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ELEVATED IgM TITERS OF SERUM ANTI-GD1a ANTIBODIES IN RELAPSING-REMITTING MULTIPLE SCLEROSIS: CORRELATION WITH NEURONAL DAMAGE

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POVIŠEN TITAR IgM SERUMSKIH ANTI-GD1a ANTITELA U RELAPSNO-REMI-TENTNOJ MULTIPLOJ SKLEROZI: KORELACIJA SA OŠTEĆENJEM NEURONA

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Key words
relapsing-remitting multiple sclerosis, neuronal damage, biomarkers, anti-GD1a antibodies,serum

Ključne reči
relapsno-remitentna multiple skleroza, oštećenje neurona, biomarkeri, anti-GD1a antitela, serum.

Abstract

In recent years it has become increasingly evident that neuronal injury considered at one time to be a late phenomenon in multiple sclerosis (MS) is now recognized to occur early in MS pathogenesis. Important strategy against MS is to identify biomarkers of early neuronal damage and to treat patient prior to the onset of any clinical symptoms. The objective of this study was to correlate the neuronal injury with serum levels of anti-GD1a antibodies in patients with relapsing-remitting multiple sclerosis (RRMS). Significantly higher IgM titers of serum anti-GD1a antibodies were detected in patients with their first attacks of RRMS in comparison with healthy subjects and RRMS patients with longer duration of the disease. These elevated titers suggest the immune-mediated neurodegeneration. Therefore, the estimation of IgM antibodies to GD1a in the serum can detect the early neuronal damage in MS, a very important indication for immediate neuroprotective treatment.

INTRODUCTION

One hundred and forty three years have passed since in 1868 Charcot [1] described the pathological and clinical features of multiple sclerosis (MS). Now, multiple sclerosis is recognized throughout the world, with around 2.5 million affected individuals. It affects females more than males. This ratio is higher (3:1) among patients in whom onset of MS is before age 15 years or after age 50 years. Multiple sclerosis most commonly affects people aged 18-50 years, but any group can be affected. The four major types of MS include relapsing-remitting MS (RRMS), primary progressive MS, secondary progressive MS and progressive relapsing MS. RRMS accounts for approximately 80-85 % of all MS cases [2].

For many years MS was considered to be primary demyelinating central nervous system (CNS) disease with preserved neuronal and axonal integrity at the onset of the disease. Recently, it has become clear that MS is a common

immune-mediated neurodegenerative disease of the CNS [2]. Neurodegeneration develops in association with inflammation and demyelination [3]. The neuronal damage begins at the earliest stages of the disease and underlies the accumulation of clinical disability. Therefore, it is of great importance to detect in the serum the early injury of brain neurons.The clinical symptoms in a variety of neurodegenerative disease are preceded by a prolonged preclinical period. It is essential to identify means of marking MS patients before the clinical onset in order to therapeutic interventions directed toward the neuronal injury.

In our recent studies a considerable increase of serum GD1a ganglioside was determined in MS patients indicating the neuronal and axonal damage [4,5]. Considerable changes of GD1a gangliosides were also detected in the CNS of Lewis rats with chronic relapsing experimental allergic encephalomyelitis (CREAE), an animal model of MS, just before the onset of the first clinical signs of the disease [6, 7, 8]. Gangliosides are a family of acidic glycosphingolipids high-

ly concentrated in the nervous system, where they represent about 10% of the total lipid content [9]. GD1a is one of the major central nervous system neuronal ganglioside fraction [10].

Over the past few years the importance of potential applicability of antibodies as biological markers for the diagnosis, classification , disease activity and prediction of clinical courses in MS has evolved [11].

The aim of this study was to correlate the neuronal injury with serum levels of anti-GD1a antibodies in patients with relapsing-remitting multiple sclerosis.

MATERIALS AND METHODS

Serum samples were obtained from 42 patients with clinically definite MS who had the relapsing – remitting form of the disease and from 20 healthy volunteers. Eight RRMS patients were evaluated during their first attack of what later was definitely diagnosed as MS. Thirty four RRMS patients were with a long duration of the disease and in clinical relapse during sample collection. None of MS patients received immunosuppressive treatment at the time of venopuncture. An informed consent was obtained from each patient.

The presence of anti-GD1a antibodies in the serum was measured by the enzyme-linked immunosorbent assay (ELISA). The ELISA protocol was selected according to modifications of the method of   Mitzutamari et al. [12]. We determined antiganglioside antibodies (AGA) of the IgM class against GD1a ganglioside. As AGA were found in low titers in some healthy subjects we estimated a reference range for the healthy controls. MS patients were considered strongly positive only if the optical density of their sera exceeded $x \pm 2$ SD of the healthy controls. The optical den-

sity was measured and read spectrometrically at 490 nm in a ELISA reader (TECAN, Sunrise TM, Austria). The Student test was used to determine statistical differences between the groups using p value of less than 0.05 as the level of confidence. The data are presented as a mean value

(M) ± standard error of mean (SEM).

RESULTS AND DISCUSSION

Statistically significant elevated IgM titers of serum anti-GD1a antibodies were detected in patients with their first attacks of relapsing-remitting MS in comparison of healthy subjects and RRMS patients with longer duration of the disease (Fig.1).These findings are in full concordance with our previous studies which have demonstrated a considerable increase of GD1a in the serum of FARRMS connected with the early neuronal damage in MS [5,13]. In humans gangliosides elicit a T-cell independent IgM response. Antiganglioside IgM antibodies can serve as a marker of axonal damage in neuropathies as multiple sclerosis [14]. Early neuronal damage in MS patients has been demonstrated in vivo by magnetic resonance spectroscopy which shows decreased levels of neuron-specific marker N-acetylaspartate in the early stages of MS [15]. Direct evidence of early neuronal injury in MS has been provided by morphological investigations [16, 17].

In conclusion, our study permits us to find, for the first time, that higher IgM titers to serum anti-GD1a antibodies reflect the early CNS neuronal injury in MS. Therefore, the estimation of IgM antibodies to GD1a in the serum can detect the early neuronal damage in multiple sclerosis, a very important indication for immediate neuroprotective treatment and its efficacy.
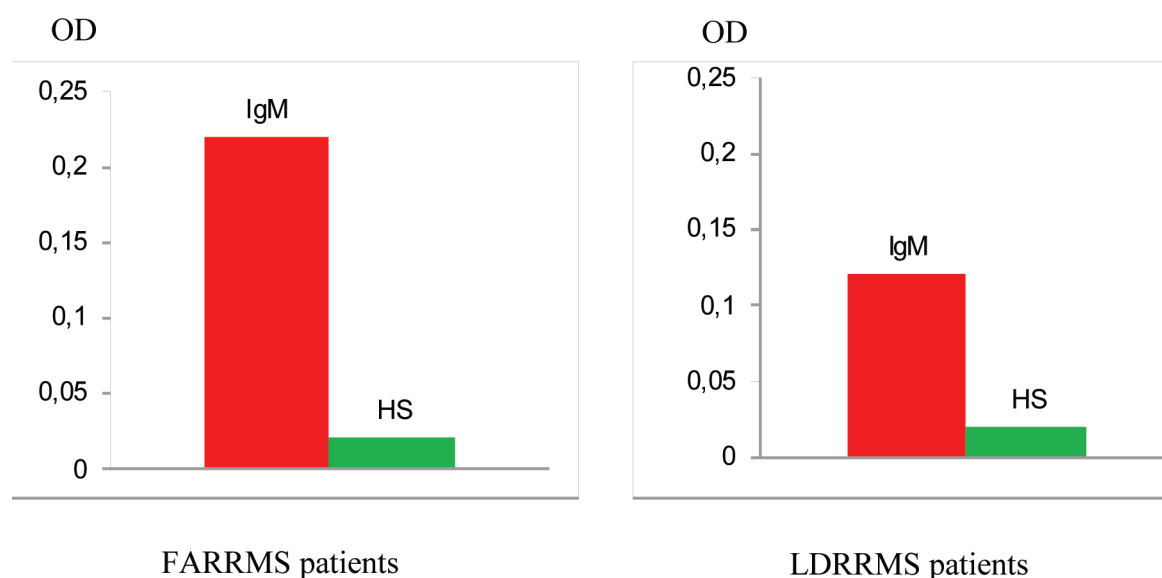

Fig. 1. Serum IgM antibodies to GD1a in RRMS patients

OD – optical density
HS – healthy subjects
FARRMS – first attacks of RRMS patients
LDRRMS – patients with longer duration of RRMS

Apstrakt

Poslednjih godina povećao se broj saznanja da je povreda neurona, ranije smatrana kasnim fenomenom u multiple sklerozi (MS), prepoznata kao rani događaj u patogenezi MS. Važna strategija u lečenju multiple skleroze je prepoznavanje biomarkera ranog oštećenja neurona i lečenje pacijenata pre pojave kliničkih simptoma bolesti. Cilj ove studije je korelacija povrede neurona sa koncentracijom anti-GD1a antitela u serumu pacijenata sa relapsno remitentnom multiple sklerozom (RRMS). Značajno povišen titar IgM serumskih anti-GD1a je otkriven kod pacijenata sa prvim pogoršanjem RRMS u poređenju sa zdravim osobama i RRMS pacijentima kod kojih je bolest duže trajala. Povišen titar ukazuje na imuno-posredovanu degeneraciju neurona. Zbog toga koncentracija IgM antitela na GD1a u serumu može da ukaže na rano oštećenje neurona u MS što je važna indikacija za pravovremeno neuroprotektivno lečenje.

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