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ORIGINAL ARTICLES

Cerebrospinal fluid and plasma concentration of soluble intercellular adhesion molecule1, vascular cell adhesion molecule1 and endothelial leukocyte adhesion molecule in patients with acute ischemic brain disease

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Background. *Leukocyte migration into the ischemic area is a complex process, controlled by adhesion molecules (AM) in leukocytes and endothelium, by migratory capacity of leukocytes and the presence of hemotaxic agents in the tissue. In this research it was supposed that in the blood and cerebrospinal fluid (CSF) of patients in the acute phase of ischemic brain disease (IBD) there were relevant changes in the concentration of soluble AM (sICAM-1, sVCAM-1 and sE-selectin), that could have been the indicators of the intensity of damaging processes in central nervous system (CNS).* ***Methods.*** *The study included 45 IBD patients, 15 with transient ischemic attack (TIA), 15 with reversible ischemic attack (RIA), and 15 with brain infarction (BI), of both sexes, mean age 66±7. Control group consisted of 15 patients with radicular lesions of discal origin, subjected to diagnostic radiculography, without the signs of interruption in the passage of CSF. Changes of selected biochemical parameters were determined in all patients in frame 72 hours since the occurence of an ischemic episode. Concentrations of soluble AM were determined in plasma and CSF by ELISA. Total number of leukocytes (TNL) in peripheral blood was determined by hematological analyzer.* ***Results.*** *The results showed that during the first 72 hrs of IBD significant increases occured in TNL and that the increase was progressive compared to the severeness of the disease. Significant increase of soluble AM concentration was shown in plasma of IBD patients. The increase was highest in BI, somewhat lower in RIA and the lowest in TIA patients compared to the control. In CSF concentrations of sICAM-1, sVCAM-1 and sE-selectin demonstrated similar increasing trend as in plasma.* ***Conclusion.*** *TNL, as well as the soluble AM concentrations in plasma and CSF, were increased during the acute IBD phase and progressive in relation to the severeness of the disease, so that they might have been the indicators of CNS inflammatory reaction intensity. Furthermore, the results indicated their role in IBD pathogenesis and offered the possibility of researching the application of antagonists and/or activity modulators of some of them in IBD therapy.*

Key words: **brain ischemia; blood; cerebrospinal fluid; leukocytes; intercellular adhesion molecule-1; vascular cell adhesion molecule-1; E-selectin; inflammation.**

Introduction

Most recent studies indicate the existence and the development of inflammatory reaction during ischemic brain disease (IBD). Two basic processes are responsible for the development of inflammatory reaction in CNS during ischemia: local reaction of brain cells and leukocyte migration from circulation into the ischemic parenchyma (1–7).

Local reaction of brain cells occurs during a few minutes (neurons and microglia), or hours (astrocytes) after ischemia. At the neuron level the central event is the increase of intracellular concentration of Ca^{2+}, uncontrolled release of neurotransmitters, increased production of free radicals, lipid mediators, derivatives of arachidonic acid, acidosis, etc. The process of exitotoxicity leads to cellular death, and to the damages of the surrounding neurons, glial and endothelial cells (8–12). Furthermore, microglia activated with ischemia and astrocytes by the secretion of large amounts of citotoxic mediators play a significant role in additional neuronal and endothelial cell damage as well as in the development of the second phase of inflammatory reaction (12–14). Pharmacological modulation of microglia effects might contribute to the improvement of structural and functional outcome of brain ischemia.

The second phase of inflammatory reaction development includes infiltration of peripheral inflammatory cells (neutrophils, monocytes and lymphocytes) in ischemic parenchyma. Leukocyte migration into the ischemic area is a complex process, controlled by adhesion molecules in leukocytes and endothelium, by migratory capacity of leukocytes and the presence of hemotaxic agents in the tissue (3, 15–18). Human data are scarce: a high number of radiolabeled polymorphonuclear leukocytes that occur in slightly perfused brain areas 6 to 12 hours after the ictus, and autopsy show intense leukocyte infiltration of brain parenchyma 2 to 3 days after the stroke (1, 3). Experimental studies suggest that the early occurence of leukocytes in to the brain parenchyma is independent from the presence of necrosis, which leads to the hypothesis that these cells have a role in the progression from ischemia to brain infarction (3, 15).

The occurence of leukocytes in the ischemic area is a multistep process (4, 5). They marginate in the venules, then adhere to endothelial cells, and finally migrate into the brain parenchyma. During each of these steps, their functions are regulated by the inflammation-related molecules that are being produced soon after the onset of cerebral ischemia. The signals mediating the entry of leukocytes into the ischemic area have not yet been completely unfolded, but a number of clues suggest that cytokines play a significant role in this process. Cytokines may have various functions during cerebral ischemia: they can attract leukocytes and stimulate synthesis of the adhesion molecules in leukocytes, endothelial cells, and other cells, thus promoting the inflammatory response of the damaged cerebral tissue (3, 5, 7, 18).

Migration of peripheral blood leukocytes requires prior adhesion to cerebral endothelial cells, which is mediated by the adhesion molecules on the surface of cerebral endothelial cells and peripheral blood leukocytes (3, 4, 16). In cerebral ischemia at least 3 different adhesion molecules have merited special interest so far: intercellular adhesion molecule 1 (ICAM-1), responsible for the adhesion of mononuclear cells and granulocytes, vascular cell adhesion molecule 1 (VCAM-1) which predominantly mediates adhesion of monocytes, and endothelial leukocyte adhesion molecule (E-selectin), expressed only by endothelial cells and facilitates adhesion of monocytes and granulocytes (3, 4, 7, 16–22).

In physiologic conditions, adhesion molecules are scarcely expressed on cell surface, but their expression might be induced by diverse pathophysiological processes (3, 4, 18–22). Circulating, soluble forms of the molecules are formed in the proteolytic release of their extracellular domains (16–18). Raised levels of soluble adhesion molecules have been described in various inflammatory and malignant diseases or infections, and it has been suggested that they were markers of diseases activity (4, 16, 18, 20, 22).

The exact pathogenic role of the mentioned adhesion molecules during brain ischemia in human has still not been completely defined. Previous studies do not offer data whether the expression of these adhesion molecules and concentration of their soluble forms is related to clinical disorders and recovering of the patients with IBD. Besides, there are no data on the dynamics of the adhesion molecules expression in patients with the different types of IBD. The question of differences between soluble adhesion molecule level in plasma and CSF in patients during the initial phase of IBD remains to be clarified. Responses to the opened questions could be significantly involved into the further therapy strategy of IBD directed at inflammatory reaction, but could also be important for clinical practice in determining the severeness of the disease and the recovery during the IBD initial phase.

In this research it was supposed that there were relevant changes in the concentration of soluble adhesion molecules (sICAM-1, sVCAM-1 and sE-selectin) in the blood and cerebrospinal fluid of patients in the acute phase of IBD. Those changes were the indicators of the intensity of damaging processes in CNS. The concentration of soluble adhesion molecules in plasma and cerebrospinal fluid, as well as the total number of leukocytes in peripheral blood was monitored in IBD patients in frame 72 hours since the formation of an ischemic episode.

Methods

Study included 45 IBD patients of both sexes, mean age 66±7. The diagnosis was established by medical history, clinical examination, and cerebral CT scans. After institutional approval, informed consent was obtained from all patients before study entry. Fifteen patients had transient ischemic attack (TIA), defined as complete recovery from clinical symptoms within 24 hours, fifteen patients had reversible ischemic attack (RIA), defined as complete recovery from neurological deficits within 7 days, and fifteen patients had brain infarction (BI), defined as neurological deficits persisting for more then 7 days. Exclusion criteria for the patients were the presence of infections, other cerebral, inflammatory, pulmonary, hepatic, renal and malignant diseases, or immunosuppressive treatment.

In all IBD patients the level of soluble adhesion molecules, as well as the total number of leukocytes was determined within the 72 hours since the occurence of ischemic episode.

Control group consisted of 15 patients of adequate age and gender, with radicular lesions of discal origin (lumbosacral area), subjected to diagnostic radiculography, without the interruption signs in the passage of cerebrospinal fluid. Only the patients with the abrupt development of motor deficit without pain and thus without analgesic therapy were included in the study, and cytochemical and immunochemical findings in CSF were within physiological limits. Patients with intensive radicular pains who used nonsteroidal analgesics, as well as patients with anamnesis and clinical data on current inflammatory, neurodegenerative and psychiatric diseases were excluded from the study.

In all the tested patients blood and CSF samples were taken in the period of 8 to 10 AM, before any therapy was given. Peripheral vein blood samples (3 ml) were collected in heparinized glasses at low temperatures. Plasma was selected by the centrifugation of the whole blood during 15 min at 3000 rpm, at +4°C.

CSF samples (1 ml) were taken during the lumbar puncture into the glasses held on ice (+4°C). Plasma and CSF samples were kept at -70°C until the biochemical analyses. The analyses were performed within 6 month after the samples colection.

Concentrations of soluble adhesion molecules sICAM-1, sVCAM-1 and sE-selectin were determined in plasma and CSF by ELISA (R&D Systems Europe, Abingdon, UK). Plasma samples were diluted with commercial diluents for detecting sICAM-1 in ratio 1:80, for s-VCAM-1 and sE-selectin in ratio 1:50, and all samples of CSF in ratio 1:1. Total numbers of leukocytes in peripheral blood were determined by hematological analyzer Technicon H-1. All samples were analyzed in duplicate, and the mean value was calculated. Intraessay and interessay precision (coefficient of variation) for all of these assay were <5.9% and <10.2%, respectively.

The values of the monitored parameters were represented as mean ± standard deviation. Statistical significance of the differences between IBD and the control values was assessed by Student's t-test (with $p<0.05$ as significant).

Results

The results showed that during the first 72 hours of IBD the significant increase in leukocyte number in peripheral blood occured being progressive according to the severeness of the disease. In BI patients values were 9.29 ± 1.89 $x10^9/l$, ($p<0.001$ compared to the control), (Figure 1).

The results showed significant increase of sICAM-1, sVCAM-1 and sE-selectin concentration in plasma of IBD patients, while the increase was the highest in BI patients, somewhat lower in RIA patients, and the lowest in TIA patients. Plasma concentration of sAM in BI patients was 693.84 ± 91.12 ng/ml for sICAM-1, ($p<0.001$); for sVCAM-1 was 687.24 ± 156.96 ng/ml, ($p<0.001$); and for sE-selectin was 51.13 ± 17.57 ng/ml, ($p<0.001$ compared to the control), (Figure 2).

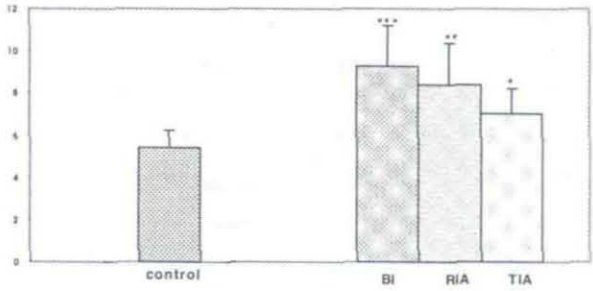

Fig. 1 – Total number of leukocytes ($x10^9/l$) in peripheral blood of patients with brain infarction (BI), reversible ischemic attack (RIA), and transient ischemic attack (TIA) during the first 72 hours after insults. Values are given as mean±SD. *($p<0.05$), **($p<0.01$), ***($p<0.001$)- significance to corresponding control values.

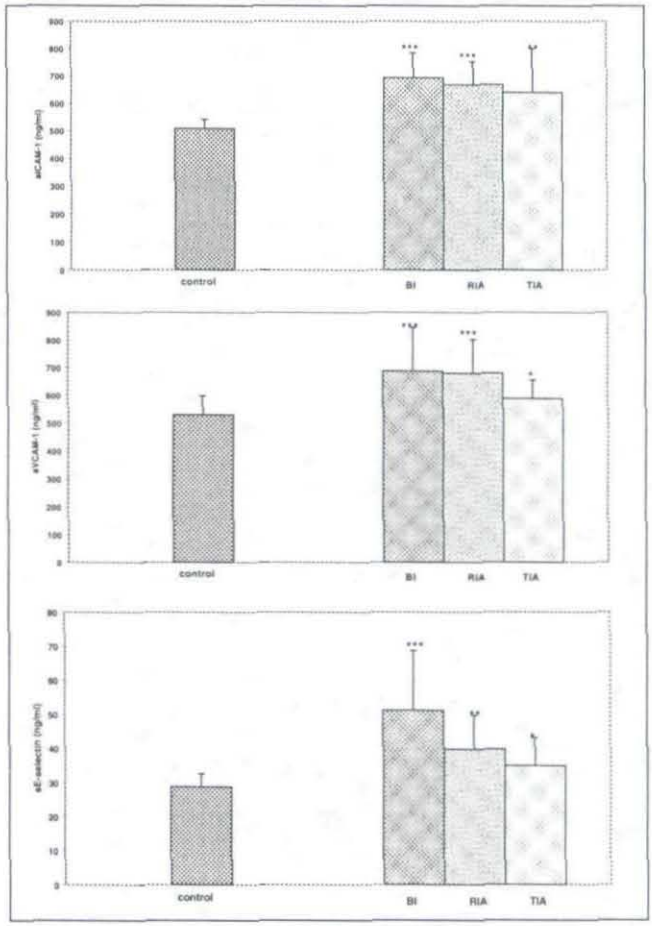

Fig. 2 – Concentration of sICAM-1, sVCAM-1 and sE-selectin in plasma of patients with brain infarction (BI), reversible ischemic attack (RIA), and transient ischemic attack (TIA) during the first 72 hours after insults. Values are given as mean± SD. *($p<0.05$), **($p<0.01$), ***($p<0.001$)- significance to corresponding control values.

In CSF concentration of sICAM-1, sVCAM-1 and sE-selectin demonstrated similar increase trend as in plasma. CSF concentration of sAM in BI patients was 3.24 ± 0.78 ng/ml, for sICAM-1 ($p<0.001$); for sVCAM-1 was 8.87 ± 2.61 ng/ml, ($p<0.001$); and for sE-selectin was 0.140 ± 0.045 ng/ml, ($p<0.05$ compared to the control), (Figure 3).

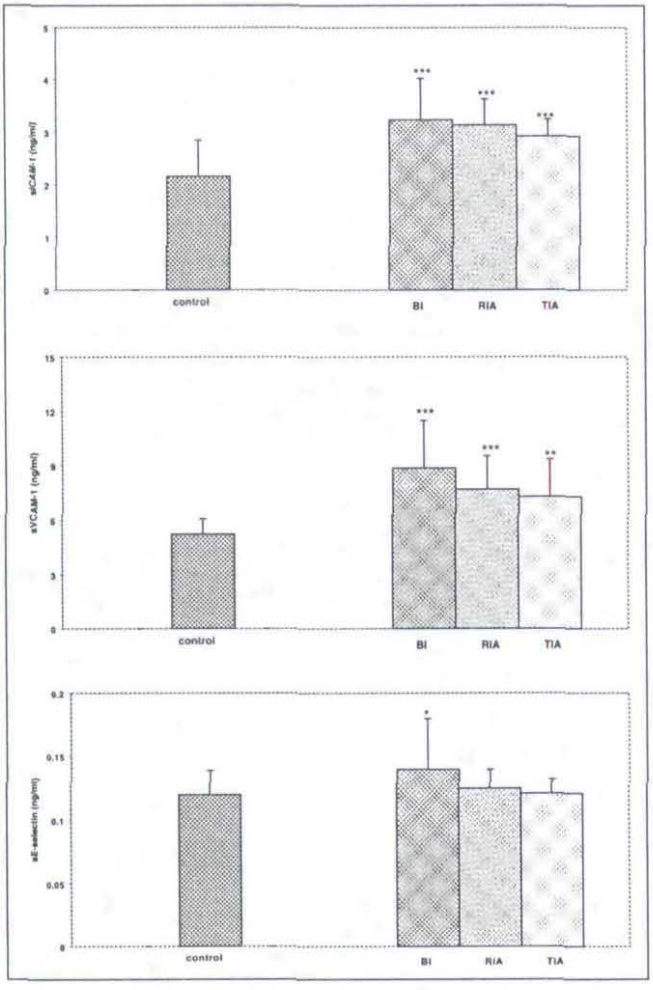

Fig. 3 – Concentration of sICAM-1, sVCAM-1 and sE-selectin *in CSF of patients with brain infarction (BI), reversible* ischemic attack (RIA), and transient ischemic attack (TIA) during the first 72 hours after insults. Values are given as mean± SD. *($p<0.05$), **($p<0.01$),***($p<0.001$)- significance to corresponding control values.

Discussion

Physiopathologically, ischemia represents a metabolic disorder in brain functioning caused by the circulation reduction that ends in morphological damages of neural elements. Namely, the primary stimulus, which triggers a cascade of metabolic changes that end in nerve cell death, is the energetic crisis or the excessive (uncontrolled) depolarization (12, 23–25). Change from reversible into irreversible neuron damage depends above all on the ischemic severeness and its duration. The efforts of contemporary acute IBD therapy are directed towards brain tissue protection before the development of irreversible damage occurs. Numerous studies showed that reversible ischemic stadium gradually progressed towards infarction. Central zone, with highly compromised blood flow is surrounded by the zone with milder ischemic level (penumbra), where electrical activity is damaged, but cell metabolism and neuron viability are partially preserved, making the final outcome in this zone is variable and dependent on numerous factors that affect time and spatial propagation of damaging and/or defense reactions after the establishing of the reperfusion. Possible therapy is directed to words the ischemic damage area because it is likely to expect that, due to the partially present circulation, the remedy reaches the targeted place. The volume of the infarction area, and consequently the clinical IBD outcome depend on the outcome of the penumbra process (25–30).

During the last few years researches were directed towards the inflammatory reaction in ischemic penumbra zone. It was demonstrated on the experimental model of IBD that this pathophysiological event was followed with *the leukocyte infiltration into the brain parenchyma and the* brain edema development (15, 31–34).

Results obtained in this research showed that in the acute brain ischemic period the important increase in number of leukocytes in the peripheral blood occured and that the increase was highest in patients with brain infarction. The mechanisms leading to leukocyte migration through the blood-brain barrier into the central nervous system, however, remained incompletely established. The inflammatory process was, thought, to be initiated by locally produced proinflammatory cytokines such as TNFα, IL-1β, IL-6 and IFNγ (4, 5, 16–18, 21). These cytokines have the capacity to induce or enhance the expression of several adhesion molecules including ICAM-1, VCAM-1, and E-selectin at least in the cultured brain vascular endothelium. Furthermore, *in vitro* stimulation of astrocytes and endothelial cells by proinflammatory cytokines resulted in the expression of chemokines - the low-molecular-weight proteins specialized to recruit specific subpopulations of leukocytes to the areas of inflammation (3–5, 35).

There are numerous mechanisms of damaging the ischemic parenchyma by leukocytes: they may clog little blood vessels and impede complete recuperation of blood flow in ischemic area after the establishing of reperfusion. Furthermore, by the release of cytotoxic enzymes, reactive oxidative species, nitric oxide and products of phospholipid cascade leukocytes could cause disturbance of brain artery vasoreactivity and contribute to the blood-brain barrier damage and the formation of brain postischemic edema. Also, the induced disorder of endothelial cells and basal membrane integrity, might facilitate erythrocyte exit into the tissue, as well as hemorrhagic infarct transformation (19, 33, 36–38).

Endothelial cells activation as the consequence of brain ischemia, caused adhesion molecules expression, like ICAM-1, VCAM-1 and E-selectin that were released into the circulation during the process of proteolysis. The results of recent *in vitro* studies showed that E-selectin was similarly upregulated only transiently during the first hours, whereas immunoglobulin-tipe adhesion molecules (ICAM-1 and VCAM-1) were persistently expressed at cellular surfaces for at least 72 hours after cytokine stimulation (4, 17, 18, 22). This corresponded with the current concepts of the initial steps of inflammation: selectins were considered to sustain the phenomenon of margination and the initial light attachment of circulating leukocytes to activated microvascular endothelium. The immunoglobulin-type adhesion molecules are particularly important for the subsequent firm attachment and transendothelial migration into the surrounding tissue (17–21). The results of this research showed significant increase of sICAM-1, sVCAM-1 and sE-selectin concentration in plasma and CSF of patients with brain infarction in the acute period of the disease. In patients with RIA and TIA, there was a significant increase of sICAM-1 and sVCAM-1 concentration, but the values were lower than in the brain infarction. Besides, sE-selectin concentration in patients with RIA and TIA was relevantly increased in plasma during first 72 hours after the ischemic attack. The obtained results pointed to different dynamics of changes in sICAM-1, sVCAM-1 and sE-selectin concentration in plasma and CSF in the acute IBD phase in dependence on IBD severeness, proving that there was different level of inflammatory reaction intensity during IBD. Brain infarction, as the severest IBD form with the highest level of morphological damages of brain parenchyma, has the most pronounced secondary inflammation as its consequence. It was confirmed in this research with the fact that the highest levels of all tested soluble adhesion molecules in plasma and CSF were obtained in the acute phase in patients with brain infarction.

The importance of leukocyte/cerebral endothelial cells interactions in regulating inflammatory responses of ischemic brain tissue was supported by following findings: a) the increased leukocyte infiltration in the brain during ischemia/reperfusion was accompanied by the increased expression of adhesion molecules in cerebral microvessels; b) depletion of circulating neutrophils (i.e. neutropenia) reduced the infarction size and edema in various experimental models of cerebral ischemia; c) the administration of antibodies against leukocyte or endothelium expressed adhesion molecules in experimental animals before and after ischemia decreased the infarction size and brain edema; d) transgenic ICAM-1-deficient mice were less susceptible to brain ischemia/reperfusion injury (39–45).

The time course of the expression of adhesion molecules and proinflammatory cytokines was similar and parallel to the time course of inflammatory cell infiltration in the ischemic brain, suggesting that this coordinated molecular mechanism was responsible for the inflammatory response in cerebral ischemia (30, 46). The exact mechanisms by which ischemia activated the expression of ICAM-1, VCAM-1 and E-selectin are still not clear. However, a possible mediator of this response might have been the nuclear factor kB (NF-kB) which played an important role in regulating the cytokine-induced transcription of endothelial adhesion molecules, and was activated by various factors induced by ischemia (47–49). Alternatively, the stimulation of adhesion molecule expression in cerebral endothelial cells by ischemia arisen from the autocrine effects of IL-1α and platelet-activating factor, since both were shown to stimulate leukocyte adhesion and were secreted by peripheral endothelial cells exposed to ischemia (48, 50).

These secondary changes (inflammation) might have potentiated already existing neuronal damage, and also led to the further damage spread on the preserved neurons. Because there was a certain time latency between the formation of the primary and the secondary damage, it was expectable that exactly this data offered the possibility of therapy application that impeded the formation and the spreading of secondary damage. According to the mentioned, it was also expectable that the relation (balance) between proinflammatory and immunoinductive (immunomodulatory) processes would determine the direction of physiopathological events in the acute IBD phase.

According to the results obtained in this research, it can be concluded that: total number of leukocytes in the peripheral blood, as well as the soluble adhesion molecules (sICAM-1, sVCAM-1 and sE-selectin) levels in plasma and CSF, were increased during the acute IBD phase progressively compared to the severeness of the disease. Thus it was concluded that they might have been the indicators of the inflammatory CNS reaction intensity. Moreover, the results indicated their role in IBD pathogenesis and offered the possibility of researching the application of antagonists and/or the activity modulator of some of them (leukocytes, ICAM-1, VCAM-1, E-selectin) in IBD therapy.

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Apstrakt

Selaković V, Čolić M, Jovanović M, Raičević R, Jovičić A. Vojnosanit Pregl 2003; 60(2): 139–146.

KONCENTRACIJA SOLUBILNOG INTERCELULARNOG ADHEZIJSKOG MOLEKULA 1, VASKULARNOG ĆELIJSKOG ADHEZIJSKOG MOLEKULA 1 I ENDOTELNOG LEUKOCITNOG ADHEZIJSKOG MOLEKULA U CEREBROSPINALNOJ TEČNOSTI I PLAZMI BOLESNIKA SA AKUTNOM ISHEMIJSKOM BOLEŠĆU MOZGA

Uvod. Migracija leukocita u područje ishemije je složen proces, kontrolisan preko adhezijskih molekula (AM) na leukocitima i endotelu, preko migratornog kapaciteta leukocita i prisustva hemotaksijskih agenasa u tkivu. U ovom istraživanju pretpostavljeno je da u krvi i cerebrospinalnoj tečnosti (CST) bolesnika sa ishemijskom bolešću mozga (IBM) u akutnoj fazi postoje značajne promene u koncentraciji solubilnih AM (sICAM-1, sVCAM-1 i sE-selektina), koje mogu biti pokazatelji intenziteta oštećujućih procesa u centralnom nervnom sistemu (CNS). **Metode.** Istraživanjem je obuhvaćeno 45 bolesnika sa IBM, oba pola, prosečne starosti 66±7 godina, od kojih je 15 imalo tranzitorni

ishemijski atak (TIA), 15 reverzibilni ishemijski atak (RIA) i 15 infarkt mozga (IM). Kontrolna grupa obuhvatila je 15 ispitanika sa radiksnim lezijama diskusnog porekla, podvrgnutih dijagnostičkoj radikulografiji, bez znakova smetnji u prolasku CST. Kod svih bolesnika odabrani biohemijski parametari određivani su prvih 72 časa nakon nastanka epizode ishemije. Koncentracije solubilnih AM u plazmi i CST određivane su pomoću ELISA. Ukupan broj leukocita (UBL) u perifernoj krvi određivan je pomoću hematološkog analizatora. **Rezultati.** Rezultati su pokazali da tokom prvih 72 časa IBM dolazi do značajnog porasta UBL i to progresivno u odnosu na težinu bolesti. Pokazan je i značajan porast koncentracije solubilnih AM u plazmi bolesnika sa IBM, s tim što je porast najveći kod bolesnika sa IM, nešto niži kod RIA i najniži kod TIA u odnosu na kontrolu. U CST koncentracije sICAM-1, sVCAM-1 i sE-selektina pokazuju sličan porast kao u plazmi. **Zaključak.** Može se zaključiti da su UBL, kao i koncentracije solubilnih AM u plazmi i CST, povećani tokom akutne faze IBM i to progresivno u odnosu na težinu bolesti, tako da mogu biti pokazatelji intenziteta inflamacijske reakcije CNS-a. Pored toga, rezultati ukazuju na ulogu solubilnih AM u patogenezi IBM, što otvara mogućnost ispitivanja primene antagonista i/ili modulatora aktivnosti nekih od njih u terapiji IBM.

K l j u č n e r e č i : **mozak, ishemija; krv; cerebrospinalna tečnost; leukociti; adhezijski molekul-1, intercelularni; adhezijski molekul-1, ćelijski, vaskularni; E-selektin; zapaljenje.**

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