinct clinical, histological, and immunopathological characteristics allow classification of the idiopathic inflammatory myopathies (IIM) into dermatomyositis (DM), polymyositis (PM) and sporadic inclusion body myositis (IBM). Clinical differences as well as discrepancies concerning histopathology suggest that the etiology, as well as the pathogenic mechanisms causing muscle inflammation, may vary among subsets of myositis (1-4).

Angiopathy on adult DM was confirmed by demonstrating active focal destruction of capillaries and the presence of complement-induced vessel injury have been established by consistent evidence of activation of the complement cascade, with capillary damage mediated by membrane attack complex (5-11). The activated complement C5a binds to endothelial cells and up-regulates adhesion molecule expression (12). ICAM 1 and VCAM 1 expressions on vessels might be induced by complement activated products in sublethal endothelial cell injury (13). Additionally, sublethal injury stimulates IL-1α production, which induces ICAM 1 expression (14, 15).

Moreover, MHC I expression on target cells is a prerequisite for antigen specific T cell mediated cytotoxicity, and MHC II expression on antigen-presenting cells (APC) is necessary to activate T helper lymphocytes and to initiate an immune response. Of note, MHC class I overexpression was observed in 10 muscles in early JDM without therapy and should indicate a muscle damage (16).

In JDM however, there are no data regarding the MHC I expression concomitantly with MHC II in early and late biopsy, as well as its association with other IIM and dystrophy. Therefore, the aim of this cross-sectional study was to evaluate MHC I and II expressions in JDM muscle fibres and to correlate with clinical, laboratorial and histological parameters, and compare to these expressions in PM, adult DM and dystrophy.

Patients and methods

Forty-eight muscle biopsy specimens from patients fulfilling Bohan and Peter definite criteria for JDM were studied (17, 18). These patients were followed in three Pediatric Rheumatology Centres in São Paulo: Instituto da Criança da Faculdade de Medicina da Universidade de São Paulo, Santa Casa de Misericórdia de São Paulo, and Hospital Menino Jesus. The mean age at disease onset was 7.1±3.0 years and the mean duration of weakness before biopsy was 9.4±12.9 months, including 14 muscle biopsies carried out in the first two months of JDM onset. Biopsy was performed prior to corticosteroids in 37 JDM patients and none of the patients used any other immunosuppressive drug prior to the muscle biopsy. The control group included 17 patients. They were distributed in three sub groups of myopathy: 8 adult PM, 5 adult DM and 4 adult dystrophy. The mean age of PM patients was 43±10.4 years, 36.6±14.9 years for DM patients, and 34.5±13.7 years for dystrophic patients. Among the latter patients, two patients with limb-girdle muscular dystrophy, specifically sarcoglicanopathy which was determined by way of immunohistochemistry findings, and two patients with molecular diagnosis of fascio-scapulo-humeral dystrophy were included in the present study. Prior to the muscle biopsy, the patients and controls were informed of the objectives of the examination and their written consent was obtained. This study was approved by the local ethics committee in all the centers.

Histopathological and Immunohistochemical methods

Muscle biopsy specimens from brachial biceps muscle and the strength score for the same muscle were obtained from all patients. A total of forty-eight muscle biopsy specimens of JDM patients and 17 samples of the comparisons subgroups were submitted to routine standard histological and histochemical techniques. Sequential frozen sections were stained with H&E, modified Gomori trichrome, periodic acid Schiff, cytochrome C oxidase, NADH-tetrazolium-reductase, succinate dehydrogenase, adenosine triphosphatase pH 4.3 and 9.4 and alkaline and acid phosphatase (19). Each muscle biopsy specimen was coded and
analyzed separately by two investigators (observer 1: AMES and observer 2: SKNM), and when any discrepancy was noted, it was reviewed concomitantly. Additionally when the consensus was not achieved, the analysis of a third observer (AW) was performed. The pathology readers were blinded to diagnosis, clinical status and therapy when the biopsies were evaluated.

Perifascicular atrophy was observed in all patients and the presence of fibre damage, inflammatory infiltrate and increased connective tissue were assessed semi-quantitatively as (-): absent, (+): minimal, (++): moderate and (+++): abundant. A visual analogue scale (VAS) was also included to score global degree of abnormality from 0 (no abnormality) to 10.0 (most abnormal).

Immunoreagents
Monoclonal antibodies (Dakopatts) MHC-I (clone W6/32 - M 0736) and II (clone CD3/43 - M0775) were used in dilution of 1:100.

Statistical analysis
Results were presented as the mean ± standard deviation (SD) or median for continuous and number (%) for categorical variables. Mann-Whitney, Kruskal Wallis, chi-square and exact Fisher’s statistical methods were used.

**Fig. 1.** Immunohistochemical preparations for MHC-I and MHC-II, 200x. The positivity of endothelium of small vessels and capillaries is observed in all of the presented samples.

A & B: Case 13, muscle biopsy performed 12 months from the onset of symptoms, showing MHC-I positivity in the majority of muscle fibres and MHC-II in more than 51% of fibres.

C & D: Case 26, biopsy done during the administration of corticotherapy after 4 months of symptoms onset, showing MHC-I positivity in almost all muscle fibres, in contrast to the positivity of MHC-II in less than 10% of fibres.

E & F: Case 48, positivity of majority of muscle fibres to MHC-I and none to MHC-II, in spite of the muscle biopsy having been performed after 64 months of the onset of symptoms.

**StreptABComplex/HRP Immunohistochemical procedure**
Serial frozen sections of 5 µm thickness were fixed for 10 minutes in acetone at 4º C. Endogenous peroxidase was blocked with H2O2 1% in absolute methanol four times, for 5 minutes each. After a rinse in distilled water followed by phosphate-buffered-saline (PBS 0.01M, pH7.4) for 5 minutes, the specimen was incubated in fetal serum in a wet chamber for 1 hour at 37º C. The primary antibody diluted in PBS and BSA 1% was applied in a wet chamber at 37º C, overnight. The slides were then washed in PBS, the prepared secondary mouse biotinylated (StreptABComplex/HRP) was applied for 30 minutes at 37ºC, and rinsed in PBS. Subsequently, the prepared StreptABComplex/HRP complex in 1:100 dilution was applied for 30 minutes at 37º C, and, after rinsing in PBS, was incubated with a chromogenic substrate solution for peroxidase 3,3'-diaminobenzidine tetrahydrochloride (DAB). After a final rinse, haematoxylin counterstaining was performed. The slides were mounted and cover-slipped with an aqueous based mounting medium. The preparation of all muscle specimens were done at the same time as a batch. Ten random fields with 400x magnification, representing almost the entire area of the specimen, and including an average of 500-1000 muscle fibres were analyzed for capillaries and large vessels on endomysial and perimysial location and muscle fibres.

**Immunohistochemical analysis**
Expressions of MHC-I and II were assessed by a semi-quantitative method where: (-): positively stained endothelial cells only; (+): 1 to 10% positively stained fibres; (++) 11 to 25% positively stained fibres; (+++) 26 to 50% positively stained fibres, (++++) 51 to 100% positively stained fibres.
The sensitivity, specificity, positive and negative predictive values were evaluated according to MHC Class I and II stains for JDM patients versus dystrophies group Observer agreement for VAS of global degree of abnormality was measured by kappa method. Significance of 0.05 (α=5%) was adopted and p-values below that were considered significant.

**Results**

The immunohistochemical analysis of MHC-I and II, clinical and laboratorial features at time of biopsy of JDM patients are in Table I.
fibres was demonstrated in 10 (20.8%) cases in each condition, and in 8 (16.7%) cases, the expression was in + of the fibres (Table I).

Of note, negative MHC I expression was observed in one patient (Case 30). This child has presented heliotrope and Gottron’s at the age of 10 years, and was submitted to biopsy 6 months from the onset of cutaneous symptoms, and evolved with gastrointestinal alterations characterized by gastro-esophageal reflux. Interestingly, the CK level at the time of the muscle biopsy was normal although LDH level was mildly elevated (718 U/L, normal range 297-537 U/L). The muscle strength by Medical Research Council (MRC) scale was grade IV in biceps muscle, and even those biopsied after 18 months of the onset presented such a positivity. More strikingly, one patient (Case 48) still showed positivity of MHC I after 64 months of the onset of symptoms. On the other hand, four patients (Cases 1, 8, 15, and 22) had less than 10% of muscle fibres with positive MHC I expression in spite of being submitted to biopsy during the first four months of symptoms and presenting increased CK and LDH levels and muscle strength compromise. In conclusion, positive MHC I expression was observed independent to the interval of disease evolution to the biopsy (p=0.704).

MHC II expression also did not correlate with time of disease to the biopsy and 2 cases with expression in ++ of the fibres corresponding to 3 and 24 months of disease. The expression of MHC I (p=0.490) and II (p=0.310), was also independent to the administration of steroid therapy previous to the muscle biopsy. Thus, all eleven patients receiving this therapy at the time of biopsy presented positive MHC I expression of +++/++++ in 64% of the cases. The overall MHC II expression was low in JDM cases; however, among 11 patients who received corticotherapy previous to the muscle biopsy 5 (45.4%) presented + expression on myofibres and 6 (54.5%) presented none.

Additionally, no significant correlation was observed between MHC I (rs=0.11, p=0.475) and II (rs=-0.22, p=0.134) expression on myofibres and the grading of muscle strength. Muscle strength was measured by MRC, one (2.08%) patient presented grade I; six (12.5%), grade II; 20 (41.6%), grade III; 17 (35.4%), grade IV and four (8.3%), grade V. The patient with grade I, presented ++++ MHC I expression; however, the patients with grades II to V presented similar expression varying from + to +++ of the analysed fibres. And 9 (64.2%) of the 14 cases that presented MHC II positive expression had grade III of muscle strength.

The age of the patient at the time of the biopsy was also not correlated to MHC I (p=0.257) and II (p=0.665) expression on fibres. Clinical systemic manifestations were observed in 7 (14.5%) JDM patients, and they were also not significantly correlated to the expression of MHC I and II as cardiac (p=0.526 and p=0.876), pulmonary (p=0.808 and p=0.099) and gastrointestinal involvement (p=0.545 and p=0.677). Calcinosi and cutaneous ulcer were observed in 12 (25%) and 4 (8.3%) patients and no statistical significance was observed with MHC I and II expressions (p=0.959 and p=0.878; p=0.816 and p=0.190; respectively).

Although increase of serum enzyme levels were observed in the majority of patients (aldolase in 90%, AST in 92%, ALT in 76%, LDH in 87% and CK in 56%) they neither correlated to the MHC I expression nor to MHC II expression.

### Table II. Immunohistochemical analysis of MHC-I and II, and age at onset in PM, DM and dystrophy muscle biopsy specimens.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at onset</th>
<th>MHC I</th>
<th>MHC II</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM</td>
<td>1</td>
<td>47y</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>35y</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>27y</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>57y</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>32y</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>46y</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>50y</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>50y</td>
<td>+</td>
</tr>
<tr>
<td>DM</td>
<td>1</td>
<td>22y</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>44y</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>24y</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>58y</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>35y</td>
<td>++++</td>
</tr>
<tr>
<td>Dystrophy</td>
<td>1</td>
<td>51y/LGMD</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>27y/FSHD</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>40y/FSHD</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>20y/LGMD</td>
<td>-</td>
</tr>
</tbody>
</table>

(-): positively stained endothelial cells only; (+): 1 to 10% positively stained fibers; (++): 11 to 25% positively stained fibers; (+++): 26 to 50% positively stained fibers; (++++): 51 to 100% positively stained fibers; y: years; LGMD: limb-girdle muscular dystrophy; FSHD: fascio-scapular-humeral dystrophy.
Inflammatory infiltration in the muscle was also detected in the majority of biopsies (46 out of 48) ranging from minimal (52% of cases) to abundant (14.6% of cases); however, it did not correlate to MHC I expression (rs=0.03 p=0.825). In contrast, it correlated to MHC II expression (rs=0.31 p=0.034). MHC II expression also correlated to increased connective tissue (p=0.001), and to VAS for global degree of abnormality of the observers 1 (rs=0.42 p=0.003) and 2 (rs=0.43 p=0.002). The agreement between the observers was good (kappa=0.74 and p=0.001).

Chronicity of the muscle involvement was inferred by the proliferation of connective tissue observed in 6 cases, from whom the muscle sample was taken 2 to 24 months from the onset of symptoms. Interestingly, MHC I expression was positive in all of them, demonstrating that MHC I expression persists longer than the acute phase of the disease.

It was found a similar expression of MHC I on JDM, PM and DM, and lower in dystrophy (p=0.009) (Table II). The majority of the analyzed PM and DM cases presented high positivity (+++++) of MHC I expression (71.4% and 60%, respectively), in contrast to few and low (+) positivity in dystrophy (25%), despite all of the included dystrophic muscle samples presented a certain degree of endomyosial or perivascular inflammatory cell infiltration. The absence of MHC I expression was observed in only one patient who presented acquired immunosuppressive disease (PM case 5), and MHC I positivity was detected in the endothelium muscle vessels of a FSHD patient.

MHC II expression was positive in up to 10% of the fibres in 7/8 (87.5%) PM patients, in all five DM, and was absent in all four dystrophy cases. The positivity of MHC II, concerning the frequency of positive staining, rather than to the degree of its expression, showed to be similar in PM and DM and lower in JDM and dystrophy (p=0.001) (Table II).

The frequencies of polyphasic or continuous course (up to 2 years) were similar in JDM patients with positivity versus negativity of MHC I and MHC II stains (19% versus 0%, p=1.0; and 7% versus 21%, p=0.4073, respectively) Of note, the sensitivity, specificity, positive and negative predictive values were elevated in MHC Class I stains for JDM patients versus dystrophies group. On the other hand, no significant statistically differences were observed in MHC Class II for JDM patients versus dystrophies group (Table III).

**Discussion**

The present study showed a high positivity of MHC I in JDM muscle biopsies. This expression was observed independent to the evolution time of disease, corticosteroid use before the muscle biopsy and to the grading of inflammation by clinical, laboratorial and histological parameters.

MHC I expression was previously demonstrated in muscle fibres of DM, PM and IBM adult patients (20-31), as well as in JDM patients (16, 22, 23, 32). MHC I expression has been considered a useful marker for the diagnosis of JDM, for its positive expression on muscle fibres even in cases presenting minimal histological abnormalities, and no inflammation (32). This finding was confirmed more recently in 10 other JDM cases (16). In this study, MHC I expression was observed independent to inflammatory infiltrate, and also in biopsy reported as normal by conventional histology, demonstrating that MHC I was over-expressed on muscles in early phase of JDM.

In addition to these observations, our results showed the persistent MHC I expression in different phases of the disease, independent to the disease duration, and also independent to the introduction of corticosteroid therapy. The continuous positive expression of MHC I from one to 64 months of onset of symptoms allows us to speculate that this expression occurs independent of the presence of active inflammation. Interestingly, the MHC I expression in this study was negative in just one case with slight muscle weakness, in contrast to the previous reported case in an amyopathic JDM (33). In adult IIM studies, MHC was also over-expressed, in the presence or absence of inflammatory infiltrates, both in early and late inactive disease and also independent to therapy with corticosteroids, similar to the results of the present study in JDM patients (26, 31).

Four large studies have been carried out to evaluate the diagnostic value of MHC I staining in myopathies (21, 22, 30, 34). Two of these studies found MHC I staining in all patients with IIM and also in muscular dystrophy. The other two found MHC I staining restricted to IIM patients, and only a few cases of muscular dystrophy showing; therefore, a high sensitivity and specificity of MHC I expression for IIM diagnosis. Our results corroborate this specificity once MHC I expression was found in JDM, PM and DM, and low expression in dystrophy.

Although the MHC II expression was observed in few cases of JDM, it was correlated to inflammatory infiltrate, increased connective tissue and VAS for global degree of abnormality, suggesting that MHC II expression is related to characteristics of local reaction. Previous reports about MHC II expression are variable, ranging from negative expression in JDM (22) to positive DR antigen detection on sarcolema of muscle fibres in PM, and DM (25-27, 28-30).
REFERENCES


PEDIATRIC RHEUMATOLOGY


