The role of electron microscopy in the assessment of dermatomyositis

Dr Hisham Alkhalidi
Assistant Professor and Consultant Pathologist Department of Pathology (32) College of Medicine King Saud University P.O. Box 2925 Riyadh 11461 Saudi Arabia Telephone: +966-533408611 Fax: +966-1-4672462
hishamkh@gmail.com

Abstract

Aim: To assess the importance of electron microscopy in the diagnosis of dermatomyositis.

Methods: A prospective review of muscle biopsy cases suspected of dermatomyositis from January 2008 to January 2012 in the Pathology Department of King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia. Samples from each case were reviewed for light and ultrastructural examination. Tubuloreticular inclusions (TRI) were considered present if these undulating tubules were detected in the endothelial cells of the capillaries.

Results: Out of ten cases that were suspected for dermatomyositis, three cases showed classical light microscopic features of dermatomyositis, two of which showed TRI. Among four cases with features suggestive of dermatomyositis, TRI were detected in two of these four cases. TRI were detected in three cases out of three where the light microscopy was not diagnostic or even suggestive.

Conclusion: The need for electron microscopy in the screening of muscle biopsies, particularly in clinically suspected inflammatory myopathies should be considered, since the presence of TRI may aid in the diagnosis of dermatomyositis.

Keywords: Dermatomyositis, myopathy, tubuloreticular inclusions and electron microscopy
**Introduction**

Muscle biopsy is an essential component, and most often, the deciding factor in the investigation and diagnosis of patients with neuromuscular disorder [1]. Electron microscopy (EM) has a strategic position improving the diagnostic accuracy of numerous muscular diseases, some not revealed by light microscopy. Together with the clinical findings, a diagnosis can be achieved based on the light and ultrastructural findings. Some pathologists suggest that light microscopy preclude the need for EM in inflammatory myopathies [2]. This direction in dealing with muscle samples is supported by immunohistochemistry as new markers are reported to help in the diagnosis and classification. The interest in muscle sample EM examination became less and less, to the degree that some pathologists don’t provide samples for EM, unless they are guided by the clinical picture of the patient to search for specific entities like metabolic storage diseases or certain congenital myopathies.

Inflammatory myopathies can be subdivided in two main groups: infectious myositis and immunogenic myositis [3-5]. Idiopathic inflammatory myopathies are immunogenic inflammatory muscle disorders of unknown origin that are classically characterized by clinical signs of proximal muscle weakness and by histopathological demonstration of inflammatory infiltrates in the clinically affected muscles [6]. Based on clinical as well as histopathological criteria, such as localization and distribution of inflammatory cells, idiopathic inflammatory myopathies have been classified into polymyositis, dermatomyositis, and inclusion body myositis.

Dermatomyositis is characterized by perifascicular atrophy and perimysial chronic inflammation. It affects the muscle in a patchy fashion and with treatment, the morphological
features -particularly the amount of inflammation- may be altered. A biopsy from a patient with dermatomyositis may lack the classical features needed for diagnosis mainly due to the focal nature of the disease or treatment effects. Electron microscopy plays a major role by detecting an important characteristic ultrastructural feature, the tubuloreticular inclusions (TRI). This prospective study was made to assess the electron microscopy contribution to the diagnostic process among patients suspected to have dermatomyositis.

Methodology

This was a prospective study conducted with muscle biopsy cases with clinical or light microscopic suspicion of dermatomyositis over four years from January 2008 to January 2012 in the Pathology Department of King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia. Cases with clinical suspicion of dermatomyositis were included. The selection of such cases was based upon the pathology request clinical information. In addition, cases with light microscopic features of degenerating/ regenerating fibers that were clustered focally in the surface of the muscle fascicle were considered. Also included were cases with mild chronic inflammation that was predominantly perimysial.

Samples from each case were submitted for light and ultrastructural examination. Three microns thick sections were made using the formalin fixed, paraffin embedded tissue of the skeletal muscle samples. They were stained using standard hematoxylin and eosin staining procedure (H&E). The different sections were studied under the optic routine microscope by a neuropathologist. The case was considered diagnostic on light microscopy if it exhibited perifascicular atrophy and perimysial chronic inflammation. The case was considered suggestive if the light microscopy revealed random (rather than perifascicular) fiber atrophy.
with mild perimysial inflammation only. The sample was considered not diagnostic if it was almost normal with no inflammation or minimal to mild random atrophy only.

Tissues submitted for electron microscopy examination were fixed in 3% glutaraldehyde. Tissues were embedded in osmium tetroxide and semi-thin sections were stained with toluidine blue. The adequacy of each sample was checked on the semi thin sections. The thin sections were stained with uranyl acetate and lead citrate. TRI were considered present if these undulating tubules were identified in the endothelial cells of the capillaries.

Results

A total of ten cases were retrieved (Table 1). Of these, three cases showed classical light microscopic features for dermatomyositis. In particular, the cases exhibited the perifascicular atrophy (Figure 1). Two of these cases contained TRI on ultrastructural examination (Figure 2). Four cases showed features suggestive of dermatomyositis (Figure 3). TRI were detected in two out of these four cases. In addition TRI were detected in three cases out of four where the light microscopy was not helpful (Figure 4).
Table 1. Light microscopy and EM findings of 10 cases studied.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age</th>
<th>Sex</th>
<th>Clinical Diagnosis</th>
<th>Light microscopy</th>
<th>TRI on EM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>F</td>
<td>Dermatomyositis</td>
<td>Suggestive</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>F</td>
<td>Dermatomyositis</td>
<td>Diagnostic</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>63</td>
<td>F</td>
<td>Myopathy</td>
<td>Suggestive</td>
<td>Present</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>F</td>
<td>Dermatomyositis</td>
<td>Not diagnostic</td>
<td>Present</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>M</td>
<td>Dermatomyositis</td>
<td>Diagnostic</td>
<td>Present</td>
</tr>
<tr>
<td>6</td>
<td>34</td>
<td>M</td>
<td>Myopathy</td>
<td>Suggestive</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>58</td>
<td>F</td>
<td>Myopathy</td>
<td>Diagnostic</td>
<td>Present</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>M</td>
<td>Dermatomyositis</td>
<td>Not diagnostic</td>
<td>Present</td>
</tr>
<tr>
<td>9</td>
<td>33</td>
<td>F</td>
<td>Dermatomyositis</td>
<td>Not diagnostic</td>
<td>Present</td>
</tr>
<tr>
<td>10</td>
<td>45</td>
<td>F</td>
<td>Dermatomyositis</td>
<td>Suggestive</td>
<td>Present</td>
</tr>
</tbody>
</table>
Figures captions:

Figure 1. Muscle biopsy showing a characteristic perifascicular atrophy (arrow), which is a classical feature of dermatomyositis (H&E, X100).

Figure 2. TRI in an endothelial cell lining a capillary (X4000).

Figure 3. In this muscle biopsy, there was mild perivascular perimysial inflammation in addition to random fiber atrophy (arrow) within the fascicle (H&E, X100).

Figure 4. Almost unremarkable muscle biopsy on light microscopy (H&E, X100).
Discussion

Several studies have described patients with myositis with pronounced muscle weakness and fatigue but without detectable infiltration of inflammatory cells in muscle tissues [7,8]. The presence of TRI remains an important clue to dermatomyositis [9]. This is significant in patients whose biopsies could not provide enough light microscopic features. Our study showed that without electron microscopy, some biopsies may not add much to the patient management course. Hence, with careful ultrastructural screening, these undulating tubules in the endothelial cells of the capillaries served its use to establish the process, regardless of the light microscopic features. This should be applied routinely on each sample if the volume of the muscle biopsy is appropriate.

TRI are not entirely specific to dermatomyositis. They can also be seen in some collagen-vascular disease [10]. Connective tissue disorders-related myositis can be induced by SLE, scleroderma and Sjogren syndrome [11]. They are also well-described in patients receiving zidovudine-associated myopathies [12-14]. TRI also have been rarely reported in inclusion body myositis [11,15]. This differential diagnosis is limited and can be narrowed by clinical correlation. For example, inclusion body myositis is predominantly a distal myopathy while dermatomyositis is a proximal one. In addition, inclusion body myositis exhibits Congo red positive inclusions on light microscopy, in addition to characteristic intranuclear and perinuclear filaments and perinuclear myelin figures on electron microscopy.

Although immunohistochemistry provides an important tool that can assist in the process of the diagnosis; its role is limited by a) non-specificity of the procedure, b) the difficulty of optimization of some antibodies and c) the difficulty in reproducibility of the results. The major issues are the non-specificity of the procedure and the difficulty of the optimization.
For example, in dermatomyositis, one of the common antibodies used to evaluate the disease is MHC class I immunostain [16,17]. However, MHC class I is found to be expressed in all forms of inflammatory myopathy [18,19]. In addition, MHC class I is reported to be not specific for categorization of inflammatory myopathies [20,21]. It is expressed on any regenerating fibers [9], and multiple other disorders including xp21 muscle dystrophy [9,19].

Our study suggests the need for electron microscopy in the screening of muscle biopsies, particularly in clinically suspected inflammatory myopathies. Together with the right clinical setting and, if available, immunohistochemistry, TRI can be a significant diagnostic feature that can be considered characteristic or at least highly suggestive of dermatomyositis.
References


