DENGUE

MUSCLE BIOPSY FINDINGS IN 15 PATIENTS

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SUMMARY — Dengue is known to produce a syndrome involving muscles, tendons and joints. The hallmark of this syndrome is severe myalgia but includes fever, cutaneous rash, and headache. The neuromuscular aspects of this infection are outlined only in isolated reports, and the muscle histopathological features during myalgia have not been described. In order to ascertain the actual neuromuscular involvement in dengue and better comprehend the histological nature of myalgia, we performed a clinical and neurological evaluation, a serum CPK level and a muscle biopsy (with histochemistry) in 15 patients (4 males), median age 23 years (range 14-47) with classic dengue fever, serologically confirmed, during the brazilian dengue epidemics from September 1986 to March 1987. All patients had a history of fever, headache and severe myalgia. Upon examination 4 had a cutaneous rash, 3 had fever, and 3 a small hepatomegaly. The neurological examination was unremarkable in all and included a manual muscle test. CPK was mildly elevated in only 3 patients. Muscle biopsy revealed a light to moderate perivascular mononuclear infiltrate in 12 patients and lipid accumulation in 11. Mild mitochondrial proliferation was seen in 3, few central nuclei in 3, rare foci of myonecrosis in 3, and 2 patients had type grouping. Dengue in our patients, produced myalgia but no detectable muscle weakness or other neuromuscular involvement. The main histopathological correlation with myalgia seems to be a perivascular mononuclear infiltrate and lipid accumulation.

KEY WORDS: dengue, muscle, myalgia.

Dengue: achados de biópsia muscular em 15 pacientes

RESUMO — A síndrome clínica produzida pelo vírus da dengue, inclui febre, exantema, cefaléia e especialmente mialgia. Entretanto, as possíveis alterações morfológicas do músculo esquelético, eventualmente relacionadas com a mialgia, ainda não haviam sido estudadas em seres humanos com dengue. Nosso objetivo foi estudar o substrato, anátomo-patológico da mialgia nesses pacientes. Foram avaliados 15 pacientes com diagnóstico de dengue, forma clássica, com idades variando de 14 a 47 anos (mediana de 23 anos), sendo 4 do sexo masculino e 11 do sexo feminino, através de exame clínico e neurológico, exames laboratoriais e biópsia muscular com histoquímica, durante a epidemia de dengue em Alagoas, em 1987. Todos os pacientes apresentavam história de cefaléia, febre e mialgia intensa, sem fraqueza muscular. Ao exame clínico observou-se exantema em 4 pacientes, febre em 3 e discreta hepatomegalia em 3. O exame neurológico foi normal em todos e a enzima CK sérica estava pouco elevada em 3 pacientes. A biópsia muscular revelou discreto infiltrado inflamatório mononuclear perivascular em 12 pacientes, acúmulo lipídico em 11, predominância de fibras do tipo I em 6, raros focos de necrose em 3, proliferação mitocondrial em 3, centralização nuclear em 3 e "type grouping" em 2. As alterações mais frequentemente observadas na biópsia muscular, infiltrado inflamatório perivascular e acúmulo lipídico, podem estar relacionadas com a mialgia.

PALAVRAS-CHAVE: dengue, músculo, mialgia.

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Dengue, caused by an arthropod-borne RNA virus [6,19], is still a major public health problem in tropical areas, mainly in Southeast Asia, the Pacific region, and more recently, in the Americas [5,8,22]. Myalgia is a characteristic symptom of dengue fever, and is also frequent in other viral infections [14,16,18] but its pathogenesis still remains poorly understood [14,16]. There are isolated experimental reports about the pathological findings in skeletal muscle in dengue [1,20], but we have been unable to find any pathological study of skeletal muscle in humans with myalgia and dengue fever.

This report describes the skeletal muscle histological abnormalities in 15 patients with dengue fever and severe myalgia, as an attempt to better understand the histological nature of that symptom.

PATIENTS AND METHODS

We studied 15 patients with dengue fever, 4 males, 11 females, median age 23 years (range 14 to 47 years), during an epidemic of classic dengue fever from September 1986 to March 1987, in the city of Maceio, state of Alagoas, Brazil. The diagnosis was based on epidemiological data, clinical manifestations and confirmed by hemagglutination inhibition method (positive in all 15 patients), IgM antibody detection by Mac ELISA (positive in all 8 studied) and virus isolation (in one patient studied).

All patients had a history of fever, headache and severe myalgia. Two patients complained also of arthralgia. All patients were submitted to clinical and neurological examinations including a manual muscle test [2]. Table 1 summarizes the age, sex, clinical signs, neurological examination and duration of symptoms before biopsy. Classic dengue fever is typically an acute febrile illness with rash, headache, retro-orbital pain, arthralgia and severe myalgia [13,21].

Blood tests that included complete blood count, sodium, potassium, lactic dehydrogenase, creatine kinase (CK), pyruvic transaminase (SGPT), and glutamic transaminase (SGOT) were unremarkable with the exception of three patients (patients 4, 7 and 13) that showed a slight increase of serum CK (2 times normal). Urinalysis and EKG were normal in all patients. Electromyography was not done.

Muscle biopsies were performed in the left superficial deltoid muscle on all patients. The biopsy technique and muscle processing used are all well established and have been described elsewhere [9]. All biopsies were stained with H&E, modified Gomori, PAS, Sudan red, ATPases 9.4, 4.3 and 4.6, NADH and SDH.

RESULTS

All biopsies presented normal staining to PAS and NADH. The histopathological findings are summarized in Table 2.

A light to moderate perivascular mononuclear infiltrate was found in 12 patients (H&E) (Fig. 1). The infiltrate consisted of mononuclear cells around small vessels mainly in the perimysium. The infiltrate was predominant in the perivascular space and did not invade muscle fibers. In rare occasions the infiltrate was also seen in the endomysium mainly around small vessels (Fig. 2.) Even when moderate the infiltrate did not invade or disrupt the vessel walls. The infiltrate was found in 11 patients within 2 to 8 days of symptomatology; only one patient had a 14 day history of dengue fever.

Eleven biopsies presented with a mild lipid accumulation (Sudan red) diffusely located in at least 50% of the fibers. Nine biopsies showed both perivascular infiltrates and lipid accumulation.

Other findings included mitochondrial proliferation located mainly in the periphery of the fibers in 3 biopsies, central nuclei in 3, rare foci of necrosis in 3, and type grouping in 2. These latter findings were always associated with either perivascular infiltrates, lipid accumulation or both (Table 2).

Except for one patient (with a 3 day history) that presented a normal muscle biopsy, 11 patients showed at least two different abnormalities in their biopsies.

Table 1. Clinical presentation of patients with classical dengue fever.

Case	Age	Sex	Clinical Signs	Neurological Examination	Duration of Symptoms (days)
1	18	f		normal	10
2	36	m		normal	08
3	15	f	rash, hepatomegaly	normal	04
4	21	f		normal	08
5	47	f	fever	normal	03
6	34	f		normal	02
7	47	m	hepatomegaly	normal	07
8	20	m	rash	normal	03
9	27	f		normal	02
10	23	f		normal	14
11	12	f	fever	normal	02
12	18	f	fever	normal	08
13	42	f	rash, hepatomegaly	normal	07
14	23	f		normal	08
15	14	m	rash	normal	06

Table 2. Muscle histopathological findings in patients with classical dengue fever.

Case	PMI*	LA#	MP@	CN∞	N§	TG•
1		+				
2	+					
3	+	+				
4	+	+				
5	+	+				
6	+	+				+
7	+	+			+	
8						
9	+	+	+			
10	+					
11	+	+			+	
12	+			+		
13		+	+	+		
14	+	+	+	+	+	
15	+	+				+

* perivascular mononuclear infiltrate
\# lipid accumulation
@ mitochondrial proliferation
∞ central nuclei
§ necrosis
• type grouping

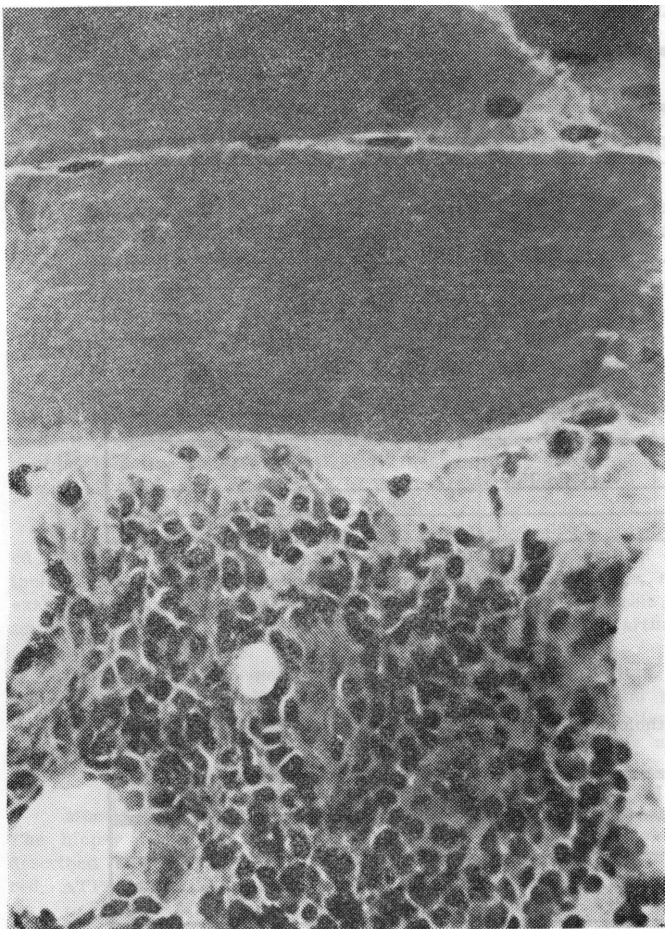

Fig. 1. A collection of mononuclear inflammatory cells around small blood vessels in the perimysium. (H&E, x500).

COMMENTS

To our knowledge this is the first report of the muscle histopathological features that accompany classic dengue fever. It is intriguing to note that we have not found any example of true myositis in spite of the fact that myositis, sometimes associated with perivascular inflammatory infiltrates, seems to be the basic histological abnormality found in skeletal muscle of animals and humans infected with other viruses like influenza and coxsackie [11,12,17].

Agarawal et al.[1] have studied metabolic changes in skeletal muscle in mice after intracerebral inoculation of Dengue virus type 2 but do not describe histological muscle abnormalities. Nath et al.[20] have reported the ultrastructural alterations in skeletal muscle of dengue infected mice showing destruction of myofibrils and sarcoplasmic reticulum, swelling of mitochondria, glycogen accumulation and the demonstration of virus-like particles in the perinuclear zone and cytoplasm. They also do not describe any histological light microscopy abnormalities.

Our findings showed perivascular mononuclear infiltrates (but no myositis) and lipid accumulation (but not glycogen). Perivascular infiltrates were described in the CNS [7], soft tissue [4] and other organs [21] in post mortem studies of patients with dengue shock syndrome but not in the skin in dengue hemorrhagic fever [24].

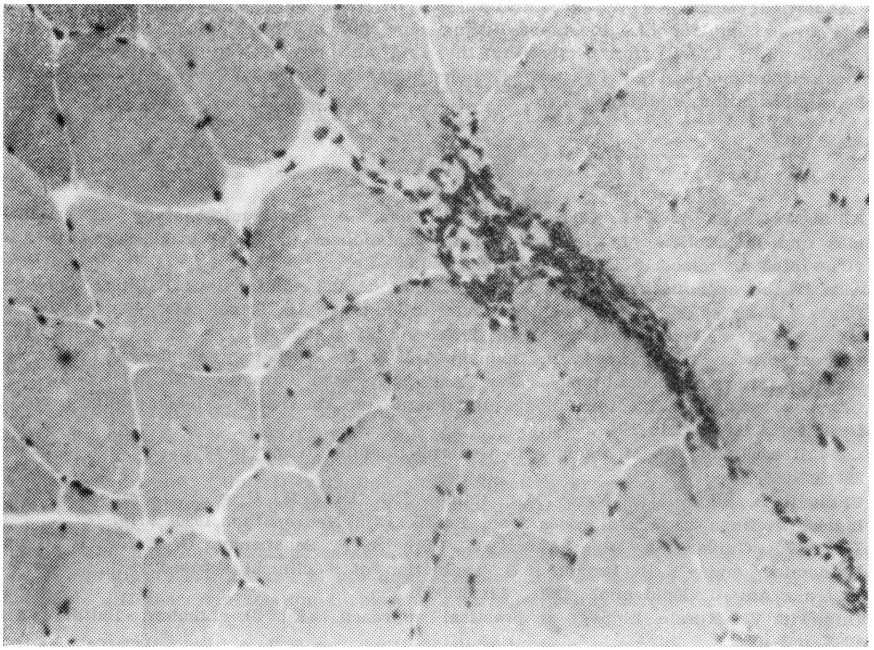

Fig. 2. Mononuclear cell infiltrates in the endomysium predominantly located around small blood vessels. (H&E, x350).

The perivascular infiltrate found in 12 of our 15 patients may be related to the pathogenesis of myalgia by producing ischaemic or metabolic changes [1,16,23] or even by local release of short-acting chemical mediators that could likely be effectors of pain [16,23]. We found lipid accumulation in 11 patients and 9 of them also showed perivascular infiltrates. The lipid accumulation could be the consequence of an ischaemic phenomenon related to the perivascular abnormality.

The mitochondrial proliferation found in three of our patients and the mitochondrial changes found by Nath et al.[20] in their ultrastructural study of skeletal muscle in mice may also reflect an abnormality in the oxidative metabolic pathway and might suggest that myalgia is due to metabolic changes [1,16,23].

Three of our patients who presented with perivascular infiltrates and lipid accumulation, also showed some foci of necrosis. Although these patients did not complain of a more severe myalgia than the others, muscle necrosis is known to produce pain [16,23]. One of these patients also showed a mildly elevated serum CK. Hence it is possible that the other two patients with elevated CK and the others without necrosis and CK abnormalities may have a metabolic muscle involvement severe enough to produce pain but not enough to produce necrosis or CK elevation.

As an indirect evidence of altered sarcoplasmic homeostasis possibly also reflecting the metabolic abnormalities already cited, three of our patients showed central nuclei [10].

We have been unable to explain the reason for the type grouping found in 2 of our 15 patients. In the superficial deltoid muscle the ATPase-checkboard pattern rarely varies [3,15] and these patients did not show any clinical evidence of peripheral nerve involvement.

New studies are needed to analyze the vascular involvement in muscle in the genesis of myalgia. Immunohistochemical and in situ hybridization techniques will help define the presence of the virus in cells around vessels and in the muscle and determine the nature of the mononuclear cells involved.

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