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Full Length Research Paper

Plasma concentration of acylated ghrelin in patients with Alzheimer's disease

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Ghrelin is a hormone produced in the stomach and tightly linked to the regulation of energy balance and cognitive processes. It may be altered in Alzheimer's disease (AD), a dementia syndrome markedly influenced by metabolic diseases. The aim of this study was to evaluate the changes in the plasma concentration of acylated ghrelin in patients with AD. The study included 71 people 37 in the case Group (23 female, 14 male) with AD and 34 controls (20 female, 14 male). The clinical symptoms of the patients based on the DAM-IV criteria were registered and cognitive function was assessed with the mini mental state exam (MMSE) which measures the severity of AD. The plasma concentration of acylated ghrelin, glucose, lipids and lipoproteins were measured by using ELISA assay kits and enzymatic methods. The plasma concentration of acylated ghrelin was significantly higher in the group with Alzheimer's disease compared with the controls and it was not associated with the biochemical parameters including glucose, lipids and lipoproteins. The results of the current study show higher plasma concentrations of AG in the people with Alzheimer's disease, and it was not associated with changes in anthropometric characteristics, plasma lipids and lipoproteins of the patients. This suggests that as a preliminary work, it could be helpful for the follow up of the disease, but more investigations are required to clarify the issue.

Key words: Alzheimer's disease, acylated ghrelin, lipids, lipoproteins.

INTRODUCTION

Alzheimer's disease (AD), the most common type of dementia, is a progressive neurodegenerative disease involving many elderly people throughout the world.

Despite its high prevalence and socioeconomic burden, the cause of the disease is unknown. This disease (AD), involve more than 50% of those who have dementia. The accepted and well known theory regarding the pathogenesis of AD is amyloid plaques in the brain (Sloe, 2001). There are different reasoning concerning the pathology of brain, vascular system and the relationship between AD and the parameters of metabolic syndrome

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(Raze et al., 2007; Vanhanen et al., 2006). Ghrelin a neuropeptide with 28 amino acids is mainly involved in the regulation of appetite through which it may influence the progression of obesity and the metabolic syndrome (Kojima and Kangawa, 2005; Higgins et al., 2007). It has also been linked to inflammation and neuromodulation and for this reason, it has been proposed to play a relevant role in AD (Gahete et al., 2011). The ghrelin gene is placed on the chromosome 3P25-26 and, it is encoded as pre-pro hormone by axons 5 (Kojima and Kangawa, 2005). It also stimulates the secretion of growth hormone, ACTH and prolactin (Muccioli et al., 2002). Two types of ghrelin are present in the blood circulation; acylated (AG) and un-acylated (UAG). Acylated ghrelin is the most biologically active type which forms about 10% of the total ghrelin in the human blood (Hosoda et al., 2004). It has a unique biological structure with an acyl side chain on the third amino acid residue (Akamizu et al., 2005; Neary et al., 2006). The esterified form is attached to octanoate on the serine 3 residue, by the help of the enzyme ghrelin O- acyltransferase (Yang et al., 2008). Alzheimer's disease first destroys the neurons and causes severe cognitive defects followed by metabolic syndrome and inflammation (Erol, 2008). On the other hand, the blood concentration of AG is related to metabolic syndrome, cognitive process, inflammation and regulation of calorie uptake (Ukkola, 2009). So there could be an association between Alzheimer's disease and blood concentration of AG but more investigations will be needed to clarify that. The aim of this study was to evaluate the changes in the plasma concentration of AG in patients with Alzheimer's disease.

MATERIALS AND METHODS

This prospective case control study included 71 people 37 in the case group (23 female, 14 male) with a mean age of 75.02 ± 13.68 and 34 controls (20 female, 14 male) with the mean age of 64 ± 10.87. We included subjects who had AD for more than two years and who had no history of chronic gastrointestinal peptic, inflammatory diseases, malignancies or any acute disease. Also, we excluded the subjects who were taking drugs that may interfere with gastrointestinal motility and visceral sensitivity. The clinical symptoms of the patients based on the DAM-IV criteria was observed and registered by medical psychiatrist and mini-mental state exam (MMSE) which measures the severity of the AD and was applied on all the subjects. The results of the test were defined as below 25 (subjects having AD) and, above 25 (healthy subjects). By a well experienced and certified AD educator, the medical history of the subjects of both the groups was obtained and in the case of any intervention, they were excluded from the study. Anthropometric parameters of the subjects including age, sex, height and weight were registered. Following a 12 h fasting period, blood samples were collected by venipuncture, and EDTA- plasma and sera were obtained by centrifugation and stored at -80°C until analysis. In order to prevent the inactivation of AG by elastase and protease enzymes in the blood (Hosoda et al., 2004; Nishi et al., 2005), 1.5 ml of the blood from each sample was immediately transferred into the vacutainer tubes containing EDTA-2 Na (1 mg/ml) and 500 U/ml of aprotenin was added to all the tubes. Then, the samples were centrifuged for 10 min at 1500 rpm, and the plasma was separated and analyzed for acylated ghrelin using ELISA Kits from Kamiya Biomedical Company and the DRG Instrument Gmbh, Germany. Meanwhile, this method measures mainly octanoylated human ghrelin. Glucose was assayed by an enzymatic (glucose oxidase) colorimetric method using a commercial kit (Pars Azmun Inc., Tehran, Iran). Serum total cholesterol, HDL-cholesterol and triglyceride were measured by enzymatic method. A Selectra-2 autoanalyser (Vital Scientific, Spankeren, Netherlands) was used for the measurements. Serum low density lipoprotein LDL-cholesterol was calculated using the Friedewald formula and uric acid was estimated by Direct, Colorimetric Test, Phosphotungestate method (ZistChem Diagnostics Tehran, Iran).

Statistical analysis

We used Kolmogrov-Smirnov test to assess distribution of variables and normality of data. Independent sample t test was used to compare quantitative variables with normal distribution. The significance of the difference in the Sex between the two groups was determined with chi-square analysis using 2 × 2 contingency tables and Fisher's exact test. Pearson's correlation coefficient was used for assessing the association of ghrelin with anthropometric parameters and biochemical variables. SPSS 15 software was used for statistical analysis and P value less than 0.05 was considered as significant.

RESULTS

There was no significant difference in demographic characteristics of the two groups including BMI and Female/male ratio ($p = 0.81$). However, with regard to age, subjects in the case group were significantly older than in the control group (Table 1).

The plasma concentration of acylated ghrelin was significantly higher in the case group compared with the controls (Table 2).

The plasma concentrations of biochemical parameters including fasting blood sugar (FBS), triglycerides (TG), cholesterol (Chol), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and uric acid showed no difference in terms of being statistically significant between the two groups (Table 2).

In multivariate analysis by multiple logistic regression method, there was a significant difference in the concentration of plasma AG (95% CI, $p < 0.0001$) after adjustment for age, sex and BMI (Table 3).

DISCUSSION

Acylated ghrelin is believed to be involved in pathophysiology of Alzheimer's disease. In this study, we have shown that the concentration of acylated ghrelin is significantly higher in the plasma of subjects with AD. Our results supports the findings from investigators suggesting that serum concentration of AG was inversely associated with cognitive functions including verbal memory, working memory and naming ,and so they showed potential role of AG in pathological cognitive

Table 1. Anthropometric characteristics of the patients with Alzheimer's disease and the control.

Parameter	Case group (n = 37)	Control group (n = 34)	P-value
Female/male ratio	23/14	20/14	0.81
Age (Years)	75.02 ± 13.68	64.75 ± 10.87	0.001
BMI(Kg/m^2)	23.51 ± 4.97	24.05 ± 4.47	0.63

The values are shown as mean ± SD, and P value is considered as < 0. 05

Table 2. Biochemical parameters in patients with Alzheimer's disease and the controls.

Parameter	Case group	Control group	P-value
FBS (mg/dl)	88.32±18.65	90.40±32.00	0.737
TG (mg/dl)	99.75±36.15	116.82±56.6	0.145
Cho (mg/dl)	186.24±41.88	201.35±44.65	0.146
HDL (mg/dl)	46.22±7.41	49.50±9.04	0.098
LDL (mg/dl)	117.3±35	129.32±35.81	0.157
Uric acid (mg/dl)	5.21±1.99	4.68±1.55	0.222
Ghrelin (pg/ml)	18.62±8.19	14.73±2.31	0.008

The values are shown as mean ± SD, and P value is considered as < 0. 05

Table 3. Unadjusted and adjusted odds ratios [OR; 95% confidence interval (CI)] between the presence of Alzheimer's disease and acylated ghrelin level.

Parameter	OR	CI	P-value
Unadjusted	0.82	0.7-0.97	< 0.023
Adjusted for age	0.76	0.63-0.93	0 < 0.009
Adjusted for age + sex	0.75	0.61-0.92	0 < 0.007
Adjusted for age + sex + BMI	0.75	0.61-0.92	0 < 0.007

decline observed in Alzheimer's disease (Spitznagel et al., 2010). However, no significant difference has been found in the plasma concentration of AG measured in 14 subjects with AD (Proto et al., 2006). Ghrelin transport depends on the primary structure of its amino acids and also presence or absence of octanole in its structure (Banks et al., 2008). The permeability of brain tissues to ghrelin depends on the affinity of transporters in passing through blood brain barrier (Diano et al., 2006). The mechanism of action of ghrelin has been given by using electrophysiological recordings, in this model; it is shown that ghrelin stimulates the activity of arcuate neuropeptide Y (NPY) neurons and acts on NPY in the para- ventricular nucleus of the hypothalamus (PVH). It has been found that the release of ghrelin at PVH may stimulate the release of orexigenic peptides and neurotransmitters, suggestive of a regulatory system of controlling the energy homeostasis (Cowley et al., 2003). Recently, investigators have identified subsets of neurons in the brain that expresses both GHSR1a and the dopamine receptor subtype -2(DRD2). They also showed that GHSR1a: DRD2 complex was present in native hypothalamic neurons that regulates appetite (Cell press, "Appetite accomplice: ghrelin receptor alters dopamine signaling" Science Daily, 26 January, 2012. Web 31 July, 2012). It has been found that apo-ghrelin receptor forms heteromers with DRD2 in hypothalamic neurons and would be essential for anorexigenic effects of DRD2 agonism (Kern et al., 2012). It has been reported that the plasma TG concentrations of more than 150 mg/dl could be a candidate for metabolic syndrome in AD ́(Kalmijn et al., 2000). Another study has shown that the concentrations of glucose and TG were higher in AD patients (Makovey et al., 2007). It has also been shown that hyperlipidemia would increase the severity of dementia (Milionis et al., 2008). However, in the present study, the plasma acylated ghrelin level was not associated with biochemical parameters including BMI, FBS, uric acid, TG, Cho, HDL-C and LDL - C in both the groups. In the present study, AD patients were significantly older than the controls. Aging and obesity are related to low concentrations of circulating ghrelin (Tschop et al., 2001). However it has been reported that these two parameters stimulate the severity of dementia which finally leads to AD (Gustafson et al., 2003). Some investigators have shown that aging may influence the

regulation of ghrelin in the blood circulation. They also observed that plasma ghrelin level is lower in healthy aged people compared to the younger subjects (Rigamonti et al., 2002). However, some investigators have not found any difference in ghrelin level between the two groups (Di Francesco et al., 2006). A clear relationship has been found between the blood concentration of acylated ghrelin and other parameters of metabolic syndrome (Mager et al., 2008; Tentolouris et al., 2004; Chu et al., 2006). However, this is not in line with the results from other investigators (Kim et al., 2008; St-Pierre et al., 2007; Barazzoni et al., 2007). One of the limitations of this study with regard to the findings is that total and unacylated ghrelin were not measured and also the age difference between the two groups was significant. Based on the present study, we conclude that the concentration of acylated ghrelin is elevated in people with Alzheimer's disease and, it is suggested that as a preliminary finding, it could be helpful for further investigations.

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