Introduction

Stroke is the loss of brain function due to a disturbance in the blood supply to the brain. According to the World Health Organization’s report, 15 million people are affected by stroke worldwide each year [1]. Of these, 5 million die and another 5 million become permanently disabled. Stroke is routinely divided into ischemic stroke (IS) and intracerebral hemorrhage (ICH). IS and ICH account for more than 80% and 8-15% of all strokes, respectively [2, 3]. IS is one of the leading causes of death and disability in the world. It is believed that ischemia-induced brain injury is mainly due to excessive release of glutamate resulting in excitotoxicity and cell death [4]. It is essential to differentiate IS from ICH earlier at the stroke onset. It is worth noting that the management of IS and ICH are different.

For instance, in patients with IS, anticoagulant therapy is vital. However, it is contraindicated in individuals with ICH [5]. The brain imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) have been employed for differentiating patients with IS and ICH [6]. Despite the progress in the brain imaging techniques, recent investigations in the IS patients or animals with traumatic brain injury demonstrated a significant alteration of blood metabolites at the time points (between 3 and 24 hours) in which no abnormality could be found by MRI [7-9]. Astrocytic glial fibrillary acidic protein (GFAP) in serum was introduced as a reliable marker of ICH in the acute phase of stroke [10]. Later studies suggested that blood biomarkers could detect damages of ischemia in nervous tissue earlier as compared to the imaging techniques [11].
Taurine or 2-ethane sulfonic acid is the semi-essential amino acid, not incorporated in the synthesis of protein and is the principal intracellular free β-amino acid [12]. Taurine is also one of the most abundant free amino acids in the heart, brain, liver, retina and skeletal muscles [12]. Taurine is also the second most abundant amino acid in the brain after glutamate [13]. Taurine has been implicated in different cell protecting and neuromodulatory functions, as well as in the central nervous system (CNS) cell migration and development. Taurine readily responds to an excess of osmotic stimuli with inward or outward movements across the membranes of different CNS cell types together with osmotically obligated water. This has proposed a role for taurine in the regulation of cell volume and cell osmolarity regulation [14]. Evidence also suggests the interaction of taurine with gamma-aminobutyric acid (GABA) and glycine receptors [15]. Elevated concentration of taurine in plasma and urine has already been reported following surgical trauma [16], muscle necrosis [17], acetaminophen overdose [18] and heroin addiction [19]. Accumulating evidence indicates that taurine might function as an inhibitory modulator or neurotransmitter in many cerebral areas [20].

Excitatory amino acids (such as aspartate, glutamate, and taurine) are linked critically to some neurological conditions, including neurodegenerative syndromes such as Alzheimer’s disease, cortical damage due to stroke and cerebral ischemia, epilepsy, amyotrophic lateral sclerosis, and schizophrenia [21]. Kimelberg et al. showed that inhibition of cell volume regulator anionic channels by tamoxifen and prevention of amino acids release could protect ischemia in brain cells in rats [22].

More recently, taurine received attention as a biomarker in the prediction of the severity of tissue damage following poisoning. In this regards, Sattari et al. reported the relation between plasma taurine concentration and prothrombin time and aspartate aminotransferase (AST) raise following paracetamol poisoning [18]. The later study suggested that taurine may be evaluated as a biomarker of liver damage in paracetamol poisoning. In a previous study, we showed that plasma concentration of taurine is up-regulated in hospitalized patients suffering from stroke. Nevertheless, the mean of plasma concentration of taurine was declined significantly in the third and the fifth day, following stroke [19]. The later study has some queries such as whether the type of stroke (IS or ICH) has any influence on the plasma taurine concentration. Also, other amino acids such as glycine and glutamic acid may rise in the plasma following stroke. To address this question, in the present study, we aimed to investigate the changes of plasma concentration of taurine, glycine and glutamic acid on the patients suffering from IS or ICH at the first, third and fifth day after hospitalization. Glycine is the smallest amino acids, which enters proteins’ structures. The normal range of plasma concentration of glycine is 10.65-22.29 mg/L in humans above 17. Glutamate is one of the nonessential amino acids with a normal range of 0.29-12.94 mg/L in plasma of humans above 17 [23]. There are several types of cellular release of taurine, of which the basal release described, as ‘leaking of taurine’ through the membrane is the commonest one. Such leaking depends on the permeability of the membrane to taurine, which in turn depends on lipid composition and several other factors [24].

The aim of present study is to evaluate plasma concentrations of taurine, glycine and glutamate in ischemic or hemorrhagic stroke patients.

Materials and Methods

Sixty patients (30 men and 30 women) suffering from acute IS (aged 37-90 years; median 71) and 60 patients (28 men and 32 women) suffering from acute ICH (aged 20-89; median 69) were admitted to the stroke unit of Imam Reza hospital of Tabriz (Iran) were recruited for the study. The control population consisted of 30 healthy individuals (aged 18-65; median 55) without antecedents of central nervous system diseases. Written informed consent to participate in the study was obtained from the subjects or their relatives and controls. The study was conducted according to the amended declaration of Helsinki (1998) and getting approval from the ethics committees of Tabriz University of Medical Sciences before implementation. The patients who had Red Bull energy drink or seafood in the last 24 h and those who received salbutamol or clenbuterol, were excluded from the study. Red Bull energy drink or seafood contains a significant amount of taurine and salbutamol and clenbuterol decrease plasma and urinary concentration of amino acids [25]. Moreover, patients with concurrent malignancy, severe cardiac, renal or hepatic insufficiency, chemotherapy history, and vitamin B6 deficiency were also excluded, because chemotherapy history and vitamin B6 deficiency depress taurine plasma concentration [26]. Three blood samples (5 mL each) were taken from the brachial vein in the first, third and fifth days of hospitalization and collected into heparinized tubes. The samples were immediately centrifuged at 4°C with 2000 g for 10 minutes. Plasma was removed using Pasteur pipette and transferred into 5 mL glass tube and kept frozen at -20°C until analysis by Ghandforoush-Sattari’s and Kamp et al.’s methods [27, 28]. The data were statistically analyzed by a one-way ANOVA using Graph Pad Prism (ver. 4). A p-value less than 0.05 was considered statistically significant.

Results

Mean plasma concentrations of taurine, glycine, and glutamate in 60 patients (30 IS and 30 ICH), in the first (before treatment) day of hospitalization were compared with those of 30 healthy control subjects by a non-paired student’s t-test (Figure 1).

Plasma concentrations of taurine ranged between 0.7 and 55.3 mg/L (16.0 ± 2.39) in the IS group (p<0.0001) and 0.4-19.1 mg/L (10.5 ± 0.84) in the ICH group (p<0.0001) compared to 1.7 – 9.0 mg/L (4.6 ± 0.38) in the control subjects. Plasma concentrations of glycine ranged between 1.69 and 53.32 mg/L (18.09 ± 2.48) in the IS group (p<0.05) and 0.97 – 92.14 mg/L (12.13 ± 2.9) in the ICH group (p<0.05) compared to 7.44 – 22.92 mg/L (13.75 ± 0.84) in the control subjects. Plasma concentrations of glutamate ranged between 0.19 and 23.13 mg/L (7.23 ± 1.14) in the IS group (p>0.05) and 0.07 – 26.35 mg/L (5.95 ± 1.11) in the ICH group (p>0.05) compared to 0.64 – 13.54 mg/L (6.18 ± 0.78) in the control subjects.
Mean of plasma concentration of taurine declined from 16.04 ± 2.39 mg/L in the first, to 12.41 ± 1.68 mg/L in the third, and to 7.22 ± 1.20 mg/L in the fifth days of hospitalization in the IS group. Whereas, it dropped from 10.51 ± 0.84 mg/L in the first, to 8.55 ± 0.84 mg/L in the third, and to 6.56 ± 1.00 mg/L in the fifth days of hospitalization in the ICH group. There was a significant difference between the plasma concentration of taurine in both groups in the first and third days (p<0.001) but not in the fifth day of the study (p>0.05) (Figure 2).

Mean of plasma concentration of glycine declined from 18.09 ± 2.48 mg/L in the first, to 15.23 ± 1.90 mg/L in the third, and to 9.61 ± 1.52 mg/L in the fifth days of hospitalization in the IS group. Whereas, it dropped from 12.13 ± 2.90 mg/L in the first, to 12.01 ± 0.99 mg/L in the third, and to 10.42 ± 0.97 mg/L in the fifth days of hospitalization in the ICH group. There were significant differences between the plasma concentration of glycine in both groups in the first and third days (p<0.05) but not in the fifth day of the study (p>0.05) (Figure 3).

Mean of plasma concentration of glutamate declined from 7.23 ± 1.14 mg/L in the first, to 5.57 ± 0.96 mg/L in the third, and to 2.32 ± 0.44 mg/L in the fifth days of hospitalization in the IS group. Whereas, it dropped from 5.95 ± 1.11 mg/L in the first, to 4.53 ± 0.82 mg/L in the third, and to 3.07 ± 0.49 mg/L in the fifth days of hospitalization in the ICH group. There was no significant difference between the plasma concentration of glutamate in both groups during five days of study (p>0.05) (Figure 4).

**Discussion**

The physiological role of Taurine is not entirely understood, but various cytoprotective properties have been attributed to taurine, including antioxidant [29], regulation of osmotic pressure [29, 30], and neuromodulator [15, 29]. Moreover, taurine deficiency is associated with various pathologies including heart dysfunction [31], abnormalities of brain development [32], and cardiomyopathy [33]. In the astrocytes and the neurons, taurine is the principal osmolyte which during hypotonic or hypertonic conditions it goes out from or accumulates inside the cells.
the cell, respectively [12]. Melani and colleagues used the brain microdialysis technique and demonstrated that after middle cerebral artery occlusion in the rat taurine was increased in the dialysate samples [34]. Interestingly, Heystad et al. ’s study on the human cerebral cortex slice indicated that during membrane depolarization and simulated ischemia, the taurine release was increased [35]. Moreover, Castillo et al. studied on 100 patients with infarction in the territory of the middle cerebral artery and reported that during the acute phase of cerebrovascular ischemia, the taurine concentration rise in the blood and cerebrospinal fluid [36]. Taurine is present in neurons and astroglial cells in multiple areas of the brain [6, 29, 37, 38]. Other studies have suggested that taurine balances glutamate activity, notably under excitotoxic conditions [39-41]. The release and immediate uptake of taurine from neurons and glial cells have been associated with this neuroprotective effect [40-44]. However, another mechanism has been proposed involving taurine as an inhibitor of depolarization through increasing membrane chloride conductance via the activation of GABA and glycine receptors [45].

Stroke is presumed to be due to the insufficient blood supply to a part of the brain. This life-threatening condition appears over a few hours and one of the leading causes of morbidity and mortality worldwide [46]. It is difficult to predict the outcome of stroke patients. If clinicians have a correct prediction of the patient outcome at the early time points, it will improve patient’s health profile with decreasing the incidence of mortality and morbidity as well as significantly facilitates clinician’s decisions. For hyperacute treatment which targets to restore perfusion and protect neuronal tissue, the acute phase, within 16 h from the stroke onset, is crucial [47]. A blood biomarker which can differentiate between hemorrhagic and in the very early stage would help to optimize acute stroke management [10]. Thus, having a near-patient test device able to the reliable differentiation between IS and ICH in the preclinical setting, would be desirable.

Previous studies showed that the concentration of some free amino acids was significantly higher in CSF [32, 48] and blood [49, 50] of patients with IS infarction and control subjects in the first 6-24h of disease, but they did not measure in plasma. In another study, glutamate and aspartate elevated to levels at least 300 times higher than average for several days after the IS event [51]. However, Brouns et al. showed no significant associations between neurotransmitter concentrations (such as glutamate, aspartate, glutamine, glycine, proline, taurine, and norepinephrine) in CSF and stroke characteristics [52]. Several neural pathologies, most particularly cerebral ischemia, hyponatremia, hepatic encephalopathy and traumatic brain injury, are associated with pronounced cell swelling [22, 53] followed by releasing taurine. We showed that the plasma concentration of taurine increases in the patients with both IS and ICH strokes probably because of brain tissue damage, which is not a simple release of all contents of cells because glycine and glutamate did not have significant changes. Plasma concentration of taurine in both groups of IS and ICH increased in the first three days of hospitalization significantly but greater in IS group probably because of larger tissue damage in IS stroke. Measurement of plasma taurine concentration could be useful as a surrogate biomarker for the detection of IS, provided that the study be performed in a larger population.

Conflict of interest
The authors declare that there are no conflicts of interest to dispose.

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Reference


16. Turner FP, Brum VC, Paquette WW, Jr., Welden RB: The Urinary Excretion of Free Taurine in Acute and Chronic Disease, Following Surgical Trauma, and in Patients with Acute Alcoholism. The Journal of surgical research 1964, 4:423-431.


