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The Gene-Treatment Interaction of FTO-rs9939609 Gene Polymorphism and Epigallocatechin-Gallate Intervention on Anthropometric Indices, Fasting Blood Sugar and Insulin Resistance/Sensitivity in Patients with Type 2 Diabetes Mellitus

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Abstract

Background: The role of FTO-rs9939609 gene variants in response to the Epigallocatechin-Gallate (EGCG) intervention remains unclear.

Objectives: The present study aimed at investigating the gene-treatment interaction of FTO-rs9939609 gene polymorphism and EGCG intervention on anthropometric indexes, fasting blood sugar, and insulin resistance/sensitivity in patients with Type 2 Diabetes Mellitus (T2DM).

Methods: This double-blind, randomized, placebo-controlled study was conducted on 66 patients (aged 20 to 60 years) with T2DM in Iran, from August 2017 to March 2018. Individuals were randomly block allocated to three groups. Group 1 received 300 mg EGCG (n = 22, TT genotype), Group 2 received 300 mg EGCG (n = 22, AA + AT genotypes), Group 3 received the placebo (n = 22). Two months following the intervention, Waist-Hip Ratio (WHR), A Body Shape Index (ABSI), Fasting Blood Sugar (FBS), and insulin levels, as well as Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) and Quantitative Insulin Sensitivity Check Index (QUICKI) were evaluated. The FTO-rs9939609 polymorphism was genotyped by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP).

Results: In both EGCG groups, a significant reduction in WHR was observed after the intervention compared with baseline ($P < 0.05$), with no significant differences in other parameters. The FTO-rs9939609 polymorphism showed no gene-treatment interaction in response to EGCG.

Conclusions: This study suggests that administration of EGCG supplement for two months may provide anti-obesity effects in patients with T2DM. However, the FTO-rs9939609 polymorphism was not associated with the change in anthropometric and glycemic status after EGCG intervention.

Keywords: Diabetes Mellitus, Epigallocatechin-Gallate, Fasting Blood Sugar, FTO, Insulin Resistance

1. Background

The International Federation of Diabetes statistics reported that the prevalence of diabetes mellitus, as one of the most common metabolic diseases, was about 415 million adults (in 2015) around worldwide and it will rise to 642 million in 2040 (1). It is estimated that about 90% of cases with T2DM are related to overweight and obesity. The extra fat tissue in obese people elevation levels of adipocytokines and factors released from adipose tissue induces insulin resistance. Also, increased free fatty acids following obesity can exacerbate insulin resistance by inhibiting the activity of glucose transporters (2).

In addition, the development of T2DM is the outcome of the interaction between environmental risk factors and genetic component. A meta-analysis estimated that the heritability of T2DM in twin pairs was 72% (3). In the last two decades, Genome-Wide Association Studies (GWASs) indicated the association of more than 100 genes with

T2DM. The study of genes associated with T2DM, in addition to recognizing the etiology, is also useful in treatment of the disease. Although medicines, diets, and lifestyle changes can improve the status of patients with T2DM, yet the genetic background of patients can affect their response to interventions (4). Recent studies have demonstrated a genotype-intervention interaction for polymorphism of diabetes-related genes (5).

The fat-mass and obesity-associated gene (FTO) is one of the genes that is strongly associated with obesity and T2DM. Numerous studies have reported on the association between the FTO variants and T2DM risk, independent of Body Mass Index (BMI), and body weight (6). Saber-Ayad et al. suggested that the individuals with FTO rs9939609 AA genotype may be more potential to insulin resistance and Impaired Fasting Glucose (IFG) in the Emirati population (7). On the other, some studies reported a genotype-intervention interaction for the FTO (rs9939609) gene polymorphism (7). Zhang et al. reported that carriers of the risk allele of the FTO had a greater reduction in body composition and fat distribution in response to a high-protein diet (8). However, the genotype-intervention interaction for the rs9939609 SNP has not yet been clarified, since some studies did not report on an interaction (9).

Epigallocatechin Gallate (EGCG) is an antioxidant that belongs to the catechins family. Furthermore, EGCG is found mainly in green tea. Anti-obesity effects of EGCG are not apparent. Basu et al. reported that green tea extracts can reduce body weight and BMI (10). In contrast, Hsu et al. suggested that green tea extract (302 mg EGCG) did not lead to weight loss (11). Previous studies have shown that EGCG improves insulin sensitivity and attenuates blood glucose and insulin resistance via restoring GLUT4 expression in skeletal muscle (12). Also, Kaneko et al. suggested that EGCG at supraphysiological concentrations has inhibitory effects on glucose-stimulated insulin secretion by the inhibition of voltage-dependent Ca (2+) channels (13). Although the effect of EGCG on T2DM has been studied in animal and in vitro studies, results of clinical trials are inconsistent. In addition, the role of genotype-intervention interaction in response to EGCG has not been evaluated.

2. Objectives

The present study aimed at investigating the gene-treatment interaction of FTO-rs9939609 gene polymorphism and EGCG intervention on anthropometric indices and glycemic profile of patients with T2DM.

3. Methods

3.1. Participants

This double-blind, randomized, placebo-controlled study was conducted on sixty-six patients with T2DM, selected from the endocrine clinic of Golestan referral and governmental Hospital of Ahvaz Jundishapur University of Medical Science, Ahvaz, Khozestan, Iran, during August, 2017 to March 2018. Randomization was stratified according to age and BMI.

Inclusion criteria were as follows: age between 20 and 60 years old, BMI of 18.5 to 35 kg/m^2, fasting blood glucose of $\geq$ 126 mg/dL, diagnosis of T2DM with disease duration of more than two years. Exclusion criteria were as follows: Diabetic foot ulcer, kidney failure, pregnant or lactating women, digestive, liver, inflammatory, cardiovascular diseases, and cancer.

Moreover, taking antioxidant supplements during the past six months, using insulin and corticosteroids and following a weight-loss diet over the past three months were also considered as the exclusion criteria (Ethical code: IR.AJUMS.REC.1395.548) (IRCT2017021612949N3).

3.2. Study Design

Prescreening was applied by interview for all volunteers and participants were selected based on the inclusion criteria (n = 200). To achieve equal number of participants in each group (based on genotype), genotyping of all eligible individuals was done. Finally, 66 individuals were selected based on the ratio of required genotype and exclusion criteria. The participants were randomly allocated to the three groups: EGCG supplement (TT genotype, n = 22), EGCG supplement (AT + AA genotype, n = 22), and placebo (A + T genotype, n = 22), according to randomized block allocation. The flowchart of the study has been presented in Figure 1.

The participants were grouped randomly by a third person and the researchers were blinded to the supplement type administered to the patients. The participants were matched for age and BMI. The intervention groups received two tablets of EGCG supplement, containing 300 mg/day EGCG of *Camellia sinensis* leaves for two months. The placebo group received the same number of placebo tablets. All subjects were contacted and monitored weekly by telephone. An observer was used to evaluate the equipment, methods, and assessment technics used in this study. During the study, all subjects were asked to maintain their dietary intake and physical activity.

The sample size was calculated with 85% power test at a significance level of 5%, and considering the Standard Deviation (SD) and difference in mean (6.3 IU/L) of serum insulin in the study by Liu et al. (14) a sample size of 17 patients was determined in each group using the Equation 1:

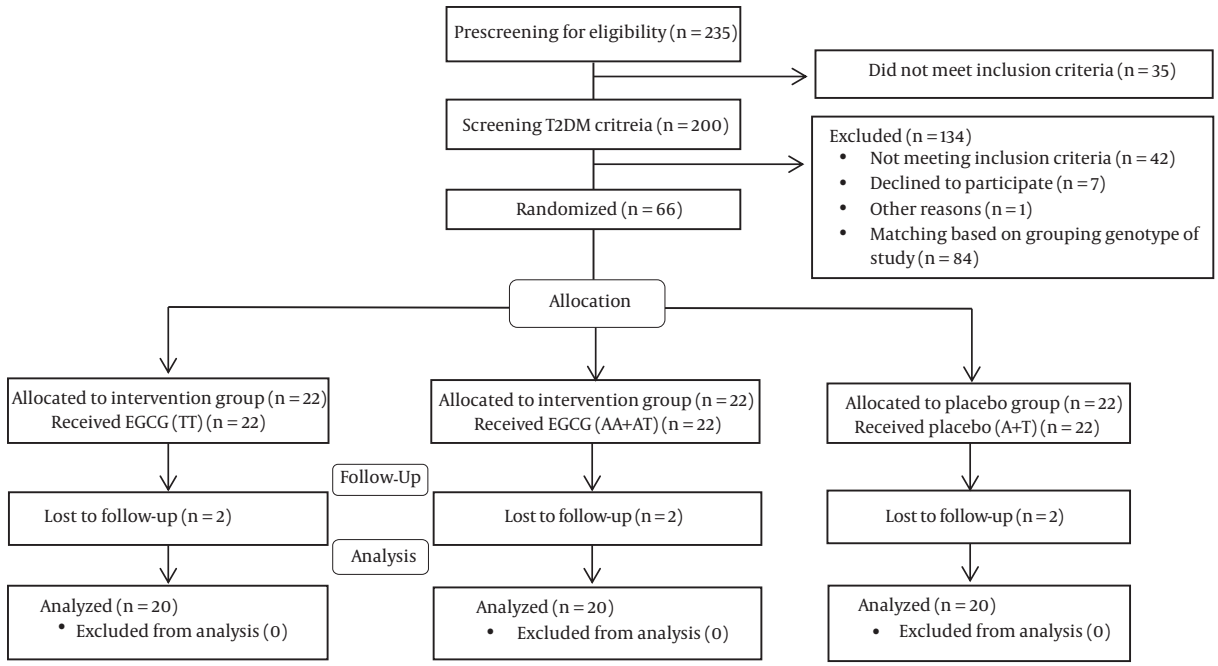

Figure 1. Flowchart of the study

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 \left(S_1^2 + S_1^2\right)}{\left(\mu_1 - \mu_2\right)^2} \quad (1)$$

The sample size was increased to 22 participants in each group, considering a possible drop out of 30%.

3.3. Dietary and Anthropometric Measurement

A 24-hour dietary recall questionnaire (two work days and one weekend) was completed to assess the dietary intakes of patients. Dietary intake was analyzed using Nutritionist 4.

Body weight (to the nearest 0.1 kg) and standing height (to the nearest 0.1 cm) were measured (Inbody230, Biospace, Korea). The scale was calibrated before each measurement. Body Mass Index was calculated with using weight (kg) divided by height (m) squared.

Waist Circumference (WC) was obtained at the level of the noticeable waist narrowing, located approximately halfway between the costal border and the iliac crest, and the level of the greatest posterior protuberance. Hip Circumference (HC) was taken at the greatest posterior protuberance of the buttocks. Waist-to-Hip Ratio (WHR) was calculated. A Body Shape Index (ABSI) is a new anthropometric index calculated according to previous studies (15).

$$A\ body\ shape\ index\ \ (ABSI) = \frac{WC}{BMI2/3 \times heigh1/2} \quad (2)$$

3.4. Biochemical Measurements

The blood samples (5 mL) were collected at the beginning and at the end of the study in an eight-hour fasting condition. The samples were centrifuged (3000 rpm for 10 minutes) to separate the serum. The FBS levels were determined immediately after sampling by a calibrated autoanalyzer (Pars Azmoon kits). The serum insulin concentration was assessed by the Enzyme-Linked Immunosorbent Assay (ELISA) kit (monoband) and Autobio microplate reader. HOMA-IR index was calculated by the following formula:

HOMA-IR = (fasting glucose) (mmol/L) × (fasting insulin) (μU/mL)/22.5).

Insulin sensitivity was calculated according to quantitative insulin sensitivity check index (QUICKI = 1/[log(I_0) + log(G_0)]).

3.5. Genotyping

A 5-mL blood sample was collected in EDTA-containing tubes and stored at -80°C for DNA extraction. Genomic DNA was extracted from whole blood samples, using DNA purification kit (Sinaclon, Iran). Single-Nucleotide Polymorphisms (SNPs) of the FTO-rs9939609 were genotyped using The Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). A DNA fragment (containing rs9939609 polymorphism) was amplified using primers. The polymerase chain reaction product of the FTO

gene was digested with *ScaI* restriction enzyme to analyze for polymorphism by the RFLP assay.

3.6. Statistical Analysis

All analyses were performed with IBM SPSS statistics for Windows, version 20.0 (IBM Corp., Armonk, N.Y., USA). Intention-to-Treat analysis (ITT) method was used for the analysis. The data were assessed for normality using the Kolmogorov-Smirnov test. According to the normality test, the data had a normal distribution. In order to assay differences before and after the intervention within groups, the paired sample *t*-test was used. Baseline characteristics were compared by chi-squared and Analysis of Variance (ANOVA). Analysis of Covariance (ANCOVA) tested differences between groups. A P value of < 0.05 was considered significant.

4. Results

Of the 235 volunteers, 200 were eligible and underwent genotyping, and anthropometric and biochemical assessments. Among them, 66 patients with T2DM were recruited in the study. After two months, there were two dropouts from each group. Consequently, the data reported here came from 60 subjects (Figure 1). There were no missing values in the study. Baseline characteristics for EGCG groups and placebo are shown in Table 1. No significant differences between any of the three group means were detected in age, gender, BMI, education, marital status, and disease duration (Table 1).

No significant differences were seen between the three groups for dietary intake, including energy, protein, carbohydrate, total fat, and cholesterol, at baseline (Table 2).

Within-group analysis after EGCG intervention on anthropometrics and glycemic variables, regarding FTO-rs9939609, is presented in Table 3. In both EGCG groups, a significant reduction in WHR was observed after the intervention compared with baseline ($P < 0.05$). However, no significant changes were observed in other variables before and after the interventions in any of the three groups.

Comparison of the modulation effects of FTO-rs9939609 on anthropometric and glycemic changes in response to EGCG are shown in Table 4. There were no significant differences in WHR, ABSI, FBS, insulin, HOMA-IR, and QUICKI changes between the groups in FTO-rs9939609 variants in response to EGCG ($P > 0.05$). In addition, after controlling for the confounding effects of age, gender, and BMI, the results did not change significantly.

5. Discussion

In the current study, the researchers observed no differential response to two months of EGCG supplementation by polymorphism of FTO-rs9939609 on anthropometric indexes and glycemic profile in patients with T2DM. However, some studies reported that response to dietary interventions in individuals with the A allele is more than TT genotype subjects (16). Also, some studies suggested that the effect of lifestyle changes on metabolic factors in genotype AA (or AT) is more compared with genotype TT (17). On the other hand, Livingstone et al. in systematic review and meta-analysis reported that differential changes in BMI, WC, and body weight after weight loss interventions (dietary, physical activity, or drug-based weight loss) were not significantly different between FTO genotypes (18). Different intervention types and characteristics of the study persons are potential reasons for discrepant findings.

The present study showed that EGCG supplements for two months in patients T2DM has no significant effect on fasting blood glucose, insulin serum, and insulin resistance/sensitivity. In this context, previous studies have shown contradictory results. Similar to the current findings, some studies did not confirm the significant effects of EGCG on glycemic profiles. The results of the study of Most et al., showed that 12 weeks of supplementation with EGCG (282 mg/day) and resveratrol (80 mg/day) cannot significantly alter the levels of glucose, insulin, and insulin resistance in obese subjects (19). In addition, Toolsee et al. in a randomized human cohort observed that three cups of green tea daily (containing 234 mg/per cup) for 14 days in pre-diabetic subjects did not lead to a significant change in FBS and hemoglobin glycosylated (20).

In contrast to the current results, some studies suggested that EGCG can significantly decrease the glycemic profile. A meta-analysis showed that green tea consumption significantly reduced the FBS (-0.09 mmol/L), fasting insulin (-1.16 μIU/mL), and hemoglobin glycosylated (-0.30%) (21). Also, a clinical trial revealed that green tea extract supplements (contains 856 mg EGCG) for 16 weeks led to significant reductions in HOMA-IR index and insulin level in obese individuals with T2DM (22). Differences in the results of studies may be due to differences in the type of population studied (the type of disease), type and dose of supplementation (EGCG or green tea), duration of intervention, sample size, and clinical features of patients at the beginning of the study.

The anti-obesity effect of EGCG may raise the potential of green tea to be effective in preventing and managing T2DM. Drinking at least four cups of tea/day can reduce the risk of developing D2TM by 16% (23). In the current study, the intervention of EGCG caused a significant reduction in WHR. In this context, most previous studies were consistent with the current results. A meta-analysis suggested that Green Tea Catechins (GTCs) significantly decreased BMI, body weight, and WC. However, did not show

Table 1. Baseline Characteristics[a]

Variables	EGCG Group, TT (N = 20)	EGCG Group, AA + AT (N = 20)	Placebo Group, A + T (N = 20)	P Value[b]
Age, y	52.25 ± 6.78	53.60 ± 8.03	55.25 ± 11.04	0.56
Male/female	8/12	9/11	7/13	0.81
BMI, kg/m^2	29.48 ± 4.48	29.59 ± 4.06	28.35 ± 3.57	0.57
Diploma or less/university	17/3	17/3	18/2	0.86
Had partner/single	19/1	19/1	18/2	0.76
Disease duration, y	8.10 ± 5.13	7.70 ± 5.00	7.95 ± 4.91	0.96

[a]Values are expressed as means ± SD.
[b]Changes between the three groups.

Table 2. Dietary Intake of the Study Patients[a]

Variables	EGCG Group, TT (N = 20)	EGCG Group, AA + AT (N = 20)	Placebo Group, A + T (N = 20)	P Value[b]
Energy, kcal	1807.83 ± 357.11	2081.50 ± 639.25	1914.07 ± 617.45	0.34
Protein, g	76.21 ± 19.10	81.67 ± 25.12	73.21 ± 16.26	0.39
Carbohydrate, g	285.48 ± 71.02	317.95 ± 118.47	295.06 ± 112.89	0.63
Fat, g	42.55 ± 18.36	54.25 ± 29.92	52.06 ± 26.26	0.34
Cholesterol, g	248.71 ± 154.48	243.45 ± 277.85	263.66 ± 314.87	0.96

[a]Values are expressed as means ± SD.
[b]For compare between groups used a ANOVA test.

that GTCs alone could positively alter anthropometric indices (24). In another study, a significant decrease was observed in weight, BMI, WC, and appetite hormone (ghrelin) levels in the EGCG group compared to the placebo group (25). In contrast, in a clinical trial, a dose 450 mg of EGCG could not significantly reduce weight, BMI, and expression of obesity-related genes (26). The difference may be due to the degree of obesity or disease nature, and this merits further investigation.

The present study is among the first clinical trials to investigate genotype-intervention interaction for the FTO-rs9939609 polymorphism in response to EGCG supplementation on anthropometric and glycemic changes in T2DM.

5.1. Main Strengths and Weaknesses

To the best of the author's knowledge, this study was the first to report the effect of the common FTO variant rs9939609 on anthropometric and insulin resistance/sensitivity in response to EGCG supplementation among patients with T2DM. Also, in the current study, to achieve an equal number of participants in each group based on genotype, screening was conducted on a population of patients with T2DM, which was an innovation and strength of the study. However, this study had some limitations, including (1) high rate of dropouts during the study; (2) Duration of intervention was relatively short (only two months), and (3) Selection of participants was only from one center.

5.2. Conclusion

Overall, the results of this study show that FTO-rs9939609 polymorphism was not associated with differential change in anthropometric indices and glycemic profile after EGCG intervention. This study suggests that administration of EGCG supplement for two months may provide anti-obesity effects in patients with T2DM.

Footnotes

Authors' Contribution: Study concept, design and acquisition of data: Meysam Alipour; clinical counseling and assistance in selecting samples: Mehrnoosh Zakerkish; guidance for biochemical and genetic analyzes, assistance in the design of the study: Pegah Ghandil; statistical analysis: Bahman Cheraghian; administrative, technical, and material support, study supervision: Seyed Ahmad Hosseini.

Conflict of Interests: The authors declare that they have no conflict of interest.

Ethical Considerations: Ethical code: IR.AJUMS.REC.1395.548 (IRCT2017021612949N3).

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Table 3. Within-Group Analysis of EGCG Effects, Regarding FTO-rs9939609[a]

Variables	EGCG Group (TT)	EGCG Group (AA + AT)	Placebo (A + T)
WHR			
Baseline	0.96 ± 0.06	0.97 ± 0.04	0.98 ± 0.05
After 2 months	0.93 ± 0.06	0.94 ± 0.06	0.98 ± 0.06
P value[b]	0.03	0.04	0.79
ABSI, $m^{11/6}$ $kg^{-2/3}$			
Baseline	0.0849 ± 0.004	0.0853 ± 0.003	0.0852 ± 0.004
After 2 months	0.0827 ± 0.007	0.0839 ± 0.006	0.0851 ± 0.002
P value[b]	0.31	0.41	0.87
FBS, mg/dL			
Baseline	181.20 ± 67.73	173.80 ± 67.42	182.50 ± 76.25
After 2 months	179.40 ± 65.38	162.05 ± 47.38	180.40 ± 63.38
P value[b]	0.80	0.19	0.78
Insulin, IU/L			
Baseline	16.26 ± 8.08	15.62 ± 7.90	11.09 ± 6.76
After 2 months	13.24 ± 5.07	13.83 ± 5.07	10.59 ± 5.20
P value[b]	0.17	0.37	0.72
HOMA-IR			
Baseline	6.84 ± 3.87	6.32 ± 3.80	5.03 ± 4.32
After 2 months	6.11 ± 3.75	5.67 ± 2.92	4.43 ± 2.06
P value[b]	0.51	0.49	0.46
QUICKI			
Baseline	0.295 ± 0.01	0.298 ±0.01	0.315 ± 0.02
After 2 months	0.303 ±0.02	0.304 ± 0.02	0.312 ± 0.02
P value[b]	0.15	0.24	0.62

Abbreviations: AA/AT/TT, the genotypes; ABSI, a body shape index; EGCG, epigallocatechin-gallate; FBS, fasting blood sugar; FTO, fat mass and obesity; HOMA-IR, homeostasis model assessment-insulin resistance; QUICKI, quantitative insulin sensitivity check index; WHR, waist-hip ratio.
[a]Values are expressed as means ± SD.
[b]Changes in Within-group, used a paired-samples *t*-test to investigate the differences within groups.

Table 4. Between-Group Analysis of EGCG Effects, Regarding FTO-rs9939609[a]

Variables	EGCG Group (TT)	EGCG Group (AA + AT)	Placebo (A + T)	P Value[b]	P Value[c]
WHR	0.03 ± 0.04	0.03 ± 0.04	0.003 ± 0.05	0.06	0.18
ABSI $m^{11/6}$ $kg^{-2/3}$	0.002 ± 0.007	0.001 ± 0.005	0.0001 ± 0.002	0.53	0.39
FBS, mg/dL	-1.80 ± 32.86	-11.75 ± 39.47	-2.10 ± 34.42	0.60	0.41
Insulin, IU/L	-3.02 ± 9.51	-1.78 ± 8.86	-0.50 ± 6.35	0.63	0.78
HOMA-IR	-0.73 ± 4.93	-0.64 ± 4.14	-0.59 ± 3.53	0.99	0.89
QUICKI	0.007 ± 0.02	0.005 ± 0.02	0.002 ± 0.02	0.31	0.55

Abbreviations: ABSI; a body shape index; AA/AT/TT, the Genotypes; EGCG, epigallocatechin-gallate; FBS, fasting blood sugar; FTO, fat mass and obesity; HOMA-IR, homeostasis model assessment-insulin resistance; QUICKI, quantitative insulin sensitivity check index; WHR; waist-hip ratio.
[a]Values are expressed as means ± SD.
[b]Changes between the three groups.
[c]Changes between the three groups adjusted for age, gender and BMI.

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