ADIPONECTIN SINGLE NUCLEOTIDE POLYMORPHISM (+276G/T) AND ITS POSSIBLE RELATION TO ADIPONECTIN LEVEL IN EGYPTIAN PATIENTS WITH CORONARY ARTERY DISEASE

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Received 3/7/2012– Accepted 15/7/2012

ABSTRACT

Increasing interest has been directed toward the role of the adiponectin gene polymorphism in the human genome and its implication in the pathogenesis of coronary artery disease. The present study was investigating the association between the single nucleotide polymorphism +276 G/T of the adiponectin gene with serum adiponectin level in patients with coronary artery disease (CAD). In this study 100 healthy controls and 100 Egyptian patients with coronary artery disease of both genders presented to the Cardiology Department of Suez Canal University Hospital were investigated. All subjects were genotyped for +276 G/T polymorphism of adiponectin gene. Lipid profile, fasting blood glucose were measured. Adiponectin and high sensitivity C-reactive protein levels were determined by ELISA technique. Polymerase chain reaction based on restriction fragment length polymorphism (PCR-RFLP) was used to determine the genotypes of the studied population. The lowest serum adiponectin value was observed in patients with CAD compared with control group. The T allele of SNP +276 G/T in the adiponectin gene was found to be associated with CAD (odd ratio 2.23; 95% CI: 1.44-3.45; P= 0.001). The significant association of the T allele (GT+TT) of this SNP with lower

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adiponectin level and higher hsCRP levels was confirmed in the study (p= 0.003 and 0.006 respectively). Our results concluded that, +276 G/T SNP in the adiponectin gene is associated with CAD. Furthermore, carriers of the at-risk T allele had lower serum adiponectin level and higher serum hsCRP, causing in turn an increased risk to develop CAD.

**Key Words:** Adiponectin gene; polymorphism; Coronary artery disease; PCR-RFLP

**INTRODUCTION**

The adipose tissue has traditionally been regarded as a silent organ that is passively stores excess energy (such as triacylglycerol). However, recent evidence suggests that adipose tissue, especially visceral fat tissue, is considered as an endocrine organ, directly involved in the pathophysiology of the metabolic syndrome and cardiovascular diseases. In fact, visceral fat accumulation has recently been recognized as a key player in the occurrence of multiple risk factors for coronary artery disease (CAD) and in vascular changes (Matsuzawa, 2005).

Adiponectin is an adipocytokine, secreted from white adipose tissue. It is a 30 kDa collagen like protein, clinically noted to be antiatherogenic and antidiabetic at elevated levels (Shibata et al., 2009). The average plasma concentration of this hormone ranges between 5 and 10 µg/ml, levels vary according to sex, body fat distribution, and metabolic status (Salmenniemi et al., 2006).

Low adiponectin has been linked to the presence of CAD (Kumada et al., 2003) and has been shown to be a risk factor for cardiovascular events (Giannessi et al., 2007). Hypoadiponectinemia is strongly linked to central adiposity, dyslipidemia, insulin resistance, and high blood pressure (Weyer et al., 2001; Trujillo and Scherer, 2005). Adiponectin also has anti-inflammatory properties affecting the nuclear factor (NF)-α pathway and inhibiting monocyte adhesion to aortic endothelial cells (Ouchi and Walsh, 2007).

Many gene association studies showed that single nucleotide polymorphisms (SNPs) of adiponectin gene affect the adiponectin production in adipose tissue and modulate circulating adiponectin, but results are controversial and inconsistent (Yang and Chuang, 2006). SNP +276 G/T (rs1501299) has been associated with low serum adiponectin level, insulin resistance, and diabetes (Hara et al., 2002).
ADIPONECTIN SINGLE NUCLEOTIDE POLYMORPHISM

One can postulate that this SNP may also play a role in regulating the risk of CAD. Therefore the aim of this study was to investigate the association between SNP +276G/T of the adiponectin gene with serum adiponectin level in patients with CAD in a case-control study among Egyptians in Suez Canal area.

MATERIALS AND METHODS

Subjects:
A total of 200 Egyptian subjects from Suez Canal area were included in this study. Hundred patients with CAD mean age 53.8 ± 5.2 years were recruited from Cardiology Department in Suez Canal University Hospital. Control subjects were 100 unrelated individuals without history of CAD and have normal ECG, mean age 55.3 ± 6.3 years. Diagnosis of CAD was done by coronary angioplasty (presence of one or more stenosis > 50% in at least one major coronary artery). Subjects with current liver or renal disease were excluded from the study. Diagnosis of type 2 diabetes mellitus was based on Fasting blood glucose >126 mg/dL and/or history of hypoglycemic treatment (American Diabetes Association, 2008). Diagnosis of hypertension was based on the presence of elevated systolic and/or diastolic Bp >140/90 mmHg and/or the current use of antihypertensive medications.

All subjects gave their written informed consent to their participation to the study. The study was approved by the Faculty of Pharmacy, Suez Canal University Ethical Committee.

Laboratory Methods:
Fasting serum glucose, total cholesterol, triglycerides and HDL-C were determined by manual enzymatic colorimetric assays (Analyticon Biotechnologies, Germany). LDL-C was calculated according to the Friedewald formula (Friedewald, 1972). Serum adiponectin and high sensitivity C-reactive protein (hsCRP) levels were measured by ELISA technique using Quantikine human total adiponectin (R & D system, USA), (Arita et al., 1999), and hsCRP (DRG International Inc., USA), (Ridker et al., 1998).

DNA analysis:
DNA was isolated from whole blood using Wizard genomic DNA purification kit (Promega, USA) according to the manufacturer's protocol. PCR-RFLP method was used to determine the distribution of genotype and alleles frequencies of the SNP +276 G/T of adiponectin.
gene. The PCR was performed in a total volume of 25 µl containing 5 U of Taq DNA polymerase (Bioron, Germany), 0.1 mmol/l of each dNTP (Bioron, Germany) and 1 µmol of each of the primers (Metabion International AG, Germany). Amplification was done by initial denaturation at 94°C for 5 min. followed by 30 cycles of 95°C for 1 min., 58°C for 1 min. and 72°C for 1 min. Final extension of 5 min at 72°C was done. PCR fragments (196bp) were digested using the restriction endonuclease BsmI (Fermentas, St Leon-Rot, Germany) as described by (Musso et al., 2008). The digestion products were separated in 1.5% (weight/volume) agarose gel along with 50-base pair (bp) marker (Axygen Biosciences, USA). The products were visualized under UV light following staining with ethidium bromide. The resultant fragments include 196 bp for T allele and 146, 50 bp for G allele.

**Statistical analysis:**

The genotype and allele frequencies for +276 G/T polymorphism of adiponectin gene were determined by direct counting. Hardy-Weinberg's equilibrium was evaluated using a chi-square test. Statistical comparisons between healthy populations and patients were performed with chi-square test for categorical variables, while independent student’s t-test used for continuous variables. Associations of genotypes with plasma adiponectin and hsCRP levels and serum lipid profile concentrations were evaluated by student t-test. To estimate the association of CAD with adiponectin gene SNP, odd ratios (OR) and 95% confidence interval (CI) were calculated. The values of P <0.05 were considered as statistically significant. All statistical analysis was performed with SPSS version 15.

**RESULTS**

**Study population and risk factors for coronary artery disease:**

Table (1) showed the clinical characteristics and biochemical parameters of study subjects. Hypertension, diabetes mellitus and family history of CAD showed significant differences between patients and healthy controls. CAD patients had significantly higher levels of total cholesterol, triglycerides, LDL-C, VLDL-C and hsCRP than in control subjects. On the other hand, serum HDL-C and adiponectin level were significantly lower in CAD patients than in control group.
Table (1): General characteristics and laboratory measures of the study population

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n=100)</th>
<th>CAD (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.8 ± 5.2</td>
<td>55.3 ± 6.3</td>
</tr>
<tr>
<td>Sex n (M/F)</td>
<td>60/40</td>
<td>55/45</td>
</tr>
<tr>
<td>BMI (Kg/m2)</td>
<td>29.7 ± 3.2</td>
<td>30.4 ± 4.2</td>
</tr>
<tr>
<td>Hypertension n (%)</td>
<td>13 (13)</td>
<td>62 (62)*</td>
</tr>
<tr>
<td>Smoking n (%)</td>
<td>26 (26)</td>
<td>39 (39)</td>
</tr>
<tr>
<td>Diabetes Mellitus n (%)</td>
<td>10 (10)</td>
<td>32 (32)*</td>
</tr>
<tr>
<td>Family history for CAD n (%)</td>
<td>19 (19)</td>
<td>40 (40)*</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>186.2 ± 25.6</td>
<td>213.7 ± 61.4*</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>161.1 ± 45.7</td>
<td>210.9 ± 77.5*</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>42.5 ± 7.9</td>
<td>40.6 ± 11.5</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>115.1 ± 21.9</td>
<td>135 ± 55.4*</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>32.6 ± 9.3</td>
<td>40.7 ± 14.4*</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>95.1 ± 29.2</td>
<td>124.8 ± 45.8*</td>
</tr>
<tr>
<td>hs CRP (mg/dl)</td>
<td>3.7 ± 2.0</td>
<td>7.6 ± 3.1*</td>
</tr>
<tr>
<td>Adiponectin level (ng/ml)</td>
<td>5.98 ± 1.8</td>
<td>4.26 ± 1.38*</td>
</tr>
</tbody>
</table>

Data are means ± SD, comparisons were performed by student t-test and chi square test. * P < 0.05 is statistically significant.

Allele frequencies and genotype distribution of adiponectin gene +276G/T polymorphism:

Allele frequency and genotype distribution were shown in Table (2) and Figure (1). Genotype and allele frequencies differed significantly between CAD patients and control subjects for the +276 G/T SNP of the adiponectin gene. Both allele frequencies and genotypes were in Hardy-Weinberg equilibrium.
Table (2): Allele frequencies and genotypes distribution of SNP +276 G/T of Adiponectin gene in control subjects and CAD patients.

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 100)</th>
<th>CAD Patients (n = 100)</th>
<th>P</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G allele</strong></td>
<td>154 (77%)</td>
<td>120 (60%)</td>
<td>0.001*</td>
<td>2.232 (1.446 – 3.445)</td>
</tr>
<tr>
<td><strong>T allele</strong></td>
<td>46 (23%)</td>
<td>80 (40%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Genotype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>60</td>
<td>39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GT</td>
<td>34</td>
<td>42</td>
<td>0.037*</td>
<td>1.901 (1.037 – 3.483)</td>
</tr>
<tr>
<td>TT</td>
<td>6</td>
<td>19</td>
<td>0.001*</td>
<td>4.872 (1.788 – 13.276)</td>
</tr>
</tbody>
</table>

Comparisons were performed by the chi-square test; (CI) = confidence interval; OR = odds ratio; a T vs. G; b GT vs. GG; c TT vs. GG. *P < 0.05 is statistically significant.

Figure (1): Adiponectin SNP +276 G/T genotype on 1.5 % agarose gel. M: 50 bp marker; Lanes (1, 2, 5, 6, 7) are GG genotypes; Lanes (3, 8) are GT genotypes; Lane (4) is TT genotype.
Anthropometric and metabolic risk factors among CAD patients according to the genotype distribution:

To test the effect of 276 G/T polymorphism on risk factors of CAD, patients were divided into two groups TT+GT genotype and GG genotype (assuming a codominant model of inheritance). As shown in table (3), among all risk factors of CAD, only hsCRP and plasma adiponectin were significantly different between patients with GG genotypes and those with GT+TT genotypes. Carriers of the T allele (GT+TT genotypes) have higher serum hsCRP and lower serum adiponectin (Figure 2) than those with the GG genotype.

Table (3): Anthropometric and metabolic risk factors among CAD patients according to the genotype distribution

<table>
<thead>
<tr>
<th>Variable</th>
<th>Carriers of GG (n=39)</th>
<th>Carriers of GT+TT (n=61)</th>
<th>P</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>55.28 ± 6.3</td>
<td>55.0 ± 6.4</td>
<td>NS</td>
<td>0.96-1.052</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>24 (61.5)</td>
<td>39 (63.9)</td>
<td>NS</td>
<td>0.621-3.368</td>
</tr>
<tr>
<td>BMI, Kg/m²</td>
<td>30.1 ± 4.5</td>
<td>29.2 ± 3.8</td>
<td>NS</td>
<td>0.97-1.09</td>
</tr>
<tr>
<td>T2DM (%)</td>
<td>8 (20.5)</td>
<td>14 (22.9)</td>
<td>NS</td>
<td>0.267-1.621</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>10 (25.6)</td>
<td>15 (24.6)</td>
<td>NS</td>
<td>0.278-1.817</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>12 (30.8)</td>
<td>21 (34.4)</td>
<td>NS</td>
<td>0.278-1.359</td>
</tr>
<tr>
<td>Family history, n (%)</td>
<td>11 (28.2)</td>
<td>19 (31.1)</td>
<td>NS</td>
<td>0.320-1.598</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>202.04 ± 76.6</td>
<td>215.7 ± 77.9</td>
<td>NS</td>
<td>0.808-1.085</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>213.9 ± 62.1</td>
<td>214.79 ± 61.6</td>
<td>NS</td>
<td>0.808-1.117</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>43.5 ± 11.4</td>
<td>37.6 ± 10.9</td>
<td>NS</td>
<td>1.04-1.29</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>136.7 ± 58.6</td>
<td>141.1 ± 57.2</td>
<td>NS</td>
<td>0.87-1.22</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>39.4 ± 13.1</td>
<td>42.5 ± 16.3</td>
<td>NS</td>
<td>0.8-1.07</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>127.7 ± 58.8</td>
<td>124.5 ± 55.6</td>
<td>NS</td>
<td>0.86-1.23</td>
</tr>
<tr>
<td>hsCRP (mg/dl)</td>
<td>6.81 ± 2.6</td>
<td>8.5 ± 3.37</td>
<td>0.006*</td>
<td>1.068-1.457</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>4.65 ± 1.27</td>
<td>3.9 ± 1.21</td>
<td>0.004*</td>
<td>1.059-1.346</td>
</tr>
</tbody>
</table>

Comparisons were performed by X² or student t-tests; CI= confidence interval; NS = non-significant. *P < 0.05 was statistically significant.
Figure (2): Adiponectin levels in T allele carriers and G allele carriers in patients with CAD. Data are presented as mean ± SD.

DISCUSSION

Coronary artery diseases (CAD) constitute a major health problem in many parts of the world and are an important cause of morbidity and mortality. It is predicted that by the year 2020, CAD will be the main cause of disability worldwide (WHO, 2008). Previous studies regarding the genetic association of adiponectin gene +276 G/T SNP and the concomitant presence of CAD have been few, with nearly no report from Egyptian population. Moreover, the overall reported associations of 276 G/T polymorphism and cardiometabolic disease were diverse. The present study provides evidence of association between the +276 G/T SNP of the adiponectin gene and CAD in this sample of Egyptian population. More precisely, a significant relation of minor T allele with the presence of CAD was approved (OR= 2.2, CI= 1.44-3.45, P= 0.001). In accordance with the findings of the present study, Filippi et al (2005) first reported that T allele of 276 G/T is associated with higher risk of CAD than G allele carriers. Another study on Saudi population confirmed the same findings (Mackawy et al., 2011). However, other studies have reported opposite results (Iacobellis et al., 2006; Leu et al., 2011) providing direct evidence that the major G allele of 276 G/T of
Adiponectin gene may lead to target organ damage.

Adiponectin serum levels have been demonstrated consistently to be reduced in patients with CAD (Kumada et al., 2003; Melanie et al., 2011); suggesting a central role in the mechanisms leading to the metabolic abnormalities present in this disorder (Filippi et al., 2005). Moreover, another study by Pischon et al (2004) has demonstrated that high adiponectin concentrations are associated with a lower incidence of myocardial infarction, suggesting that low adiponectin could be a causal risk factor for CAD. In favor of this hypothesis is the study done in animal model of atherosclerosis which demonstrates that adenovirus mediated increase adiponectin significantly suppressing the progression of atherosclerotic lesions (Okamoto et al., 2002). The mechanism by which Adiponectin affects CAD may be explained by the fact that adiponectin has been shown to exert several effects on vascular structure and function, including inhibition of endothelial thickening, induction of arterial vasodilatation, inhibition of foam cell formation and suppression of adhesion molecules (Arita et al., 2002; Fernandez-Real et al., 2004).

Previous studies focused on the association between plasma adiponectin level and the adiponectin gene 276 G/T SNP were not consistent and occasionally discrepant (Leu et al., 2011; Fillippi et al., 2005). Our findings support the hypothesis that the +276 G/T SNP of the adiponectin gene may determine a reduced expression of the protein (Yang et al., 2003), as patients with T allele in homo- and hetero-zygotes forms (GT+TT) have a lower serum adiponectin levels than patients with GG genotype. Another possibility is that this SNP is in linkage disequilibrium with another mutation either within or in other genes close to the adiponectin gene that determines its negative effects. In support of our finding, genome wide association study (GWAS) using plasma adiponectin as a quantitative trait demonstrated the adiponectin gene as the only major gene for plasma adiponectin in Caucasian population (Heid et al., 2010).

hs-CRP has been demonstrated to contribute to vascular inflammation by inhibiting nitric oxide (NO) production (Verma et al., 2002). Increased plasma hs-CRP and hypoadiponectinemia might be related to the progression of CAD. Interestingly, our data demonstrated that serum hsCRP was higher in carriers of the T allele (GT+TT genotypes) than those with the GG genotype.
In conclusion, +276 G/T SNP in the adiponectin gene is associated with CAD. Furthermore, carriers of the at-risk T allele had lower serum adiponectin level and higher serum hsCRP, causing in turn an increased risk to develop CAD. Further larger-scale high-throughput genotyping is needed to investigate the other polymorphic sites of adiponectin gene that may affect its serum level.

REFERENCES


WHO (October 2008): The top 10 causes of death. Fact Sheet No. 310.

الملخص العربي

دراسة العلاقة المحتملة لتعدد البذور (G/T) 276 ناجمة عن الادبوبونتين
ومرض الشريان التاجي في منطقة قناة السويس

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لاقتراب الاهتمام بدور تعدد البذور في جين الادبوبونتين لمشاركته في التسبب
بمرض إنسداد الشريان التاجي، تحت أمراض القلب من الأسباب الرئيسي للفوات كنتيجة
حتي لعوامل الخطورة المختلفة وعليه فقد هدفت هذه الدراسة أولا: إلى التحقق من إمكانية
العلاقة بين تعدد البذور 276 ناجمة عن الادبوبونتين ومرض إنسداد الشريان التاجي
ثانيا: تقدير الارتباط بين مستوى تركيز هرمون الادبوبونتين والبدل الجيني السابق
الذكرون. اشتملت هذه الدراسة على 100 من الأفراد الأصحاء و100 من المرضى المصابين بإنسداد
الشريان التاجي المصري من الجنسين على حد سواء، والمترادفين على قسم أمراض القلب
في مستشفى جامعة قناة السويس. تم إجراء التحليل الجيني لتعدد البذور بواسطة تقنية
التفاعل التسلسلي التكيفي، كما تم قياس مستوى الدهون والسكر في الدم وقياس مستوى
تركيز هرمون الادبوبونتين وبروتين ج النشط ذو الحساسية العامة بواسطة تقنية الإلزام للكلا
المجموعتين. بعد التحليل الإحصائي للنتائج أوضحت الدراسة ارتباط مستوى تركيز
هرمون الادبوبونتين وزيادة مستوى بروتين ج النشط في مجموعة المرضى مقارنة
بالمجموعة الضابطة من الأصحاء. كما أظهرت الدراسة أيضاً ارتباط الإيل (T) بمرض
إنسداد الشريان التاجي. وعلى ذلك أدى تأكيد هذه الدراسة أن هناك علاقة ارتباط ذو دلاله
إحصائية وثيقة بين البذور الجينية (G/T) 276 ناجمة عن الادبوبونتين وانخفاض مستوى تركيز
الهرمون عند الأليل (G) والموروثان (GT+TT) في المرضى بمرض إنسداد الشريان التاجي المصري في منطقة القناة.