Elevated serum neopterin and homocysteine increased the risk of ischemic stroke in patients with transient ischemic attack

Abstract: Objective: To investigate the correlation between serum neopterin, homocysteine (Hcy) and the risk of developing ischemic stroke (IS) in patients with transient ischemic attack (TIA).

Methods: Ninety-two TIA patients were prospectively recruited at the First Affiliated Hospital of the Medical College, Shihezi University, Xinjiang Autonomous Region China. Of the included patients, 27 developed ischemic stroke (IS group) and other 65 cases did not (TIA group). Peripheral venous blood was obtained within 24 hours of TIA diagnosis. Serum neopterin, Hcy and serum lipid levels were tested and compared between IS and TIA groups.

Results: Serum neopterin (6.38±1.76 ng/mL vs 5.39±1.51 ng/mL), Hcy (17.95±4.10 μmol/L vs 15.30±3.66 μmol/L), TG (1.82±0.92 mmol/L vs 1.40±0.71 mmol/L) and LDL (3.31±0.67 mmol/L vs 2.69±0.62 mmol/L) of IS group were significantly higher than those of TIA group (p<0.05). The AUC of serum neopterin, Hcy, TG and LDL for predicting the risk of developing IS in patients with TIA were 0.68 (95%CI: 0.55-0.81), 0.68 (95%CI: 0.57-0.80), 0.64 (95%CI: 0.51-0.78) and 0.75 (95%CI: 0.63-0.86), respectively.

Conclusion: Serum neopterin, Hcy, TG and LDL are promising serological markers for predicting the increased risk of developing IS for patients with TIA.

Keywords: neopterin; homocysteine; ischemic stroke; transient ischemic attack; biomarker.

Introduction

Transient ischemic attack (TIA) is a central nervous ischemia with highly variable triggers including arterial thrombotic, embolic events or other local hemodynamic changes including vasospasms, but not exclusively caused by vasospasms. TIA is more common in elderly patients, especially those with hypertension. TIA is an important indicator for the risk for developing ischemic stroke (IS). Clinical epidemiological data has shown that the incidence of acute IS in TIA patients within one week is about 10%, while the risk of stroke one month post repeated TIA is more than 25% [1]. A multicenter large sample short-term study in the United States showed that 9.5% -14.6% of patients developed cerebral infarction within 90 days of a TIA [2].

Neopterin is an intermediate in the biosynthesis pathway of guanosine triphosphate (GTP) and tetrahydrobiopterin in vivo. Neopterin is also a coenzyme in the hydroxylation of phenylalanine, tyrosine and tryptophan. Previous studies have suggested that neopterin is associated with various diseases related to the activation of cellular immune responses such as infections [3], trauma [4], tumors [5, 6], autoimmunity and inflammatory diseases. In recent years, studies have reported that serum neopterin levels are also related to atherosclerosis [7 , 8]. Since McCully [9] first found that hyperhomocysteinemia was associated with cerebrovascular disease in 1969, many studies have confirmed that hyperhomocysteinemia is an independent risk factor for ischemic cerebrovascular disease [10, 11]. High concentrations of Hcy in the plasma can significantly increase the risk of cerebral infarction. Additionally, more than 40% of patients with cerebral infarction have hyperhomocysteinemia.
Material and methods

Patients

Ninety-two TIA patients were prospectively recruited at the First Affiliated Hospital of the Medical College, Shihezi University, Xinjiang Autonomous Region China. For the included 92 patients, 27 developed ischemic stroke (IS group) while the other 65 cases did not (TIA group). Patients inclusion criteria were as follows: (1) TIA was diagnosed according to the guidelines and confirmed by a brain computerized tomography (CT) scan or magnetic resonance imaging (MRI) scan; (2) Non-cerebral vascular malformation; (3) Signed informed consent was obtained from all the included patients; (4) The study design was reviewed and approved by the ethics committee of the First Affiliated Hospital of the Medical College. Exclusion criteria was as follows: (1) Cardiogenic TIA; (3) Previous hemorrhagic or IS history; (4) Severe liver and kidney dysfunction; (5) Combined with acute infection; (6) HIV positive status; (7) Hematological diseases; (8) Patients with cancer.

Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the ethics committee of the Medical College, Shihezi University, Xinjiang Autonomous Region China.

Serum neopterin, Hcy and serum lipid measurement

Peripheral venous blood was obtained within 24 hours of TIA diagnosis. Serum lipids such as TG, Tch, HDL and LDL were tested by Automatic biochemical analyzer (Abbott, c8000); Serum neopterin and Hcy were examined by enzyme linked immunosorbent assay (ELISA) (Immuno-Biological Laboratories Co., Ltd). The testing procedure was conducted in strict accordance to the manufacturers instructions.

Statistical analysis

Serum neopterin, Hcy and serum lipid levels were expressed as the mean ± SD. Student-t test (two tailed, independent) was used to find the significance of parameters between the IS and TIA groups. The enumeration data was expressed as relative number (n, %). Chi-square or fisher’s exact test was used to detect the difference between each parameter. The prediction markers of serum neopterin and Hcy were calculated according to the Bayesian theorem with the equation of sensitivity=true positive/(true positive+false negative), specificity=true negative/(true negative+false positive). The area under the receiver operating characteristic (ROC) curve was used to evaluate the feasibility of serum neopterin, homocysteine (Hcy) as biomarkers for ischemic stroke prediction. We used SPSS version 15.0 (SPSS, Inc., Chicago, IL, USA) for statistical analyses and p<0.05 (two tails) was considered statistically significant.

Results

Main character of the two groups

Clinical characteristics such as age, gender, hypertension and symptom were not statistical different between the two groups (p>0.05) (Table 1).

Serum neopterin, Hcy and serum lipid

The serum concentrations of neopterin, Hcy, triglycerides and cholesterol are shown in Table 2. Serum neopterin, Hcy, TG and LDL of the IS group were significantly higher than those of TIA group (p<0.05).

Association of serum neopterin, Hcy, TG and LDL with ischemic stroke

The AUC of serum neopterin, Hcy, TG and LDL for predicting the risk of developing IS in patients with TIA were 0.68 (95%CI: 0.55-0.81), 0.68 (95%CI: 0.57-0.80), 0.64 (95%CI: 0.51-0.78) and 0.75 (95%CI: 0.63-0.86), respectively (Table 3 & Figure 1).

Discussion

TIA is an important risk indicator for the development of ischemic stroke [12]. Clinical manifestations of TIA are transient ischemic symptoms, including, but not limited to, temporary deep sensory impairment, speech disorder
Table 1: The main characteristics of the included patients [n, (%)].

| Characteristic                              | IS (n=27) | TIA (65) | chi-square | p-value |
|---------------------------------------------|-----------|----------|------------|---------|
| Age (years)                                 |           |          | 0.29       | 0.59    |
| ≤60                                         | 10(37.04) | 28(43.08)|            |         |
| >60                                         | 17(62.96) | 37(56.92)|            |         |
| Gender                                      |           |          | 0.34       | 0.56    |
| Male                                        | 14(51.9)  | 38(58.46)|            |         |
| Female                                      | 13(48.1)  | 27(41.54)|            |         |
| Hypertension                                |           |          | 0.08       | 0.77    |
| Yes                                         | 20(74.07) | 50(76.92)|            |         |
| No                                          | 7(25.93)  | 15(23.08)|            |         |
| Diabetes mellitus                           |           |          | 0.08       | 0.77    |
| Yes                                         | 7(25.93)  | 15(23.08)|            |         |
| No                                          | 20(74.07) | 50(76.92)|            |         |
| Coronary atherosclerotic heart disease       |           |          | 0.02       | 0.88    |
| Yes                                         | 9(33.33)  | 19(29.23)|            |         |
| No                                          | 18(66.67) | 46(70.77)|            |         |
| Smoking                                     |           |          | 0.92       | 0.34    |
| Yes                                         | 12(44.44) | 36(55.38)|            |         |
| No                                          | 15(55.56) | 29(44.62)|            |         |
| Family history of stroke                    |           |          | 0.005      | 0.94    |
| Yes                                         | 4(14.81)  | 10(15.38)|            |         |
| No                                          | 23(85.19) | 55(84.61)|            |         |
| Symptom                                     |           |          |            |         |
| Limb weakness                               |           |          | 0.11       | 0.73    |
| Yes                                         | 16(59.26) | 36(55.38)|            |         |
| No                                          | 11(40.74) | 29(44.62)|            |         |
| Aphasia                                     |           |          | 0.21       | 0.65    |
| Yes                                         | 8(29.63)  | 16(24.62)|            |         |
| No                                          | 19(70.37) | 49(75.38)|            |         |
| Disorder of consciousness                   |           |          | 0.02       | 0.88    |
| Yes                                         | 1(3.70)   | 2(3.08)  |            |         |
| No                                          | 26(96.30) | 63(96.92)|            |         |
| Duration of symptoms                        |           |          | 0.25       | 0.62    |
| >10 min                                     | 14(51.85) | 30(46.15)|            |         |
| ≤10min                                      | 13(48.15) | 35(53.85)|            |         |

Table 2: Serum neopterin, homocysteine and serum lipid comparison between IS and TIA groups.

| Characteristic | IS (n=27) | TIA (65) | t   | p-value |
|----------------|-----------|----------|-----|---------|
| Neopterin (ng/mL) | 6.38±1.76 | 5.39±1.51 | 2.72| 0.008   |
| Hcy (μmol/L)     | 17.95±4.10| 15.30±3.66| 3.05| 0.003   |
| TG (mmol/L)      | 1.82±0.92 | 1.40±0.71 | 2.37| 0.02    |
| Tch (mmol/L)     | 5.36±1.21 | 5.48±1.09 | 0.47| 0.64    |
| HDL (mmol/L)     | 1.16±0.33 | 1.20±0.31 | 0.55| 0.58    |
| LDL (mmol/L)     | 3.31±0.67 | 2.69±0.62 | 4.26| 0.00    |
and visual impairment [13, 14]. Some TIA patients suffer from repeated attacks of the above symptoms in a short period of time, however, brain CT or MIR scans often do not identify any infraction lesions [15]. TIA is an important risk marker for acute cerebral ischemic stroke, although it does not cause permanent limb or language dysfunction. Clinical epidemiological data has shown that the incidence of acute ischemic stroke in TIA patients within one week of the TIA attack(s) was approximately 10%. Alarmingly, the risk of stroke within one month of repeated TIA attacks is more than 25% [1]. Attention should therefore be paid to TIA patients and appropriate treatment strategies should be implemented, including blood pressure and blood sugar control. Furthermore, policy relating to anticoagulation therapy should be

| Characteristic      | AUC   | Std    | 95%CI Lower limit | 95%CI Upper limit | p-value |
|---------------------|-------|--------|-------------------|-------------------|---------|
| Neopterin (ng/mL)   | 0.68  | 0.066  | 0.55              | 0.81              | 0.007   |
| Hcy (μmol/L)        | 0.68  | 0.061  | 0.57              | 0.80              | 0.006   |
| TG (mmol/L)         | 0.64  | 0.069  | 0.51              | 0.78              | 0.030   |
| LDL (mmol/L)        | 0.75  | 0.057  | 0.63              | 0.86              | 0.000   |

Figure 1. ROC curve of serum neopterin, Hcy TG and LDL as biomarker for predicting the risk of developing IS in patients with TIA (a: serum neopterin; b: serum Hcy; c: serum TG; d: serum LDL).
strengthened throughout healthcare systems in order to reduce the risk of developing acute stroke.

Neopterin is a low molecular weight alkaline substance which has been detected only in humans and primates [16]. Neopterin has been implicated in the pathogenesis of various diseases related to the activation of cellular immune responses, including infection, trauma, tumors, autoimmunity and other inflammatory diseases [17, 18]. As such, neopterin may well be used as a biological marker to predict the course and prognosis of diseases such as malignant diseases and infections caused by the human immunodeficiency virus (HIV). Neopterin also has potential as a predictor of cardiac events in patients with acute coronary syndrome (ACS) [19, 20]. However, studies relevant to neopterin and cerebrovascular disease are thus far rarely reported. In our work, we investigated the serum levels of neopterin in TIA patients and found that elevated serum neopterin is a risk factor for ischemic stroke in TIA patients with the prediction performance of AUC=0.68.

The correlation between hyperhomocysteinemia and the increased risk of cerebrovascular disease had been widely discussed in the literature. The exact mechanism of hyperhomocysteinemia in cerebrovascular disease is still unclear. Possible pathological mechanisms include [21]: (1) Dysfunction of vascular endothelial cells by the production of peroxides and oxygen free radicals; (2) Stimulation of atherosclerotic plaque formation by promoting vascular smooth muscle cell proliferation has been correlated with lipid metabolism; (3) Enhanced adhesiveness of platelets in the blood, disturbs the balance of coagulation and fibrinolysis leading to cerebrovascular disease. Our research has found that serum Hcy is elevated in ischemic stroke patients compared to TIA patients thereby providing evidence that Hcy is a potentially promising maker for predicting ischemic stroke in TIA patients. This result is in accordance with the previous relevant publication [22].

Conclusion

Serum neopterin, Hcy, TG and LDL were significantly elevated in ischemic stroke patients compared to TIA subjects in our study. The combined detection of serum neopterin, Hcy, TG and LDL can provide appropriate assessment of the risk of stroke in patients with TIA.

Conflict of interest: Authors state no conflict of interest.

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