ORIGINAL ARTICLE

Genetic Variability of Omentin-1 Gene in Apparently Healthy Population

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ABSTRACT

Objective: Omentin-1 is a recently identified adipokine, highly expressed in visceral omental tissue with anti-inflammatory, antiatherogenic, and antidiabetic properties. Several studies have reported the association of Omentin-1 gene +326 A/T variant with different diseases such as type 2 diabetes, coronary artery disease, rheumatoid arthritis, psoriasis, and breast cancer in different populations. Therefore, the present study was designed to assess the frequency of omentin-1 gene +326 A/T variant in the apparently healthy Pakistani population.

Methodology: This cross-sectional analytical study was conducted at two tertiary care hospitals of Karachi. Participants were recruited from January 2016 to August 2016. The study group comprised of 110 apparently healthy individuals including doctors, nurses, lab technicians, and patient's attendants. Omentin-1 gene +326 A/T variant was determined by polymerase chain reaction-restricted fragment length polymorphism method.

Results: In this study, the wild type genotype (AA) was observed to be 53.6%, mutant genotype (TT) was found to be 6.4%, while mutant genotypes (AT) was found in 40 %. However, the frequency of (AT) mutant genotype was found in 75% males.

Conclusion: The prevalence of AT genotype in 75% of apparently healthy males is a significant finding of the current study. This data may help in the evaluation of population-based risk factors for a number of diseases associated with Omentin-1 gene +326 A/T variant.

Key words: Adipose tissue, Genotype, Omentin-1, Polymorphism

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عنوان: بظاہر صحت مند نظر آنے والے افراد میں omentin-1 جین کا جینیاتی تغیّر۔

تعارف: اس تحقیق کا مقصد پاکستان کے بظاہر صحت مند نظر آنے والے افراد میں omentin-1 +326 A/T جین کی موجودگی اور اس خاص جین کے مختلف پیرامیٹرز کے ساتھ تعلق کی جانچ کرنا ہے۔اس سے حاصل ہونے والی معلومات کا دنیا کے مختلف آبادیوں سے موازنہ بھی اس تحقیق کا حصّہ ہے۔

طریقہ کار: کراچی کے دو بڑے تیسرے درجے کے تدریسی ہسپتالوں میں جنوری 2016 سے اگست 2016 کے دوران یہ تحقیق کی گئی۔جس میں بظاہر صحت مند نظر آنے والے ڈاکٹرز، نرسز، لیب ٹیکنیشنز اور تیمارداروں پر مشتمل 110 افراد کو انکی اجازت سے شامل کیا گیا۔ Omentin-1 gene polymorphism کا تخمینہ PCR-RFLP طریقے سے کیا گیا۔

نتائج: مشاہدات میں genotype AA کی مقدار 53.6%، genotype TT 6.4% اور genotypes AT 40.0% ریکاڈ کی گئیں۔جبکہ مرد حضرات میں AT mutant genotype کی مقدار 75% ریکاڈ کی گئی۔ AT genotype اور triglycerides levels اور waist circumference کے ساتھ خاندانی دل کے امراض کے درمیان مثبت تعلق پایا گیا۔

حاصلِ مطالعہ: مرد حضرات میں AT mutant genotype کی 75% مقدار کے نتائج اس تحقیق کی اہم معلومات ہیں۔حاصل ہونے والی معلومات آیندہ نسل میں Omentin-1 جین سے منسلک کئی بیماریوں کے تحفظ میں مددگار ثابت ہوسکتی ہے۔

INTRODUCTION

Adipose tissue is no longer considered to be an inert tissue that just stores excess energy but it serves as a main endocrine tissue with miscellaneous functions and cellular configuration[1]. Adipose tissue is capable of secreting several biologically active hormone like proteins called adipokines[2]. The association of these proteins has been established in various physiological

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processes including inflammation, hunger, energy metabolism, insulin resistance, immunity, and angiogenesis[3]. There is a greater possibility of developing cardiac disease, diabetes mellitus, and other metabolic disorders with expansion of visceral fats as compared to subcutaneous fats; however, both types of this tissue are involved in production of a unique profile of adipokines[4]. Omentin-1, a 34kDa protein consisting of 295 amino acids, is a newly identified adipokine which is mainly secreted by stromal vascular cells of visceral omental fat[5,6]. Omentin-1 is an anti-inflammatory protein that is highly expressed in epicardial adipose tissue (EAT) around the heart and coronary arteries[7,8]. The expression of omentin-1 in the heart, lungs, ovary, and placenta has also been reported, but its role in these organs is not yet completely known. Omentin protein exists in two forms: omentin-1 and omentin-2; however the major circulating isoform is omentin-1. Its plasma level is 100 ng/ml to 1 ug/ml[8,9]. Omentin-1 has anti-inflammatory, anti-atherogenic, and anti-diabetic properties. It may improve insulin sensitivity in human adipocyte, myocytes, and hepatocytes via activation of AMPK/Akt pathway[10]. Furthermore, in vitro studies have shown that omentin-1 causes inhibition of tumor necrosis factor Alfa (TNFá) induced degradation of IkB and NF-kB (nuclear factor kappa B) activity to reduce inflammation[11]. Omentin-1 serum level is observed to be lower in obese subjects[12] and type 2 diabetic patients[13]. The Omentin-1 gene contains 1269bp, with 8 exons and 7 introns, localized at 1q22-q23 position[14]. In 2007, Val109Asp single nucleotide polymorphism in exon 4 of omentin-1 gene was reported and +326 A/T nucleotide was declared to be polymorphic[15]. Several studies have discussed the association of this single nucleotide polymorphism (SNP) in different diseases such as type 2 diabetes[15], coronary artery disease[20], rheumatoid arthritis[19], psoriasis[9], and breast cancer[17]. The frequency of +326 A/T nucleotide variant has been described from several world populations so, it is quite important to know the prevalence of such a clinically significant gene polymorphism in the healthy population of Pakistan. Current study is the first study, to the best of our knowledge, that has observed the prevalence omentin-1 gene +326 A/T variant in apparently healthy and non-symptomatic individuals of Pakistan. The data will help in future studies in finding other disorders associated with omentin-1 gene +326 A/T variant.

METHODOLOGY

The current study was approved from the ethical review board of Dr. Abdul Qadeer Khan Institute of Biotechnology and Genetic Engineering (KIBGE). A total of 110 healthy individuals were recruited in the Civil hospital Karachi (CHK) and the Karachi Institute of Heart Diseases (KIHD) from January 2016 to August 2016. Sample size was calculated using open epi software. Convenient sampling was used to select the participants. All the selected healthy participants, including doctors, nurses, lab technicians, and patients' attendants, were informed about the methods and significance of study. Written Consents were taken from all participants. Information about age, gender, family history, ethnicity, and past medical history was recorded through proforma. The blood pressure of the patients was measured via defined protocol. Weight, height, and waist circumference were also measured. Weight and height were measured to the nearest kilogram and centimeter, respectively. BMI was calculated by standard formula (kg/m^2). Blood sample was drawn from brachial vein in early morning and transferred to blood collection tube containing anticoagulant EDTA (ethylene diamine tetra acetate).

Subjects with history of infectious diseases in the previous four weeks, taking anti-inflammatory drugs, statins, diabetes mellitus, heart disease, malignancy, known renal or hepatic disorder were excluded from the study.

DNA Extraction: Whole blood was used to extract genomic DNA via salting out extraction method. Nano-drop (Thermo Scientific USA) was used for quantifying the extracted DNA whereas integrity was checked by horizontal gel system by resolving 2 µl genomic DNA samples on 0.8% agarose gel.

PCR Analysis: Polymerase chain reaction[16] was performed using F-primer5-GAGCCTTTAGGCCATGTCTCT-3' and the R- primer 5'- CTCTCCTTCTTCTCCAGCCCAT-3'[15]. Total volume of 50 µl was prepared for PCR, consisting of genomic DNA, 2 units of Taq DNA polymerase, 1.5mM MgCl, 0.2 mM dNTPs, and 1X PCR buffer of pH-8.3. Initial genomic DNA pre-denaturation was done at the temperature of 94°C for 5 minutes. Denaturation phase consisted of 35 cycles at 94°C, annealing phase had 40 seconds at 62°C followed by 60 second extension phase at 72°C. The final extension time was 5 minutes at 72°C. Amplified PCR product of 471 bp was resolved on 2% of agarose gel and visualized by gel doc system (Fig. 1).

Restriction Fragment Length Enzyme Analysis: PCR products were purified and treated with 10U of restriction enzyme AccI (Molecul-on, New- Zeeland) and placed in incubator for 16 hours at 37 ^{o} C. Digested product of PCR was analyzed by Gel documentation system. TT homozygous individuals had shown two

fragments of 274 and 197 bp, AA homozygous individuals had single fragment of 479 bp while AT heterozygous individuals displayed three bands of 471, 294 and 197 bp (Fig 2).

Statistical analysis: Data were stored and analyzed using IBM-SPSS version 23.0. Count with percentages given for gender, ethnicity, genotype, history of diabetes, hypertension, smoking, and family history of heart disease. Spearman rank correlation was used to see the relationship of genotype and studied parameters. P-values less than 0.05 were considered significant.

RESULTS

A total of 110 participants were recruited for the current study. Seventy seven (75%) males while 33 (25%) females with mean age 49.22 ±6.40 years were included in this study. Total 51.8% data was received from Urdu speaking ethnic group, 26.4% was from Pashto speaking, 4.5% were Balochi speaking, and 17.3% belonged to the Punjabi ethnic group. The mean body mass index was 22.90 ±3.48 kg/m^2, whereas, mean waist circumference was 44.68± 9.95 inches (Table 1).

Table 1: Baseline Characteristics of Studied Subjects (n=110)

Characteristics		n	%
Ethnicity	Urdu	57	51.8
	Pashto	29	26.4
	Balochi	5	4.5
	Punjabi	19	17.3
Gender	Male	77	70.0
	Female	33	30.0
Age (Years)	Mean ±SD	49.22 ±6.40	
BMI (Kg/m2)	Mean ±SD	22.90 ±3.48	
WC (inches)	Mean ±SD	44.68 ±9.95	
Exercise	Yes	37	33.6
Smoking	Yes	54	49.1
Family with heart disease	Yes	61	55.5

In the total of 110 samples, frequency of AA, AT and TT genotypes was found to be 53.6%, 40%, and 6.4% respectively, whereas the frequency of A and T alleles was 78% and 22% respectively (Table 2).

Table 2: Frequency Distribution of Genotype (n=110) and Alleles of Omentin-1 Gene

Gene	n	%
AA	59	53.6
AT	44	40.0
TT	7	6.4
Allelic frequency		
A allele		78
T allele		22

The Spearman's Rank Correlation was applied to analyze the correlation between heterozygous mutant AT genotype and studied parameter. It was found that AT mutant genotype positively correlated with waist circumference, serum triglyceride levels, and family history of heart diseases, however, negative correlation of AT genotype was observed with BMI, FBS, and HDL-C but it was not statistically significant. No correlation was found with gender, ethnicity, junk diet, exercise, and smoking (Table 3 and 4).

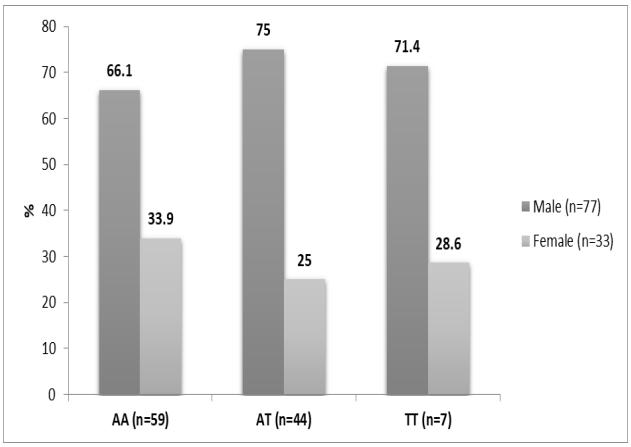
Table 3: Correlation of AT Genotype with Studied Parameters

Parameters	AT Genotype Correlation	p-value
Age, years	0.008	0.93
Waist circumference, inches	0.197	<0.01*
BMI, Kg/m2	-0.010	0.91
FBS mg/dl	-0.088	0.36
T/G mg/dl	0.273	<0.01*
Cholesterol mg/dl	0.040	0.67
HDL mg/dl	-0.032	0.72
LDL mg/dl	0.088	0.36

Quantitative parameters	Genotype Correlation	p-value
Gender	0.086	0.37
Ethnicity	0.124	0.19
Exercise	0.100	0.29
Diet	0.058	0.55
smoking	0.042	0.66
Family history of CAD	0.209	0.02*

*p<0.05 was considered significant for Spearman Rank Correlation

Bar chart 1 is showing the frequency of AA, AT, and TT genotypes in males and females. Seventy-five percent males were found with AT mutant genotype.



Bar chart 1: Genotype distribution of omentin-1 gene with respect to gender

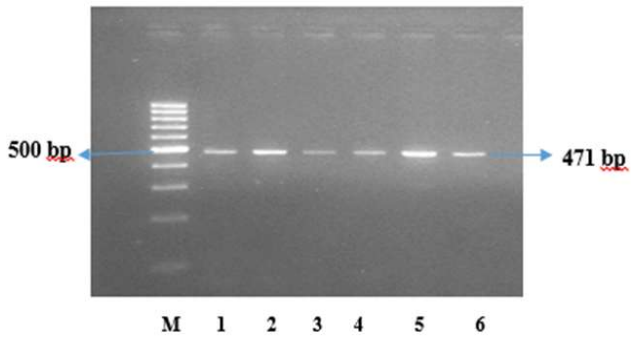


Figure 1| Lane 1-6 PCR product of omentin-1 gene (471 bp)
M = DNA molecular weight marker (100 bp)

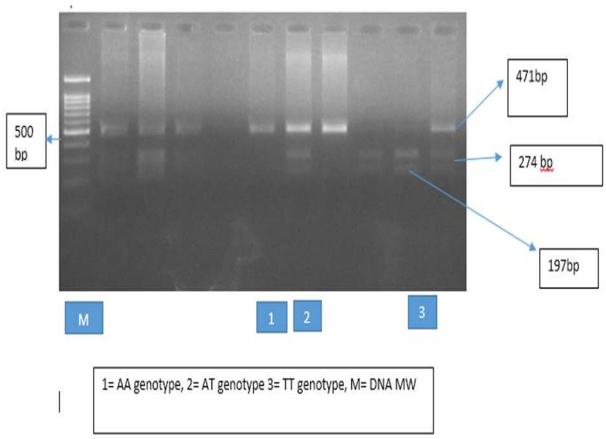


Figure 2. RFLP-PCR analysis

DISCUSSION

In the present study, 110 healthy subjects (33 females and 77 males) were recruited and analyzed for +326 A/T variant in exon 4 of omentin-1 gene. The *omentin-1 +326 A/T* revealed the substitution polymorphism in which there is replacement of A nucleotide by T at position no 326. Allele A is the wild type and allele T is the mutant type. It was found that 59 healthy subjects had wild type homozygous AA genotype, 7 individuals had homozygous TT genotype while the rest of the 44 individuals had heterozygous AT mutant genotype.

The current study revealed the prevalence of 53.6% AA as compared to 40.0% AT genotype in healthy individuals. Surprisingly, AT genotype was found in 75% of male population, so, this data is showing that males are at more risk of developing disorders associated with 326 A/T variant.

While finding correlation between AT mutant genotype with different studied parameters, it was observed that high T/G and waist circumference was statistically associated with AT genotype. Shaffler et al. have revealed the association of AT genotype with T/G in diabetic patients[15], but, we were unable to find any literature which defines this relationship in healthy population. So, our study is the first study that has observed the status of T/G in apparently healthy individuals with AT mutant genotype. No data was found that has stated the association of AT genotype of omentin-1 gene, with waist circumference in apparently healthy individuals.

The current study has observed positive correlation between AT genotype with family history of coronary heart disease. Therefore, this data is determining the fact that subjects with family history of heart disease are at high risk.

While scrutinizing the global publications on omentin-1 gene polymorphism, it was found that the frequency of 'T' allele observed in the present study, was 0.22%, that was close to the result of the study conducted in Turkey (0.20%). Kyrgyz population had shown 0.44%, Iran had 0.28% and 0.37% in two different studies and Caucasian population was found with 0.76% T allele frequency. These results have revealed that the mutant T allele is less prevalent in healthy subjects of various populations. Bahadori et al. have investigated the association of (+326 A/T) polymorphism with breast cancer in Iranian women and reported that TT+AT genotype frequency was 45.6% in cases as compared to 32.7% in control[17]. Boron et al. have identified that genotype AA was more frequent in healthy women of reproductive ages and after menopause, while increased genotype TT was found in unhealthy women of the same age groups[18]. Turan et al., discovered that T allele was high in the psoriatic patients as compared to controls who showed 'A' allele[9]. Yaykasli et al. identified no TT genotype in control group of rheumatoid arthritis[19]. Yourk et al. had identified 2.5 folds increase in TT homozygous mutant genotype in patients with coronary artery disease[20]. These results reveal that overall mutant 'T' allele was found more frequently in diseased subjects as compared to wild type A allele.

CONCLUSION

The objective of the current study was to evaluate the occurrence of the clinically important omentin-1 gene variant (+326 A/T) in apparently healthy population of Pakistan. The most significant finding of this study is prevalence of AT genotype in 75% of male healthy individuals. This gene variant also proved its association with high T/G and waist circumference. So, we may conclude that our apparently healthy population may be future candidates of clinical manifestation related with this gene variant. This data will help in future

studies searching for other disorders associated with omentin-1 gene +326 A/T variant. Further studies are needed to find out whether there is a change in omentin-1 protein physiological function with mutant AT genotype of omentin-1 polymorphism among healthy individuals.

Author's contribution: Dr Shazia Nazar conceived the idea, worked on literature search, data collection, data analysis and review, worked on introduction, discussion and result, drew the conclusion from the discussion and edited the manuscript. Dr Ambreen Qamar and Dr Shayan Zoufishan worked on literature search, results, and discussion. Dr Sara Rafique reviewed the literature, worked on discussion, and edited the manuscript. All authors discussed the results and contributed to the final manuscript.

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