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Prognostic evaluation of sex difference in adenosine deaminase and homocysteine activities of acute ischemic stroke patients

Akut iskemik inme hastalarının adenozin deaminaz ve homosistein aktivitelerindeki cinsiyet farkının prognostic değerlendirmesi

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Abstract

Aim of the present study was to investigate serial changes in adenosine deaminase (ADA) and total homocysteine (tHcy) activities in male and female acute ischemic stroke (AIS) patients to determine their prognostic value. ADA and tHcy activities were measured in follow-up blood sample collected from AIS patients at admission, 24 hrs, 48 hrs, 72 hrs, and 144 hrs after admission and in control subjects. We did not observe any significant difference in ADA and tHcy activities between male and female of AIS and control subjects. ADA activity was significantly high in male AIS patients as compare to female throughout the follow-up. There were no significant difference in tHcy activity between male and female of AIS patients. Similarly there were no significant differences in ADA and tHcy activities on comparing follow-up samples of AIS patients with that of admission value. In addition, m-RS scale in female was better than male AIS patients. Findings of this preliminary study suggest that increase in ADA activity in male AIS patient may reflect poor outcome than female. The tHcy activity is not related with the severity and out come after AIS. Further prospective studies are needed to see if ADA is clinically useful in outcome prediction after AIS.

Keywords: Acute ischemic stroke, adenosine deaminase, homocysteine, prognosis

Özet

Bu araştırmanın amacı erkek ve kadın akut iskemik inme hastalarında adenozin deaminaz (ADA) ve total homosistein (tHcy) aktivitelerindeki seri değişimin prognostik

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değerlerini belirlemektir. ADA ve tHcy aktiviteleri kontrol bireylerden ve kabulde, kabulden 24, 48, 72 ve 144 saat sonra akut istemik inme hastalarından ölçüldü. Kontrol ve akut iskemik inme olgularında kadın ve erkek bireylerin ADA ve tHcy aktiviteleri benzer bulundu. ADA aktivitesi erkek AIS hastalarında kadınlara göre belirgin şekilde yüksektir. Erkek ve kadın AIS hastalarında tHcy aktivitelerinde önemli bir fark yoktur. Benzer şekilde AIS hastalarının kabul ve takip ADA ve tHcy aktivitelerinde belirgin bir fark yoktur. Ayrıca kadın hastaların m-RS ölçüleri erkek AIS hastalarından daha iyidir. Bu öncül çalışmanın bulguları erkek AIS hastalarındaki ADA aktivitesinin artışı bayanlara göre daha önemsiz olmasıdır. tHcy aktivitesi AIS sonrası sonuç ve ölçüsü olamadığını gösterir. AIS sonrası tahminler için ADA'nın klinik olarak kullanılıp kullanılmayacağı için daha fazla prospektif çalışmaya ihtiyaç vardır.

Anahtar sözcükler: Akut istemik inme, adenozin deaminaz, homosistein, prognoz

Introduction

During ischemia, there is increase in intracellular degradation of ATP to adenosine due to energy demand, which results in a rapid elevation of extra cellular adenosine level [1]. Adenosine deaminase (ADA, EC 3.5.4.4) is the key enzyme in regulation of extra cellular adenosine catalyzes the irreversible hydrolytic deamination of adenosine and 20-deoxyadenosine to inosine and 20-deoxyinosine, respectively [2].Serum ADA activity has also been shown to be elevated in many disease conditions [3]. However very limited information is available regarding role of serum ADA activity in stroke patients [4].The aim of the present study was to investigate serial changes in ADA activity in male and female acute ischemic stroke (AIS) patients to determine their prognostic value. In addition to that, we also investigated total homocysteine.

Materials and Methods

Subjects

Fifteen patients (11 Male, 4 Female), aged 23-83 years, admitted, within 24 hrs of the onset of symptoms of AIS were included in the present study. Diagnosis was based on WHO definition. CT scan was performed in all the cases for the confirmation of the AIS. Patients with transient ischemic attacks and other types of brain injury were also excluded from the present study Neurological deficit was assessed as per National Institute of Health Stroke Scale (NIHSS) score during the hospitalization and functional recovery was assessed by Modified Rankin scale (mRS) at the time of discharge. All the patients were kept in the Intensive Care Unit (ICU), where the ambient temperature was between 20- 25° C. Among the study group etiological distribution of AIS include 9 (60%) were of undetermined etiology, 3 (20%) were of having cardioembolic stroke and 3 (20%) were having atherosclerotic stroke. All patients received standard medical treatment which include anti-platelet agents (aspirin 150 mg, clopidregel 75 mg once a day); three patients were thrombolyzed using intravenous recombinant tissue-plasminogen activator; others received anti-edema measures mannitol (20%, 0.25–0.5 g/kg) over 20 min, with symptoms of raised intracranial pressure; and other supportive measures for the treatment of concurrent illnesses such as hypertension and diabetes mellitus. Out of the 15 patients 2 (1male and 1 female) patient expired during the treatment were also excluded from the study. The protocol of this study was reviewed and approved by the Institutional Ethics Committee.

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Samples

Venous blood sample of the AIS patients were collected in plain and EDTA tube immediately at the time of admission (0 hrs) 24 hrs, 48 hrs, 72 hrs and 144 hrs after admission. Blood sample from age and sex-matched healthy individuals (n=14) were also taken as control group.

Determination of the serum ADA activity

ADA activity in serum was determined by the method of Guisti and Galanti [5] based on the Berthlot reaction, which is the formation of colored indophenol complex from ammonia liberated during deamination of adenosine. Colure developed was quantified by spectrophotometer (Systronics India). One unit of ADA is defined as the amount of enzyme required to release 1 mMol of ammonia per minute from adenosine at standard assay conditions. Results were expressed as units per liter per minute (U/L/min)

Determination of plasma total homocysteine

Plasma sample for tHcy estimation was separated in cold conditioned and stored at −20°C. tHcy estimation in plasma was done by ELISA as per manufacturer's instruction (Axis Shield Diagnostic Limited; U.K.) Results were expressed as µMol/L.

Statistical analysis

Data were expressed as ±SD. Statistical analyses were performed using MedCalc for Windows, version 9.5.0.0 (MedCalc Software, Mariakerke, Belgium). Student t-test was used to detect the difference in ADA and tHcy activities and m-RS score between males and females from AIS patients and control subjects. A p value <0.05 was considered as statistically significant.

Results

After excluding two expired cases, the study population consists of the 13 AIS patients (10 males and 3 females) and 14 controls (4 males and 10 females). Mean NIHSS score for severity in males and females were 12.9 ± 5.0 and 14.7 ± 0.6, respectively. Similarly mean m-RS scale for outcome in male and female were 3.7 ± 1.1 and 2.3 ± 0.6, respectively. Although females had a high severity score on admission, m-RS scale for outcome was good as compare to males but it could not reach to statistical significance (*P>0.05*), i.e. *P=0.0685*. (Table 1)

The mean value ADA and tHcy activities in the follow-up AIS samples collected at admission (0 hrs), 24 hrs, 48 hrs, 72 hrs, 144 hrs and control groups are shown in the Table 2.

We did not observe any significant difference in ADA and tHcy activities between the males and females of the AIS and control subjects. ADA activity was significantly *(P<0.05)* high in the male AIS patients as compare to female ones throughout the follow-up. There were no significant difference in tHcy activity between the males and females of AIS patients. Similarly there were no significant differences in ADA and tHcy activities on comparing the follow-up samples of AIS patients with that of admission values.

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Table 1. Clinical details of study subjects.

Subjects	Age	NIHSS on admission	m-RS Score at discharge
Female (mean±SD)	59.3±31.7	14.7±0.6	2.3 ± 0.6
Lt. MCA territory infarct	23	15	2
Lt. PCA infarct	80	14	3
Lt. MCA Infarct	75	15	2
Male (mean±SD)	60±17.5	12.9±5.0	3.7±1.1
Rt. MCA infarct	72	13	4
Lt. MCA infarct	37	7	2
Rt. Side MCA Infarct, ICA occluded	54	17	4
Rt. MCA infarct,	81	15	5
Rt. Peripheral MCA Infarct	80	10	3
Lt. MCA infarct	45	18	4
Rt. Side MCA infarct	70	9	4
Lt. MCA territory infarct	64	8	2
Lt. MCA territory infarct	70	20	5
Rt. MCA Infarct	35	12	4

Table 2. ADA and tHcy activity in follow-up samples of study subjects.

	n	Male		n	Female	
		ADA (U/L/min)	tHcy (µM/L)		ADA (U/L/min)	tHcy (µM/L)
Controls	4	15.3±1.7	15.5±3.1	10	12.8±2.5	12.7±6.6
AIS patients	10			3		
Admission		19.4±5.1*	16.8±8.7		11.63±1.68	11.3±7.4
24 hrs		18.7±3.7*	15.5±6.6		12.65±1.65	10.9±7.1
48 hrs		19.7±5.7*	16.1±5.6		11.42±2.19	13.3±8.8
72 hrs		19.5±5.4*	13.2±4.2		11.39±2.92	15.6±9.9
144 hrs		20.9±5.6*	13.7±3.2		10.97±5.29	11.1±1.5
Values are mean ± SD; *p<0.05 vs. female AIS patients.						

Discussion

In the current study, we investigated serial change in ADA and total tHcy activity in male and female AIS patients to determine their prognostic value. We did not observe any significant difference in ADA and tHcy activity between the male and female of the AIS and control subjects. ADA activity was significantly high in male AIS patients as compare to female throughout the follow-up. There were no significant difference in tHcy activity between male and female of AIS patient. Similarly, there were no significant differences in ADA and tHcy activity on comparing the follow-up samples of AIS patients with that of admission value.

During disease conditions involving the reduction in the cerebral blood flow, even in smaller reductions in blood flow triggers adenosine release by the neuronal cells [6]. Adenosine is an endogenous neuroprotectant it inhibits a glutamate release in nerve cells, which is a major excitotoxic amino acid, involve in the pathophysiology of AIS [7]. ADA is the key enzyme in the regulation of the extracellular level of the adenosine [2]. In our

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study mean ADA activity were significant high in male AIS patients as compare to female throughout our follow-up. Comparatively stable ADA activity in female AIS patients could be an adaptation mechanism for making availability of neuroprotective adenosine for longer time. Interestingly Outcome m-RS Scale of the female were good then male AIS patients. Thus in male AIS patient high ADA activity may reflect poor outcome than female.

Homocysteine is sulfur containing amino acid and an intermediate in methionine metabolism. Homocysteine activates N-methyl-D aspartate receptor, which leads to cell death. It also converts into homocysteic acid, which has a toxic effect on the neurons of cerebral cortex [8]. Recent report shows that rise in the plasma tHcy was independently associated with AIS [9]. We did not observe any significant difference in tHcy activity between the male and female of the AIS and control subjects. Similarly, there were no significant differences in tHcy activity on comparing the follow-up samples of AIS with that of admission value indicating that tHcy is not related with the severity and outcome.

In conclusion, results of this preliminary study suggest that increase in ADA activity in male AIS patient may reflect poor outcome than female. The tHcy activity is not related with the severity and out come after AIS. Further prospective studies are needed to see if ADA is clinically useful in outcome prediction after AIS.

Conflicts of interest

The authors stated no conflicts of interest.

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