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# The Role of Electron Microscopy in the Assessment of Dermatomyositis: A Retrospective Pilot Study on Skeletal Muscle Biopsies

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## Author's contribution

The author performed the whole research work. Author HA wrote the first draft of the paper. Author HA read and approved the final manuscript.

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## ABSTRACT

**Aims:** To assess the contribution of electron microscopy in the process of muscle biopsies evaluation for dermatomyositis.

**Study Design:** Retrospective review of muscle biopsy cases.

**Place and Duration of Study:** Pathology Department of King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia from January 2008 to January 2012.

**Methodology:** Samples from cases suspected to have dermatomyositis were reviewed for light and ultrastructural morphological 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 non-specific light microscopic features that can be seen in dermatomyositis, TRI were detected in two of these four cases. Among three cases with non-contributory light microscopy, TRI were found in all of these three cases.

**Conclusion:** Electron microscopy -if feasible- may be useful in the screening of muscle biopsies, when clinically or morphologically suspected inflammatory myopathies are considered. Further studies to assess the significance of TRI with a larger number of

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cases, clinical data correlation and immunostains are needed.

*Keywords: Dermatomyositis; tubuloreticular inclusions; electron microscopy.*

## 1. 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 certain 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. Electron microscopes are relatively an expensive hardware to install. But if they are already installed in the institution, ultrastructural screening of the muscle samples can provide valuable information with minimal amount of cost. However, 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 recent 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 simply classified into polymyositis, dermatomyositis, and inclusion body myositis. Pestronk has recently revised the classification of acquired immune and inflammatory myopathies, using a different scope that can provide additional diagnostic clarification in these myopathies [7].

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 [8]. It is classically described that electron microscopy can help in detecting an ultrastructural characteristic feature, the tubuloreticular inclusions (TRI) [9-14]. We tried to assess the electron microscopy contribution to the diagnostic process among patients suspected to have dermatomyositis.

## 2. MATERIALS AND METHODS

This was a morphological 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. The age, the sex, the creatine kinase level and the status of immunosuppressive recent therapy at the time of the biopsy were documented. The clinical diagnosis after the biopsy was recorded.

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 routine stains that were reviewed include Gomori trichrome, NADH, SDH, COX, PAS, ORO and the ATPases. Congo red was carefully assessed for the presence of inclusions. The sample was considered "typical" on light microscopy if it exhibited perifascicular atrophy and perimysial chronic inflammation. The sample was considered "not specific" if the light microscopy revealed random (rather than perifascicular) fiber atrophy with mild perimysial inflammation only. The sample was considered "not helpful" if it was almost normal with no inflammation or minimal 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. Careful screening of the samples skeletal muscle cells and blood vessels walls was performed. TRI were considered present if these undulating tubules were identified in the endothelial cells of the capillaries.

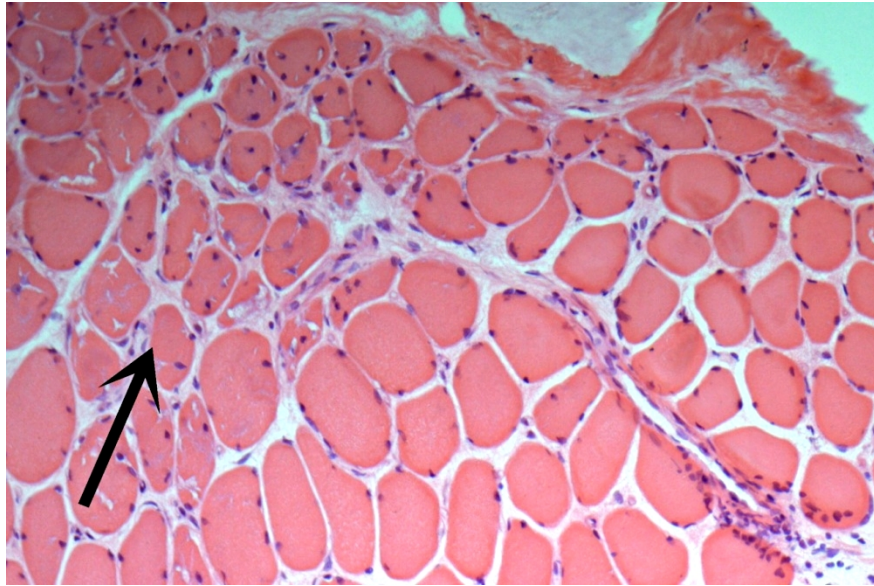
### 3. RESULTS

A total of ten cases were retrieved (Table 1).

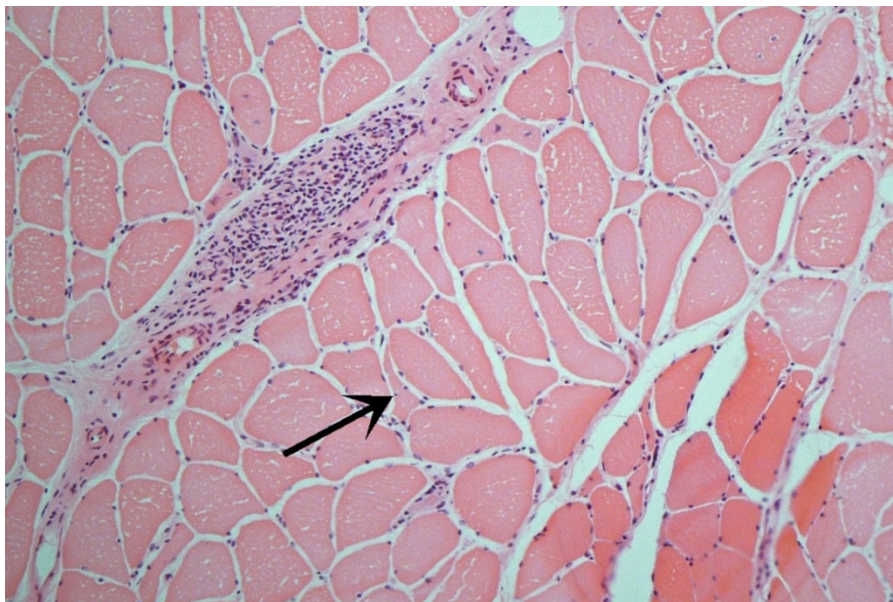
**Table 1. Light microscopy and EM findings of 10 cases studied**

Case no.	Age	Sex	Clinical suspicion	Light microscopy	TRI on EM
1	47	F	Dermatomyositis	Not specific	Negative
2	25	F	Dermatomyositis	Typical	Negative
3	63	F	Myopathy	Not specific	Present
4	38	F	Dermatomyositis	Not helpful	Present
5	29	M	Dermatomyositis	Typical	Present
6	34	M	Myopathy	Not specific	Negative
7	58	F	Myopathy	Typical	Present
8	30	M	Dermatomyositis	Not helpful	Present
9	33	F	Dermatomyositis	Not helpful	Present
10	45	F	Dermatomyositis	Not specific	Present

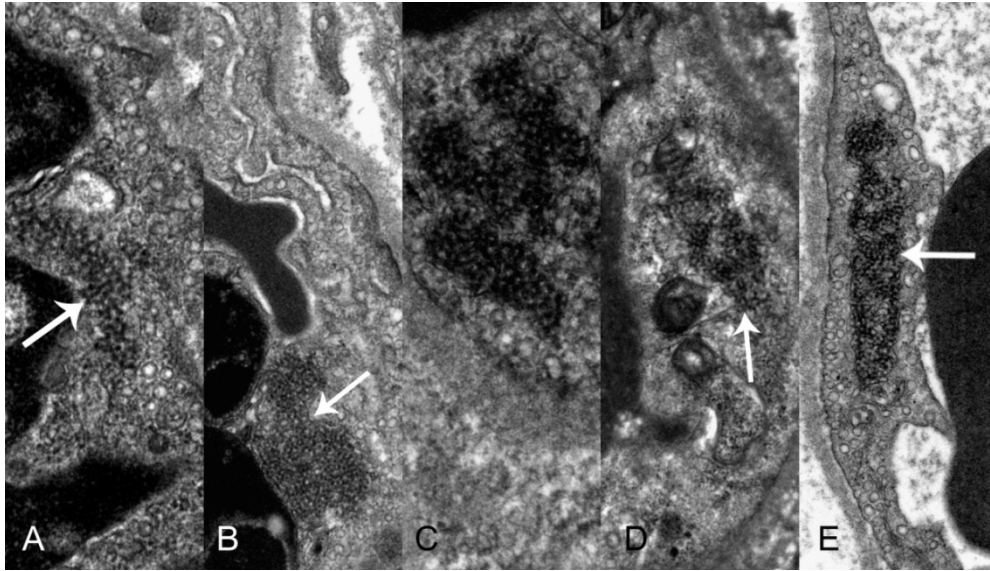
Of these, three cases showed classical light microscopic features for dermatomyositis. In particular, these cases exhibited the perifascicular atrophy (Fig. 1). Two of these cases contained TRI on ultrastructural examination. Four cases showed non-specific morphological features that can be seen in dermatomyositis (Fig. 2). TRI were detected in two out of these four cases (Figs. 3A and 3E). TRI were detected in three cases out of four (Figs. 3B, 3C and 3D) where the light microscopy was not helpful (Fig. 4). None of the cases showed nuclear or cytoplasmic sarcoplasmic inclusions or pseudo-myelinic membranes.



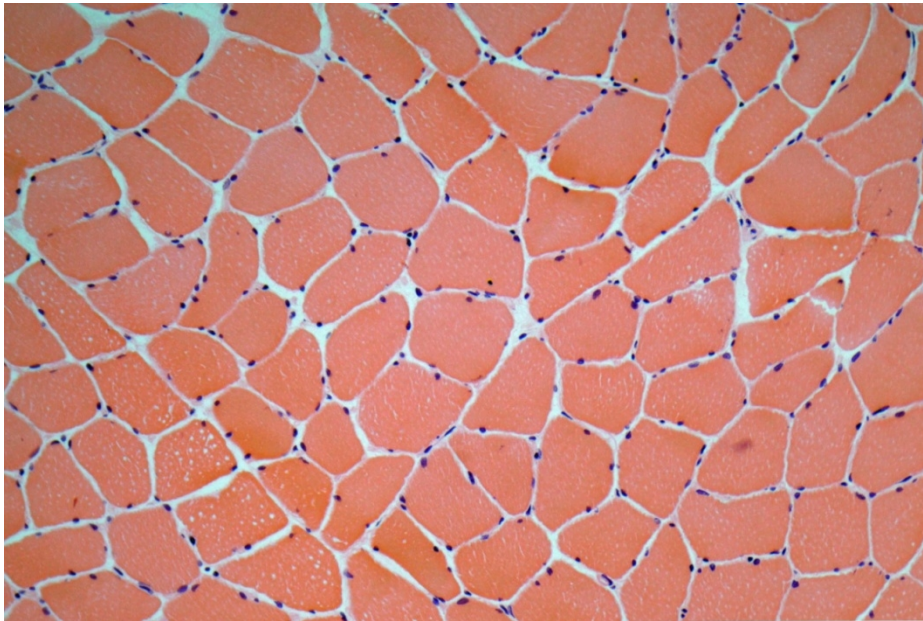
**Fig. 1. Muscle biopsy showing a characteristic perifascicular atrophy (arrow), which is a classical feature of dermatomyositis. The atrophic area exhibits myofiber necrosis and regeneration , myofiber splitting, occasional internal nuclei and irregular sarcoplasmic vacuolation (H&E, X100)**



**Fig. 2. In this muscle biopsy, there was mild perivascular perimysial inflammation in addition to random fiber atrophy (arrow) within the fascicle (H&E, X100)**



**Fig. 3. TRI (arrows) in endothelial cells lining capillaries. A) Case 3 (X4000). B) Case 4 (X4000). C) Case 8 (X8000). D) Case 9 (X4000). E) Case 10 (X4000)**



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

Screening Trichrome and Congo red stained sections didn't reveal the presence of inclusions in any of these cases. NAD, SDH and COX showed uneven staining or lobulation in some patients (cases 2,3,5 and 7). Otherwise, they were non-contributory. ATPases stains revealed type II atrophy in one of the patients who received steroids before the biopsy (case 3). PAS and ORO were non contributory.

Creatine kinase level was increased at the time of the biopsy three to ten folds the normal control levels in seven patients (1,4-7,9 and 10). It was normal in two patients (2 and 3). The data about one of the patients (case 8) is not available. It was an outside referral for tissue assessment only. Six of the patients (cases 1,3,4,6,7 and 9) received an immunomodulating treatment prior to the biopsy (usually in the form of oral steroids). This is attributed due to delays in the muscle biopsy procedure. Three patients (cases 2,5 and 10) didn't receive any treatment. The data about one of the patients (case 8) was not available.

The clinical final diagnosis was dermatomyositis in 8 patients. One patient (case 6) was found to have scleroderma. One patient (case 8) clinical data was not available.

#### **4. 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]. As mentioned above, it is classically described that the presence of TRI can be an important clue to dermatomyositis. This could be useful in patients whose biopsies could not provide enough light microscopic features. Our findings suggest that without electron microscopy, some of these biopsies may not add much to the patient management course (cases 3,4,9 and 10). This is particularly true if the immunohistochemical stains are not available. Hence, with careful ultrastructural screening, these undulating tubules in the endothelial cells of the capillaries served its use to assess a well-described feature in dermatomyositis. Such feature may help to support the diagnosis of dermatomyositis in the right clinical context. If an electron microscope is already available in the institution, screening for TRI could be applied routinely on each sample, considering that the volume of the muscle tissue is appropriate.

However, TRI are not entirely specific to dermatomyositis. They can also be seen in some collagen-vascular disease [15]. These include connective tissue disorders-related myositis that can be induced by SLE, scleroderma and Sjogren syndrome [16]. They are also well-described in patients receiving zidovudine-associated myopathies [17-19]. TRI also have been rarely reported in inclusion body myositis [16,20]. 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. 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.

Immunohistochemistry provides an important tool that can assist in the process of the diagnosis. The analysis of inflammatory cells, MHC class I expressions and MAC deposits may help to rule in an idiopathic inflammatory myopathy [21,22]. This role can be 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. In dermatomyositis, one of the common antibodies used to evaluate the disease is MHC class I immunostain [23,24]. MHC class I is found to be expressed in all classic forms of inflammatory myopathy [25,26]. MHC class I is reported to be not specific for categorization of inflammatory myopathies [22,27]. It is expressed on any regenerating fibers [10], and multiple other disorders including xp21 muscle dystrophy [10,26]. Similarly, MAC immunostaining was found to lack the ability to differentiate the individual subtypes of inflammatory myopathy [22].

Our study suggests that electron microscopy may play a role in the screening of muscle biopsies, particularly in clinically suspected inflammatory myopathies. Further studies assessing the clinical pattern and outcome and comparing TRI with the immunohistochemical staining pattern are needed before assuming any solid conclusions. Considering the high cost of an electron microscope installation; screening for TRI could be considered practical if the institution has one already (e.g. for renal biopsies examination).

## **5. CONCLUSION**

Electron microscopy (if feasible) may be useful in the screening of muscle biopsies, particularly when clinically suspected inflammatory myopathies are considered, since the presence of TRI may aid in the diagnosis of dermatomyositis. Further studies to assess the significance of TRI with a larger number of cases, clinical data and immunostains are required.

## **CONSENT**

Not applicable.

## **ETHICAL APPROVAL**

Not applicable.

## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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