Investigation for toxoplasmic infection in muscles of patients with idiopathic inflammatory myopathies: An immunocytochemical study of 56 cases

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Abstract: The aim of the present study was the immunohistochemical investigation of muscle specimens for toxoplasmic infection in patients with confirmed idiopathic inflammatory myopathies. For this purpose muscle specimens were obtained from 35 patients suffered from polymyositis, 11 from dermatomyositis, 4 from juvenile dermatomyositis, 2 from polymyositis and lung cancer, 1 from polymyositis and sarcoidosis and 1 patient from dermatomyositis and bowel cancer. The findings from the histological and histochemical investigation were, in all cases, compatible with the clinical diagnosis. Immunolabeling for Toxoplasma gondii was negative for all idiopathic inflammatory myopathy and control patients. In conclusion, our findings are not in support of the view that toxoplasmosis has a causative relation with the pathogenesis of the idiopathic inflammatory myopathies.

Key words: Toxoplasma, muscle, immunocytochemistry, inflammatory myopathy

The idiopathic inflammatory myopathies are a group of acquired myopathies of childhood and adulthood that includes polymyositis, dermatomyositis and inclusion body myositis. The aetiology is unknown but the involvement of immunological mechanism is certain [1]. Toxoplasma gondii, a sporozoan parasite that encysts in skeletal muscle among other organs, has implicated in the pathogenesis of inflammatory myopathies, although its presence in diseased muscle has rarely been detected [2-5].

Aim of the present study is the immunohistochemical investigation for the evidence of toxoplasmic infection in muscle specimens of patients with confirmed idiopathic inflammatory myopathies.

Materials and Methods

Muscle specimens were obtained, by means of open biopsy, from 56 patients who suffered from diagnosed idiopathic inflammatory myopathies. Thirty five patients suffered from polymyositis, 11 from dermatomyositis, 4 from juvenile dermatomyositis, 2 from polymyositis and lung cancer, 1 from polymyositis and sarcoidosis and 1 patient from dermatomyositis and bowel cancer. All patients had proximal muscle weakness, elevated levels of serum muscle enzymes, myopathic or myopathic plus denervation findings on electromyography. In addition, the patients with dermatomyositis presented with or had a history of erythematous skin rash of the face. Clinical details of the patients are presented in Table 1.

The muscle biopsy was done for all patients to carry out for diagnostic purposes in brand new cases or re-evaluation of the diagnosis. Frozen sections were processed for histology and histochemistry. The following routine histological and histochemical stains were applied [6]. Hematoxylin-eosin, Gomori’s modified trichrome, Oil-red 0, Periodic acid Schill’s reaction, Myofibrillar adenosine triphosphatase at pH 9,4 and Nicotinamide adenine dinucleotide tetrazolium reductase. The immunolabeling of
Toxoplasma gondii was performed in frozen sections with a monoclonal antibody (immunoglobulin class IgG1 Kappa, clone G II, Biogeney Laboratories), using a peroxidase-antiperoxidase technique. At least 250 muscle fibers were examined in each muscle sample. Morphometric analysis was performed by an automatically image analysis system (Image-Pro Plus, version e4.5.1-Media Cybernetic).

The above mentioned procedure was also applied in muscle samples from 35 patients suffering from muscular dystrophy (17 patients), spinal muscular atrophy (8 patients), metabolitic myopathy (4 patients) or mitochondrial encephalomyopathy (6 patients) for control purposes.

**Results**

The findings from the histological and histochemical investigation were compatible with the clinical diagnosis in all cases. The main findings in muscle biopsies were degeneration, phagocytosis and regeneration of muscle fibers. Endomysial fibrosis was seen in some areas. Endomysial inflammatory reaction was present in most of the cases and perivascular inflammation in 43 of them confirming the diagnosis of myositis. Evidence of neurogenic atrophy was seen in 31 of muscles. The zones of atrophic muscle fibers showing signs of degeneration were often localized to the peripheral part of the fascicles (perifascicular atrophy). The last finding was confirmed with the complete morphometric study.

The immunolabeling for Toxoplasma gondii was negative in all cases of idiopathic inflammatory myopathy as well as in all control cases.

**Discussion**

The pathogenesis of the idiopathic inflammatory myopathies is associated with immune-mediated mechanisms that may be triggered by infectious agents, such as viruses [7]. This mechanism is seen in patients who develop a myopathy after being infected with the human immunodeficiency virus [8]. Muscle is a common localization site for the sporozoan parasite Toxoplasma gondii which has been implicated in the pathogenesis of inflammatory myopathies. Although its actual isolation in muscles of patients with polymyositis or dermatomyositis is rare [2-5]. The possibility of an indirect immune mechanism in these cases has also been discussed in the literature [5].

In our study, there was no morphological or immunocytochemical evidence of toxoplasmic infection. It was evident in all 56 cases of various types of inflammatory myopathies. It should also be noted that our cases had a wide age range (from 3.5 to 73 years) while the duration of illness was also diverse (from 20 days to 22 years). Quite a few of our patients had a history of immunosuppressive therapy for several years. Despite immunosuppression in patients with inflammatory myopathies, our results did not answer our expectations of the possibility of secondary development of skeletal muscle toxoplasmosis. Interestingly, skeletal muscles involvement by Toxoplasma gondii in patients with AIDS appears to be rather uncommon, although systemic toxoplasmosis is a usual opportunistic infection in this disease [9].

In conclusion, the findings of our study are not consistent with the thought that toxoplasmosis has a causative relation with the pathogenesis of

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of patients</th>
<th>Age (years)</th>
<th>Duration of illness</th>
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<tr>
<td></td>
<td>Total Males Females</td>
<td>Mean Range</td>
<td>Mean Range</td>
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<tr>
<td>Polymyositis</td>
<td>35 12 23</td>
<td>40.8 3.5-71</td>
<td>3.1 20 days-22 years</td>
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<tr>
<td>Dermatomyositis</td>
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<td>33.7 19-62</td>
<td>0.6 15 days-2 years</td>
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<tr>
<td>Juvenile dermatomyositis</td>
<td>4 1 3</td>
<td>10.2 5-14</td>
<td>1.4 20 days-2 years</td>
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<td>16 - 9</td>
<td>-</td>
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<tr>
<td>Polymyositis/Neoplasm</td>
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<td>68 63-73</td>
<td>1.2 6 months-2 years</td>
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<td>59 - 2</td>
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<td>57 - 30</td>
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<tr>
<td>Dermatomyositis/Neoplasm</td>
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<td>47 - 2 months</td>
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the idiopathic inflammatory myopathies. Since the precise nature of the pathogenetic mechanism in idiopathic inflammatory myopathies is still unclear, future histochemical studies are required to provide further information on the possible role of toxoplasmic infection.

References