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RESEARCH

ASSOCIATION OF PERIPHERAL VISFATIN LEVELS AND VISFATIN G-948T GENE POLYMORPHISM WITH ALZHEIMER'S DISEASE IN A TURKISH POPULATION

ABSTRACT

Introduction: Visfatin is a pleiotropic cytokine implicated in several physiological and patho-physiological processes such as innate immunity, cellular metabolism, longevity, and inflammation. Altered visfatin levels and visfatin gene polymorphisms have been reported in various human diseases, including type 2 diabetes mellitus, rheumatoid arthritis, obesity, coronary artery disease and stroke.

Materials and Method: In this study, we investigated whether serum visfatin levels were changed in volunteers with Alzheimer's disease (AD) (n = 40) or healthy controls (n=40) and whether visfatin gene G-948T polymorphism was associated with the disease.

Results: Visfatin levels were not significantly different in AD patients compared with healthy aged control subjects. In this relatively small population, we found a significant, but weak association between GT heterozygous genotype at position -948 of visfatin gene and AD. However, there was no association between G-948T polymorphism and visfatin levels, and any demographic, anthropometric or biochemical parameters.

Conclusion: Further studies in larger and different populations are needed to fully elaborate the involvement of visfatin gene in AD.

Key Words: Nicotinamide Phosphoribosyltransferase; Alzheimer Disease; Polymorphism; Inflammation; Aging.



ARAŞTIRMA

TÜRK POPULASYONUNDA PERİFERAL VISFATİN DÜZEYİNİN VE VISFATİN G-948T POLİMORFİZMİNİN ALZHEİMER HASTALIĞI İLE İLİŞKİSİ

Öz

Giriş: Visfatin, doğuştan immünite, hücre metabolizması, uzun yaşam ve inflamasyon gibi pek çok fizyolojik ve patofizyolojik olaya katılan bir pleiotropik sitokindir. Yükselen visfatin düzeyi ve visfatin gen polimorfizmlerinin tip 2 diyabet mellitus, romatoid artrit, obezite, koroner arter hastalığı ve inme gibi çeşitli hastalıklarla ilişkisi daha önce gösterilmiştir.

Gereç ve Yöntem: Çalışmamızda; Alzheimer Hastalığı tanısı almış gönüllüler (n=40) ve sağlıklı kontrollerde (n=40), serum visfatin düzeyinin değişimi ve visfatin G-948T gen polimorfizminin AH'na yakınlıktaki rolü araştırılmıştır.

Bulgular: AH hastalarının serum visfatin düzeylerinde sağlıklı kontrollere kıyasla anlamlı bir fark bulunamamıştır. Bu göreceli küçük grupta; visfatin geninin -948 pozisyonundaki GT heterozigot genotipi ile AH arasında belirgin ancak zayıf bir ilişki bulunmuştur. Ancak yine de G-948T polimorfizmi ile serum visfatin düzeyleri, veya herhangi bir demografik, antropometrik ya da biyokimyasal veri arasında bir ilişki bulunamamıştır.

Sonuç: Visfatinin AH ile ilişkisini kesin olarak belirleyebilmek için daha geniş ve farklı populasyonlarda yapılacak ileri araştırmalara ihtiyaç duyulmaktadır.

Anahtar Sözcükler: Nikotinamid Fosforibosiltransferaz; Alzheimer Hastalığı; Genetik Polimorfizm; İnflamasyon; Yaşlanma.



INTRODUCTION

Alzheimer's disease (AD), the most common cause of dementia, is characterized by loss of memory and cognitive function associated with deposit of amyloid fibrils and neurofibrillary tangles in specific areas of the brain (1). Definitive diagnosis of AD can only be achieved by post-mortem pathological examination of the brain. Thus, there is a significant need for reliable biological markers that bring added value to the clinical diagnosis. AD is the most prevalent neurodegenerative disease and the interplay between multiple environmental and genetic susceptibility factors play a key role in the pathogenesis (2). Among various factors, inflammation seems to play a major role in the pathogenesis of AD (3). Polymorphism of pro-inflammatory genes could be a risk factor for disease progression as was supported by an association between proinflammatory cytokine tumor necrosis factor alpha (TNF- α) polymorphism and the risk of AD (4). Metabolic dysfunction may also increase disease risk as suggested by epidemiological and basic science evidence of a comorbidity and possible shared pathophysiology between type 2 diabetes mellitus (T2DM) and AD (5). Thus, genetic dissection of inflammatory and metabolic pathways may shed light on the pathogenesis of AD.

Visfatin [also known as pre-B cell colony-enhancing factor (PBEF) or nicotinamide phosphoribosyltransferase (NAMPT)] is a novel pro-inflammatory adipokine expressed mainly in visceral fat. Besides its role in innate immunity, visfatin also has a key role in the regulation of cellular metabolism, as a rate-limiting enzyme that catalyzes the first step in the biosynthesis of nicotinamide adenine dinucleotide (NAD) from nicotinamide (6). Plasma visfatin levels were found to be elevated in several diseases, including T2DM (7), rheumatoid arthritis (8) and inflammatory bowel disease (9). Nucleotide variations in visfatin gene were evaluated in obesity, T2DM and coronary artery disease to find out whether it may affect disease risk in humans (3,10,11). The visfatin promoter polymorphisms have been reported as a linkage with chronic, low-grade inflammation in T2DM (11). One of the most frequently studied single nucleotide polymorphisms (SNPs), G-948T polymorphism was reported to be associated with the increased high-density lipoprotein (HDL) in obese subjects and higher plasma fibrinogen levels and higher C-reactive protein (CRP) levels in T2DM (10,11).

To the best of our knowledge, no data have been published on visfatin levels in AD and an association of the visfatin gene polymorphisms with the risk of AD. Here, we postulated that visfatin levels would be different in patients with

AD from controls, making it a potential biological marker. To test this hypothesis, serum visfatin levels were measured in patients with clinically diagnosed AD and aged control subjects. We also investigated the possible association between G-948T polymorphism in visfatin gene and its influence on the risk of AD.

MATERIALS AND METHOD

Study Groups

Forty patients diagnosed with AD according to NINCDS-ADRDA (12) criteria from dementia outpatient clinic at Dokuz Eylul University Hospital and 40 healthy elderly subjects were included. Healthy aged subjects were enrolled as control cases (n = 40). All subjects received complete neurological and medical examinations. In order to exclude the presence of cognitive impairment, all control subjects underwent neuropsychological evaluation (MMSE: mini mental stage examination, RAVLT: Rey auditory verbal learning test, Benton facial recognition test and category fluency). Comprehensive cognitive evaluation was also performed in patients with AD. Blood samples were taken after written informed consent had been obtained from the participants or their representatives. As an anthropometric parameter, body mass index (BMI) was measured in all subjects. The protocol of this study has been approved by Local Ethical Committee with 190/2009 protocol number (11/06/2009) and all participants provided written, informed consent.

Laboratory Measurements

Fasting blood glucose, low-density lipoprotein (LDL), HDL, triglyceride, total cholesterol were measured by standard commercial methods on autoanalyzer (Hitachi 902 Autoanalyzer, Tokyo, Japan) and CRP levels were determined by nephelometric method (Beckman Coulter 800 Immage, Fullerton, CA)

Measurement of Serum Visfatin Level

Blood samples were centrifuged for 10 minutes at 3,000 rpm, and the sera were frozen at -80° C until assay. Serum visfatin levels were determined by a commercially available ELISA kit (BioVision, Mountain View, CA, USA) according to the manufacturer's protocol.

Genotyping of Visfatin SNP

DNA was extracted from blood samples using the Nucleospin Blood Kit (Macharey-Nagel, Düren, Germany) according to



the manufacturer's procedure. Visfatin G-948T polymorphism was genotyped by real-time PCR and high-resolution melting curve analysis on the Light Cycler 1.5 System according to the Light Cycler Easy Start DNA Master Hybprobe Kit (Roche Diagnostics, Switzerland). The primers used for melting curve analysis are Forward primer: 5'-ttgatcctttgagagatg-gtttgac-3', Reverse 1 primer: 5'-agcaaagagcctgcgttg-3' and Reverse 2 primer: 5'-tggcccttgcatcttct-3'. Briefly, 5 μ l of the DNA extract was mixed with 15 μ l of Light Cycler Easy Start DNA Master Hybprobe, 2 μ l of 0.1 μ mol/l primers and 2 μ l of 0.4 μ mol/l probes for a 20 μ l single reaction according to the manufacturer's protocol. The following PCR protocol was used: denaturation at 95 °C (10 s), annealing at 57 °C (10 s), and extension at 72 °C (6 s) for 45 cycles with final melting conditions at 95 °C (30 s) and 40 °C (2 min). Each allele melts at a specific temperature, therefore heterozygote samples showed two peaks, one at each temperature representing the combination of both alleles.

Statistical Analyses

All statistical analyses were done using SPSS, version 18.0. The results were expressed as mean \pm SD. Group comparisons were analyzed using an unpaired two tailed t test. Differences in genotype and allelic distribution among the patients and healthy controls were determined using Pearson Chi-squared test, with the OR and 95% CI calculated subsequently. Spearman rank correlation was used to determine correlations of visfatin levels with other variables. Additionally, variable selection for multivariate linear regression was used to test for significant relations in visfatin levels with adjustment for possible confounders. The level of significance in all tests was set at $p < 0.05$.

RESULTS

In this case control study, a total of 80 subjects, which included 40 AD patients and 40 controls, were studied for G-948T polymorphism in visfatin gene. Serum visfatin levels were also measured in all subjects. Table 1 shows the demographic, clinical and biochemical profiles of the control subjects and AD patients. Although the control group included elderly people, the control subjects were younger than the patients ($p=0.01$). Other known risk factors, such as BMI, lipid profiles, fasting glucose, and CRP levels were not different between the study groups (Table 1).

Table 1— Demographic, Neurocognitive and Biochemical Features of Study Groups.

Variable	Control (n=40)	AD Patients (n=40)	p
Gender (Female/Male)	28/12	27/13	0.809
Education (year)	7.37 \pm 4.43	7.14 \pm 5.14	0.825
MMSE	28.58 \pm 1.55	18.65 \pm 7.49	0.001<
RAVLT	102.38 \pm 13.25	43.58 \pm 22.82	0.001<
Benton Facial Recognition Test	45.95 \pm 5.75	33.35 \pm 15.04	0.001<
Category Fluency	18.30 \pm 4.91	10.23 \pm 5.53	0.001<
Body Mass Index	28.76 \pm 5.30	27.02 \pm 4.16	0.095
Fasting Blood Glucose (mg/dL)	101.05 \pm 16.89	109.72 \pm 31.95	0.134
LDL (mg/dL)	124.30 \pm 31.51	113.60 \pm 34.41	0.153
HDL (mg/dL)	56.28 \pm 17.46	51.07 \pm 16.25	0.172
Triglyceride (mg/dL)	156.60 \pm 62.73	171.10 \pm 93.52	0.417
Cholesterol (mg/dL)	214.42 \pm 39.31	206.02 \pm 45.35	0.379
CRP (mg/dL)	4.60 \pm 1.00	12.02 \pm 7.47	0.329
Visfatin (pg/ml)	873.00 \pm 1630.46	775.90 \pm 815.3	0.736

Data are presented as mean \pm SD.

Visfatin Gene G-948T Polymorphism

The association of visfatin gene G-948T polymorphism with the risk of developing AD was assessed. The genotype distributions were in Hardy-Weinberg equilibrium in both groups. We found only a weak association between GT heterozygous genotype and AD. GT heterozygous genotype was significantly more frequent in AD patients than the control group ($p = 0.048$). Additionally, minor allele carriers were compared with participants homozygous for the major allele, studying possible associations with clinical parameters because of the small number of participants homozygous for the minor allele ($n=2$). We found that duration of illness was longer in AD patients carrying the major allele ($n=22$) than those carrying the minor allele ($n=18$) (5 ± 0.75 versus 2.89 ± 0.41 , $p=0.016$). In addition, category fluency scores of AD patients carrying the major allele were lower than those carrying the minor allele (8.59 ± 1.2 versus 12.2 ± 1.1 , $p=0.025$).

Serum Visfatin Levels

There was no significant difference in serum levels of visfatin between AD patients and control subjects (Table 2). We evaluated the relation between the clinical and laboratory findings and serum visfatin protein levels. We did not find any



Table 2— Visfatin G-948T Allele and Genotype Frequencies in AD Patients and Control Subjects

	Control (n=40)	AD (n=40)	χ^2	OR	95% CI	p
Allele						
G	68 (0.85)	60 (0.75)	2.500	0.529	0.239-1.173	0.114
T	12 (0.15)	20 (0.25)				
Genotype						
GG	30 (0.75)	22 (0.55)	3.897	0.367	0.133-1.008	0.048
GT	8 (0.20)	16 (0.40)				
TT	2 (0.05)	2 (0.05)	0.000	0.733	0.096-5.616	1.000

Figures in parentheses indicate frequencies. OR = Odds ratio; CI = confidence interval.

correlation between serum visfatin levels and the clinical or laboratory parameters in each study group. In the multivariate analysis with age-, sex-, BMI-, lipid profile-, fasting glucose-, and CRP- adjusted basal visfatin concentration as a dependent variable, there were no independent predictors in the model ($p>0.05$). In addition, no association between serum visfatin levels and visfatin gene G-948T polymorphism was observed ($p>0.05$).

DISCUSSION

In this study, we investigated whether serum visfatin levels significantly differed in AD patients in a Turkish population. We found that visfatin levels were not different in patients and healthy aged controls. Moreover, there was no significant association between serum visfatin levels and clinical or biochemical data in both study groups. Visfatin is an adipocytokine with predominant expression in the visceral fat that exerts insulin-like action on glucose metabolism through direct interaction with the insulin receptor (13). Although, visfatin is mainly secreted by adipocytes, various cell types throughout the body such as lymphocytes, monocytes, neutrophils express and secrete this adipocytokine. Alterations in the circulating visfatin levels have been demonstrated in several human diseases, including T2DM (7), rheumatoid arthritis (8), inflammatory bowel disease (9) and stroke (14). Although several studies in patients with obesity or T2DM found a positive or negative correlation between visfatin levels and HDL and LDL levels (15-17), there was no significant association between serum visfatin levels and biochemical parameters in our study. Neither AD patients nor control subjects in our study showed any glucose and lipid profile abnormalities. Lu et al. reported a positive association between plasma visfatin and CRP levels in stroke patients

(14). Since CRP is an acute phase protein, lack of any correlation between visfatin and CRP levels in AD patients may be a reflection of the differences in acute and chronic inflammatory processes.

In this case-control study, we found a significant, but weak association between GT genotype at position -948of visfatin gene and AD. However, there was no association between this polymorphism and biochemical parameters. In addition, no association between serum visfatin level and visfatin G-948T polymorphism was observed. The human visfatin gene is located on chromosome 7q22.2, which is composed of 11 exons and 10 introns, spanning 34.7 kb of genomic DNA, and its promoter contains several transcription factor binding sites (18). Several visfatin gene polymorphisms were studied in various human diseases, such as coronary artery disease (11,19), obesity (10,20) and T2DM (11). In contrast to our findings, various effects of different visfatin gene polymorphisms on lipid profile and glucose metabolism were shown in several studies. A correlation was shown

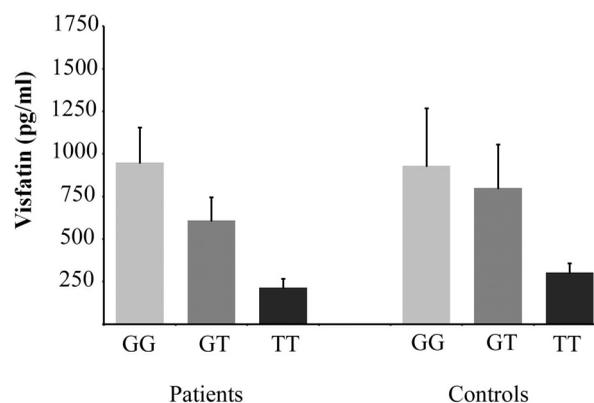


Figure 1— Serum visfatin levels in controls and AD patients for each genotype separately.



between the variety of promoter of visfatin gene and increase of plasma insulin and plasma glucose levels (21). Moreover, Johansson et al. showed that obese visfatin G-948T variant allele carriers had a higher HDL level (10). But, G-948T polymorphism had no major effect on phenotypes associated with obesity. A correlation was shown between plasma insulin and glucose levels with the increase of the T-allele carriers and G-948T polymorphism (10).

Current understanding of the physiological role of visfatin in the central nervous system (CNS) is very limited. A very recent study by Wang et al. has demonstrated that visfatin is expressed in rodent brain tissue (mainly neuronal) and cultured neurons and astrocytes (22). In murine brain, visfatin is highly expressed in neurons, but not in glial cells (23). In humans, visfatin is also present in cerebrospinal fluid (CSF) at concentrations that are 10% of those in plasma and decreases with rising body fat (24). It is not yet known whether the peptide penetrates the CNS from the circulation or is secreted by the CNS cells. Assessment of protein concentrations (eg, amyloid beta 1-42, total tau, phosphorylated tau) in CSF are diagnostic tools that are not widely available. AD biomarker discovery studies emphasize the analysis of CSF because of its close association with the brain (7). Thus, measurement of CSF visfatin levels as a reflection of ongoing neurodegenerative process in the CNS might be more realistic. In the present study, we could not evaluate CSF visfatin levels because of the invasive nature of lumbar puncture procedure due to ethical concerns and low patience levels of the AD patients and elderly people.

The role of visfatin in neurological diseases still remains elusive. Elevated visfatin levels are associated with ischemic stroke in the Chinese population (14). Two experimental stroke studies revealed the neuroprotective action of visfatin in ischemic stroke (22,23). In vivo cerebral ischemic injury and oxygen-glucose deprivation in cultured neurons upregulate visfatin expression, possibly as a compensatory process (19). These findings suggest prosurvival action of intraneuronal visfatin via energy balance regulation, overcoming its systemic inflammatory action in the stroke setting. As a multifunctional protein, visfatin plays an important role in immunity, metabolism, aging, inflammation, and responses to stress. Both intracellular (enzymatic) and extracellular (cytokine like) functions are responsible for its relevance in immune, metabolic and stress responses. Its intracellular functions concentrate on the regulation of the activity of NAD-consuming enzymes such as various sirtuins, thereby also promoting cell survival. Its cytokine functions are main-

ly pro-inflammatory as it potently induces various other pro-inflammatory cytokines such as TNF α , interleukin-1 beta (IL-1 β) or interleukin-6 (IL-6). Indeed, intracerebroventricular visfatin injection induces sickness responses in rats, reflecting its proinflammatory role in the brain (25). More basic research is needed to clarify the definite role of visfatin in neurological diseases such as AD.

Several studies in human diseases found alterations in circulating visfatin levels suggesting that this adipocytokine could be a promising biomarker with diagnostic and prognostic significance (13). Current peripheral blood biomarkers are primarily based on the detection of components derived from amyloid plaques and NFTs. Since a chronic inflammatory process has been implicated in the pathogenesis of AD, putative peripheral inflammatory markers are also extensively investigated. As a pro-inflammatory adipocytokine, visfatin has been associated with several inflammatory diseases (8,9). However, circulating visfatin levels have not been investigated in AD. In this study, we found that visfatin levels were not different in the patients and healthy aged controls. Independent replication of this finding with further studies in a larger population is necessary before excluding the possible use of circulating levels of visfatin as a biomarker in AD.

To our knowledge, this is the first study investigating a possible association of circulating visfatin levels and visfatin G-948T gene polymorphism with AD. We found a significant, but weak association between GT genotype at position -948 of visfatin gene and AD. In addition, duration of the disease was longer and category fluency scores were lower in AD patients carrying the major allele than those carrying the minor allele. Although the results of the present study suggest that visfatin G-948T gene polymorphism might confer a minor risk for the development of AD, further confirmation studies with larger groups in different populations are needed before considering these findings conclusive.

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