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Clinical value of homocysteine, cystatin C and FFA levels in patients with ischemic cerebrovascular disease

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Abstract: **To investigate the clinical value of combined detection of plasma homocysteine (Hcy), cystatin C (CysC) and free fatty acid (FFA) in patients with ischemic cerebrovascular disease. Hcy, CysC and FFA in 42 patients with transient cerebral ischemic attacks(TIA), in 57 patients with ischemic stroke and 51 normal persons(controls) were detected respectively by circulating enzymatic, immune transmission turbidimetric and enzymatic. In TIA patients, the levels of Hcy 9.29+3.80 umol/L, CysC 0.83+0.16 mg/L, FFA(0.40+0.17) mmol/L were significantly higher than those in controls, which were 7.78+1.79 umol/L, 0.68+0.082 mg/L, 0.35+0.30 mmol/L P<0.05; In ischemic stroke patients, the levels of Hcy 11.52+6.56 umol/L, CysC 0.97+0.27 mg/L, FFA 0.50+0.30 mmol/L were significantly higher than those in controls, respectively(P<0.01). The levels of CysC and FFA in ischemic stroke patients were significantly higher than those in TIA patients (P<0.05), but Hcy levels between the two groups had no significant difference (P<0.05). In TIA patients, there were positive correlation between Hcy and CysC (r=0.607, P<0.01), CysC and FFA(r=0.469, P<0.01); In ischemic stroke patients, Hcy levels correlated positively with CysC levels. Hcy, CysC and FFA may play an important role in the occurrence of ischemic cerebrovascular disease, and the detection of combining Hcy, CysC and FFA may help the diagnosis, the prognosis and monitoring in ischemic cerebrovascular disease.**

Keywords: Brain ischemia； Homocysteine； Cystatin C； Free fatty acids

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1. Introduction

Ischemic cerebrovascular disease is one of the three major diseases leading to human death, second only to heart disease and cancer, with high incidence and high morbidity, mortality is high. Numerous studies have confirmed that high homocysteine levels is one of the independent risk factors in atherosclerosis (AS) disease, and is closely associated with ischemic cerebrovascular disease[1-2]. Domestic and international studies show that CysC actives in the formation of the artery atheromatous plaque, metastasis of the tumor and so on, so CysC has important application value in heart cerebrovascular disease, tumor, and the diagnosis of renal disease[3]. As a lipid intermediate metabolite, under conditions of FFA overload, they become toxic, inducing ROS （reactive oxygen species）production, ER stress (endoplasmic reticulum stress), apoptosis and inflammation, being closely associated with obesity, free fatty acid (FFA) can also reflect the state of the body lipid metabolism, diabetes and atherosclerosis [4-5]. Based on detecting plasma Hcy, CysC and FFA levels, his study intend to explore their correlation with ischemic cerebrovascular diseases.

2. Method

2.1 Patients

The study objects were hospitalized patients in the affiliated hospital of Qingdao university neurology and emergency neurology from October 2013 to March 2014. Inclusion criteria: all the patients were in line with the diagnostic criteria in the fourth national cerebrovascular disease conference and confirmed by CT and/or MRI, eliminated suffering from severe disease of heart, liver and kidney, malignant tumors, scleroderma, thyroid disease.42 cases of transient ischemic attack (TIA) group, 22 males and 20 females patients, aged 45 to 86 (an average of 61 + 12); 57 cases of ischemic stroke group, 31 males and 26 patients, aged 30-84 (an average of 65 + 12). 51 heathy volunteers were selected as the control group, 27 males and 24 females, aged 41 to 74 years old (an average of 61 + 11), without disease of heart, liver and kidney,

malignant tumors, scleroderma, thyroid disease, with normal CT, MRI and so on. Each group had no significant differences in age, sex, constitute.

2.2 ***Follow-up***

Blood samples were collected from patients who had been in fasting state next morning after admission, centrifuged (15 min, 3000 r/min) and separated serum within two hours. Homocysteine (Hcy) (circulating enzymatic method), CysC (immune turbidimetry method) and free fatty acid (FFA) (enzymatic method)were measured with an automatic biochemical analyzer (HITACHI7600-210, Japan's Hitachi).

2.3 ***Statistical analysis***

We used SPSS20.0 software for statistical analysis. The measurement data were presented as mean+ SD. The t-test of two independent samples was used for comparison in each two groups. The correlations in analysis of indicators were determined by Pearson correlation analysis. P<0.05 was considered statistically significant.

3. Result

3.1 Levels of plasma Hcy, CysC and FFA count

The plasma Hcy, CysC and FFA count in the three groups are shown in table 1. Hcy, CysC, FFA levels of ischemic stroke group were higher than those of healthy control group, and the difference had statistical significance; Hcy, CysC and FFA levels of transient ischemic group were higher than those in healthy control group; Hcy, CysC and FFA levels of ischemic stroke group were higher than those in transient ischemic group, the difference was statistically significant.

3.2 Correlation between CysC and Hcy

In every subgroup with ischemic cerebrovascular disease, There was significant correlation between Hcy and CysC (r = 0.607, P<0.01) in TIA group, and Hcy significantly related to CysC in ischemic stroke patients (r = 0.469, P<0.01).

Table 1groups of Hcy, CysC and FFA levels (mean±SD)

groups	Cases	Hcy (umol/L）	CysC （mg/L）	FFA (mmol/L)
control	51	7.78+1.79	0.68+0.082	0.35+0.17
TIA	42	9.29+3.802	0.83+0.161	0.40+0.173
ischemic stroke	57	11.52+6.561	0.97+0.271	0.50+0.301

1): compared with control group, P < 0.01; 2): compared with control group, P < 0.05; 3): compared with ischemic stroke group, P < 0.05.

4. Discussion

Homocysteine (Hcy) is a sulfur-containing amino acid that is generated during methionine metabolism, high levels of Hcy is called hyper-homocysteinemia(hHcy). HHcy is recognized as a comorbid risk factor of human stroke, it also aggravates the ischemia-induced injury by increasd production of reactive oxygen species, and by the homocysteinylation and thiolation of functional proteins[6-10].Elevated Hcy plasma levels in patients with ischemic cerebrovascular disease are considered to be involved in the progress of cerebral infarction through the mechanism of atherosclerosis and thrombosis, so hHcy is widely considered as one of the risk factors of ischemic cerebrovascular disease[11-14].With increased levels, Hcy has direct and toxic effect on vascular endothelial cells, and can directly damage the vascular endothelial cells[15-16]. Wang Zheng[17] and so on, measured Hcy levels by fluorescence polarization immunoassay method in 35 patients with acute ischemic stroke and 20 normal control persons, obtained a conclusion that Hcy may be associated with ischemic cerebrovascular disease. Li Yajuan[18], used enzyme-linked immunosorbent

method, tested plasma Hcy levels in 90 cerebral infarction patients, in 44 cases of TIA and 40 cases of health examination(control group), the analysis results showed that in patients with ischemic cerebrovascular disease group，cerebral infarction group and TIA group plasma Hcy levels were higher than that of control group ($P < 0.05$), plasma Hcy levels in cerebral infarction patients group have no significant difference with TIA group ($P > 0.05$). It is concluded that the plasma Hcy levels may have nothing to do with the traditional risk factors and may be an independent risk factor in ischemic cerebrovascular disease, but the levels of Hcy do not depend on the type and clinical serious degree in ischemic cerebrovascular disease. Our study used circulating enzymatic method to detect Hcy plasma levels, the results showed TIA and ischemic stroke group Hcy levels were higher than those of healthy controls, and ischemic stroke group Hcy levels were higher than those of the TIA group, but there is no statistically significant difference, which are in conformity with Wang Zheng‘s research conclusion.

CysC is a cysteine protease inhibitor, adjusts the extra and intracellular protease hydrolysis. The concentration of serum CysC can reflect the stability of the artery atheromatous plaque. As cysteine protease inhibitors, CysC has strong inhibitory effect on half-and-half cystineprotease, cathepsin and matrix metalloproteinases. With reduced CysC concentrations, cathepsin and matrix metalloproteinases activity could be enhanced, leading to increase extracellular matrix degradation and reduced plaque collagen fiber, finally fibrous cap would be weak[19]. Salemi G[20] ever reported in acute artery stroke Hcy and CysC plasma levels were positively related to each other, the results also showed that CysC levels of TIA and ischemic stroke group were higher than that of healthy control group, CysC levels of ischemic stroke group was higher than that of TIA group, and in TIA and ischemic stroke patients there is significant positive correlation between Hcy and CysC, which was consistent with the domestic and international research results, prompting CysC not only reflected the sensitive index of the glomerular filtration rate, but also played an important role in plaque rupture, restenosis and forming pathogenesis of aneurysm.

Free fatty acid (FFA), known as the esterification of fatty acids (nonestesterified fatty acid, NEFA), is composed of oleic acid, palmitic acid, linoleic acid and so on, most of FFA combined with albumin, presented in blood. The concentration of serum FFA has relation with lipid metabolism, glucose metabolism and endocrine function. In recent years, researches have shown that free fatty acid is one of the substances involved in oxidative stress[4]. The consequences of high free fatty acid (FFA) stimulation are highly active reactive oxygen species(ROS) and increase formation of reactive nitrogen species (RNS), which launched the oxidative stress mechanism (highly active reaction molecules and long-term imbalances between antioxidant effect and cause tissue damage)[5]. At the same time, high free fatty acid (FFA) promoted the interaction of white blood cells and endothelial cells, damaged endothelial and mitochondrial oxidatie function, promoted vascular endothelial inflammatory response, and then participated in the occurrence of cerebrovascular disease[4]. Our study showed that FFA levels of ischemic stroke group was higher than those of transient ischemic group and healthy controls, and the difference was statistically significant, it was likely to lead to ischemic stroke by injuring endothelial cells, involved in the pathogenesis of cerebrovascular disease.

5. Conclusion

Obviously, increased concentrations of Hcy, CysC and FFA are closely associated with ischemic cerebrovascular disease, so the detection the serum Hcy, CysC and FFA concentrations in patients with ischemic cerebrovascular disease should be necessary. The detection has certain clinical practical value and will help looking for the treatment methods of reducing Hcy, TO monitor the pathological changes and treatment effect, to prevent and reduce the morbidity and mortality of cerebrovascular.

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