

## Original Article

# Assessment of serum and urine ghrelin levels in patients with acute stroke

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**Abstract:** Introduction: Ghrelin is a novel brain-gut peptide hormone consisted of 28 amino-acid. In the plasma, it exists in two major molecular forms, acylated and des-acyled ghrelin, filtered in glomeruli or secreted by nephrons. Primary biological effects of hormones are regulating appetite, foods intake and energy metabolism. We investigated the changing and relationships between serum and urine ghrelin levels in acute stroke patients to provide more information whether diagnostic parameter. Methods: Thirty acute stroke patients and thirty consecutive volunteers included in study prospectively. To analyze serum and urine ghrelin levels, at the time of diagnose, all of participant blood and fresh urine (1 ml serum, 2 ml urine respectively) samples were obtained. Serum ghrelin levels analyzed ELISA technique, and urine ghrelin levels studied by validation technique. To compare quantitative data student's t test, and for qualitative data chi-square and Fisher's Exact Chi-square test was used.  $P < 0.05$  was considered statistically significant. Results: Urine acyl ghrelin levels found statistically significant between patient and control groups ( $P = 0.001$ ), but there were no statistically significant differences between both groups ( $P > 0.05$ ) in serum acyl ghrelin, des-acyl ghrelin and urine des-acyle ghrelin levels. Conclusions: The results indicate that urine acyl ghrelin levels may be considered as a diagnostic parameter in acute ischemic stroke patients. Further studies delineating the mechanism of these observed results are warranted.

**Keywords:** Stroke, acute, ghrelin, serum, urine

## Introduction

Ischemic stroke is the second most common cause of death and major cause of disability worldwide. The stroke is generally defined as any disease process that interrupts blood flow to the brain and causes focal neurologic syndroms [1, 2]. Injury is related to the loss of oxygen and glucose substrates necessary for high-energy phosphate production and the presence of mediators of secondary ischemic cellular injury. When a cell becomes sufficiently depleted of oxygen and nutrients, pro-apoptotic genes involved in cell death are activated. There are three principal types of cell death induced by cerebral ischemia and hypoxic injury; apoptosis, necrosis, and autophagy [2]. The more delayed cell death that occurs in the ischemic penumbra (3-24 hours and beyond) via princi-

pally apoptotic and autophagic mechanisms, against which ghrelin may be useful [3]. Relationship between stroke and endocrine system has been investigated for many years. It is known that stroke may causes transient or permanent changes of hormones secreted by circadian rhythm [4, 5].

Ghrelin is a novel brain-gut peptide hormone consisted of 28 amino-acid was discovered by Japan scientists Kojima et al. in 1999 [6, 7]. In the plasma, it exist in two major molecular forms, acyl and des-acyl ghrelin, the latter of which is the result of post-translational octanoylation of pro-ghrelin by the enzyme ghrelin-O-acyltransferase (GOAT) [8, 9]. Circulating ghrelin consists of more than 90% of des-acyl ghrelin and less than 10% acyl ghrelin. They are secreted in a pulsated manner as its level

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increases before the onset of meal, during fasting, and decreases after feeding [10]. Both ghrelin forms have been reported to prevent cell death by increasing mitochondrial function in cultured neurons exposed to oxygen and glucose deprivation (an *in vitro* model of ischemia) [2, 11, 12]. Ghrelin also protects against inflammation that plays a significant role which contributes to brain injury in hours to day after ischemic events [13]. Mean circulating half life of total ghrelin (acyl + desacyl ghrelin) was in the range of 35 min [10]. Ghrelin circulates into the bloodstream bound to lipoproteins [14], and filtered in the glomeruli or secreted by nephrons [15].

Circulating ghrelin is predominantly produced by specific cells in the stomach's oxyntic glands X/A-like cells of the fundus mucosa representing about 20% of gastric mucosal cells in humans [16], where 65-90% of the circulating ghrelin is synthesized [17-19]. The small intestine is the second major source of ghrelin synthesis and secretion but there are other sources of ghrelin, for example in the hypothalamus, pituitary, arcuate nucleus and various parts of the central nervous system (CNS), salivary and thyroid glands, breast, small intestine, kidney and heart that should be taken into consideration [20]. The major biological functions of ghrelin include the secretion of growth hormone, the stimulation of appetite and food intake, the modulation of gastric acid secretion and motility, and the modulation of the endocrine and exocrine pancreatic secretions. The hormone exerts biological effects via the activation of growth hormone secretagogue receptor-1a (GHSR-1a), the presence of which was confirmed in different parts of the gastrointestinal (GI) tract and midbrain structures [9, 10, 21]. Only acyl form of ghrelin stimulates growth hormone (GH) release. In contrast, des-acyl ghrelin is the most abundant form in plasma and does not bind GHSR-1a. It has no GH stimulating activity or any effect on other anterior pituitary function. A desacyl ghrelin not only a reservoir but also both types of ghrelin exhibit similar biological activities [22]. Some of variety biological effects of hormones are regulating appetite, foods intake and energy metabolism via mediating endogenous GH [23, 24]. Ghrelin also exerts numerous peripheral effects such as direct effect on endocrine and exocrine pancreatic functions, the cardiovascular functions, gastric secretion, gastrointestinal (GI)

motility, glucose release, cell proliferation, reproductive functions, and sleep [9, 20].

It is also now evident that ghrelin can be a powerful neuroprotective agent in experimental models of cerebral ischemia. Exogenous ghrelin also improves neurological deficit, infarct size, and survival of cortical neurons in rodents after transient focal ischemia-reperfusion (middle cerebral artery occlusion; MCAO) [25, 26]. Local expression of ghrelin receptor has also been shown in the cerebral cortex, and ghrelin has been shown to inhibit apoptosis, stabilizing the mitochondrial membrane potential, and improving mitochondrial function by regulating reactive oxygen species (ROS), respiration, enzyme activity and mitochondrial gene expression in transient focal cerebral ischemia [12, 25, 27]. Middle cerebral artery occlusion (MCAO) rat models had significantly showed lower gastric kinetic and elevated serum ghrelin levels in the early stage of cerebral ischemia. This can be explained as a compensatory reaction in the early ischemic stage. The up-regulation of serum ghrelin may prevent brain damage and impose decreased intestinal motility so as to decrease injury to the mucosal barrier [28].

We conducted this study to investigate prospectively serum and urine ghrelin levels in patients with acute stroke.

### Materials and methods

#### *Study design*

This prospective study was conducted between June 22 and December 5, 2007 in the ED of a tertiary care hospital. The study was approved by the local ethics committee. Informed consent approval was obtained from each patient before study enrollment. The informed consent was composed of four parts: patient name, aim and expected benefits of the study, and the rights of patients during the study.

#### *Study setting and population*

Consecutive 30 patients with acute ischemic stroke presenting to ED during the study period and 30 healthy volunteers for control group who were at the same age group with patients was included study. Mean admission time to the ED of patients with acute stroke is three hours (range 0-36 h). Because ghrelin values may be affected from one or more of the follow-

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**Table 1.** Shows study exclusion criteria

Some disease and conditions	Some hormones, drug and nutrients
Gastric ulcer	Acetylcholine agonists
Hyperthyroidism	Glucose load
Severe liver disease	Arginine
Chronic renal impairment	GH, GHRH, or GHRH plus
Hemorrhagic stroke	Oxyntomodulin
Subarachnoid hemorrhage	Leptin
Ischemic stroke beyond 48 hours	Testosterone
Chronic obstructive pulmonel disease	Insulin
Congestive heart failure	Urocortin
Hypogonadism	Somatostatin
Cushing's syndrome	Oestrogen
Polycystic ovarian syndrome	Thyroid hormones
Anorexia or Bulimia nervosa	Glucocorticoids
Fasting and chronic food restriction	Cortistatin
Obesity	Sex steroids
Type 1 DM, Type 2 DM	Oral protein load
Cardiac or cancer related cachexia	
Presence of active infection	
Recent antineoplastic therapy (chemotherapy, radiation therapy or surgery)	

Abbreviations: DM, Diabetes mellitus; GH, Growth hormone; GHRH, Growth hormone releasing hormone.

ing conditions, this patients were excluded from the study: the presence of active infection or gastric ulcer; recent antineoplastic therapy such as chemotherapy, radiation therapy or surgery; other primary cachectic states such as thyroid disease or severe liver disease, and chronic renal impairment, hemorrhagic stroke, subarachnoid hemorrhage (SAH), intracranial mass, ischemic stroke beyond 48 h, chronic obstructive pulmonary disease, congestive heart failure, cardiac or cancer related cachexia, hyperthyroidism, hypogonadism, cushing's syndrome, polycystic ovarian syndrome, anorexia or bulimia nervosa , fasting and chronic food restriction, obesity, type 1 DM, type 2 DM and the patients who ingesting some hormones, drug and nutrients (**Table 1**). The eligibility of patients for the study was determined by an attending emergency physician between 8:00 a.m. and midnight and by a senior emergency medicine (EM) resident from midnight to 8:00 a.m.

**Table 2.** Shows demographic data

	Patient (n=30)	Control (n=30)	P
Age (years)	69.77±1.76	66.23±2.35	P>0.05
Gender	Female 13 (43.3%)	14 (46.7%)	P>0.05
	Male 17 (56.7%)	16 (53.3%)	P>0.05

**Table 3.** Serum and Urine acyl ghrelin levels

	Patient (n=30)	Control (n=30)	P
Serum (pg/ml)	19.88±1.44	26.63±6.26	P>0.05
Urine (pg/ml)	18.20±1.96	9.80±1.19	P=0.01

### Study protocol

Data of study were collected by EM residents. The diagnose was confirmed with patient history, physical examination, cranial computerized tomography, carotid doppler ultrasound, magnetic resonance imaging (MRI) and diffusion imaging in all patients. MRI was used as the gold standard test for diagnosed with acute ischemic stroke. All patients were treated in the neurologic care unit of the university hospital.

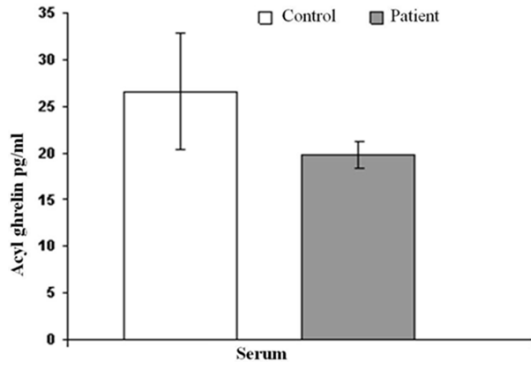
### Laboratory measurements

Blood and fresh urine (1 ml serum, 2 ml urine respectively) samples of all participants were obtained for analyze serum and urine ghrelin levels at the time of diagnose simultaneously. To prevent peptide hormones from cellular protease for each 1 ml of blood 20-30 µl aprotinin was added to each samples tube. After centrifugations, 1/10 volume ratio 1N HCL was added to derived samples, and stored at -80°C until they were assayed [10, 19, 29]. Ghrelin plasma levels were measured using an enzyme-linked immunosorbent assay (ELISA) technique with Biotec L 800 device and urine ghrelin levels were studied by validation technique.

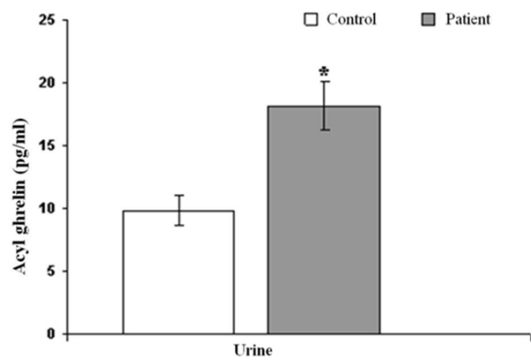
### Data analysis

The study data were analyzed by descriptive statistical methods (mean ± standard deviation). To compare quantitative data student's t test, and for comparison of qualitative data, chi-square and Fisher's Exact Chi-square test was used. P value <0.05 was considered significant.

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**Figure 1.** Shows variation between patient and control groups serum acyl ghrelin levels.



**Figure 2.** Shows variation between patient and control group's urine acyl ghrelin levels.

### Results

A total of 30 patients with acute ischemic stroke were admitted to the ED during the study period. The patients included 13 females (43.3%) and 17 men (56.7%). The control group included 14 females (46.7%) and 16 men (53.3%). The study subjects had a mean age of  $69.77 \pm 1.76$  (50-86) for patient and  $66.23 \pm 2$  (35-88) for control group, there were no significant statistical age and gender differences between both groups ( $P > 0.05$ ) (**Table 2**).

The serum acyl ghrelin levels were  $19.88 \pm 1.44$  pg/ml in patients and  $26.63 \pm 6.26$  pg/ml in control group. There were no statistically significant differences between both groups ( $P > 0.05$ ) (**Table 3; Figure 1**). Urine acyl ghrelin levels were  $18.20 \pm 1.96$  pg/ml in patients, and  $9.80 \pm 1.19$  pg/ml in control group. Urine acyl ghrelin levels were determined statistically significant between patient and control group ( $P = 0.001$ ) (**Table 3; Figure 2**).

**Table 4.** Serum and Urine des-acyl ghrelin levels

	Patient (n=30)	Control (n=30)	P
Serum (pg/ml)	$937.80 \pm 144.14$	$705.30 \pm 102.19$	$P > 0.05$
Urine (pg/ml)	$299.25 \pm 183.74$	$127.67 \pm 31.57$	$P > 0.05$

The serum des-acyl ghrelin levels were  $937.80 \pm 144.14$  pg/ml in patients and  $705.30 \pm 102.19$  pg/ml in control group. There were no statistically significant differences between both groups ( $P > 0.05$ ) (**Table 4; Figure 3**). Urine des-acyl ghrelin levels were  $299.25 \pm 183.74$  pg/ml in patient group, and  $127.67 \pm 31.57$  pg/ml in control group. There was no statistically significant difference between both groups ( $P > 0.05$ ) (**Table 4; Figure 4**).

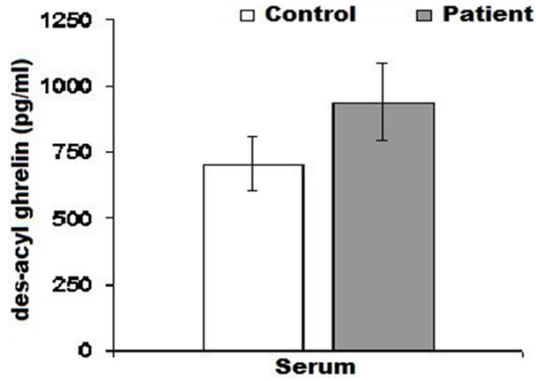
### Discussion

In present study, we observed that urine acyl ghrelin levels were determined statistically significant between patient and healthy control subjects, whereas there were no statistically significances between urine des-acyl ghrelin levels and serum acylated and des-acylated ghrelin values in both groups. To the best of our knowledge, because this is first study in literature analyzing of urine acyl and des-acyl ghrelin levels in patients with acute ischemic stroke, these results may be important.

Takeda et al. found that administration of ghrelin subcutaneously to acute renal ischemic rats showed that the ghrelin protective effects realized through increasing serum IGF-1 levels [30]. El Eter et al. investigated acute gastric ischemia and reperfusion injury generated by clamping rat celiac artery and reported that ghrelin protects gastric mucosa against ischemic injury of the stomach through its antioxidant properties [31]. Miao et al. found that the expression of ghrelin's receptor GHSR-1a in cerebral cortex of rat were obviously decreased by ischemia/reperfusion injury and increased by ghrelin (i.v.). They also showed that ghrelin (i.v.) prevented cortical neurons from injury induced by in vivo ischemia/reperfusion [25]. Massadi et al. reported that, pre-administration of a GHSR1a antagonist completely reversed the neuroprotective effect of acylated ghrelin but not des-acyl ghrelin, suggesting that des-acylated ghrelin has neuroprotective effects independent of GHSR1a [18].

Krazewski et al. conducted a study concerning with ischemia and reperfusion injury on ani-

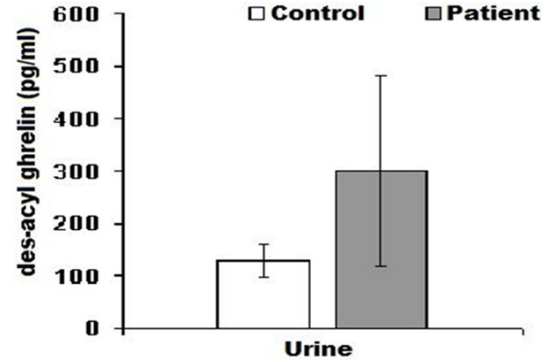
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**Figure 3.** Shows variation between patient and control groups serum des-acyl ghrelin levels.

mals, and found that ghrelin significantly reduced in cortical neuronal apoptosis [32]. Hwang et al. examined the role of proapoptotic gene the expression of which is increased after ischemic injury, in ghrelin-mediated neuroprotection during middle cerebral artery occlusion (MCAO). Finally they demonstrate that peripheral administration of acylated and des-acylated ghrelin protected cortical neurons from ischemic injury caused by MCAO. The protective effects of ghrelin and des-acyl ghrelin appear to be related with an inhibition of the expression of Par-4. Another probable mechanism of ghrelin and deacyl ghrelin is anti-apoptotic effects by targeting Bcl-2 protein family, inhibiting cytochrome c release and caspase-3 activity. Their data suggest that ghrelin, independent of its acylation, may have therapeutic potential for the treatment of stroke and can function as a neuroprotective agent [9]. William et al. conducted a rat study and showed that ghrelin can cross the blood-brain barrier (BBB) and plays neuroprotective role in ischemic area of the brain [33].

Cheyuo et al. studied the effect of ghrelin in permanent focal cerebral ischemia and the role of the vagus nerve in its action in rat models underwent right-sided permanent middle cerebral artery occlusion (MCAO) with or without prior bilateral truncal vagotomy and they said that human ghrelin is a neuroprotective agent that inhibits inflammation, nNOS activity, and apoptosis in focal cerebral ischemia through a vagal pathway [25]. Ersahin et al. investigate to elucidate the putative neuroprotective effects



**Figure 4.** Shows variation between patient and control groups urine des-acyl ghrelin levels.

of ghrelin in subarachnoid hemorrhage (SAH)-induced brain injury, and declared that ghrelin alleviates SAH-induced oxidative brain damage, and exerts neuroprotection by maintaining a balance in oxidant-antioxidant status, by inhibiting proinflammatory mediators, and preventing the depletion of endogenous antioxidants evoked by SAH [34].

The circulating concentration ghrelin has been found to be related to a meal pattern learning independent of the nutrient status. Total plasma ghrelin levels increase pre-prandially and decrease post-prandially [16, 22]. The mechanisms responsible for preprandial surges in ghrelin are not known completely, but they are probably triggered by the sympathetic nervous system. In rats and humans, ghrelin secretion varies markedly throughout the day, with peaks preceding food intake. The nocturnal increase in plasma ghrelin concentration is blunted in obese subjects and by sleep deprivation. It is suggested that the depth and duration of prandial suppression of ghrelin concentration is related to the number of calories ingested, and that ingested carbohydrates proteins and lipids suppress ghrelin levels which latter less effectively than others [35]. Increases in circulating ghrelin expression have also been correlated with the disease severity and sequelae. Additionally ghrelin levels correlated with the expression of proinflammatory cytokines, more specifically, TNF- $\alpha$ , IL-6 and IL-1 $\beta$  as classic markers of inflammation. Elevated levels of S-100 in plasma after head trauma, subarachnoid hemorrhage, and stroke have been correlated with the extent of brain damage and indicate blood-brain barrier dysfunction [16].

Various hormones, drugs and condition also affect serum and plasma ghrelin levels. Insulin, either oral or intravenous glucose, somatostatin and its natural analogue cortistatin, and GH suppress systemic concentrations of ghrelin. It has been recently demonstrated that plasma ghrelin is decreased in obesity and negatively correlated with BMI. However, little was known about circulating ghrelin levels in patients with stroke [29, 35].

Some studies suggested that ghrelin levels are reduced in clinical stroke populations. Miao et al. outlined that the expression of ghrelin's receptor (GHSR-1a) in cerebral cortex of rat were obviously decreased by ischemia/reperfusion injury and increased by ghrelin infusion (i.v.). In particular, ghrelin levels have been reported to be lower in male patients after cardio embolic stroke compared with the healthy controls [36, 37]. Age may be an important indicator and parallel to differences in the circulating levels of total and acylated ghrelin. Two recent studies in elderly subjects have revealed diminished levels of ghrelin in people 70 years or older and that these lower levels correlated with a decline in nutritional status [22].

Circulating ghrelin is lower in patients who have experienced stroke than in the general population when controlling for factors such as age and obesity [2]. In this study, the time of acute stroke period we found that the patients' serum and plasma ghrelin level did not changed secondary to against ischemia when compared with control subjects. Given the apparent neuroprotective properties of ghrelin, restoring circulating ghrelin levels after stroke therefore may produce significant beneficial outcomes on neurological and cognitive functions [38, 39]. The mechanisms of these effects are unclear; however, a combination of the anti-apoptotic and inflammatory modulatory effects of ghrelin may play a role.

### Conclusion

Recently, the studies about ghrelin being popular marker have increasingly continued. We suggest that ghrelin having neuroprotective properties should be infused to the patients, especially aged peoples, who experienced acute stroke episode to improve prognosis. Also, our results demonstrate that urine acyl ghrelin level elevated in patients with acute stroke. But

we do not know whether these results may be used as diagnostic or prognostic scale, comprehensive studies are needed to support these results.

### Disclosure of conflict of interest

None.

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