Association of Dietary Total Antioxidant Capacity and MC4R Polymorphisms in Metabolically Healthy and Unhealthy Overweight/Obese Women

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Abstract

Background: 3-15% of people with obesity display a metabolically healthy phenotype. An inflammatory diet impairs metabolic signaling pathways and eliciting metabolic syndrome. The main purpose of this study was to investigate how the Dietary Total Antioxidant Capacity (DTAC) and the MC4R variants affect the metabolic health status in overweight and obese Iranian women.

Methods and results: This cross-sectional study was conducted on 237 overweight-obese Tehrani women with mean age of 36 years. The DTAC was calculated using the following indices: total reactive antioxidant potential (TRAP), Trolox equivalent antioxidant capacity (TEAC), and the ferric-reducing ability of plasma (FRAP). The Metabolic health was evaluated using the Karelis criteria. The MC4R genotypes were determined by the restriction fragment length polymorphism (PCR-RFLP) method.

Approximately 43% of participants were identified as Metabolically Unhealthy Obesity (MUO), 6% Metabolically Healthy Obesity (MHO) including 42.5% of the total had T/T genotype, 23.8% had the C/T genotype, and 33.5% had the C/C genotype ($P=0.05$). In the C/C genotype people 75% were unhealthy whereas it was lower in T/T and T/C genotype people. Further, The C/C genotype with higher DTAC quartile had higher Karlis score than other DTAC quartile. A liner model test supported that the probability of MHO was significantly higher in subjects with the T/C genotype ($\beta=-0.001$, 95% CI= -0.001-4.796; $P \leq 0.1$).

Conclusions: Our results indicated that the C/C genotype with higher DTAC had favorable lipid profile and were metabolically healthier. It is plausible that dietary modification targeting antioxidant intake may attenuate metabolic impairments associated with genetic predisposition.

Introduction

The global prevalence of obesity and its upcoming metabolic complications and chronic diseases such as insulin resistance, dyslipidemia, hypertension, inflammation, cardiovascular diseases (CVD), type 2 diabetes mellitus (DM2) continue to rise [1–3]. Interestingly, 3–15% of people with obesity do not present diagnosed metabolic complications [4–6]. Although, people with obesity considered metabolically healthy have a higher risk of CVD compared to normal weight individuals without metabolic alterations, the risk appears less than metabolically unhealthy obese people [7–9]. It is suggested that a nutrigenomic factor or mechanism affording some protection against metabolic syndrome [10].

Rare mutations in the melanocortin-4 receptor (MC4R), the second SNPs for obesity, are related with lower energy expenditure and more likely to experience hyperphagia. According to estimates, polymorphisms in this gene are responsible for 2.4–9% of significant obesity and has a tight interaction with environmental factors [11–13]. There are also some variants in the *MC4R* gene, which may have preventive effects on obesity [7, 14]. Several variants in the *MC4R* gene have been identified which rs17782313 polymorphism (T/C) is one of those frequently described [15–21]. A higher BMI and energy intake in people with the common SNP rs17782313 near MC4R gene have also been observed [22–24]. Further, an association of

rs17782313 polymorphism (T/C) with increased risk of obesity among subgroups of Europeans and East Asians, adults and children has been well reported [8, 22, 25].

The interaction of the MC4R polymorphisms and metabolism may be moderated by the adaptive response to oxidative stress. Oxidative stress represents an integral component of metabolic complications. Dietary total antioxidant capacity (DTAC) is a reliable indicator of diet quality and is typically used to estimate the cumulative effect of antioxidants in the diet overall [26]. It can also provide insight into synergistic effects that may exist between diverse antioxidants in a mixed diet [14, 15]. The overproduction of reactive oxygen species may impair insulin signaling pathways and lead to endothelial damage. This causes insulin resistance and promotes acceleration of the atherogenic processes underlying metabolic syndrome, obesity, type 2 diabetes and CVD [18, 19]. The relationship between DTAC and MC4R polymorphism has been investigated in limited studies mostly with healthy people. To our knowledge, there have been few human based studies in Middle East countries with different genotype structure and diet pattern, evaluating this relationship regarding metabolic health status. As high incidence of metabolic disorders continue to burden the region, the aim of this cross-sectional study was to assess the interaction between DTAC and MC4R among overweight and obese Iranian women with regard to their metabolic health status.

2. Method

2.1. Study population

In this cross-sectional study, 218 out of 288 healthy women with overweight and obesity who were referred to community health centers of Tehran university of medical science were randomly selected based on the following inclusion criteria: 1) body mass index (BMI) 25-40 kg.m² - 2) aged 18-50 years old. The primary exclusion criteria included the following: 1) menopause 2) pregnancy 3) cardiovascular diseases 4) diabetes 5) Cancer 6) kidney disease 7) thyroid disease 8) acute or chronic diseases 9) use of dietary supplements for weight loss 10) follow up diet during the past year 11) use of lipid lowering drugs 12) use of blood glucose lowering drugs. More over, participants who did not complete more than 35 items of the food frequency questionnaire and those who reported total daily energy intakes ≤ 800 or ≥ 4500 kcal/d were excluded. This study carried out in accordance with the recommendations of the ethics committee of institution (Ethical number IR.TUMS.VCR.REC.1398.619) with written informed consent from all participants .

2.2. Anthropometric assessment

For each participant, the height, the weight, waist, and hip circumferences were measured. These measurements were made in compliance with WHO recommendations and the assessments were performed by an experience nutritionist. Participants were weighed without shoes and in minimal clothing on a solar digital scale recorded to the last 0.2 kg. The standing height was measured on a free-standing portable height meter with a precision of 0.5cm without shoes. BMI was calculated by weight (kg)/height (m) squared [10].

2.4. Dietary assessment and DTAC evaluation

Detailed dietary information was obtained through the use of the validated semi quantitative food frequency questionnaire (FFQ) [27]. The FFQ includes 147 items with a standard serving size commonly consumed by Iranians. The reported frequency for each food item was then converted to a daily intake. The nutrient content of foods were computed by the Nutritionist 4 software based on United States Department of Agriculture (USDA) food composition table modified for Iranian foods. DTAC was calculated using the following indices: total reactive antioxidant potential (TRAP), Trolox equivalent antioxidant capacity (TEAC), and the ferric-reducing ability of plasma (FRAP). Since no databases were available to calculate the quantity of antioxidants in Iranian foods, given the available resources, we chose databases that contained most of the foods that are consumed by the Iranian population. TRAP and TEAC values were obtained from published databases for Italian foods. For FRAP, we used a database developed by Halvorsen et al. Total antioxidant capacity values for each food item in the FFQ were matched to an equivalent food in each of the databases. If any food was not directly matched with a corresponding food in a database, a proxy estimation was used based on the mean value of a similar food or the value of a raw food as a substitute for the cooked food [19, 21]. DTAC for every participant was obtained by multiplying the daily intake of each selected food item by its corresponding antioxidant value per food portion and summing the final values. Antioxidants from supplements were not included in the calculation of DTAC reported as micromole of Trolox Equivalents per day ($\mu\text{mol TE/day}$).

2.3. Biochemical analysis

Venous blood was drawn at 8:00 AM to avoid variations due to circadian rhythm and after a fast equal to or >12 h. Samples were collected in two tubes, one containing sodium citrate and the other without anticoagulant centrifuged at 3000 rpm for 15 min at 4 °C, and aliquots of plasma and serum were prepared for testing. Glucose, total cholesterol, HDL-C, and triglycerides levels were determined by the semiautomatic chemical analyzer Ekem Kontrolab. LDL-C serum concentration was calculated with Fried Ewald's formula. Insulin resistance was evaluated through homeostasis model assessment : $\text{HOMA-IR} = \text{insulin (mU/mL)} \times \text{fasting glucose (mmol/L)} / 22.5$ [28].

2.4. Definition of metabolically healthy and unhealthy phenotypes

There are no general definition of metabolically healthy obesity (MHO). Throughout this paper, the MHO was described using adapted Karelis criteria. In the case of the Karelis criteria : total cholesterol ≤ 200 mg/dL, triglycerides ≤ 150 mg/dL (≤ 1.7 mmol/L), high-density lipoprotein cholesterol (HDL_C) ≥ 50 mg/dL and no treatment, low-density lipoprotein cholesterol (LDL-C) ≤ 100 mg/dL and no treatment, and HOMA-IR ≤ 2.8 ; that ≥ 4 positive score criteria defined as Metabolic Healthy. Thus, participants were classified into 4 groups according to metabolic health: Metabolically Healthy Over Weight (MHOW), Metabolically Unhealthy Over Weight (MUOW), Metabolically Healthy Obesity (MHO) and Metabolically Unhealthy Obesity (MUO) [8, 10].

2.5. The Genotype determination

The chromosome 9p21 rs17782313 SNP (genotypes C & T) was genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The Genomic DNA was extracted from 200 mL of whole blood using the Mini Columns Type G kit manual (GeneAll, South Korea). The extracted DNA was used to assess two reported SNPs near MC4R gene, rs17782313 and rs17700633 SNPs. Polymerase chain reaction (PCR) was performed on the rs17782313 and rs17700633 SNPs using the following primers: forward primer 5AAGTTCTACCTACCATGTTCTTGG-3 and reverse primer 5-TTCCCCCTGAAGCTTTTCTTGTCATTTTGAT-3. PCR was carried out on a total volume of 20 μ L, containing 1 μ L extracted DNA, 0.5 μ L primers F, 0.5 μ L primers R, 10 μ L Permixon (Amplicon, Germany), and 8 μ L Distilled water, with the following conditions in a DNA thermocycler: 1- primary denaturation at 95°C for 2 min; 2- Thirty-five cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 30 seconds; 3- final extension at 72°C for 5 min; 4- final step at 4°C. The amplification protocol consisted of a primary denaturation step at 94 ° C for 5 min, followed by 35 cycles of denaturation at 60 ° C for 1 min, annealing at 94 ° C for 45 s, and extension at 72 ° C for 1 min, and final extension at 72 ° C for 10 min. The digestion products were stained with ethidium bromide on a 2% agarose gel and imaged. 10% of the samples were directly sequenced for confirmation the PCR-RFLP results. The sequencing process performed using the ABI PRISM 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA) [29]. Amplified DNA (7 μ L) was digested with 0.5 μ L of BclI restriction enzyme (Fermentase, Germany) at 56 °C overnight. All products were visualized by agarose gel electrophoresis. Then, fragments containing three genotypes were distinguished: CC, CT, and TT.

2.6. Statistical analyses

Statistical analyses were performed using SPSS 20 (SPSS Inc., Chicago, IL, USA). Normal data distribution was determined using the Kolmogorov-Smirnov test. The ANOVA test and general linear model were used to assess differences in biochemical measurements and characteristics between groups. A general linear model adjusted for MC4R, DTACT as confounder effects was used. Coding of the SNPs was performed using an additive model. The study population characteristics are reported as mean $\pm$ standard deviation (SD). The Statistical significance was defined as $p \leq 0.05$ for all analyses.

3. Results

The mean and standard deviation (SD) of age of the participants were 36.23 ± 8.2 years, range 18–50 years. The characteristics of participants are summarized in Table 1. Participants were 43% MUOB, 6.3% MHOB, 29.5% MUOW and 20.6% MHOW, according to Karelis criteria. In the C/C genotype people 75% were unhealthy where as it declined to 67.1% and 67.4% in T/T and T/C genotype people respectively.

Table 1
Characteristics of the study participants

	Mean ± SD	Min	Max
AGE(y)	36.23 ± 8.22	18	50
BMI(Kg/m ²)	30.69 ± 3.69	25.00	40.70
WHR	0.93 ± 0.05	0.81	1.08
TG(mg/dL)	120.94 ± 68.69	37.00	512.00
HDL(mg/dL)	46.36 ± 10.58	18.00	82.00
LDL(mg/dL)	94.42 ± 23.81	34.00	156.00
Insulin(μIU/mL)	15.56 ± 6.02	6.67	65.89
HOMA INDEX	3.40 ± 1.52	1.29	16.59
DTAC (mmol TE/100 g)	1167.28 ± 764.66	9.11	4179.96
Abbreviations: SD, standard deviation; Min, minimum; Max, maximum; BMI, body mass index; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; HOMA, homeostatic model assessment; TG, triglycerides; DTAC, Dietary total antioxidant capacity			

There was a significant difference between groups in metabolic health status. All Subjects in the metabolically unhealthy groups had higher BMI, TG, LDL-C, HOMA-Index, insulin and lower HDL-C than those in the metabolically healthy groups significantly ($P \leq 0.05$) (Table 2).

Table 2
The Metabolic characteristics of healthy and unhealthy groups

The metabolic characteristics of healthy and unhealthy groups					
BMI (Kg/m2)		MH-OW	MU-OW	MHO	MUO
	Mean ± SD	27.55 ± 1.60	27.72 ± 1.36	32.42 ± 2.80	33.33 ± 2.69
	p-value	≤ 0.05			
HDL(mg/dL)	Mean ± SD	80.20 ± 26.50	117.05 ± 49.38	80.00 ± 20.84	149.13 ± 84.10
	p-value	≤ 0.05			
TG(mg/dL)	Mean ± SD	51.69 ± 9.97	43.98 ± 8.05	53.00 ± 7.33	44.47 ± 11.60
	p-value	≤ 0.05			
LDL(mg/dL)	Mean ± SD	86.10 ± 19.84	95.97 ± 22.93	81.75 ± 18.77	99.73 ± 25.06
	p-value	≤ 0.05			
INSULIN(μIU/mL)	Mean ± SD	11.73 ± 3.62	15.92 ± 4.68	12.33 ± 3.92	17.67 ± 7.13
	p-value	≤ 0.05			
HOMA INDEX	Mean ± SD	2.45 ± 0.85	3.38 ± 1.03	2.52 ± 0.89	3.96 ± 1.84
	p-value	≤ 0.05			
Abbreviations: SD, standard deviation; MH-OWBMI, body mass index; HDL, high-density lipoprotein cholesterol; LDL,low-densitylipoproteincholesterol;HOMA,homeostaticmodelassessment;TG,triglycerides;MH-OW, metabolically healthy overweight; MU-OW, metabolically unhealthy overweight; MHO, metabolically healthy obesity; MUO, metabolically healthy overweight, ANOVA test (P ≤ 0.05)					

Table 3 represented that the mean DTAC was 1167.28 mEq with ± SD 764.66. The TG, LDL, HOMA-INDEX values had inverse relation to DTAC values, albeit insignificant ($P \leq 0.05$) (Table 3).

Table 3
The Mean $\pm$ SD of metabolic values in DTAC tertile groups

Variables	DTAC tertile	Mean $\pm$ SD	P-value
BMI (Kg/m ²)	low	30.32 $\pm$ 3.59	0.61
	median	30.87 $\pm$ 3.42	
	high	30.68 $\pm$ 3.92	
	Total	30.62 $\pm$ 3.64	
TG(mg/dL)	low	122.86 $\pm$ 72.58	0.80
	median	117.78 $\pm$ 73.38	
	high	115.12 $\pm$ 67.06	
	Total	118.51 $\pm$ 70.67	
HDL(mg/dL)	low	47.17 $\pm$ 10.52	0.75
	median	45.92 $\pm$ 10.77	
	high	47.12 $\pm$ 11.73	
	Total	46.75 $\pm$ 11.00	
LDL(mg/dL)	low	93.45 $\pm$ 24.74	0.97
	median	92.75 $\pm$ 22.51	
	high	92.64 $\pm$ 23.04	
	Total	92.94 $\pm$ 23.33	
INSULIN (μ IU/mL)	low	15.76 $\pm$ 5.07	0.88
	median	15.21 $\pm$ 4.69	
	high	15.45 $\pm$ 8.38	
	Total	15.48 $\pm$ 6.32	
HOMA-INDEX	low	3.43 $\pm$ 1.20	0.90
	median	3.31 $\pm$ 1.31	
	high	3.37 $\pm$ 2.14	
	Total	3.37 $\pm$ 1.618	

Data presented as SD, standard deviation;

BMI, body mass index; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; HOMA, homeostatic model assessment; IR, insulin resistance; TG, triglycerides; DTAC, Dietary total antioxidant capacity, ANOVA test ($P \leq 0.05$)

Furthermore, the T/T genotype was identified in 42.5%, 23.8% the C/T genotype, and 33.5% the C/C genotype. The C/C genotype with higher DTAC quartile had higher Karlis score than other DTAC quartile. The T/C genotype had higher TG, LDL-C and HOMA-INDEX but not statistically different from other genotypes. Although, meaningful correlation was observed between this genotype and insulin ($P \leq 0.05$) and HOMA-INDEX ($P \leq 0.05$) (Table 4).

Table 4
The Mean & $\pm$ SD of variables in MC4R rs17782313 Polymorphism groups

Variables	MC4R polymorphism	Mean $\pm$ SD	P-value
BMI (Kg/m ²)	TT	30.74 $\pm$ 3.65	0.91
	TC	30.51 $\pm$ 3.61	
	CC	30.74 $\pm$ 3.70	
TG(mg/dL)	TT	125.09 $\pm$ 81.36	0.33
	TC	126.60 $\pm$ 68.60	
	CC	110.71 $\pm$ 55.15	
HDL(mg/dL)	TT	45.34 $\pm$ 10.34	0.33
	TC	47.30 $\pm$ 11.32	
	CC	47.45 $\pm$ 10.96	
LDL(mg/dL)	TT	91.36 $\pm$ 23.04	0.34
	TC	97.00 $\pm$ 26.07	
	CC	95.31 $\pm$ 24.93	
INSULIN (μ IU/mL)	TT	14.63 $\pm$ 4.58	0.04
	TC	17.25 $\pm$ 9.21	
	CC	15.65 $\pm$ 4.96	
HOMA-INDEX	TT	3.14 $\pm$ 1.08	0.02
	TC	3.88 $\pm$ 2.42	
	CC	3.37 $\pm$ 1.25	

Data presented in SD, standard deviation;

BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HOMA, homeostatic model assessment; IR, insulin resistance; TG, triglycerides; DTAC, Dietary total antioxidant capacity; MC4R Gene, melanocortin-4 receptor polymorphism, ANOVA test ($P \leq 0.05$)

To assess the interaction between particular polymorphisms and the MUO and MHO, a liner model test was applied (Table 5). The test revealed that the probability of being MHO was lower in subjects with the C/C genotype than two other genotypes. The test value was statistically significant ($\beta = 0$, 95% CI: 0.000–0.001; $P \leq 0.05$). Interestingly, it was observed that C/C genotype with higher DTAC were metabolically healthier. In contrast, T/C genotype with lower DTAC values were metabolically healthier (Fig. 1). No significant correlation detected between the T/T genotype and DTAC level.

Table 5
The Interaction between MC4R Gene Polymorphism and DTAC

Independent Variables	B	Std. Error	CI	P-Value
MC4R CC* DTAC	0.00	0.00	0.00–0.00	0.05
MC4R TT* DTAC	0.00	0.00	0.00–0.00	0.28
MC4R TC * DTAC	0.00	0.00	0.00–4.79	0.05
Liner model test, reference = MC4R CC				
Dependent variable: Karlis				
For interactions $p < 0.1$ was considered significant.				
a. set to zero because this parameter is redundant				
b. maximum likelihood estimate.				

4. Discussion

This cross-sectional study investigated how the DTAC and MC4R gene variants were related to being MHO/MUO in overweight and obese women. Interactions between genetic and environmental factors play an important part in the metabolic health status and obesity. Our results showed that a significant correlation was between the DTAC and the MC4R SNPs and the MHO. MUOW-OB diagnosed on the basis of the Karelis criteria was observed in 67.4% of these overweight-obese women. It was slightly more common in the subgroup with the C/C genotype (75%). The liner model test showed that the C allele raises the risk of metabolically unhealthy significantly. For the first time, this study provided evidence for a significant relation between the MC4R gene and the DTAC level. The C/C genotype subjects with higher DTAC had favorable lipid profile and were metabolically healthier. Conversely, T/C genotype subjects with lower DTAC values were metabolically healthier. These results supported the interaction was found between the T/C genotype and the metabolism in some previous studies[5].

MHO/OW incidence in this study was similar previous studies [4, 8, 30, 31]. The recent Hosseinpanah cohort study showed that 26.9% of male and 39.4% of female Iranian people were MHO [31].

Pervious studies have demonstrated that metabolically healthy obese people (MHO) have a lower risk of CVD compared to the MUO population [7, 8]. Although the causes of this metabolic alterations are still not

clearly understood, evidence suggests that interactions between the genetic and life style factors including diet can influence such phenotypes in various population.

The MC4R SNPs prevalence in the Iranian ow/ob women was similar to the results of Mollahosseini et al study with the prevalence of the C/C allele 52.85% [3]. Furthermore, we observed a close connection between (SNP) of this gene and increased BMI. In line with our findings, previous studies showed that the C/C allele carriers' BMI was higher by 0.2 kg.m² on average [5, 22, 23]. German authors reported Comparable results, who claimed that the presence of the C/C allele entailed a considerably higher BMI. Even though we were unable to demonstrate a significant correlation between the DTAC values and TG, LDL, HOMA-INDEX values, earlier researches revealed an inverse relationship between these factors. This apparent lack of correlation could be attributed to hard to control entity of the DTAC and method of study.

Previous investigations revealed that higher DTAC positively affected obesity related disorders [8, 21]. Mousavizadeh's study suggested that the healthy diet interacts with MC4RSNP (rs17782313) which decrease the risk of general obesity in C-allele carriers [24].

In another similar study, high DTAC were significantly associated with a reduced odds ratio for the prevalence of chronic diseases and metabolic syndrome components [32]. Besides, the findings of longitudinal Tehran Lipid and Glucose Study showed that higher DTAC were positively associated with a lower occurrence of metabolic complications, hence being healthy[33]. The European Prospective Investigation into Cancer and Nutrition study pointed out the DTAC might play an essential role in reducing the risk of diabetes in women [34]. MC4R SNPs changes metabolic profile through uncertain mechanisms. Their phenotypic expression is affected by environmental factors, such as quantitative and qualitative composition of diet [13].

In line with previous studies, a statically significant association between MC4R genotype and Insulin and HOMA-INDEX was observed[1, 4, 8]. This to some extent confirms that insulin resistance may be the mechanism through which the MC4R polymorphisms affect metabolism leading to be metabolically healthy or unhealthy obese. In other word, HOMA-INDEX could likely be important predictor in general population [4, 35, 36]. Achilike et al. study suggested that lipid profile and insulin resistance are two key factors that predict the development of metabolically healthy or unhealthy obesity [35].

The main strengths of this study include assessment of MC4R SNPs regarding Metabolic Health OW-OB in Iranian women (A Middle East countries). The first limitation of the study was small sample that could not examine the exact odds ratios. Other limitation were monogender and cross-sectional method of study. Hence, it is suggested that other scientists perform similar studies with larger sample sizes and the case-control method. Beside, we did not have normal weight subjects for better comparison.

In Conclusion, Our study supported that higher dietary antioxidant intake have favorable effects on metabolic indexes in the C/C genotype MHO people. Therefore, we could suggest that the encouragement of lifestyle modification including the consumption of antioxidant rich diet, is an important factor in nutritional interventions aimed at preventing metabolic complications. Furthermore, our results revealed

that insulin resistance might be the mechanism through which the MC4R polymorphisms affect metabolism. HOMA-INDEX could likely be used as predictor of being MHO or MUO in investigations.

Declarations

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Availability of data and material “ applicable”

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Authors' contributions [Khadijeh Mirzaei], Formal analysis and investigation: [Habib Yarizadeh], Writing - original draft preparation: [Maryam ElhamKia]; Writing - review and editing: [Krista Casazza , Leila Setayesh, Maryam ElhamKia], Supervision: [khadijeh mirzaei], Methodology: [Zahra Veisy, Vajihe Aghamohammadi, Sara Pooyan]

Ethics approval Ethical number IR.TUMS.VCR.REC.1398.619

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Figures

Figure 1

The association between Karelis scores and DTAC level tertile and the chromosome 9p21 rs1333048 SNP Polymorphisms L, low level of DTAC; M, median level of DTAC; H, high level of DTAC, P-value $\leq$ 0.05.