

# Assessment of Adiponectin Gene Variants and Protein Level in Osteoarthritis among Egyptian Population

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## ABSTRACT

**Background:** Osteoarthritis (OA) is a common joint disease influencing the majority of individuals over the age of 65. The most affected joints in OA are knees, hands, hips, and spine leading to impaired mobility in the elderly.

**Objectives:** To analyze and correlate the level of plasma adiponectin and the adiponectin G/T (rs1501299) single nucleotide polymorphism in a group of Egyptians with knee osteoarthritis (OA) and a control group.

**Materials and methods:** 130 unrelated subjects with knee OA and 120 healthy subjects were enrolled in this study. Peripheral blood was used for extraction of DNA which was used to genotype adiponectin gene G/T (rs1501299) polymorphism utilizing polymerase chain reaction followed by digestion with restriction endonuclease. Plasma adiponectin level was estimated using enzyme linked immunosorbent assay (ELISA).

**Results:** Plasma adiponectin concentration was higher in the OA subjects than the controls ( $P = 0.01$ ). For the adiponectin G/T (rs1501299) polymorphism, there was no significant difference in the genotype distribution and allele frequency of the adiponectin between the control subjects and the OA subjects ( $P > 0.05$ ).

**Conclusion:** The results suggested adiponectin G/T (rs1501299) polymorphism might not be related to the susceptibility of OA among the Egyptian subjects.

**Key words:** gene polymorphism; osteoarthritis; adiponectin; Polymerase chain reaction; restriction enzyme; ELISA

## INTRODUCTION

Osteoarthritis (OA) is a frequent degenerative irreversible joint disorder and affects most of the individuals above the age of 65 and its incidence is lower before 40.<sup>(1,2)</sup> The most affected joints in OA are knees, hands, hips, and spine leading to impaired mobility.<sup>(3,4)</sup> The major symptoms are persistent pain, unstable joint, stiffness, deformity of joints, swelling and narrowing of joint space radiographically.<sup>(5,6)</sup> The exact

pathogenesis of OA is still indefinite, but numerous risk factors are suggested to predispose to OA such as genetic factors, aging, obesity, and joint malalignment.<sup>(7,8)</sup> Accordingly, the interactions between the environmental factors and genetic elements are associated with the susceptibility of OA.<sup>(9,10)</sup>

The joint pathological changes are triggered by aging and injury and are induced by inflammatory molecules such as

those released from adipose tissue. Several adipokines such as adiponectin, visfatin, chemerin and leptin which are released from adipose tissue and have an important role in bone formation.<sup>(11)</sup> Beside the role of these adipokines in inflammation together with other inflammatory cytokines like tumor necrosis factor and interleukin-6, they play a regulatory function in the metabolism of different joint cells such as osteoblasts, osteoclasts and chondrocytes.<sup>(12)</sup>

Additionally, some adipokines are released from the fat under the patella into the synovial fluid. Adipokines bind their receptors located in many cell types in the joints leading to inflammation of the joint, degeneration of the cartilage and induce the release of matrix metalloproteinase.<sup>(13)</sup>

Genetically, polymorphisms in the adiponectin gene have been associated with OA. Biochemically, it was shown that the adiponectin is markedly increased in plasma and in joint tissues of subjects with OA and rheumatoid arthritis, and so it is reported to be a biochemical marker of arthritis.<sup>(14-16)</sup> It has been shown that aged individuals have increased amount of fat under the patella in comparison with younger individuals resulting in increased release of adipokines during aging.<sup>(17,18)</sup> Furthermore, fibroblasts in the synovium can release prostaglandin E2 and interleukin (IL)-6/8 in response to adipokines and this contributes to the progression of OA.<sup>(19)</sup> Recently, the risk of knee OA has been associated with adipokine gene polymorphisms. The human adiponectin gene is present on chromosome 3q27 and consists of three exons and two introns.<sup>(20)</sup> Several investigators have explored the association between the adiponectin gene polymorphisms and OA risk, but the results were inconsistent.<sup>(21-23)</sup> Thus, this study was carried out to assess the relationship between the adiponectin G/T (rs1501299) single nucleotide polymorphism and the risk of OA in Egyptian population. To our knowledge, this is the first research studying the ADIPOQ gene polymorphism in OA in Egyptian population.

## **MATERIALS AND METHODS**

### **Study design and Participants**

A total of 250 subjects (130 unrelated patients affected with knee OA and 120 healthy control subjects) have been selected from the outpatient clinic, Department of Orthopedics and Traumatology, Faculty of Medicine, Tanta University, Egypt. All the participants were Egyptian individuals. Both groups had been matched regarding the gender and age. The inclusion criteria for selection of controls were the absence of any signs or symptoms of OA, other types of arthritis or any other articular disease. The exclusion criteria include gouty arthritis, ankylosing spondylitis, systemic lupus erythematosus, rheumatoid arthritis, psoriasis, previous knee injury or infection, presence of autoimmune diseases, or any type of chronic or malignant illness. All subjects were selected from the same ethnic and geographical area. The diagnosis of primary knee OA was carried out according to the criteria of the American College of Rheumatology. The severity of knee OA was determined according to Kellgren-Lawrence (KL) grading system 1, 2, 3, or 4 that correspond to radiographic signs.<sup>(24)</sup> Ethical approval was obtained from the ethical committee of the Faculty of Medicine, Tanta University, Egypt. These guidelines of the IRB follow the Egyptian and International ethics and patient protection guidelines that follow the Declaration of Helsinki in 1995. Signed informed consents were obtained from all participants in the study.

### **Blood specimen collection**

Venous blood specimens were collected from all subjects in K<sub>3</sub>EDTA (tripotassium ethylene diamine tetraacetic acid) coated tubes. Blood was centrifuged at 1200 g for 5 min and the plasma was separated into a new tube. The buffy coat was utilized for preparation of genomic DNA. All samples were kept at -20°C until the assay time.

### Adiponectin ELISA assay

Adiponectin was measured in plasma utilizing commercial human adiponectin ELISA kit (ABCAM, Cambridge, USA). Assay was carried out according to the maker's instructions.

### Genomic DNA extraction

Peripheral blood was used for extraction of genomic DNA utilizing DNA preparation kit (QIAamp DNA Blood Mini Kits, Qiagen, Hilden, Germany) according to the manufacturer's instructions. Aliquots of DNA were used for PCR experiment.

### Adiponectin G/T (rs1501299) genotyping

Adiponectin polymorphism was determined utilizing the PCR-RFLP according to the procedure described previously.<sup>(25)</sup> The primers used were: 5'-ACACTGATATAACGCC ATGAA -3' as forward primer and 5'-GCAGCAAAGCCAAAGTCTTC-3' as reverse primer. The PCR program consisted

of a denaturation step at 95 °C for 5 min, 35 cycles: 94 °C 30 sec, 50 °C 30 sec, 72 °C 1 min and a final extension at 72 °C for 10 min. The amplified fragment (168 bp) was digested with BglII (Thermo Fisher Scientific) and then separated on 3.0 % agarose gel and visualized under UV light. Genotypes pattern were: T/T genotype gave a single band of 168 bp; G/G gave 2 bands of 147 bp and 21 bp and GT gave 3 bands of 168 bp, 147 bp and 21 bp.

### Statistical analysis

SPSS version 21 (SPSS Inc, Chicago, IL, USA) was utilized for data analysis. Continuous variables were analyzed using Student's t-test, while Chi-squared test was used to analyze the categorical data. Odds ratio (OR) was calculated with 95% confidence interval (CI). A *P* value <0.05 was considered statistically significant for all analyzes.

## RESULTS

### Characteristics of the study population

Table 1. Demographic, clinical data and ADIPOQ level in the study population

Characteristics	Control group (120)	Patient group (130)	<i>P</i> value
Age (years)	54.53±5.97	55.29±5.82	0.30
Gender (F/M)	83/37	91/39	0.89
BMI (kg/m <sup>2</sup> )	24.62±1.34	25.29±1.55	0.01
K-L grade			
1	-	18 (13.85%)	
2	-	45 (34.62%)	
3	-	51 (39.32)	
4	-	16 (12.30)	
Plasma ADIPOQ (µg/ml)	5.89±1.93	6.63±2.02	0.01

Data are shown as mean ±SD. *P*-value <0.05 was considered as significant.

Table 1 summarizes the demographic data, K-L grades of OA subjects and plasma adiponectin level of the study population. The mean ages of the controls and OA subjects were 54.53±5.97 and 55.29±5.82 respectively. The women to men ratios were 69.16 % and 30.84 % in the controls and were 70% and 30 % in the OA subjects. The mean value of the BMI was 24.62±1.34 and 25.29±1.55 in the controls and OA group respectively. There were no significant differences in gender, age between the controls and the OA group (*P*>

0.05). The BMI was significantly higher in the OA than the controls (*P*=0.01). The adiponectin plasma level was significantly higher in the OA than the controls (*P*=0.01).

### Adiponectin G/T (rs1501299) polymorphism

The distribution of the genotypes and allele frequency of adiponectin G/T (rs1501299) polymorphism in both control and the OA groups are summarized in Table 2. The distribution of adiponectin genotypes was in Hardy-Weinberg equilibrium in both

groups. For the control subjects, the genotypes GG, GT and TT were 52.5 %, 38.33 % and 9.17 % respectively and were 45.38 %, 43.08 % and 11.54 % respectively in the OA subjects. The percentage of G allele was 71.67 % and 66.92 % while T

allele was 28.33 % and 33.08 % in the controls and OA subjects respectively. The genotype and allele distributions of the adiponectin in both groups were indifferent ( $P > 0.05$ ).

**Table 2 Genotype and allele frequencies of adiponectin G/T (rs1501299) Single Nucleotide Polymorphism in the study groups**

Adiponectin polymorphism	Control (n=120)		OA Patients (n=130)		P value	Odds ratio	95% CI
	No	%	No	%			
<b>Genotypes:</b>							
GG	63	52.5	59	45.38		1	
GT	46	38.33	56	43.08	0.35	1.3	0.767-2.203
TT	11	9.17	15	11.54	0.518	1.456	0.619-3.425
<b>Alleles:</b>							
G	172	71.67	174	66.92		1	
T	68	28.33	86	33.08	0.311	1.33	0.809-2.188

Chi-square analysis of genotypes between patients with OA and healthy controls. \*P-value < 0.05 was considered as significant.

### Plasma adiponectin level in different genotypes

**Table 3 Comparison between adiponectin G/T (rs1501299) genotypes with respect to plasma adiponectin concentration in the study group**

Study group	Adiponectin polymorphism (rs1929992)	Number (%)	Plasma adiponectin concentration	$\chi^2$	P
Control group	GG	63 (52.5)	5.86±1.95	1.339	0.512
	GT	46 (38.33)	5.99±1.95		
	TT	11 (9.17)	6.4±1.75		
OA group	GG	59 (45.38)	6.45±2.02	1.601	0.449
	GT	56 (43.08)	6.68±2.08		
	TT	15 (11.54)	7.14±1.73		

\*Kruskal-Wallis test,  $P < 0.05$  considered as statistically significant. Data are shown as mean±SD.

Table 3 shows the adiponectin concentrations in different genotypes of the study group. The level of the adiponectin in the GG, GT and TT genotypes of the controls were 5.86±1.95, 5.99±1.95 and 6.4±1.75 while they were 6.45±2.02, 6.68±2.08 and 7.14±1.73 in the OA group respectively. The adiponectin level was indifferent between different genotypes in the controls ( $P = 0.512$ ) and the OA group ( $P = 0.449$ ).

### DISCUSSION

Osteoarthritis is a complex joint disorder, resulting from interaction of several factors including environmental and genetic factors. Strong evidence supported that genetic factors play a role in the OA risk.<sup>(10)</sup> Moreover, there is increasing evidence indicating that some adipokines released in the serum or synovial fluid are associated with the pathogenesis of OA. One of these mediators is adiponectin, which has been shown to be related OA.<sup>(26)</sup> The adiponectin single nucleotide

polymorphisms (SNPs) and their relationship with OA have been studied in some ethnic populations but the results were inconclusive.<sup>(21-23, 25)</sup> The objective of this study was to assess the relationship between the adiponectin G/T (rs1501299) polymorphism and the susceptibility to OA in a group of Egyptian subjects from Tanta environ. Moreover, we studied the impact of the genotypes of adiponectin G/T (rs1501299) polymorphism on the plasma concentration of adiponectin. To our knowledge, this is the first report that studied the relationship between adiponectin G/T (rs1501299) polymorphism and OA among Egyptians. The plasma adiponectin level was statistically higher in the OA subjects compared with the controls while the genotype and allele frequencies of the adiponectin G/T (rs1501299) polymorphism showed no difference between both OA subjects and the controls. Zhan D et al (2017) studied two single nucleotide polymorphisms, T/G (rs2241766) and G/T (rs1501299), in adiponectin gene and found

no significant association between the genotype and the allele frequencies of both SNPS between the OA group and the control group in Thai population. They observed that the plasma adiponectin level was significantly lower in OA than the controls.<sup>(22)</sup> Hamalainen et al (2018) did not find association between adiponectin G/T (rs1501299) polymorphism and the risk of hand OA in Finnish population.<sup>(21)</sup> These results were consistent with our results regarding the adiponectin G/T (rs1501299) gene polymorphism.

Shang H et al (2019) studied the association between adiponectin G/T (rs1501299) polymorphisms and found that the TT genotype and T allele were related to the risk of OA in Chinese individuals.<sup>(25)</sup> They found also that the plasma adiponectin level is statistically higher in OA group than the control group. Another recent study carried out by Jiang L et al (2018) who studied three SNPS in adiponectin gene and showed that only the rs182052 SNP may have susceptibility to knee OA in the Chinese population.<sup>(23)</sup> This contradiction in the results regarding the genotype susceptibility and the plasma adiponectin level may be explained by different genetic background between different ethnic populations, the clinical stage of the disease, different affected joints (knee and hand) and the sample size.

Furthermore, The adiponectin gene polymorphism was studied in several articular and non-articular diseases such as rheumatoid arthritis,<sup>(27)</sup> obesity,<sup>(28)</sup> Cardiovascular Disease<sup>(29)</sup> and type 2 diabetes.<sup>(30)</sup>

Nonetheless, this study is not without limitations particularly the sample size was not large enough and we studied only one SNP in the adiponectin gene. So, further research will be required using a large OA cohort including other SNPS and haplotype analysis to understand the role of the adiponectin in the pathogenesis of OA.

## CONCLUSION

We concluded that the adiponectin G/T (rs1501299) polymorphism might not

be related to the susceptibility of OA among Egyptian population, however more genetic analysis with larger sample size are necessary to ascertain the role of adiponectin in the development of OA.

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## Conflict of Interest

No conflict of interest exists.

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