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Blood serum TNF-α concentration in patients with ischaemic and haemorrhagic brain stroke

Stężenie TNF-α w surowicy krwi pacjentów z udarem niedokrwiennym i krwotocznym mózgu

Tumor necrosis factor alfa (TNF-α) is a glycoprotein produced by monocytes, glial cells and endothelial cells (5, 10). It shows different immunomodulating activity: intensifies proliferation and differentiation of lymphocytes T and B, induces IL-1 synthesis, activates neutrophils, increases an expression of the histocompatibility complex antigens on the cells, exhibits cytotoxic activity against neoplastic cells. The secretion is increased during ischaemia, infection, surgical operation or injury. Animal experimental studies have revealed that brain concentration of TNF-α is considerably increased within 6 hours after laboratory induced ischaemia, next it decreases within 12 hours to control levels, and temporarily it increases after 24 hours again. Longer periods of ischaemia correspond with this cytokine higher concentration (8). Studies of TNF-α activity mechanism show that cytokine causes the expression of proadhesive molecules on the endothelium, intensifies leukocytes adherence and migration from capillaries into the brain thus enlarging the infiltration area and tissue damage.

TNF-α through glial cells activation regulates gliosis process and scar formation (3). Experiments with TNF-α binding protein (soluble receptor I TNF-α) prove that the augmentation of TNF-α concentration is a factor which influences the progression of ischaemic changes in the brain, thus increasing an extent of necrosis and oedema (1, 6, 7). Immunohistochemical examinations of human autopsy brains show TNF-α production increase is the early inflammatory response in patients after an acute brain stroke (9). There are single reports in the literature of the subject on TNF-α activity after brain stroke. The aim of the study was to determine if haemorrhagic or ischaemic stroke cause the increase in TNF-α concentration in patients' peripheral blood and if its levels differ with regard to the stroke type.

MATERIAL AND METHODS

The study analysed blood serum samples from 31 patients with brain stroke admitted to Neurology Clinic. The clinical diagnosis of infarct and intracerebral haemorrhage was determined on the basis of generally accepted criteria, considering anamnesis and clinical picture. All diagnoses were confirmed by computerised tomography (CT). General history, chest X-rays and standard laboratory investigations (erythrocyte sedimentation rate, white blood cells count, urinalysis) were performed to exclude coexistence of pathologic conditions which influence TNF-α concentration (e.g. infections, malignancies). The study group included 20 (64.5%) patients with ischaemic stroke and 11 (35.5%) with haemorrhagic stroke (Fig. 1), the group consisted of 16 women (51.6%) and 15 men (48.4%) aged between 44 and 86 years.

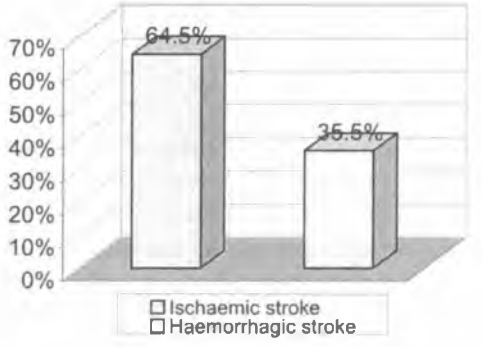

Fig. 1. The percentage of patients with ischaemic and haemorrhagic stroke

The control group consisted of 15 persons in good health, aged between 46 and 70. The samples of peripheral venous blood obtained on the 1st , 3rd and 7th day following the onset of the stroke were examined. Blood was allowed to clot at room temperature and after centrifugation the obtained serum was frozen for further use. The serum level of TNF-α was determined by ELISA immunoenzymatic method with a commercial Predicta TNF-α ELISA Kit (Genzyme, USA). The kit contained microtiter plate with an immobilised mouse monoclonal antibody to human TNF-α, which formed complex with TNF-α present in the standards or samples. A biotinylated polyclonal antibody to TNF-α and peroxidase-labelled streptavidin were added to produce the immune complex, which was detected after adding a substrate solution for peroxidase producing a blue colour. The colour reaction was stopped by the addition of acid which changes the blue colour to yellow. The intensity of the yellow colour was proportional to the amount of TNF-α present in the samples or standards. The absorbance was read at 450 nm. On the ground of it a standard curve was constructed to quantify TNF-α concentrations in the samples.

RESULTS AND DISCUSSION

The sensitivity threshold of the used test is 12 pg/ml, which corresponds with the absorbance 0.14 (Fig. 2). Only in the first examination the increase in blood serum TNF-α concentration was found in 3 patients.

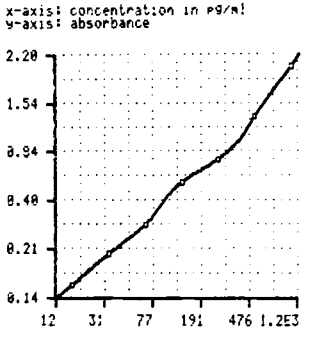

Fig. 2. Dependence between TNF-α concentration and absorbance value

The following concentrations were obtained: 1st patient- 155.7 pg/ml, 2nd patient - 19.8 pg/ml and 3rd patient - 19.8 pg/ml. The first patient was a woman aged 84 with brain ischaemic stroke. She was admitted to hospital in the less serious general condition. The first period of the disease was without complications. The patient regained little range of the active movement of the lower limb. TNF-α concentrations increased during the first twenty-four hours and decreased below the indeterminable value. The second patient was a man aged 64 with intracerebral haemorrhage with affected lateral ventricle as well as the third and fourth ventricles. The patient was admitted in severe general condition and died after 4 days without regaining conciousness. The increased concentration of TNF-α was found in the first examination. The third patient with the diagnosis of intracerebral haemorrhage, admitted to hospital in a very severe condition and unconscious, died within twenty-four hours.

The absorbance values obtained during the tests were below 0.14 and were beyond the assumed test threshold. The concentrations of TNF-α in those serum samples were lower than 12 pg/ml and did not differ significantly from the obtained control values. Similar results were obtained by other authors who determined that cytokine level in the serum samples from 19 patients with cerebral ischaemic stroke. According to their reports, the TNF-α concentration on 1st day following the onset of stroke was 10.32 +/- 1.93 pg/ml, on 3rd day it was 7.13 +/- 1.67 pg/ml and on 7th day 8.97 +/- 2.90 pg/ml (2). Gilad et al. found no essential increase in TNF-α concentration in the blood serum and cerebrospinal fluid of patients who had suffered brain stroke (4). The maintained low

TNF-α concentration in the blood serum may be explained by its large dilution in the peripheral blood.

In the animal studies the blood samples were collected directly from the brain blood vessels, which explains its considerably increased concentration (8).

CONCLUSIONS

The increased blood serum TNF-α concentration is not found in the patients with haemorrhagic or ischaemic stroke on 1st , 3rd and 7th day following the onset of the disease.

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STRESZCZENIE

TNF-α (tumor necrosis factor alpha) jest glikoproteiną wytwarzaną przez monocyty, mikroglej i komórki endotelium, komórki NK, limfocyty T i B. Doświadczenia z wykorzystaniem białka wiążącego TNF-α (rozpuszczalny receptor I TNF) dowodzą, że wzrost stężenia TNF-α jest czynnikiem wpływającym na progresję zmian niedokrwiennych w mózgu, zwiększającym zasięg martwicy i obrzęku. Badania immunohistochemiczne autopsyjne móz-gów ludzkich wskazują na wzrost produkcji TNF-α jako wczesnej odpowiedzi zapalnej u pacjentów po wystąpieniu ostrego udaru mózgu. Celem pracy było ustalenie, czy u chorych z udarem mózgu występuje wzrost stężenia TNF-α we krwi obwodowej oraz czy jego wartości wykazują różnicę w zależności od typu udaru. Poddano analizie próbki surowicy krwi 31 chorych z udarem mózgu w wieku od 44 do 86 lat (16 kobiet i 15 mężczyzn), u których wykluczono współistnienie innych stanów patologicznych mających wpływ na poziom TNF-α. Grupę kontrolną stanowiło 15 zdrowych osób w wieku od 46 do 70 lat. Oznaczenia wykonano w próbkach krwi żylnej pobranej do badań na czczo w pierwszej, trzeciej i siódmej dobie. Oznaczenia poziomu TNF-α wykonano metodą immunoenzymatyczną ELISA, stosując komercyjne zestawy firmy Genzyme USA. Dokonano oznaczeń stężenia TNF-α w próbkach surowic krwi 20 chorych z udarem niedokrwiennym mózgu i 11 z udarem krwotocznym mózgu. Tylko w 3 pojedynczych oznaczeniach u różnych pacjentów stwierdzono wzrost stężenia TNF-α. W pozostałych próbkach stężenie TNF-α w surowicy nie różniło się istotnie od wartości otrzymanych w grupie kontrolnej.

Udar krwotoczny i niedokrwienny mózgu nie powoduje wzrostu stężenia TNF-α w surowicy krwi chorych w 1, 3 i 7 dobie choroby.