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Original Article

Does apolipoprotein A-II polymorphism interact with the association of obesity and serum inflammatory biomarkers in type 2 diabetes patients?

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Article History
Received:
14/06/2014
Revised:
02/09/2014
Accepted:
11/12/2014

Keywords:
Inflammation,
Obesity,
Apolipoprotein A-II,
Type 2 diabetes,
Gene,
Polymorphism

A B S T R A C T

Background: The objective was to investigate the relationship between serum interleukin-18 (IL-18), pentraxin 3 (PTX3), and high-sensitivity C-reactive protein (hs-CRP) levels with body mass index (BMI) and abdominal obesity and also the interaction between genetic variants of apolipoprotein A-II (Apo A-II) and obesity on the levels of these factors in type 2 diabetes patients (T2D).

Methods: A comparative cross-sectional study was conducted in 21 diabetes centers in Tehran. Totally, 180 (35-65 years) T2D patients were divided into two groups of 90 obese (BMI ≥ 30) and 90 non-obese (BMI < 30), according to the BMI with equal numbers of each genotype of Apo A-II: 30 TT, 30 CC, and 30 TC. Serum IL-18, PTX3, and hs-CRP concentrations were compared between two obese and non-obese groups and between subjects with and without central obesity. To investigate the interaction of Apo A-II genetic variants and BMI with inflammatory factors, general linear model was used.

Results: After adjusting data with confounding factors, the mean of serum PTX3 was significantly lower ($p < 0.050$) in the obese diabetes than non-obese diabetes subjects. Moreover, obese diabetes had higher serum hs-CRP level that on obese subjects ($p < 0.010$). No significant interaction between Apo A-II 265 T > C polymorphism and BMI on inflammatory biomarkers was observed.

Conclusion: There was a significant difference in inflammatory markers (PTX3 and hs-CRP concentration) between obese and non-obese diabetes. In addition, there was no interaction of Apo A-II 265 T > C genotypes and BMI on inflammatory markers. Weight control may be recommended to modulate inflammation and its complications in obese patients.

Citation: Keramat L, Eshraghian M, Djalali M, Sotoudeh G, Sadrzadeh-Yeganeh H, Fariba Koohdani. **Does apolipoprotein A-II polymorphism interact with the association of obesity and serum inflammatory biomarkers in type 2 diabetes patients?** J Nutr Sci & Diet 2015; 1(2): 107-13.

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Introduction

Type 2 diabetes (T2D) as a public-health problem is one of the most common and growing metabolic diseases in humans. According to the International Diabetes Federation, the number of

diabetes people worldwide in 2011 had been 366 million and the proportion of Asian race of this figure is continuously increasing. In Iran, the prevalence of diabetes is expected to be 2/5 million by 2025 [1, 2]. Tehran lipid and glucose study results in 1381 suggest the percent of people with glucose intolerance are 5.14-5.22 in Tehran over 30 years and nearly a quarter of them will be diabetic in the future [3].

One of the major complications in patients with T2D is the cardiovascular disease that is an important cause of death in Western countries. At first, this risk was mainly attributed to hyperglycemia, dyslipidemia as traditional risk factors, but recently attention has focused on the levels of inflammatory factors [4].

One of the major causes of diabetes and insulin resistance is obesity and overweight. Obesity is a growing global public health problem, which is closely associated with chronic diseases, including dyslipidemia, metabolic syndrome, T2D, atherosclerosis, and cardiovascular diseases [1, 2]. Today's, adipose tissue is known as an endocrine tissue because of its role in inflammation and the production of various cytokines which is leading to inflammation and changing in metabolic status [5, 6]. The accumulation of abdominal obesity seems involved in atherosclerosis by creating an inflammatory situation [7, 8].

Inflammatory factors such as interleukin-18 (IL-18), C-reactive protein (CRP), and pentraxin 3 (PTX3) have been investigated in many studies as factors associated with overweight and obesity and inflammation caused by them; on the other hand, they can be used to predict the levels of inflammation lead to the occurrence of chronic diseases like cardiovascular disease [9-11]. In recent years, many strategies have been conducted to identify genetic factors that determine the prevalence of obesity. One of the candidate genes which is involved in this field is the APOAII gene [12]. APOAII is the major structural proteins component of high-density lipoprotein (HDL) are [13]. Studies have shown that the T to C substitution in upstream of the apolipoprotein A-II (Apo A-II) gene transcription start site results in 30% reduction of Apo A-II expression [14]. Some studies have shown that the incidence of obesity is higher in individuals with the CC genotype than in carriers of the T allele (TT + TC) [15-17].

In a study by Lara-Castro et al., the ApoA-II polymorphism had significant relationship with amount of abdominal fat deposition in women [18] and in another study, an association was observed between the ApoA-II polymorphism with the waist circumference (WC) in men [14].

Regarding the high prevalence of obesity in patients with T2D [19, 20], this study has been designed to investigate the relationship between serum IL-18, PTX3, and high-sensitivity CRP (hs-CRP) levels with body mass index (BMI) and abdominal obesity and also the interaction between genetic variants of Apo A-II and obesity on the levels of aforementioned factors in T2D patients.

Methods

Subjects

This comparative cross-sectional study was conducted on obese and non-obese (35-65 years) patients with diabetes. The study was evaluated and approved by the Ethics Committee at the Tehran University of Medical Sciences. Among the 816 patients with T2D, who had participated in the earlier study [21], 180 patients who had the inclusion criteria were enrolled.

Inclusion criteria

All patients were T2D patients with no insulin injection, did not take narcotic, smoking and alcohol, absence of cardiovascular disease, stroke, liver disease, kidney failure, thyroid diseases, cancer, and inflammatory illnesses did not receive anti-inflammatory medicines, and multivitamin/mineral supplement. In the earlier study, required information such as age, sex, duration of diabetes, taking oral hyperglycemic agents, and lipid-lowering medications had been obtained using a self-administered questionnaire.

Anthropometrics and dietary assessments

The information on dietary intakes was collected in the earlier study [21] by trained dietitian using validated semi-quantitative food frequency questionnaire (FFQ) [22]. Physical activity was evaluated by MET questionnaire [23]. At baseline, a trained dietician was measured weights, heights, and waist measures according to standard protocols [24]. BMI was calculated by dividing weight (in kg) by the square of height (meters). WC $\geq$ 102 cm in men and WC $\geq$ 88 cm in women was considered as central obesity [25]. Obesity was defined as BMI $\geq$ 30 kg/m^2 [26]. The patients were divided into two groups of 90 obese (BMI $\geq$ 30) and 90 non-obese (BMI < 30), according to the BMI with equal numbers of participants in each genotype of Apo A-II: 30 TT, 30 CC, and 30 TC.

Laboratory investigation

A venous blood sample of each subject was obtained at 08.00 AM after 12 hours fasting and was collected in labeled micro-tubes. Total cholesterol, low-density lipoprotein cholesterol (LDL-c), HDL-cholesterol, and triglyceride (TG) levels had been determined by using Pars Azmun Company kits. Genomic DNA was extracted using salting out method and Apo A-II genotypes were determined using Step One Plus Real-time PCR system (Applied BioSystems) [27]. For determination of serum concentration of inflammatory factors including IL-18 and PTX3 using ELISA kit (Shanghai Crystal Day Biotech Co., Ltd.) and hs-CRP using an hs-CRP ELISA kit (Diagnostics Biochem Canada Inc. London, Ontario, Canada).

Statistical analysis

Statistical Package for the Social Sciences (SPSS), version 20 (SPSS Inc., Chicago, IL, USA) was used for data analyses. $p < 0.050$ were considered statistically significant for all tests. All data were reported as mean ± standard error unless stated otherwise. For analyzing FFQ Nutritionist III (version 7.0, N Squared Computing) was used. To check the normality distribution of variables, Kolmogorov–Smirnov test was examined. In all analyses, we used log-transformed TGs, total cholesterol, and hs-CRP values. Independent t-test was used to compare means and chi-square test to compare qualitative variables. ANCOVA test was performed to evaluate the confounding effects of variables. Among confounding variables, since R obtained from the collinearity test between mono-unsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) intake was < 0.2, only the effect of MUFA intake was adjusted.

To investigate the interaction of Apo A-II genetic variants and BMI on the inflammatory factors, general linear model was applied

Results

Totally, 180 T2D patients participated in this study, which included 116 women (64.4%) and 64 men (36.6%). The related results to mean age, anthropometric indicators, physical activity, nutrient intake, lipid indices, and lipid-lowering drugs intake in obese and non-obese groups have been shown in table 1. The average of age ($p \leq 0.010$), BMI and WC ($p < 0.001$), MUFA and PUFA intakes ($p \leq 0.010$) indicated significant difference between two groups, but physical activity, lipid profile, and lipid-lowering drugs intake had no significant difference between two groups.

Table 1. Characteristics of study participants

Characteristics	Obese	Non-obese	p-value
Study population, n	90	90	-
Lipid-lowering medications, n	44	52	0.230*
Oral hyperglycemic agents, n	86	81	0.150*
Age, years	52.87 ± 0.74	55.10 ± 0.63	0.020†
BMI, kg/m^2	33.02 ± 0.34	25.99 ± 0.23	< 0.001†
WC, cm	98.88 ± 0.95	85.62 ± 0.83	< 0.001†
Physical activity, MET.time/day	37.74 ± 0.51	39.10 ± 0.63	0.090†
Food intake			
Energy, Kcal/day	2576.83 ± 95.16	2643.64 ± 130.36	0.680†
Carbohydrate, g/day	347.44 ± 8.14	363.22 ± 6.69	0.250$^{\#}$
Protein, g/day	80.64 ± 1.88	84.04 ± 2.04	0.170$^{\#}$
Fat, g/day	110.11 ± 3.18	100.97 ± 3.44	0.120$^{\#}$
PUFA, g/day	26.83 ± 1.17	22.15 ± 1.19	0.020$^{\#}$
MUFA, g/day	28.76 ± 1.37	23.82 ± 1.29	0.010$^{\#}$
SFA, g/day	27.06 ± 1.18	26.58 ± 1.09	0.370$^{\#}$
Vitamin E, mg/day	24.17 ± 1.56	19.92 ± 1.37	0.170$^{\#}$
Vitamin C, mg/day	182.95 ± 10.26	180.73 ± 11.83	0.490$^{\#}$
Vitamin A, µg/day	715.73 ± 37.13	716.46 ± 44.26	0.700$^{\#}$
Fiber, g/day	39.85 ± 1.80	45.32 ± 3.41	0.110$^{\#}$
Lipid profile			
TGs, mg/dl	190.75 ± 13.39	171.52 ± 11.85	0.280†
Total cholesterol, mg/dl	200.40 ± 7.78	194.76 ± 9.79	0.650†
HDL-c, mg/dl	53.48 ± 1.36	53.24 ± 1.25	0.890†
LDL-c, mg/dl	112.75 ± 4.13	106.82 ± 3.91	0.290†

*Data are presented as mean ± SE or number of subjects. *p-value was calculated using chi-square. The †p value was calculated using an independent t-test. The $^{\#}$p value was calculated using ANCOVA and adjusted for energy by ANCOVA. SFA = Saturated fatty acid, MET = Metabolic equivalent, BMI = Body mass index, WC= Waist circumference, PUFA = Polyunsaturated fatty acid, MUFA = Monounsaturated fatty acid, HDL-c = High-density lipoprotein-cholesterol, LDL-c = Low-density lipoprotein cholesterol, TG = Triglycerides, SE = Standard error*

Table 2. Mean value of inflammatory markers in obese and non-obese diabetes patients

Inflammatory factors	Obese	Non-obese	p-value*	p-value†
IL18, pg/ml	255.06 ± 3.49	243.35 ± 2.84	0.010	0.090
PTX3, ng/ml	2.54 ± 0.04	2.70 ± 0.05	0.010	0.020
hsCRP, mg/L	2.55 ± 0.14	1.90 ± 0.16	0.004	0.004

*Data are presented as mean ± SE. *p-value was calculated using an independent t-test. †adjusted for WC, age, sex, MUFA by ANCOVA. IL-18 = Interleukin 18, PTX3 = Pentraxin 3, hsCRP = High-sensitivity C-reactive protein, SE = Standard error, MUFA = Monounsaturated fatty acid, BMI = Body mass index, WC = Waist circumference*

Table 3. Mean value of inflammatory markers according to waist circumference categories

Waist circumference	IL-18, pg/ml	PTX3, ng/ml	hsCRP, mg/l
Men			
WC <102 cm	245/73 ± 4.02	2/63 ± 0.06	2.20 ± 0.21
WC ≥102 cm	246/09 ± 10.86	2/58 ± 0.07	2.07 ± 0.35
p-value*	0.850	0.670	0.760
p value†	0.590	0.850	0.810
Women			
WC <88 cm	250.76 ± 3.91	2.65 ± 0.06	2.12 ± 0.20
WC ≥88 cm	250.96 ± 3.79	2.63 ± 0.06	2.43 ± 0.19
p-value*	0.970	0.840	0.270
p value†	0.940	0.800	0.530

*Data are presented as mean±SE. *p-value was calculated using an independent t-test. †Adjusted for BMI by ANCOVA. IL-18 = Interleukin 18, PTX3 = Pentraxin 3, hsCRP = High-sensitivity C-reactive protein, SE = Standard error, BMI = Body mass index*

Table 4. Interaction between the genetic variants of Apo A-II and BMI with inflammatory markers

Inflammatory factors	Obese		Non-obese		p-value*	p-value†
	CC n = 30	TT/TC n = 60	CC n = 30	TT/TC n = 60		
IL-18, pg/ml	256.89 ± 5.21	254.56 ± 4.64	245.31 ± 4.71	242.37 ± 3.57	0.870	0.790
PTX3, ng/ml	2.44 ± 0.09	2.59 ± 0.05	2.59 ± 0.06	2.76 ± 0.06	0.580	0.750
hsCRP, mg/L	3.06 ± 0.29	2.28 ± 0.15	2.59 ± 0.30	1.55 ± 0.17	0.550	0.370

*Data are presented as mean ± SE. *p-value was calculated using general linear model, †Adjusted for WC, age, sex, and MUFA by ANCOVA. IL-18 = Interleukin 18, PTX3 = Pentraxin 3, hsCRP = High-sensitivity C-reactive protein, SE = Standard error, MUFA = Monounsaturated fatty acid, WC = Waist circumference, Apo A-II = Apolipoprotein A-II*

In table 2, the mean values of inflammatory factors (IL-18, PTX3, hs-CRP) were compared between two groups. In this analysis, the mean concentrations of IL-18, hs-CRP ($p \leq 0.050$) and PTX3 ($p \leq 0.001$) in the two groups were significant. The significant differences were remained for hs-CRP ($p \leq 0.001$) and PTX3 ($p \leq 0.010$) after adjusting of age, gender, WC, and MUFA intake while the significant difference of IL-18 in the two groups was removed.

Table 3 shows that the mean of inflammatory factors in T2D men with a waist 102 ≤ and 102 > cm and T2D women with waist 88 ≤ and 88 > cm. In men with WC of > 102, the PTX 3 concentration was higher than those with WCs < 102, but this difference is not statistically significant. There was no significant difference in the mean concentration of IL-18 and hs-CRP in men between two categories of WCs. Furthermore, the mean concentrations of IL-18, hs-CRP and PTX3, in women with WC of > 88 than those with WC of ≤ 88, were not statistically significant. After adjusting data for BMI, also no significant difference was found in any of the studied groups.

Table 4 examines the interaction between obesity and Apo II genotype on inflammatory factors. On the basis of the previous studies [2-4], TT and TC individuals were grouped and compared with CC individuals. According to the results, no significant association between Apo A-II genetic variants and BMI with inflammatory factors was observed. After adjusting data for age, gender, WC, and MUFA intake, these associations remained no significant.

Discussion

According to the results, the mean concentration of hs-CRP and PTX3 was statistically different in obese than non-obese diabetes patients.

The our results are consistent with studies which showed obesity not only in subjects with the normal blood sugar levels [28-30] but also in

subjects with diabetes [7] or in the metabolic syndromes [31] causes inflammatory response. However, our results are in contrast with findings from those studies which showed that in non-obese T2D patients, concentrations of some inflammatory factors like CRP are higher than matched normoglycemic subjects [32, 33]. Indeed, the results of these studies emphasize the higher effect of diabetes on the increasing level of inflammation while our findings suggest the further effect of obesity in this area. The reason for this discrepancy might be attributed to the different ethnic background of the subjects in the different studies [34, 35]. Some studies have shown differences in adipose tissue mass distribution and the level of insulin resistance in different races that these differences can be effective on the levels of inflammatory cytokines [6, 36]. Furthermore, the lack of studies on patients with diabetes in this area can be noted. More studies have compared obese and non-obese healthy subjects to assess the degree of inflammation [28-30], few studies have been done on obese and non-obese diabetes patients [7].

Several mechanisms have been proposed for increasing inflammation associated with obesity. In hypertrophic-hyperplastic adipocytes, insulin receptors density is lower and beta-3 adrenergic receptor density is higher, which facilitates the diapedesis of monocytes to the visceral adipose stroma, initiating a proinflammatory cycle between adipose and monocytes [37].

On the other hand, increase of free fatty acids concentration, especially saturated fatty acids, coming from adipose tissue overflow, buildup in the liver. The fat accumulation in the liver leads to over production of LDLs, IL-6, and CRP [6].

Another reason for the effect of obesity on increasing of inflammatory levels may be related to the increase in body fat mass and serum leptin concentration [38]. Leptin is known as a pro-inflammatory cytokine that causes inflammation by stimulating the secretion of tumor necrosis factor-α and IL-6 from monocytes and other immune cells [38, 39].

After adjusting findings with BMI, no significant relationship was observed between the inflammatory factors levels in men and women with and without abdominal obesity. These results are consistent with those in which no significant relationship between levels of inflammatory markers such as CRP [7] and IL-18 [29], and WC was observed in diabetes patients. Probably the difference between our findings with other studies [8, 29, 31] is due to the different study population. In our study, similar to Zirlik's study [29], all samples were T2D. Studies have shown that inflammatory cytokines levels can be effective on insulin resistance in T2D patients [40, 41].

We also examined the effect of obesity and Apo A-II genotype interaction on the inflammatory factors levels (Table 4). The interaction effects of these two factors had no significant effect on the increasing levels of inflammation. It was also observed that in the CC group compared to the TT/TC group, the mean PTX3 concentration was lower while the mean IL-18 and hs-CRP concentrations were higher. It can be concluded that inflammation is higher in obese and non-obese subjects with the CC genotype as compared with the carriers of the T-allele. A functional polymorphism representing a T-to-C substitution at the 265 position of this gene has been associated with reduces Apo A-II expression in liver cells and thus reduces its secretion into plasma [14, 18]. Some studies have shown that lower concentration of Apo A-II is associated with a decrease in leukocytes and inflammatory cytokines [42]. To interpret this result, further investigation is needed in this area.

It can be noted that not measuring the concentration of adiponectin and leptin, and lack of healthy samples for comparison with diabetes patients are limitations of this investigation.

Conclusion

There was a significant difference in inflammation markers between obese and non-obese diabetes subjects. There was no interaction of Apo A-II 265 T > C genotypes and BMI on inflammatory markers.

Acknowledgments

This research was funded by the Research Council of Tehran University of Medical Sciences (project number 20413). The authors thank all of the diabetes patients who participated in this project.

Conflict of interest

The authors declare no conflict of interest.

Sources of funding

This research has been supported by Tehran University of Medical Sciences Grant No: 20414.

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