Vojnomedicinska akademija, Beograd
Institut za medicinska istraživanja

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NEURON-SPECIFIČNA ENOLAZA U CEREBROSPINALNOJ TEČNOSTI I PLAZMI BOLESNIKA SA AKUTNOM ISHEMIJSKOM BOLEŠĆU MOZGA

NEURON-SPECIFIC ENOLASE IN CEREBROSPINAL FLUID AND PLASMA OF PATIENTS WITH ACUTE ISCHEMIC BRAIN DISEASE

Vesna SELAKOVIĆ

Sažetak - Cilj istraživanja bio je da kod bolesnika sa akutnom ishemijskom bolešću mozga u cerebrospinalnoj tečnosti i plazmi odredimo dinamiku promene koncentracije neuron-specifične enolaze. Istraživanje je obuhvatilo 103 bolesnika oba pola, prosečne starosti 58-66 godina. Kontrolna grupa je obuhvatila 16 ispitanika, odgovarajuće starosne i polne strukture, sa radikularnim lezijama diskalnog porekla, podvrgnutih dijagnostičkoj radikulografiji. Koncentracija neuron-specifične enolaze merena je fluoroimunometrijskom metodom. Rezultati su pokazali da je koncentracija neuron-specifične enolaze u cerebrospinalnoj tečnosti i plazmi bolesnika sa ishemijskom bolešću mozga tokom prvih sedam dana statistički značajno povećana u odnosu na kontrolu. Najveći porast koncentracije zabeležen je kod infarkta mozga, nešto niži kod reverzibilnog ishemijskog ataka, a najniži kod tranzitornog ishemijskog ataka. Maksimalna koncentracija zabeležena je 3-4 dana nakon infarkta mozga. Koncentracija neuron-specifične enolaze u cerebrospinalnoj tečnosti i plazmi može biti pokazatelj patofizioloških zbivanja u akutnoj fazi ishemije mozga i značajna je u ranoj dijagnostici i terapiji ovog oboljenja.
Ključne reči: Cerebralna ishemija; Fosfopiruvat hidrataza; Cerebrospinalna tečnost

Summary - *The objective of this research was to determine the dynamics of change of neuron-specific enolase concentration in patients with acute ischemic brain disease in cerebrospinal fluid and plasma. The study included 103 patients, their mean age 58-66 years. The control group consisted of 16 patients, of matching age and sex, with radicular lesions of discal origin, subjected to diagnostic radiculography. Concentration of neuron-specific enolase was measured by a flouroimmunometric method. The results showed that the concentration of neuron-specific enolase in cerebrospinal fluid and plasma of patients with brain ischemic disease within first seven days significantly increased compared to the control. The highest increase of concentration was established in brain infarction, somewhat lower in reversible ischemic attack, and the lowest in transient ischemic attack. Maximal concentration was established on the 3rd-4th day upon the brain infarction. Neuron-specific enolase concentration in cerebrospinal fluid and plasma may be an indicator of pathophysiological processes in the acute phase of brain ischemia and is significant in early diagnostics and therapy of the disease.*
Key words: *Cerebral Ischemia; Phosphopyruvate Hydratase; Cerebrospinal Fluid*

Uvod

Ishemijska bolest mozga je etiopatogenetski složen proces, a po kliničkoj fenomenologiji raznovrsna, pa su prepoznavanje, potvrda i lečenje ovog kliničkog sindroma izuzetno kompleksni. Ishemijsko oštećenje neurona je najčešće praćeno sekundarnim oštećenjem, pa je u ranom periodu ishemijske bolesti mozga veoma važno razlikovati tranzitorni ishemijski atak od reverzibilnog ishemijskog ataka i razvoja ishemijske lezije moždanog parenhima po tipu infarkta mozga [1-5].

Tokom ranog perioda nakon ishemijskog insulta, teško je klinički oceniti i jasno razlikovati pojedine oblike ishemijske bolesti mozga, kao i reverzibilne od ireverzibilnih promena. Za jasnu diferencijalnu dijagnostiku najčešće je neophodna višednevna klinička opservacija. Zato se intenzivno traga za pouzdanim biohemijskim markerima oštećenja, čije bi određivanje u krvi i cerebrospinalnoj tečnosti u ranom periodu ishemije mozga bilo jednostavno i pouzdano za praćenje toka i prognozu oboljenja i za blagovremenu primenu odgovarajuće terapije. Kao mogući veoma pouzdan marker oštećenja moždanog

Introduction

Ischemic brain disease represents a complex etiopathogenetic process, and according to clinical phenomenology it is diverse, and thus recognition, confirmation and treatment of this clinical syndrome is extremely complex. Ischemic neuronal damage is most frequently followed by secondary damage, so in the early period of ischemic brain disease it is extremely important to distinguish transient ischemic attack from reversible ischemic attack and development of ischemic lesion of brain parenchyma according to the type of brain infarction [1-5].

During the early period after the ischemic insult, it is difficult to clinically assess and clearly distinguish certain forms of ischemic brain disease, as well as to distinguish reversible from irreversible changes. Clear differential diagnostics, very often requires clinical observation that lasts a few days. Thus, we are searching for reliable bio-chemical markers of damage, whose determination in blood and cerebrospinal fluid in the early period of brain ischemia would be simple and reliable, both for monitoring the course of

Adresa autora: Naučni saradnik dr sc. med. Vesna Selaković, Vojnomedicinska akademija, 11000 Beograd, Crnotravska 17

tkiva u novije vreme spominje se neuron-specifična enolaza.

Neuron-specifična enolaza (γ-enolaza) je intracelularni protein, prisutan u citoplazmi neurona i u neznatnoj količini u neuroendokrinim ćelijama [6, 7]. To je izoenzim glikolitičkog enzima enolaze, specifičan za fosfoglicerat i fosfoenolpiruvat (2-fosfo-D-glicerat-hidrolaza). Visoko je solubilan protein, pa se lako oslobađa u cerebrospinalnu tečnost i krv nakon tkivnog oštećenja i ima biološki poluživot 48 časova [8-11].

Uloga neuron-specifične enolaze u centralnom nervnom sistemu još uvek nije u potpunosti rasvetljena. Pokazano je da tokom razvoja mozga učestvuje u formiranju membranskih struktura i svim energetski zavisnim procesima u ćeliji [10-13]. Takođe, ovaj enzim je neophodan za održavanje ekscitabilnosti neuronskih membrana, a dokazano je i učešće u reparativnim procesima mozga [10,14]. Eksperimentalne studije su pokazale da nakon fokalne i globalne ishemije mozga dolazi do porasta koncentracije neuron-specifične enolaze u cerebrospinalnoj tečnosti i plazmi eksperimentalnih životinja [14-16].

Cilj istraživanja je bio da kod bolesnika sa ishemijskom bolešću mozga u akutnoj fazi u cerebrospinalnoj tečnosti i plazmi izmerimo koncentraciju neuron-specifične enolaze i odredimo vremensku dinamiku promena ovog parametra.

Materijal i metode

Istraživanje je obuhvatilo 103 bolesnika sa ishemijskom bolešću mozga u akutnoj fazi, oba pola, prosečne starosti od 58 do 66 godina. Svi bolesnici su dali pristanak za uključenje u studiju. Dijagnoza je postavljana na osnovu anamnestičkih podataka, kliničkog nalaza i kompjuterizovane tomografije glave. Bolesnici su podeljeni u grupe prema težini: grupa sa tranzitornim ishemijskim atakom (24 bolesnika), grupa sa reverzibilnim ishemijskim atakom (24 bolesnika) i grupa sa infarktom mozga (55 bolesnika). Na osnovu vremena proteklog od nastanka ishemijskog ataka, bolesnici sa infarktom mozga su podeljeni na podgrupe: 1-2 dana nakon lezije (21 bolesnik), 3-4 dana (14 bolesnika) i 5-7 dana (20 bolesnika). U grupama sa reverzibilnim i tranzitornim ishemijskim atakom u svakoj podgrupi je bilo po 8 bolesnika.

Kontrolna grupa je obuhvatila 16 ispitanika, odgovarajuće starosne i polne strukture, sa radikularnim lezijama diskalnog porekla, podvrgnutih dijagnostičkoj radikulografiji, bez znakova smetnji u pasaži cerebrospinalne tečnosti. U istraživanje su uključeni samo ispitanici sa naglim razvojem motornog deficita, bez bolova, tako da nisu koristili antidoloroznu terapiju. Osobe sa anamnestičkim i kliničkim podacima o aktuelnim inflamatornim, malignim, bubrežnim, hepatičnim, plućnim, neurodegenerativnim i psihijatrijskim bolestima isključene su iz studije.

tion of relevant therapy. Nuron-specific enolase has recently been mentioned as a possibly very reliable marker of brain parenchymal damage.

Neuron-specific enolase (γ-enolase) is an intracellular protein, present in neuronal cytoplasm and with insignificant amount in neuroendocrine cells [6,7]. It is an isoenzyme of glycolytic enzyme of enolase, specific for phosphoglycerate and phosphoenolpyruvate (2-phospho-D-glycerate-hydrolase). It is a highly soluble protein, so it is easily released in cerebrospinal fluid and blood after tissue damage and has biologic half-life of 48 hours [8-11].

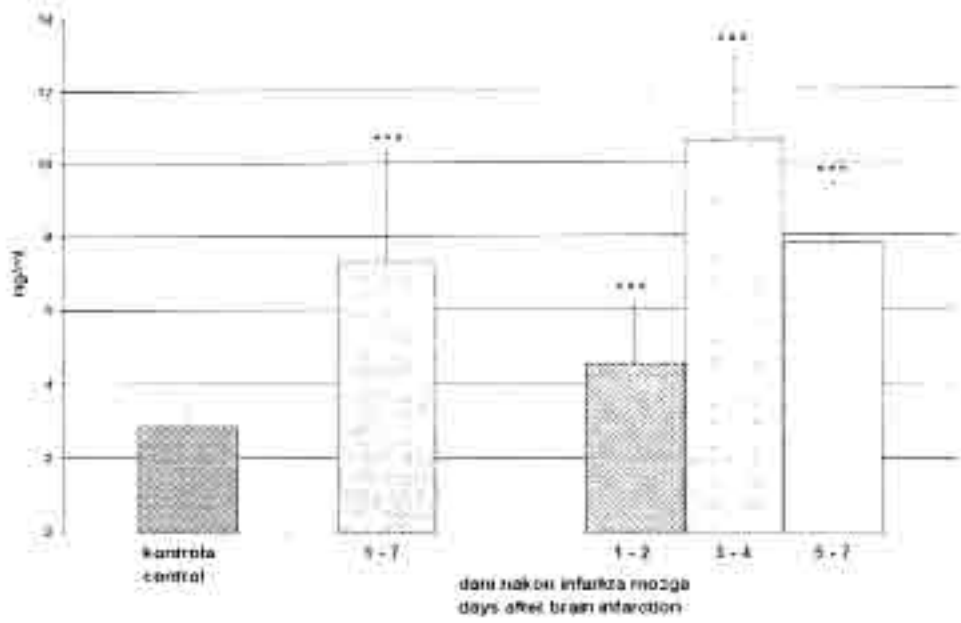
The role of neuron-specific enolase in the central nervous system is not yet clarified. It has been established that during brain development it participates in formation of membrane structures and all energy dependent processes in the cell [10-13]. Also, this enzyme is necessary in maintenance of neuron membranes excitability, and participation in the reparative brain processes [10.14]. Experimental studies have shown that after focal and global brain ischemia there is an increase of neuron-specific enolase concentration in cerebrospinal fluid and plasma of experimental animals [14-16].

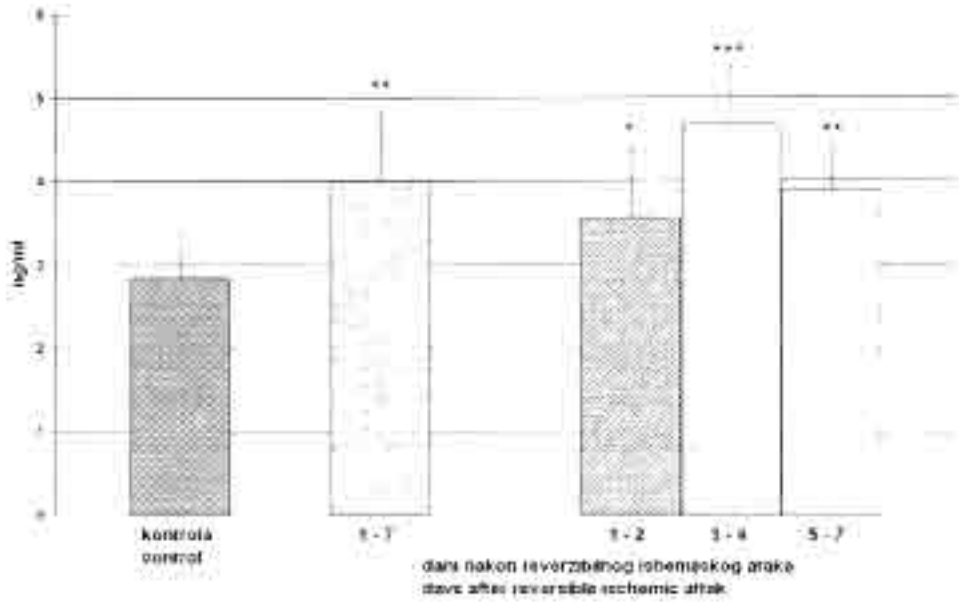
The aim of this research was to measure the concentration of neuron-specific enolase in patients with acute brain ischemic disease in cerebrospinal fluid and plasma and to determine the time dynamics of changes regarding this parameter.

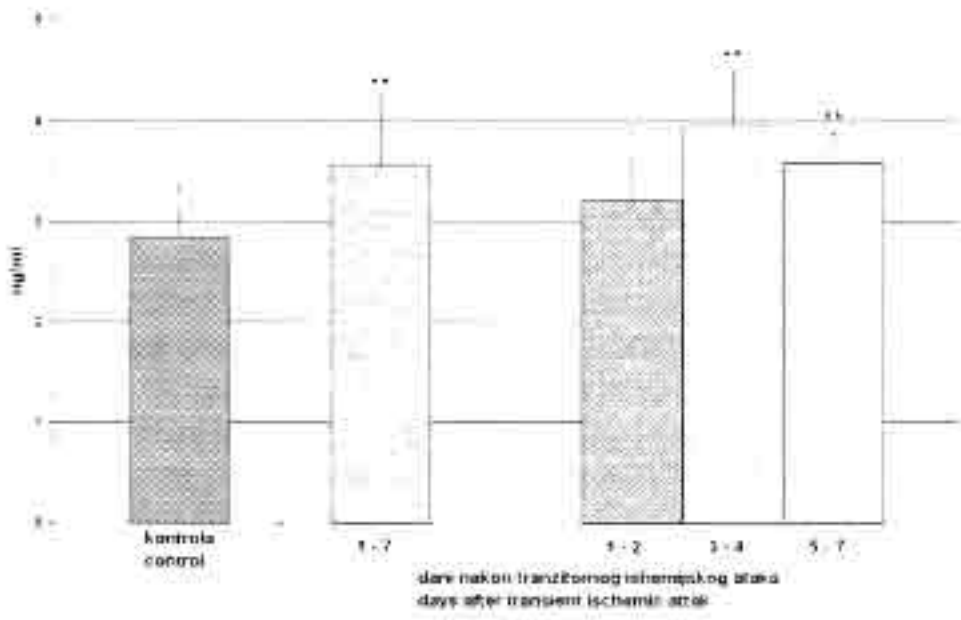
Material and methods

This investigation included 103 patients with acute brain ischemic disease - of both sexes, aged 58 - 66 years. All patients gave informed consent for being included into the study. The diagnosis was made according to the anamnestic data, clinical findings and computerized head tomography. Patients were divided into groups by severity: transient ischemic attack (24 patients), reversible ischemic attack (24 patients) and brain infarction (55 patients). Taking into account the time since occurrence of ischemic attack, patients with brain infarction were divided into subgroups: 1-2 days after the lesion (21 patient), 3-4 days (14 patients) and 5-7 days (20 patients). In groups with reversible and transient ischemic attacks, there were eight patients in each subgroup.

The control group consisted of 16 patients of corresponding age and sex, with radicular lesions of discal origin. They were subjected to diagnostic radiculography, without signs of obstruction in the passage of cerebrospinal fluid. Only patients with abrupt development of motor deficit, without pain and analgesic therapy were included into the study. Persons with anamnesis and clinical data on current inflammatory, malignant, renal, hepatic, pulmonary, neurodegenerative and psychiatric diseases were excluded from the study.







Slika 1. Koncentracija neuron-specifične enolaze u cerebrospinalnoj tečnosti bolesnika sa infarktom mozga, reverzibilnim ishemijskim atakom i tranzitornim ishemijskim atakom. Vrednosti su prikazane kao srednja vrednost ± standardna devijacija. *(p<0,05), **(p<0,01), ***(p<0,001) - statistički značajna razlika u odnosu na kontrolnu vrednost

Fig. 1. *Concentration of neuron-specific enolase in cerebrospinal fluid of patients with brain infarction, reversible ischemic attack and transient ischemic attack. Mean values and standard deviation: *(p<0.05), **(p<0.01), ***(p<0.001) statistically significant difference in regard to control values*

Plasma was selected from samples of peripheral vein blood that were collected into heparinized tubes at low temperatures. Samples of cerebrospinal fluid were taken during lumbar puncture and after centrifugation they were kept at -70°C as well as plasma samples until adequate biochemical analysis.

Concentration of neuron-specific enolase was measured by flouroimmunometric method (LKB, Wallac Oy, Finland). This method is based on direct "sandwich" technique, with two monoclonal antibodies to separate antigens which determine neuron-specific enolase molecule. Fluorescence of samples was measured using a radioimmunofluorometer (TR Fluorometer 1230E, Wallac). All samples and standards were analyzed in duplicate. The concentration of neuron-specific enolase (ng/ml) was measured by means of standard curve based on values of absorbances of the well-known increasing standards of concentration. The results were shown as mean value and standard deviation and analyzed using Student t-test.

Results

During the first seven days concentration of neuron-specific enolase in cerebrospinal fluid in patients with brain infarction was 7.284±2.977 ng/ml; in patients with reversible ischemic attack it was 3.999±0.844 ng/ml and in patients with transient ischemic attack 3.553±0.658 ng/ml of cerebrospinal fluid, that is a statistically remarkable increase ($p<0.001$, $p<0.01$, $p<0.01$) compared to values in the control group (2.838±0.504 ng/ml of cerebrospinal fluid), (Fig. 1). The highest concentration was established in the period 3-4 days upon the infarction, when it was 10.605±2.258 ng/ml (Fig. 1).

During the first seven days concentration of neuron-specific enolase in the plasma of patients with brain infarction was 9.946±3.495 ng/ml; in patients with reversible ischemic attack it was 6.052±1.515 ng/ml and in patients with transient ischemic attack 5.585± 0.862 ng/ml of plasma, that is a statistically significant increase ($p<0.001$, $p<0.01$, $p<0.05$) compared to values in the control group (4.479± 0.893 ng/ml of plasma), (Fig. 2). Maximal concentration was perceived in the period 3-4 days upon the infarction, when it was 14.617±1.8 ng/ml of plasma ($p<0.001$), (Fig. 2).

Discussion

The extent, course and outcome of biochemical and functional changes in the brain during ischemia above all depends on the type and lenght of ischemia [17-19]. Evolution of ischemic lesion may last for minutes and hours, and it is usually related to secondary brain cell damage, that additionally increases the amount and extent of initial damage [20]. Consequently, in order to apply appropriate therapy, early diagnostics of particular forms of brain ischemia re-

Plazma je izdvajana iz uzoraka periferne venske krvi koji su prikupljani u heparinizirane epruvete na hladnom. Uzorci cerebrospinalne tečnosti uzimani su tokom lumbalne punkcije i nakon centrifugiranja, kao i uzorci plazme, čuvani su na -70°C do odgovarajuće biohemijske analize.

Koncentracija neuron-specifične enolaze merena je fluoroimunometrijskom metodom (LKB, Wallac Oy, Finland). Radi se o metodi zasnovanoj na direktnoj *sendvič* tehnici, pri čemu su korišćena dva monoklonska antitela na odvojene antigene determinante molekula neuron-specifične enolaze. Fluorescenca uzoraka je očitavana na radioimunofluorimetru (TR Fluorometer 1230E, Wallac). Svi uzorci i standardi su analizirani u duplikatu. Koncentracija neuron-specifične enolaze (ng/ml) je izračunavana pomoću standardne krive koja je konstruisana na osnovu vrednosti apsorbanci poznatih rastućih koncentracija standarda. Rezultati su prikazivani kao srednja vrednost ± standardna devijacija i analizirani primenom Studentovog t-testa.

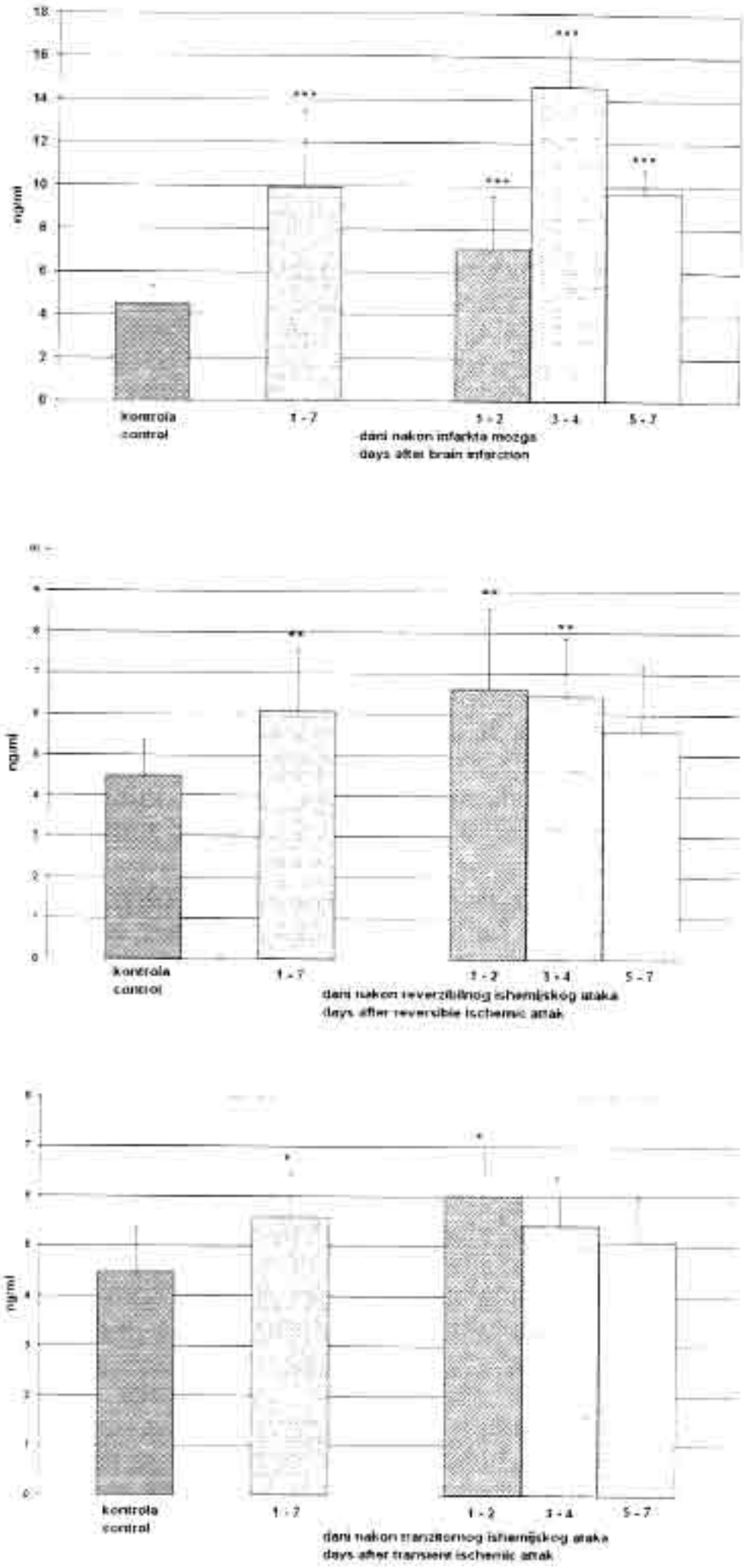
Rezultati

Koncentracija neuron-specifične enolaze u cerebrospinalnoj tečnosti bolesnika sa infarktom mozga tokom prvih sedam dana iznosila je 7,284±2,977 ng/ml, kod bolesnika sa reverzibilnim ishemijskim atakom 3,999±0,844 ng/ml i kod bolesnika sa tranzitornim ishemijskim atakom 3,553±0,658 ng/ml cerebrospinalne tečnosti, što je statistički značajno povećanje ($p<0,001$, $p<0,01$, $p<0,01$) u odnosu na vrednost u kontrolnoj grupi ispitanika (2,838±0,504 ng/ml cerebrospinalne tečnosti) (Slika 1). Najviša koncentracija je zabeležena u periodu 3-4 dana nakon infarkta, kada je iznosila 10,605±2,258 ng/ml (Slika 1).

Koncentracija neuron-specifične enolaze u plazmi bolesnika sa infarktom mozga tokom prvih sedam dana iznosila je 9,946±3,495 ng/ml, kod bolesnika sa reverzibilnim ishemijskim atakom 6,052 ±1,515 ng/ml i kod bolesnika sa tranzitornim ishemijskim atakom 5,585±0,862 ng/ml plazme, što je statistički značajno povećanje ($p<0,001$, $p<0,01$, $p<0,05$) u odnosu na vrednost u kontrolnoj grupi ispitanika (4,479±0,893 ng/ml plazme) (Slika 2). Maksimalna koncentracija je zabeležena u periodu 3-4 dana nakon infarkta, kada je iznosila 14,617±1,8 ng/ml plazme ($p<0,001$) (Slika 2).

Diskusija

Stepen, tok i ishod biohemijskih i funkcionalnih promena u mozgu tokom ishemije prvenstveno zavise od tipa i dužine trajanja ishemije [17-19]. Evolucija ishemijske lezije može trajati minutima i satima, a gotovo uvek je povezana sa sekundarnim oštećenjem ćelija mozga, što dodatno povećava veličinu i stepen početnog oštećenja [20]. Shodno tome, radi blagovremene primene odgovarajuće terapije, rana dijagnostika pojedinih oblika ishemije



Slika 2. Koncentracija neuron-specifične enolaze u plazmi bolesnika sa infarktom mozga, reverzibilnim ishemijskim atakom i tranzitornim ishemijskim atakom. Vrednosti su prikazane kao srednja vrednost ± standardna devijacija. *($p<0,05$), **($p<0,01$), ***($p<0,001$)- statistički značajna razlika u odnosu na kontrolnu vrednost

Fig. 2. *Concentration of neuron-specific enolase in plasma of patients with brain infarction, reversible ischemic attack and transient ischemic attack. Mean values and mean standard deviation: *($p<0.05$), **($p<0.01$), ***($p<0.001$) statistically significant difference in regard to control values*

mozga zahteva, pored kliničkih ispitivanja i određivanje pouzdanih biohemijskih markera oštećenja nervnih ćelija.

Neuron-specifična enolaza je u fiziološkim uslovima prisutna u veoma maloj koncentraciji u cerebrospinalnoj tečnosti i plazmi [21]. Eksperimentalne studije su pokazale da ishemija mozga u trajanju od 5 minuta izaziva značajan porast koncentracije neuron-specifične enolaze u serumu 24 časa nakon ishemije, a kod 15-minutne ishemije porast se detektuje već nakon četiri časa [14-16]. Takođe, detektovane su i histopatološke i imunohistohemijske promene u tkivu mozga koje odgovaraju vremenu izlaska neuron-specifične enolaze iz citoplazme neurona u ekstracelularni prostor [22,23]. Pokazano je da se visoka koncentracija ovog enzima u cerebrospinalnoj tečnosti i serumu eksperimentalnih životinja održava u zavisnosti od dužine trajanja ishemije i da maksimum dostiže u periodu od drugog do četvrtog dana nakon insulta [16].

Dosadašnja klinička istraživanja su pokazala postojanje visoke koncentracije neuron-specifične enolaze u cerebrospinalnoj tečnosti i plazmi bolesnika sa akutnom ishemijom mozga [24-28]. U ovim istraživanjima se radilo o pojedinačnim slučajevima infarkta mozga, malom broju bolesnika bez uvida u tip akutne ishemije mozga, ili je koncentracija neuron-specifične enolaze određivana samo u plazmi [24-28].

Rezultati našeg istraživanja su pokazali da je koncentracija neuron-specifične enolaze u cerebrospinalnoj tečnosti i plazmi bolesnika sa ishemijskom bolešću mozga tokom prvih sedam dana statistički značajno povećana u odnosu na kontrolu. Najveći porast zabeležen je kod infarkta mozga, nešto niži kod reverzibilnog ishemijskog ataka, a najniži kod tranzitornog ishemijskog ataka. Objašnjenje za ovakav nalaz daju činjenice da tokom ishemije mozga prvenstveno stradaju neuroni pa se neuron-specifična enolaza, kao intraneuronalni protein oslobađa u cerebrospinalnu tečnost u zavisnosti od stepena oštećenja neurona. Visoka koncentracija u plazmi bolesnika sa infarktom mozga je posledica kako masivnog oštećenja neurona tako i oštećenja krvnomoždane barijere, što omogućava prelazak ispitivanog enzima iz cerebrospinalne tečnosti u perifernu krv. Međutim, kod bolesnika sa reverzibilnim i tranzitornim ishemijskim atakom stepen oštećenja neurona je znatno manji, pa je i koncentracija neuron-specifične enolaze srazmerno niža nego kod infarkta mozga. Takođe, rezultati su pokazali da se koncentracija ispitivanog enzima u plazmi već od petog dana kod bolesnika sa reverzibilnim i trećeg dana kod bolesnika sa tranzitornim ishemijskim atakom, približava kontrolnim vrednostima.

Dobijeni rezultati pokazuju da tokom prvih sedam dana nakon akutne ishemije postoji vremenska dinamika promene koncentracije neuron-specifične enolaze u cerebrospinalnoj tečnosti i plazmi, što ukazuje na različit stepen izraženosti, kako primar-

quires, besides clinical research, determination of reliable biochemical markers of nerve cells damage.

In physiological conditions neuron-specific enolase is present in very small concentrations in cerebrospinal fluid and plasma [21]. Experimental studies have shown that brain ischemia lasting 5 minutes, causes a significant increase of neuron-specific enolase concentration in serum 24 hours upon ischemia occurrence, whereas in a 15-minute ischemia the increase is detected already after four hours [14-16]. Also, histopathological and immunohistochemical changes in brain tissue correspond with the time of loss of neuron-specific enolase from cytoplasma into the extracellular space [22, 23]. High concentration of this enzyme in cerebrospinal fluid and serum of experimental animals is maintained depending on the duration of ischemia and the maximum is reached in the period from the second till the forth day after insult [16].

Clinical studies indicate existence of high concentrations of neuron-specific enolase in cerebrospinal fluid and plasma in patients with acute brain ischemia [24-28]. This research included particular cases of brain ischemia, a small number of patients without taking into account the type of brain ischemia and concentration of neuron-specific enolase was determined only in plasma [24-28].

Results of our research have shown that concentration of neuron-specific enolase in cerebrospinal fluid and plasma in patients with ischemic brain disease within the first seven days was statistically significantly increased compared to the control. The highest increase was established in brain infarction, somewhat lower in reversible ishemic attack, and the lowest in transient ischemic attacks. The explanation for such findings is found in data that during brain ischemia primarily neurons are damaged so neuron-specific enolase, as well as intraneuronal protein, is released into the cerebrospinal fluid depending on the extent of neuronal damage. High concentration in plasma in patients with brain infarction is the consequence both of massive neuronal damage and of blood-brain barrier damage and transition of examined enzyme from cerebrospinal fluid into peripheral blood. However, in patients with reversible and transient ischemic attacks the extent of neuron damage is remarkably smaller, so the concentration of neuron-specific enolase is proportionally lower than in brain infarction. Also, results have shown that concentrations of monitored enzyme in plasma on the fifth day in patients with reversible and the third day in patients with transient ischemic attack, reach control values.

The obtained results show that during the first seven days after acute ischemia there is a time-dependent dynamics of change of neuron-specific enolase concentration in cerebrospinal fluid and plasma, reveling different extent of primary and secondary ischemic brain lesions. Brain infarction,

ne, tako i sekundarne ishemijske lezije mozga. Infarkt mozga, kao najteži oblik ishemije mozga, sa najvećim stepenom morfološkog oštećenja moždanog parenhima, ima za posledicu masivno oslobađanje neuron-specifične enolaze u cerebrospinalnu tečnost, što je najizraženije u periodu 3-4 dana nakon insulta.

Zaključak

Na osnovu rezultata dobijenih tokom ovog istraživanja možemo zaključiti da je koncentracija neuron-specifične enolaze u cerebrospinalnoj tečnosti i plazmi značajno povećana u akutnoj fazi ishemijske bolesti mozga i to progresivno u odnosu na težinu oboljenja, što može imati dijagnostički i prognostički značaj. Maksimalna koncentracija neuron-specifične enolaze je zabeležena u periodu 3-4 dana nakon infarkta mozga, što ukazuje da se u tom periodu dešavaju najintenzivnija oštećenja neurona. Takođe, koncentracija neuron-specifične enolaze u cerebrospinalnoj tečnosti i plazmi može biti pokazatelj patofizioloških zbivanja u akutnoj fazi ishemijske bolesti mozga, koji odražava stepen oštećenja centralnog nervnog sistema, pa određivanje ovog pokazatelja ima značaj u ranoj dijagnostici i terapiji ovog oboljenja.

as the severest form of brain ischemia, with highest extent of morphological damage of brain parenchyma, consequently causes massive release of neuron-specific enolase into cerebrospinal fluid, mostly 3-4 days after insult.

Conclusion

Results of this research indicate that concentrations of neuron-specific enolase in cerebrospinal fluid and plasma significantly increase in the acute phase of brain ischemic disease and it occurs progressively in relation to severity of the disease, which may have diagnostic and prognostic importance. Maximal concentrations of neuron-specific enolase are noticed in the period 3-4 days after brain infarction, meaning that the most intense neuronal damage occurs in this period. Also, of neuron-specific enolase concentration in cerebrospinal fluid and plasma may be the indicator of pathophysiological occurrences in acute brain ischemic disease, reflecting the extent of central nervous system damage, so determination of this indicator is crucial in early diagnostics and therapy of the disease.

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