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Association of G-174C Polymorphism of the Interleukin-6 Gene Promoter with Obesity in Iranian Population

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***Abstract*—**Expression and secretion of inflammation markers are disturbed in obesity. Interleukin-6 reduces body fat mass. The common G-174C polymorphism in the promoter of IL-6 gene has been reported that effects on transcriptional regulation. The objective was to investigate association of the common polymorphism G-174C with obesity in Iranian population. The present study is cross sectional association study that included 242 individuals (110 men and 132 women). Serum IL-6 levels, C-reactive protein, fasting blood glucose and blood lipids profile were measured .BMI and WHR were calculated. Genotyping is carried out by PCR and RFLP. The frequencies of G and C allele were 64.5% and 35.5%, respectively. The G-174C polymorphism was not associated with BMI and WHR. However in obese individual, fasting blood glucose was significantly higher in carrier of C allele compared with the non-carrier. The IL-6 G-174C polymorphism is not a risk factor for obesity in Iranian population.

***Keywords*—**Interleukin 6, Polymorphism genetic, Obesity.

I. Introduction

THE prevalence of obesity has been increasing rapidly in most of the world [1]. Numerous studies indicate that excess body fat induce a multitude of co-morbid conditions such as diabetes, cardiovascular disease metabolic syndrome and cancer [2]. The importance of genetics factors to control of human body weight and composition, in concert with environmental effects has been well established. It has been reported that chronic low-grade activation of the immune system plays a central role in the etiology of obesity [2] [3].Obesity can be expressed as an inflammation condition [1]. In the obesity state the expression and secretion of inflammation markers are disturbed. So, one of the best ways for identification of body fat regulative mechanisms has been exploration of the genetic relationship between pro-inflammation cytokines and adiposity [2]. IL-6, a major pro-inflammation cytokine expressed in several tissues such as, adipose tissue, muscles, immune cells and hypothalamus are associated with regulatory of energy balance in human [3]. High circulation and adipose tissue levels of IL-6 have been correlated with obesity and visceral fat [4]. Several SNPs (Single nucleotide polymorphism) have been identified in the IL-6 gene. The common G-174C polymorphism in the promoter of IL-6 gene has been reported that affects on transcriptional regulation [2], [3]. Several studies suggest that -174 G-containing haplotypes are stronger enhancer of IL-6 transcription than those containing C allele [5]. Whereas, other studies observed that polymorphism G-174C was not associated with obesity and IL-6 levels. However, studies on the effects this polymorphism on circulating IL-6 and gain weight generated conflicting result.

Based on these finding, we examined the association of the common polymorphism G-174C (rs1800795) whit obesity in obese and non-obese from prospective cohort.

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II. Methods

A. Study subject

The sample for the present study included 242 individuals (110 men and 132 women) overall, who were selected for the study from the Tehran Lipid and Glucose Study (TLGS) cohort. Peripheral blood, blood clot and blood with anti-coagulation EDTA were obtained. Hip and waist circumference and hypertension were measured .Body Mass Index (BMI) was calculated with the formula: weight (kg)/height (m^2). Waist to hip ratio **(**WHR) was calculated as waist circumference divided by hip circumference. BMI≥30 and WHR (> 0.85 for women; > 1.0 for men) were used as indices of obesity.

B. Biochemical analyses

The serum concentration of triglyceride (TG), total cholesterol and glucose were measured by commercial kits (Pars Azmoun, Tehran, Iran).The HDL-C of serum was determined by apolipoprotein sediment with phosphotangestenic acid. LDL-C in subjects with TG < 400 mg/dl was assayed used of the Friedewald formula. The serum IL-6 and high sensitive CRP (hsCRP) were measured by using

an ELISA kits (Diaclone, Besancon, France and dbc, Ontario, Canada**)** respectively.

C. DNA isolation and genotyping:

Genomic DNAs were obtained from peripheral blood leukocytes by salting out method. The polymorphism of the IL-6 promoter region at -174 was studied by PCR-RFLP. The region of interest was amplified by PCR using primers 5-TGACTTCAGCTTTACTTTGT- 3 and 5-AATCTTAATAAGGTTTCCA- 3. The reaction was carried out in a final volume of 25 µL containing 1.5 mmol/L of MgCl2, 0.2 mmol/L of each dNTP, 0.2 mmol/L of each primer and 1 unit of Taq polymerase (Cinagene Co, Tehran, Iran). DNA was amplified during 30 cycles with an initial denaturation of 10 minutes at 94°C and a final extension of 10 m at 72°C. The cycle program consisted in 1 minute denaturation at 94°C, 1 minute and 35s annealing at 62°C and 1 m extension at 72°C. The PCR product was digested by adding 10U of Nla III restriction enzyme at 37°C overnight, separated the digested fragments by Agarose Gel Electrophoresis and detected by ethidium bromide staining. The polymorphism is due to a replacement of G by C at position -174. The identified genotypes were named according to the presence or absence of the enzyme restriction sites, so Nla III (GG), (GC) and (CC) are homozygote for the presence of the site (153/40 bp), and heterozygote for the presence/absence of the site (193/153/40) and homozygote for the absence of the site (198 bp), respectively.

D. Statistical analysis:

To analysis data using the SPSS program (version 16.0). To compare of variables between the three different genotypes and carriers of G-174C polymorphism respectively, we used the Kruskal–Wallis (K–W one-way) and Mann-Whitney due to non-normally distributed data. X^2 analysis was used for the investigation of the presence of Hardy–Weinberg equilibrium. Results were expressed as means ± SE.

III. Result

In this study, the genotypes were in Hardy-Weinberg equilibrium. The frequencies of G and C allele in the G-174C IL-6 polymorphism were 64.5% and 35.5%, respectively. In all, 156 subjects(64.5%) had a GG genotype, 71subjects (30.3%) a GC genotype and 15 subjects(6.2%) had a CC genotype. all of the groups(men , women and obese, non-obese) frequency of carrying G allele were more than carrying C allele exception in obese men that frequency of C allele was more than G allele (Table I). The relation between G-174C genotype and various vital, blood chemistry parameters and geometric of adiposity measures (MBI, Waist circumference and Waist to hip ratio) is shown in table II. The G-174C polymorphism was not associated with difference in age, blood pressure, fasting blood glucose, total cholesterol, LDL, HDL, triglycerides, serum IL-6, hsCRP. Moreover, the G-174C polymorphism was not associated with BMI and WHR. However in obese individual, fasting glucose blood was significantly higher in carrier of allele C compared with the non-carrier (P=0.04) (Table III). There was a tendency in obese individuals that carrying C allele has higher BMI, WHR and weight than carrying G allele and has lower IL-6 concentration (Table 2).

IV. Discussion

The present study shows that the IL-6 G-174C polymorphism is not significantly associated with increased BMI in Iranian population. Several studies examined the associations between the common polymorphism in the IL-6 gene promoter in human. Some of these studies expressed that, the IL-6 polymorphism G-174C have been related with obesity especially central adiposity [5], [6]. Some surveys have reported that meaningful relation between IL-6 G-174C gene polymorphism and adiposity were not observed [7], [8]. Some other studies have reported that IL-6 increase energy expenditure in rodents and human, suppresses body fat and limits late-onset obesity [9]. The C allele appears to be related with low concentration of IL-6 in variant cell systems and decrease body temperature and energy expenditure [10], [11], [12]. Decrease of IL-6 concentration and its association with the G-17C allele has been reported in some studies [11], [13], not all of them [14], [15]. In this study, we observed that obese individual carrying the G allele had higher IL-6 concentration than the C allele carrier obese individuals but this difference was not significant. Circulating IL-6 is derived from different cell types such as adipose tissue, muscle cells and hypothalamus, that all of them contribute to the control of energy balance [4]. In addition, there are considerable irregular variations of circulating IL-6 levels in during the day [16]. In other hand, genetically determined decrease in IL-6 production per weight adipose tissue probably in theory cause increased adiposity [17]. The high concentration of IL-6 in obese individuals could involve resistant of IL-6 in a like path as they are patterned to be Leptin resistant [18].

Nevertheless, evidence have been shown that the effects of IL-6 on adipose tissue and energy expenditure are exerted at the level the central nervous system (CNS) instead of the periphery [9], [19], [20]. As well as, the IL-6 level in the CNS seem to be controlled in a various way than circulating levels of IL-6 [21], raising the possibility level of circulating IL-6 do not return the anti-obesity potential of IL-6 [3]. Obesity, insulin resistant and metabolic syndrome seems to be related with serum IL-6 levels [22]. In 1990, it was reported that treatment with high amount of IL-6 increase insulin resistant [23], [24]. However, some more studies have not shown that after IL-6 treatment any increase in blood glucose [25] [26]. Interestingly, anti-IL-6 receptor antibody treatment has been shown to cause increased level of blood glucose in some patient with rheumatoid arthritis [27]. In the present study, we observed that fasting blood glucose in obese individuals carrying C allele was higher than obese individuals carrying G allele. So, the effect of IL-6 appears complex and both increased and decreased activity of IL-6 cause enhanced insulin resistant. In conclusion, as indicated in

the study, the C allele is probably not affecting gain weight in Iranian population, maybe due to interaction with unknown environmental and genetic factors.

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TABLE 1
FREQUENCY OF G AND C ALLELE

	Female		Male		Total
	obese	Non-obese	obese	Non- obese	
Allele G	15(38/4)	40(72/2)	25(64.1)	76(69.7)	156(64.5)
Allele C	24(61.6)	15(27/7)	14(35.9)	33(30.3)	86(35.5)

Data are shown as no (%).

TABLE II
CLINICAL AND BIOCHEMICAL CHARACTERISTICS ACCORDING TO THE G-174C GENOTYPE

	-174 IL-6 genotype			Pvalue (K-W)
	GG	GC	CC	
Subject(No. /%)	156/64	71/29	15/6	
Age	38.43±19.06	34.58±16.99	42.20±14.07	
BMI	25.28±15.24	2539±4.91	26.57±6.60	0.17
WHR	0.86±0.08	0.86±0.09	0.85±0.08	0.95
Weight	64.66±15.24	65.94±13.98	69.70±13.57	0.50
Ln SBP(mmHg)	4.73±0.13	4.70±0.13	4.70±0.12	0.22
DBP(mmHg)	73.21±8.13	72.49±8.71	73.73±8.74	0.75
Fb-glucose	88.5±28.75	87.50±55.86	91.00±20.76	0.11
Total cholesterol(mM)	191.58±41.47	186.90±42.97	193.33±43.99	0.62
LDL(mM)	120.79±34.95	116.34±34.08	120.28±41.14	0.65
HDL(mM)	43.12±9.94	42.14±9.54	45.86±11.31	0.54
LnTriglycerides (mM)	4.82±0.44	4.79±0.58	4.78±0.52	0.65
IL-6(pg/ml)	1.49±0.97	1.48±0.99	1.71±0.68	0.55
hsCRP(ng/ml)	6.73±1.48	6.50±1.56	6.32±1.91	0.53

SBP=systolic blood pressure, DBP=diastolic blood pressure, Fb-glucose=fasting blood glucose, IL-6=interleukin 6, hsCRP=high sensitive C-reactive protein. The *p*-value is for comparison of the three genotypes (Kruskal -Wallis). Data are shown as mean ± SEM

TABLE III
CLINICAL AND BIOCHEMICAL CHARACTERISTICS ACCORDING TO ALLELE CARRIERS IN G-174C SNP

Carrier	obese		p-value	Non-obese		p-value
	G	C		G	C	
BMI	31.53±2.78	32.49±4.09	0.63	23.97±2.11	23.90±2.91	0.84
Weight	75.57±8.16	80.74±11.49	0.15	62.37±15.41	63.18±12.29	0.65
WHR	0.89±0.05	0.92±0.07	0.20	0.85±0.09	0.84±0.08	0.88
Fb-glucose (mg/dl)	89.5±14.9	95.00±33.4	**0.04**	88.5±30.8	88.5±30.8	0.05

The *p* -value are for comparison of the carriers (Mann-Whitney). Data are shown as mean ± SEM