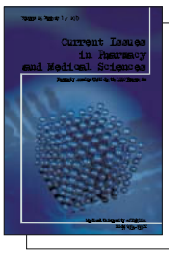

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Blood serum amino acids profile in patients with Multiple Sclerosis

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ABSTRACT

Multiple sclerosis is the most common demyelinating disease of the central nervous system, affecting mostly young people. There were many risk factors for MS identified, however a direct cause of the disease is still unknown. Pathological changes in the SM lead to the myelin sheath damage around axons, what prevents proper transmission of nerve impulses in the central nervous system. The aim of this study was analyzing and comparing the amino acids profile in the blood serum of MS patients to control group of healthy individuals and evaluating the relationship between them. Significant ($p<0.05$) differences in the level of glutamate, aspartate and taurine in the blood serum of MS patients were revealed. A positive glutamate and aspartate level correlation in the serum has been demonstrated. Gender is significant only in the case of glutamate level in blood serum. The studies highlight the important role of neurotransmitters in MS and are the initial step in proteomic research.

Keywords: Multiple sclerosis (MS), amino acid, aspartic acid, glutamic acid, taurine

INTRODUCTION

Multiple sclerosis (MS) is the most common demyelinating disease of the central nervous system affecting mostly young people between 20 and 40 years of age. It affects twice as many women as men [1, 3]. MS is a chronic, inflammatory, multi-phase disease with periods of exacerbations and improvement. The changes of nerve cell in multiple sclerosis cause damage to the myelin sheath around axons, which prevents proper transmission of impulses along nerve tracts in the brain and spinal cord. Multifocal nervous tissue damage is created as a result of neuronal dysfunction [12]. Many risk factors for MS has been identified but the direct cause of the disease is still unknown. MS develops probably as a result of interaction between environmental factors and genetic predisposition [5]. Various theories try to explain the origin and the development of the disease. The most popular is the autoimmune theory saying that it could be caused by participation of a viral infection or a reactivation of latent retroviral infection, which initiates later an immune response.

The role of Epstein-Barr virus in the pathogenesis of MS and other unknown environmental factors contributing to the development of demyelinating lesions in the brain tissue is also considered. [16, 9].The similarity in the molecular structure between the virus antigen and self-antigen of the cells of central nervous system could explain the immune response directed against own cells.

Many other hypotheses tell about inadequate response of the immune system to one or more environmental factors [21]. The further from the equator the more frequent is the rate of MS development. A reduced exposure to sunlight and lower synthesis of vitamin D3 was investigated in the pathogenesis of MS [13, 4]. This theory is supported by recent findings indicating that vitamin D is an important regulator of the immune system [15]. Study carried out on a large group of participants in 2006 by the Harvard School of Public Health show a higher incidence rate of MS when the level of vitamin D is low. However, it applies only to the white not black race [13]. Research in 2007 showed that in the absence of genetic predisposition, exposure to the light during childhood reduces the risk of developing MS [4]. New alternative theories are proposed for other factors being direct purpose of neurodegenerative changes. Research is conducted in almost all fields of science such as genetics, molecular biology, immunology and microbiology etc..

Degeneration of the myelin covering axons of nerve cells is characteristic for MS. Subsequent relapses lead to insufficient remyelinisation efficiency, which causes heavy damage to the myelin sheath and irreversible dam-

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age to the axons [11]. Oligodendrocytes responsible for producing myelin sheath disappear and are replaced with macrophages, which induce inflammation [14, 21]. In the place of injury, there develops a scar consisting of the astroglial. According to the immune theory of MS, the regulatory T cells which have several times less ability to inhibit division of other lymphocytes play the major role in the initiation of the inflammatory process [23]. Lymphocytes recognize myelin as a foreign substance and try to destroy it. Initiated inflammation involves also the other cells of immune system as well as cytokines and antibodies. Blood-brain barrier is injured, which promotes the damaging processes such as swelling, activation of macrophages, stronger activation of cytokines and tissue destroying enzymes such as metalloproteinases [19, 23]. Pathology of multiple sclerosis is more complex. It has been proven that mitochondria, which are the energy center of the cells, play the crucial role in demyelination of axons on the cellular level in all stages of the disease.

Literature data suggests that changes in the level of neurotransmitters, biogenic amines and amino acids are also important in the development of motor dysfunction (including spasticity) which are the most common development symptoms of the disease [1]. MS symptoms develop as an expression of cumulative process as the numerous lesions in the brain and spinal cord. Their variable number and the location is the reason why individual patients may present a variety of symptoms. Multiple sclerosis can cause a variety of symptoms and syndromes: most common are movement, sensory, cerebral (balance disorder), visual, autonomic disorders, pain and psychiatric symptoms such as cognitive impairment and mood disorders. One of the common symptoms is also chronic fatigue. Multiple sclerosis is one of the most frequent causes of disability amongst young people but many patients may experience a mild course [10]. Multiple sclerosis can take one of the following forms: Relapsing Remitting MS (RRMS), Secondary Progressive MS (SPMS), Primary Progressive MS (PPMS) and Progressive Remitting MS (PRMS) [8, 21]. The degree of severity of the disease can be assessed by classifying clinical symptoms in patients with MS in 10-point Kurtzky's scale (Extended Disability Status Scale - EDSS).

The purpose of the test was to determine the majority of amino acids in patients with diagnosed MS and comparing the level of amino acids with the control group. Correlation with the clinical stage of the disease can give a wider view of the importance of amino acids in SM and their possible use in manufacturing of the medicinal preparations.

MATERIAL AND METHODS

The material for this study was blood serum obtained from 23 patients with MS aged 18 to 62 years (median = 41, SD = 11.04 years). The population consisted of 20 female patients (86.95%) and 3 men (13.04%), in different clinical stages of the disease, rated on a scale of Kurtzky. Eleven patients (36.6%) with a mild form of MS (stage 1-3 in EDSS), 2 patients (6.66%) with symptoms typical of 4 and 5 stage of motor disability and 10 patients (33.3%) of severe clinical stage (6-8 in Kurtzky scale). Control group consisted of seven healthy men. Level of 19 amino acids in serum was marked with HPLC and UV detection.

STATISTICA 10.0 program was used for statistical presentation.

Test of choice to evaluate the results in the studied group was a Mann-Whitney U test. In order to determine differences in the distribution r Spearman correlation factor was applied. Only significant differences in the amino acid level between patients with MS and control group were presented and discussed.

RESULTS

There was a significant difference shown in the group of MS patients in the levels of glutamic acid ($p = 0.00008$), aspartic acid ($p = 0.024$) and taurine ($p = 0.027$) compared to the control group of healthy individuals. The data is contained in Table 1.

Table 1. The level of aspartic acid, glutamic acid and taurine in MS patients and the control group (μmol/l)

Amino acid		Aspartic acid (μmol/l)	Glutamic acid (μmol/l)	Taurine (μmol/l)
Multiple Sclerosis (MS)	Median ±1 SD	0,044 (0,027–0,061)	0,081 (0,04–0,122)	0,674 (0,506–0,842)
Control group	Median ±1 SD	0,032 (0,024–0,04)	0,130 (0,108–0,152)	0,085 (0,074–0,06)

Aspartic acid level in MS patients was significantly higher (median 0.044 μmol/l ± 0.017) than in control group (median 0.032 μmol/l ± 0.008) (Figure 1).

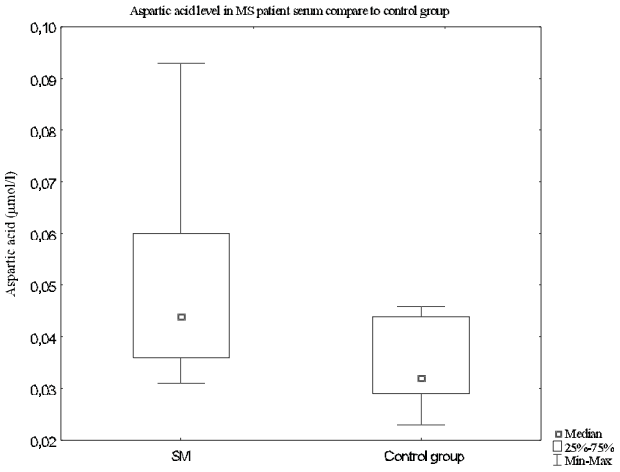

Fig. 1. Aspartic acid level (μmol/l) in patients with MS as compared to the control group

The same relation in comparison to control group as above was shown at a concentration of glutamic acid. Patients with MS demonstrated significantly higher values (median 0.674 μmol/l ± 0.168) than the healthy population (median 0.085 μmol/l ± 0.011) (Figure 2).

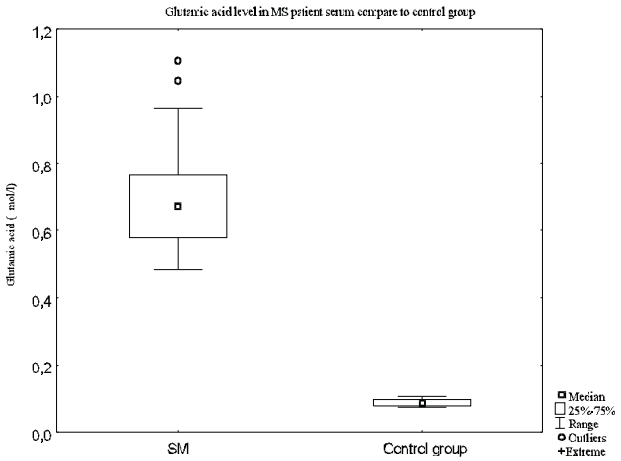

Fig. 2. The level of glutamic acid (μmol/l) in a population of MS patients compared to the control group

A significant difference in the level of taurine ($p = 0.027$) compared to the control group was shown. Serum of MS patients showed substantially lower level of taurine (median 0.081 μmol/l ± 0.041) comparing to control (median 0.130 μmol/l ± 0.022) (Figure 3).

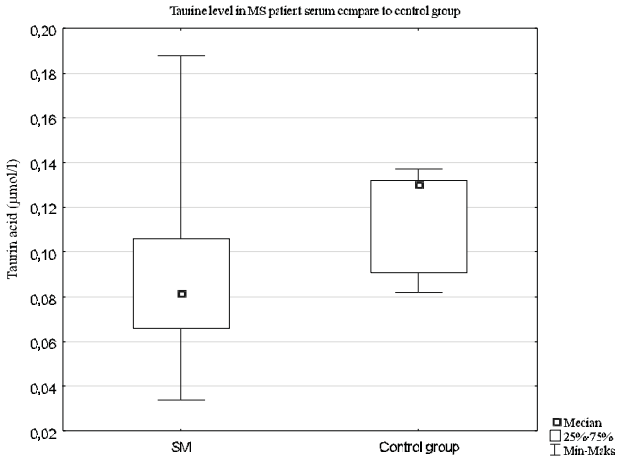

Fig. 3. The level of taurine (μmol/l) in a population of MS patients compared to the control group

No significant differences in the level of the other amino acids in the human serum of MS patients comparing to the control was discovered.

No significant differences were obtained at the amino acid level in the blood serum of patients presenting different clinical stage at EDSS scale.

However studies have shown a significant correlation ($p = 0.002$) in the level of glutamic acid in men and women. In women's serum there was a higher level of glutamic acid determined (median 0.656 μmol/l ± 0.156) comparing with men's serum (median 0.0945 μmol/l ± 0.343). Results are shown in Figure 4.

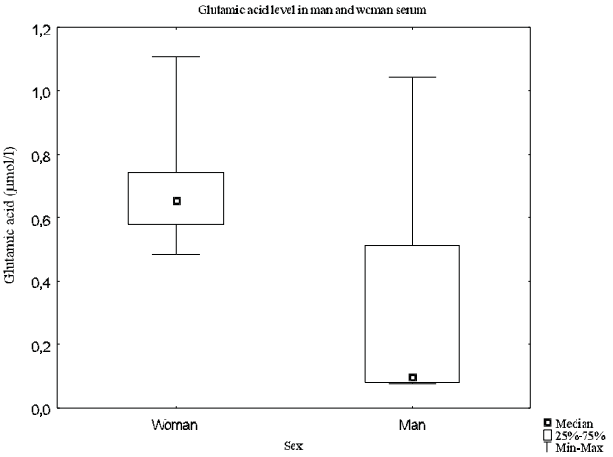

Fig. 4. Glutamic acid level (μmol/l) in the serum of women and men

The study revealed a significant positive correlation ($p = 0.002$, $R = 0.538$) in the level of aspartic and glutamic acid in the blood serum.

Conducting this research on a larger group and additional statistical correlation with other clinical and laboratory parameters, could give a more complete picture of changes in the amino acid profile of MS patients be also useful in the treatment of these patients.

DISCUSSION

Glutamate is a major excitatory amino acid neurotransmitter acting on receptors located in the membrane of the postsynaptic neuron. Its level is controlled by glutamate transporters present in astrocytes, oligodendrocytes and microglia cells that capture the released glutamate and convert it into glutamine with the participation of glutamine synthetase [24].

The resulting glutamine is directed to neurons by Na^+-dependent amino acid transporters and participates into a series of changes leading to formation of active glutamate. Kimmy G. Su in his work states that the high level of glutamate is toxic in the synapses and its reuptake is inadequate by cells associated with neurons. As an example, he lists excessive glutamate production by activated macrophages and microglial cells in the inflammatory demyelinating process accompanying acute multiple sclerosis [21]. For comparison in chronic demyelinating foci, elevated level of glutamate were not observed. Srinivasan et al. suggest that monitoring of the level of this neurotransmitter in the various stages of MS can be useful [19].

Aspartic acid as well as glutamic acid is the neurotransmitter in the nervous system. It works by NMDA receptors

located in postsynaptic membrane and opens channels for Ca^{2+} ions flowing into the cell. Derivative of aspartic acid N-acetyl-aspartate (NAA) is produced by neuronal mitochondria and reflects their activity in axons. Since mitochondria play an important role in degeneration of axons in all stages of MS, NAA is considered as indirect marker of axonal damage.

According to Su Kimmy G. et al., NAA level is significantly reduced in acute inflammatory demyelination foci then partly increases as the inflammatory process subsides [21]. Jeffery D. Haines suggests that the concentration of N-acetyl-aspartate is reduced both in pathologically changed cells as in the some part of the brain white matter not covered by the demyelination process [6].

In the course of neurological diseases causing the damage to the blood - brain barrier, its permeability rises, which allows the passage of proteins by diffusion or active transport. The level of amino acid neurotransmitters in the serum may vary from the level of these substances in the cerebrospinal fluid and brain tissue.

Another substance undergoing quantitative changes in MS is taurine. Taurine is a final degradation product of sulfuric amino acid cysteine. It plays many different roles in the body: one of them is taking part in immuno- and neuromodulation processes [21]. Taurine helps transporting creatine into muscles, what causes more effective and efficient using of it and promotes muscle recovery after physical effort. In the course of physical effort, the body stops producing sufficient amount of taurine causing its deficiency. Taurine affects the central nervous system, attributed to the neurotransmitter functions. It is the GABA(A)- receptors agonist. It is believed that the very high concentration of taurine is presented in the developing brain, and highly decreases immediately after the development process. Sternberg and associates suggest that there is possibility of using taurine and its derivatives in the treatment of MS in the future [21].

CONCLUSION

Research carried out in our studies indicates low level of taurine in the course of multiple sclerosis, which can be compensated by an increase in the level of other neurotransmitters such as glutamic and aspartic acid, playing an important role in the central nervous system. Analysis of the results gives a wider picture of the changes taking place in the demyelinating process. It could also be the initial step in MS proteomic research.

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