

ELECTROPHYSIOLOGICAL ASPECTS IN MASTICATORY MUSCLE MYOSITIS IN DOGS ASPECTE ELECTROFIZIOLOGICE ÎN MIOZITA MUȘCHILOR MASTICATORI LA CÂINI

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ABSTRACT | REZUMAT

The purpose of the current study was to describe electrophysiological tests applied in masticatory muscle myositis and analysis of the electrodiagnostic abnormalities recorded in dogs with those conditions, because neuroelectrophysiology has a crucial role in the diagnosis of diseases, being noninvasive. 5 dogs with muscular signs presented in Internal Medicine Clinic of Faculty of Veterinary Medicine Iași were included in the study. Electric biopotentials were recorded using the Electromyography (EMG) program of a Neuropack S, MEB 9400 K (Nihon Kohden) electric diagnosis device. The examined areas were shaven, degreased using alcohol and Skine Pure (Nihon Kohden) abrasive paste, and the surface electrodes were applied using Elefix gel. The electrophysiological tests, which revealed defects in masticatory muscles supported the prognosis and guided the therapy. Thus, the electroneuromyography revealed high insertion activity, fibrillation potentials, positive slow waves and high frequency discharges. The electric activity in the appendicular muscles consisted of electric silentium. The electroneurogram revealed normal amplitudes and nerve conduction velocities for the sciatic/tibial nerves in all of the patients. In conclusion, masticatory muscle myositis diagnosis was set by corroborating various data collected by anamnesis, neurological examination, conventional and advanced imaging techniques and electrodiagnosis tests. Electroneuromyography was used for the periodic assessment of the clinical status of these patients, and for therapy guidance and support.

Keywords: dog, electrophysiology, masticatory muscle myositis, eosinophilic myositis

Studiul curent are drept scop descrierea testelor de electrofiziologice aplicate în miozita mușchilor masticatori și analiza aspectelor electrodiagnostice înregistrate la câinii cu aceste tulburări, neuroelectrofiziologia aplicată având rol crucial în stabilirea diagnosticului de boală. Au fost incluși în studiu 5 câini prezentați la Clinica Medicală a FMV Iași, care la examenul neurologic au prezentat semne ale afectării musculare. Achiziția biopotențialelor electrice s-a realizat cu ajutorul sistemului de electrodiagnostic Neuropack S, MEB 9400 K (Nihon Kohden), în programul Electromyography (EMG). Ariile investigate au fost tunse, rase, degresate cu alcool și pastă abrazivă Skine Pure (Nihon Kohden) iar electrozii de suprafață au fost aplicați cu gel Elefix.

Testele de electrofiziologie au relevat anomalii care obiectivează afectarea mușchilor masticatori. Astfel, electroneuromiografic, s-a înregistrat o activitate de inserție crescută, potențiale de fibrilație, unde lente pozitive și descărcări cu frecvență înaltă. Activitatea electrică în mușchii apendiculari a prezentat silențiu electric. Electroneurograma a relevat amplitudini și viteze de conducere motorii normale pentru nervii sciatic/tibial la toți pacienții.

În concluzie, diagnosticul miozitei mușchilor masticatori a fost pus prin coroborarea datelor anamnetice, a examenului neurologic, tehnicilor convenționale și avansate de imagistică și a testelor de electrodiagnostic. Cu ajutorul electroneuromiogramei s-a putut evalua periodic statusul clinic al acestor pacienți, ghida și justifica tratamentul.

Cuvinte cheie: câine, electrofiziologie, miozita mușchilor masticatori, miozita eozinofilică

Masticatory muscle myositis (MMM) is an autoimmune local inflammatory myopathy, with clinical symptoms limited to some of the muscles of mastication (Fig. 1) represented by digastric rostral, tempo-

ral, masseter, pterygoid (lateral, medial) or in all the muscles innervated by the mandibular branch of the trigeminal nerve (1, 9). The muscles of the limbs are not usually impaired. Auto-antibodies against type 2M fibers are associated with MMM and they represent specific diagnosis indicators (2, 6).

In the past, this condition was called eosinophilic myositis (4) or atrophic myositis.

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Although these names suggest a different pathogenesis, it seems, in fact, that they represent the acute and chronic stage of the disease (3). During the acute stage, the patient's clinical symptoms consist of maxillary pain, trismus (lockjaw) and swelling of the involved areas, whereas muscle atrophy is a characteristic of the chronic stage. Unless the disease is early detected and an aggressive therapy is initiated, the myofibril loss and muscle fibrosis may cause irreversible maxillary dysfunctions and severe muscle atrophy (5, 6).

Despite the fact that it used to be considered a form of polymyositis, it has been proven over time that MMM is actually a unique form of myopathy. Although both the muscles in the limbs and the muscles of mastication contain type 1 and 2 muscle fibers, the limb muscles contain type 1A (for slow contraction) and 2A (for fast contraction) muscle fibers, whereas the muscles of mastication are made of type 2M and type 1 variant fibers. The biochemical tests carried out to evaluate myosin isoforms by electrophoresis helped distinguishing them from the fetal, limb and mastication muscles. Thus, it has been proven that type 2M isoform myofibrils are closely linked to various motor nerve branches, which develop during embryonic life. The immunohistochemical tests revealed autoantibodies against type 2M fibers in the mastication muscles of dogs suffering from MMM. More important, these antibodies were not reactive either with other muscle groups in MMM, or with other myopathies, such as polymyositis, polymyopathies or degenerative illnesses. These results confer the autoimmune nature of the disease. Immunohistochemical staining using staphylococcal protein A conjugated horseradish peroxidase revealed mobile and fixed antibodies (Ig G) in 85% of the dogs with MMM (7, 8).

The cause of autoantibodies formation and why these autoantibodies are directed against the type 2M fibers is still unknown. Some theories suggest that an important role is played by the molecular mimicry to the antibodies or T cells produced in response to an infectious agent, which later suffer a cross-reaction with the auto-antigens. In this "scenario", the peptide sequence or the conformational structure of bacterial antigens is similar to some of the 2M myofibril components (10). The antibodies against these antigen bacteria enhance the cross-reaction with these myofibrils. This theory is also supported by the literature for humans, more precisely by Kresher's research (1985) which proved that the auto-antibodies against *Streptococcus pyogenes* attacked the skeletal and

cardiac muscles. Other human diseases, such as pericarditis and rheumatoid arthritis, also involve autoantibodies against certain specific myofibrils (3).

MATERIAL AND METHODS

The research was conducted between October 2012 and December 2016 and included 5 dogs (Table 1) that were brought for examination to the Internal Medicine Clinic of the Faculty of Veterinary Medicine from Iași. Each patient underwent both clinical and neurological examinations. A standard neurological examination was conducted in all the patients, in comfortable thermal and mental conditions, after a period of accommodation with the examining veterinarian. It consisted of the assessment of their gait, postural reactions, spinal and cranial nerve reflexes. Both their personal and their family medical history were considered. In order to rule out the dogs suffering from motor conditions of extra muscular origin, all the patients underwent blood tests: complete blood count, hepatic (transaminases, gamma-glutamyl transpeptidase, alkaline phosphatase, total protein, glucose, direct and indirect total bilirubin, cholesterol, total lipids, triglycerides, iron), renal (creatinine, urea nitrogen), ionic (total serum calcium, potassium, phosphorus, magnesium) and thyroidal (TSH, FT4) biochemical profiles (11).

Table 1

Nr. crt.	Breed	Sex	Age (years)	Creatine-phosphokinase (UI/L)
P1	Husky	Male	1.5	192
P2	German shepherd	Female	2	63
P3	German shepherd	Female	1.7	134
P4	Crossbreed	Male	2	112
P5	Crossbreed	Female	4	103

Electric biopotentials were recorded using the Electromyography (EMG) program of a Neuropack S, MEB 9400 K (Nihon Kohden) electric diagnosis device. The examined areas were shaven, degreased using alcohol and Skine Pure (NihonKohden) abrasive paste, and the surface electrodes were applied using Elefix gel. The motor nerve conductive velocity (mNCV) was recorded electroneurographically by testing the tibial and peroneal nerves of the pelvic limb and the ulnar, radial and median nerves for the thoracic limb.

Bipolar surface electrodes were used to record the mNCV. The current intensity was increased progressively up to a level where the amplitude of the motor response remained constant. In order to make sure that we recorded the maximal response, we increased current intensity by an additional 25 – 50% (11). The electromyographical test was conducted on the following muscles: digastric rostral, temporal, masseter, pterygoid deltoid, triceps, extensor carpi radialis and brachial biceps in the anterior limb, and femoral biceps, quadriceps and cranial tibial in the posterior limb. The active electrode, i. e. the electrode that collects the bioelectrical data, consisting of a single-use bipolar concentric needle (50 x 0.45), was inserted through the skin in various areas and at different depths in the examined muscle, whereas the reference electrode was placed in the motor point of each muscle. In order to gather informations as accurate as possible on the clinical status of each of the examined muscles, they were tested in 5-10 areas.

RESULTS AND DISCUSSIONS

Anamnesis and physical examination.

One month before the examination, 3 patients exhibited left ($n = 2$) or right ($n = 1$) cheek hypertrophy associated with an inflammatory reaction of the local regional lymph nodes. These clinical symptoms apparently partially improved after the administration of an amoxicillin and clavulanic acid antibiotic. Then, the dogs had difficulties in eating food and drinking liquids, and consequently loosed a lot of weight.

The other two patients from the study started having difficulties drinking liquids five weeks before the examination, which also caused a loss of appetite for food. They started experiencing ptyalism and feeling pain when petted on the face and head. The attending veterinarian recommended a 10-day therapy with multivitamins, but the patient's clinical status did not improved. The general physical examination in all patients revealed severe bilateral atrophy of the temporal and masseter muscles (Fig. 1), associated with lockjaw. No heart and respiratory rate or body temperature changes were detected.

The neurological examination revealed no appen-

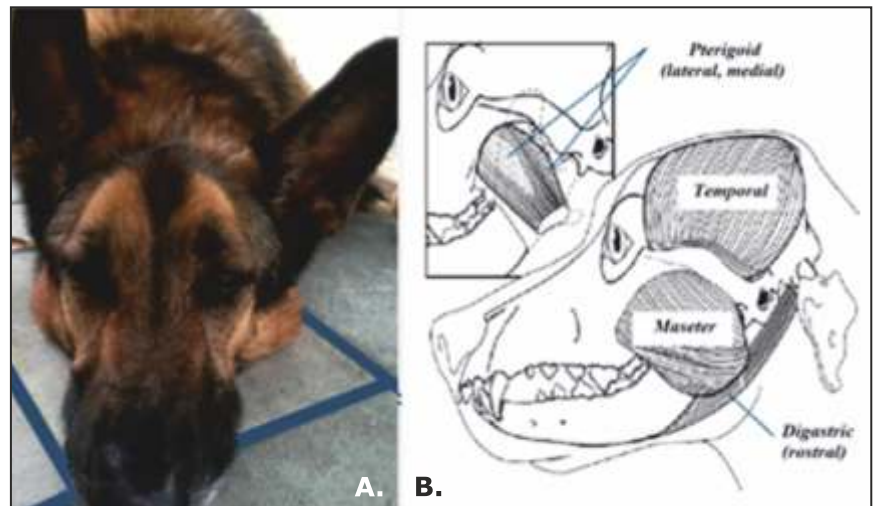


Fig. 1. A. Muscular atrophy localized in the temporal and masseter muscles. A two-year-old female German shepherd (original).
B. Muscles involved in mastication (after Shelton, 2004)

dicular deficits. Except for the dogs' inability to open and close their mouths, the cranial nerves were normal. The sensory function of the trigeminal nerves was normal: physiological blink reflex and normal response to nasal mucosa and cutaneous mandibular area stimulation.

The possible diagnoses were myositis /myopathy of the muscles of mastication, an osteoarticular condition of the temporal-mandibular joint or bilateral neuropathy of the motor part of the trigeminal nerve.

The mean age of MMM onset is three years, although cases of four-month patients with this disease were reported (9). This disease may occur in any dog, regardless of its sex or breed, although a higher occurrence rate has been noted in big dogs like the German shepherd, Labrador, Doberman and Golden Retriever. The Cavalier King Charles breed seems to have a genetic predisposition to this disease.

The biochemical blood tests revealed relatively high creatinine phosphokinase (CK) levels, but no other changes, which made the veterinarian to suspect a myopathy (CK: P1 – 192 UI/L, P2: 63 UI/L, P3: 134 UI/L, P4: 112 UI/L and P5: 103 UI/L; reference range: 0-50 UI/L) (3). The CK levels are frequently high during the acute stage of MMM and reach physiological values when the disease has already passed to the chronic stage. The elevated enzyme levels, if any, are lower than those of polymyositis patients, since a smaller muscular mass is impaired (3). In patients with MMM, serum biochemistry may also reveal hyperglobulinemia, moderate anemia and proteinuria.

The electromyogram of the masseter and temporal muscles revealed high insertion activity, fibrillation

potentials, positive slow waves (Fig. 2) and high frequency discharges (Fig. 3). The electric activity in the appendicular muscles consisted of electric silence (Fig. 4). The electroneurogram revealed normal amplitudes and nerve conduction velocities for the sciatic /tibial nerves in both patients.

The spontaneous electromyographic activity of the muscles of mastication confirmed a neuromuscular impairment. The conduction velocity study allowed the veterinary to rule out the possibility of a generalized neuropathy. The EMG at rest conducted on the appendicular muscles led the diagnosis towards myositis of the muscles of mastication. The relatively rapid mastication muscle atrophy may also be caused by diseases impairing the trigeminal nerve, especially neuritis and tumors. However, patients with trigeminal nerve neuritis do not experience pain and their maxillary tonus is flaccid. Conditions of the temporal-mandibular joint were ruled out by means of an X-ray. The electromyographic results were specific to the acute stage of the disease and supported a positive prognosis, since normal EMG would have only been recorded during the last MMM stage, after severe atrophy onset or muscle fibers loss and fibrosis onset (1, 9). The dog owners refused any mastication muscle sampling for biopsy, histological examination and immunological analysis (testing the

autoantibodies against type 2M muscle fibers with 85-90% sensitivity and 100 % specificity) since this procedure is very expensive; these tests are carried out only in the Comparative Neuromuscular Laboratory of the University of California, San Diego.

Histological testing is an important diagnosis setting stage, it does not provide any data on disease etiology yet. It reveals the presence of a necrosis focus and muscle fiber phagocytosis with perivascular infiltrate of mononuclear cells. Biopsy helps to determine muscle fiber loss severity, as well as fibrosis severity, which is important for determining the long-term prognosis and the likelihood of a successful therapy. The absence of an inflammatory lesion does not rule out MMM. The symptoms that may be due to masticatory muscle myositis and the positive results of the tests on the autoantibodies against type 2M muscle fibers confirm the diagnosis.

However, false negative results may occur if the patients were administered immunosuppressive corticoids as early as 7-10 days before testing or during the terminal stage of the disease, due to myofibril loss and fibrosis onset (9).

Therapy.

A corticoid therapy with degressive immunosuppressive doses of prednisolon was administered, initially with 2mg/kg/day for 3 days then the prednisolon dose of the following 3 days was reduced

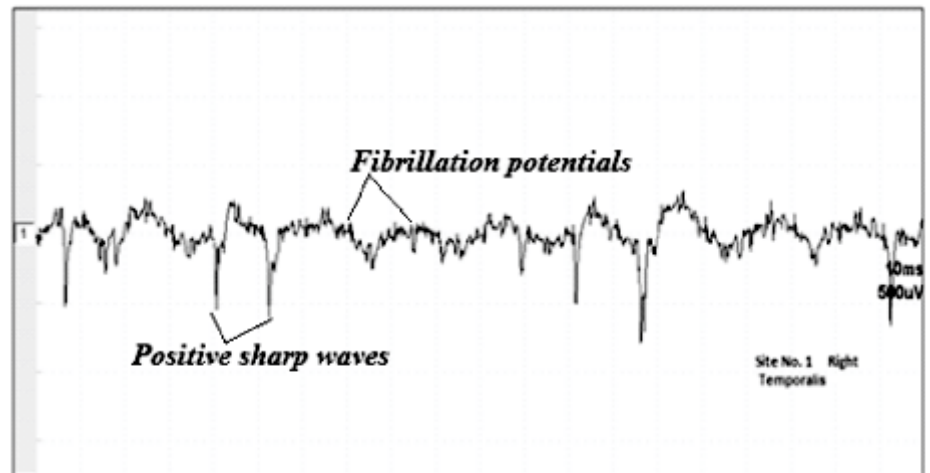


Fig. 2. Right temporal muscle electromyogram. 1.5-year-old male Husky. Fibrillation potentials and positive sharp waves.

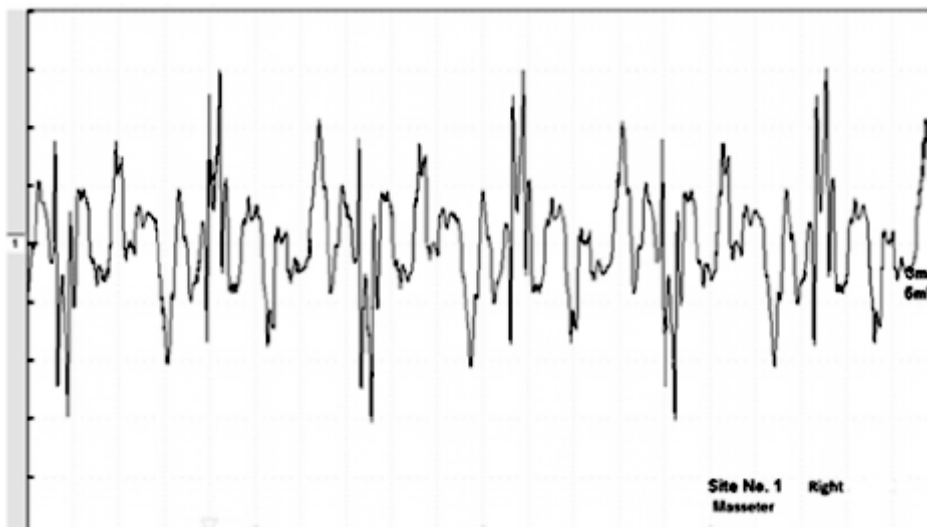
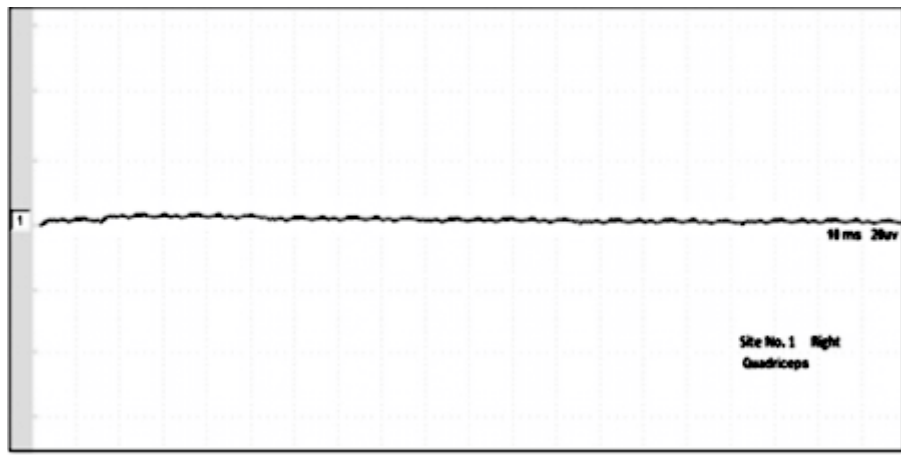


Fig. 3. Right masseter muscle EMG. 2.5 year old female German Shepherd. Complex repetitive discharges.

to 1 mg/kg/day, and then to 0.5 mg/kg/day - for the following 2 weeks. Then, the patients were administered the same dose every 48 hours for one month. After the first two weeks of therapy, the patients started to open their mouths again, and by the end of the therapy the "ankylosis" of the temporal-mandibular joint had gone and the muscles of mastication has started to regenerate.



CONCLUSIONS

The electrodiagnosis study allowed the confirmation of a neuromuscular condition and the localization of the lesions in the muscles of mastication.

It ruled out several possible diagnoses (polymyositis and polyneuropathy) and, in the absence of any specific examination methods (histological and serological tests), guided the therapy towards the most effective prognosis.

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Fig. 4. Physiological EMG on quadriceps muscle. 1.5-year-old male Husky.