Research Square

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Genetic Investigation of the Trail Mechanism in Diabetic and Non- Diabetic Obese Patients

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Abstract

Background

Obesity is an important healthcare issue caused by abnormally increased adipose tissue because of energy-intake overcoming energy expenditure. Disturbances in the physiological function of adipose tissue mediate the development of diabetes. It is a metabolic disease that results from decreased insulin-levels and/or changes in the insulin action mechanism. Tumor Necrosis Factor-Associated Apoptosis-Inducing Ligand(TRAIL), which is a member of the Tumor Necrosis Factor(TNF)-family with an important role in adipose tissue biology, is included in many studies with its ability to induce apoptosis in cancer cells, but the number of human-studies conducted on the gene related to its protective-role against diabetes and obesity at this level is insufficient.

Methods

Our study was carried out as a case and control and included 3 groups (80 diabetic obese, 80 non-diabetic obese, and 80 healthy individuals as the control group). The Real-Time-PZR(RT-qPZR), and DNA Sanger-Sequencing Methods were used for gene expression and gene squences.

Results

As a result of the analyses, TRAIL gene expression level was found to be higher in the controls than in the diabetic-obese and non-diabetic-obese group. This change in TRAIL gene expression suggests that TRAIL maybe a protective factor against diabetes.

Conclusions

The presence of rs781673405, rs143353036, rs1244378045, rs767450259, rs759369504, rs750556128, and rs369143448 mutations, which was determined with the Sequencing-Method, was shown for the first time in the present study. In addition, it is the first study in which human TRAIL gene-expression and sequencing were performed together. We believe that these data will make an important contribution to the literature.

1 Introduction

Obesity is among the most common chronic inflammatory diseases on a global scale. Obesity can cause adipose hypertrophy in the body because the calories taken are more than the calories burned. Increasing fat accumulation with obesity plays roles as a risk factor for various health problems, including cardiovascular diseases, chronic kidney disease, and diabetes mellitus because of insulin resistance. It is already known that the socioeconomic levels, nutritional habits, and physical activities of individuals, especially genetic and environmental factors, affect the development of obesity [1–4].

Diabetes is a metabolic disease that is characterized by elevated blood glucose levels because of impaired insulin secretion and/or activity in cells in the Langerhans Islets of the pancreas [5]. Studies have shown that the relationship between diabetes and obesity is the main cause of insulin resistance which results from body fat distribution, TNF-α, free fatty acids, and gene defects of the β3-adrenergic receptor in the body [6, 7].

“Tumor Necrosis Factor-Associated Apoptosis-Inducing Ligand (TRAIL(TNSF10)(Apo2L))” is a member of the “Tumor Necrosis Factor (TNF) Superfamily” and is defined as a 281 amino acid Type II transmembrane protein or a soluble cytokine [8, 9]. The TRAIL gene family consists of 4 transmembranes (TRAIL-R1, TRAIL-R2, TRAIL-R3, and TRAIL-R4) and one soluble (osteoprotegerin (OPG)) receptor [10]. It was reported previously that TRAIL induces caspase activation in adipocyte cells, impairs insulin-dependent glucose uptake and lipogenesis, and triggers apoptosis with TRAIL-R1 and TRAIL-R2-mediated caspase activation, causing insulin resistance [11]. Whether TRAIL has a protective role in diabetes is still under investigation. It was reported in a previous study that described the relationship between TRAIL and Type II Diabetes (T2DM) that TRAIL deficiency significantly affected glucose tolerance and might be associated with β-cell dysfunction. Among the protective mechanisms of TRAIL against the development of T2DM, it is considered that TRAIL has anti-adipogenic and anti-inflammatory effects [12]. Apoe −/−, Trail −/− Apoe −/−, and Trail −/− mice that were fed with a high-fat diet were used in the present study, and their pancreatic islets were examined for insulin and beta cell dysfunction. As a result of the study, Trail −/− Apoe −/−, and Trail −/− mice showed hyperglycemia, decreased plasma insulin level, impaired glucose tolerance, macrophage infiltration, and beta cell dysfunction; and plasma cholesterol levels, triacylglycerol, VLDL and LDL cholesterol synthesis and expression of genes involved in lipogenesis increased. The effect of TRAIL on insulin resistance in the presence of obesity and the lack of studies conducted on Diabetes Mellitus developing as a result of it, and the presence of fewer studies in humans focusing on the relationship of these data with anthropometric and/or biochemical parameters made us conduct the present study on this subject. For this purpose, the study aimed to examine the expression and gene sequencing of the TRAIL gene in diabetic and obese individuals.

2 Methods

Participants

The current study consisted of 3 groups (80 diabetic obese, 80 non-diabetic obese, and 80 healthy individuals as the Control Group). The samples were selected from individuals who applied to Istanbul University, Faculty of Medicine, Department of Internal Medicine, Department of Endocrinology and Metabolic Diseases, diagnosed with obesity/diabetes, but did not have any chronic diseases other than these. Informed consent forms were obtained from the individuals in the study groups and the purpose of the study was explained.

Analysis of Biochemical Parameters

The sera were obtained from the blood samples obtained from the study groups by centrifugation at 4.500×g for 15 minutes at + 4°C. The analysis of glucose, urea, creatinine, LDH, total cholesterol, triglyceride, HDL, LDL, and HbA1c from the serum samples was performed by using AU5800 Series Clinical Chemistry Analyzers (Beckman Coulter Inc., Brea, CA, USA).

Nucleic Acid Extraction

The Robotic Device (RINA™ M14) (Bioeksen, Turkey) was used to obtain fully automatic nucleic acid extraction from the blood samples. The principle of the device is based on keeping the genetic materials on the magnetic bead surface and isolating them by washing them after they are taken out of the cell by physical and chemical degradation, heat treatment, and enzymatic applications. Nucleic acid extraction was performed in line with the literature data [13].

cDNA Synthesis and RT-qPCR Analysis

GAPDH_Human_F (5'-GAAGGTGAAGGTCGGAGT-3') and GAPDH_Human_R (5'-GAAGATGGTGATGGGATTTC-3') Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was chosen as the housekeeping gene to determine the gene expression level in the study [14]. The exons of the human TRAIL gene were analyzed in the hg38 genome version for primer design. The primers (TRAIL_F: GTCAAGTGGCAACTCCGTCA, TRAIL_R: TGAATGCCCACTCCTTGATG) were designed by using the Primer3 Program.

The cDNA synthesis and RT-qPCR analysis were made in one single step in a Bio-Rad C1000TM Thermal Cycler device. To complete the total reaction volume of the mixture, mix prepared for cDNA and RT-PCR analysis to 10 µL, 5 µL 2X Prime Script Mix, 2.5 µL Oligo Mix, and 2.5 µL Template Nucleic Acid was taken. The reverse transcription steps involved 2.5 µL isolated RNA samples and µL 2X Prime Script Mix, respectively; and the incubation was performed at 52°C for 10 min, Real-Time (RT)-qPCR was performed with the continued processing steps from the cDNAs that were obtained after the incubation.

High-Resolution Melt Curve (HRM) Analysis and Sanger Sequencing

The Bio-Speedy Device was used for HRM analysis and Minitab for dendograms. As a result of the Dendrogram Analysis, 5 groups that had < 99% similarity were obtained, sequence analysis was performed and differences were identified [15].

The annealing temperatures of the primers (TRAIL_S_F: GGACCCCAATGACGAAGAGA, TRAIL_S_R: TCCCAGTTATGTGAGCTGCT) that were designed in the Sanger Sequencing were chosen close to each other, the purine-pyrimidine base numbers were chosen evenly, and PCR products reproduced as templates were used. The Sequence Analysis was made on the ABI Prism 377 DNA Sequencer (Applied Biosystems, USA) with the ABI Prism Big Dye Terminator Cycle-Sequencing Ready Reaction Kit [16, 17].

Statistical analyses

For statistical analyses, $p < 0.05$ was taken as significant by using the SPSS (Statistical Package for Social Sciences for Windows) [18] 22.0 software program. Numerical data were shown as Mean ± Standard Deviation (Mean ± SD) and median, the number of individuals participating in the study (n) and/or (%) in the data. The descriptive data between biochemical parameters were calculated and their conformity to the normal distribution was determined with the Shapiro-Wilk Test. The Student's t-test was used in pairwise comparisons in case of normal distribution, and Mann-Whitney U-test was used if not. Similarly, the ANOVA Test was used if the triple comparisons fit the normal distribution, and the Kruskal-Wallis Test if they did not. The Ct values obtained as a result of the gene expression were normalized by using Fold Change ($2^{-\Delta\Delta Ct}$).

3 Results

Analysis of Biochemical Parameters

As a result of the data analysis of the biochemical parameters, a high degree of significance was detected in the diabetic obese group in terms of glucose, urea, creatinine, triglyceride, BMI, and HbA1c values when compared to the non-diabetic obese group and the control group ($p \leq 0.001$). The HDL and LDH values were higher and more significant in the non-diabetic obese group when compared to the other groups ($p < 0.05$). When evaluated in terms of total cholesterol and LDL, no statistical differences were detected between the groups ($p > 0.05$) (Table 1.).

TRAIL Gene Expression

The TRAIL expression level was calculated as $2^{-\Delta\Delta Ct}$ with reference to the GAPDH gene, and its statistical significance was explained with the Kruskal-Wallis Test. As a result, it was determined that TRAIL gene expression decreased in the diabetic obese group, increased in the non-diabetic obese group and the control group, and was statistically highly significant (0.25; 1.68; 10.95, respectively; $p < 0.001$) (Table 2.).

TRAIL gene expression was compared according to gender in the diabetic obese, non-diabetic obese, and control groups as male and female and it was shown that ΔCT expression level in women in the diabetic obese group was higher and more significant than in men ($p = 0.015$). Although the TRAIL ΔCT expression level was higher in men in the non-diabetic obese group than in women, statistically significant differences could not be detected. TRAIL ΔCT expression level was found to be higher and more significant in males than females in the Control Group ($p = 0.015$) (Fig. 1.).

When the TRAIL gene expression in the Diabetic Obese, Non-Diabetic Obese, and Control Groups was compared in terms of biochemical parameters, no statistically significant differences were detected in the parameters other than the total protein value. It was found that the total protein level was higher and more significant in those with increased expression when compared to those with decreased gene expression ($p = 0.007$).

HRM Analysis and Sanger Sequencing Method

In the current study, High-Resolution Melting (HRM) Analysis was made before the Sanger Sequencing. The samples that were selected from the groups determined as a result of the HRM Analysis were sequenced and their sequences were determined. The mutations were determined by using dendogram in the study groups (Table 3.)

Discussion and Conclusion

Obesity is an important healthcare issue on a global scale. Adipocyte hypertrophy developing with the effect of unhealthy nutrition causes adipose tissue dysfunction. Disturbances in the physiological functioning of adipose tissue play important roles in the development of diabetes [19, 20]. Diabetes is a metabolic disorder caused by the deficiency of insulin released from beta cells in the Langerhans Islets in the pancreas [21]. TRAIL, which is a member of the Tumor Necrosis Factor Superfamily, is a 5-exon gene located at position

3q26 of the chromosome. It was reported that TRAIL, which is known to have prognostic properties in cardiovascular diseases, might have effects against adipose tissue dysfunction and insulin resistance [22].

The patients that were included in the current study were evaluated in terms of somatic and biochemical parameters (BMI, albumin, total protein, Cl, K, Na, HbA1c, LDL, HDL, total cholesterol, triglyceride, AST, ALT, LDH, creatinine, urea, and glucose). Consistent with the literature data, BMI, glucose, urea, creatinine, triglyceride, and HbA1c were found to be higher in the diabetic obese group than in the non-diabetic obese group and the control group ($p \leq 0.001$) [23–25]. In addition to this, HDL, Na, and Cl values were found to be higher and statistically significant in the non-diabetic obese group when compared to the diabetic obese group and the control group ($p < 0.05$).

In our study, when TRAIL gene expression was compared according to gender, the $2^{-\Delta\Delta CT}$ level was found to be higher and more significant in men in the diabetic obese group than in women ($p = 0.015$). Also, when TRAIL gene expression was compared between the groups (Fold Change diabetic obese: 0.25; non-diabetic obese:1.68; control 10.95, respectively), it was found that the gene expression level increased 10-fold in the control group ($p < 0.001$). Consistent with the literature data, Chang et al. reported that TRAIL expression and serum levels were significantly decreased in the patient group when compared to patients with diabetes and diabetic nephropathy and the control group ($p < 0.005$) [18]. In line with the study of Chan et al., the expression of TRAIL at the protein and mRNA level was examined in Type-I diabetes and control groups, and it was shown that TRAIL expression increased in the patient group at significant levels [26]. In a study that was conducted in the Turkish population, serum TRAIL levels of patients with Type-II diabetes were examined and it was shown that the sTRAIL level was significantly lower in the patient group when compared to the control group [27]. Similar to these studies, it was reported in the literature that autoimmune diabetes is accelerated in TRAIL deficiency and that TRAIL might play protective roles against diabetes [28–31]. Consistent with these studies, Koliaki C and Katsilambros N reported that direct effects of TRAIL on several tissues involved in diabetes pathophysiology such as pancreatic islets, sketal muscle, adipose tissue, liver, kidney, immune and vascular cells in their review [32].

In the patient group in this study, 8 different mutations in the TRAIL gene were identified with the Sanger Sequencing after HRM analysis. The mutations obtained as a result of the study were rs781673405 (ATGAA[C/G]AGCCC), rs143353036 (TGGCAAG[T/C]), rs1244378045 (TTAGAAAG[A/T]), rs201137623 (TTAGAAAGAT[G/C,A]), rs767450259 (ATTTTG[A/G]), rs759369504 (TTTTGAGAA[C/A]), rs750556128 (TGAGAACCTC[T/C]), rs369143448 (AAGAGGTC[C/T]). As a result of the literature review, no study was found regarding the mutations identified here, and it is shown for the first time in our study. Based on this point of view, we think that our data will make an important contribution to the literature.

All these results obtained suggest that the change in TRAIL gene expression may be a protective factor of TRAIL against diabetes. More studies are needed to elucidate the biological basis of TRAIL's relationship with anthropometric and biochemical parameters and to develop effective treatment methods for TRAIL against diabetes and its complications.

Declarations

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Istanbul University Clinical Research Ethics Committee (Date: 09.12.2019 / Issue: 1479) and was supported by the Istanbul University Scientific Research Projects Unit (Project Number: TDK-2020-36578). This study is the doctoral thesis of Saadet Busra Aksoyer Sezgin.

Conflict of interest The authors declare no competing interests.

Consent to participate Informed consent was obtained from all individual participants included in the study. Patients signed informed consent regarding publishing their data.

Authors' Contributions All authors contributed to the study conception and design. Study concept and design: Saadet Busra Aksoyer Sezgin and Umit Zeybek; acquisition of data: Saadet Busra Aksoyer Sezgin, Sermin Durak, Ramazan Cakmak; analysis and interpretation of data: Saadet Busra Aksoyer Sezgin, Umit Zeybek, Sermin Durak, Faruk Celik and Arezoo Gheybi; drafting of the manuscript: Saadet Busra Aksoyer Sezgin, Sermin Durak and Faruk Celik; critical revision of the manuscript: Umit Zeybek, Ali Osman Gurol and Ilhan Yaylim; statistical analysis: Murat Diramali; study supervision: Umit Zeybek.

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Tables

Table 1
Anthropometric and biochemical parameters of the diabetic and non-diabetic obese groups

	Diabetic Obese n = 80			Non-diabetic Obese n = 80			Control n = 80			*p*
	Min	**Max**	**Mean ± SD***	**Min**	**Max**	**Mean ± SD***	**Min**	**Max**	**Mean ± SD***	
Glucose	48	422	187.73 ± 75.19	75	105	89.95 ± 8.55	72	111	91.88 ± 8.07	**< 0.001**
Urea	16	105	35.44 ± 16.74	13	37	24.16 ± 8.11	12	47	26.78 ± 7.66	**< 0.001**
Creatinine	0.4	2.66	0.86 ± 0.35	0.34	0.9	0.60 ± 0.17	0.38	1.07	0.68 ± 0.15	**< 0.001**
LDH	133	298	187.48 ± 32.80	138	330	188.05 ± 44.07	118	263	173.06 ± 30.58	**0.048**
T_Chol	112	301	203.26 ± 42.78	145	333	207.18 ± 47.48	96	359	209.38 ± 51.43	*0.79*
Trig	50	815	195.19 ± 129.79	29	936	132.27 ± 184.63	31	364	112.74 ± 62.56	**< 0.001**
HDL	23	66.5	44.04 ± 9.94	20	111	54.68 ± 17.32	32	80	54.16 ± 11.27	**< 0.001**
LDL	58	193	121.40 ± 32.90	54	242	130.36 ± 41.07	52	256	132.26 ± 42.69	*0.396*
HbA1c	5.1	13.7	8.56 ± 1.97	4.5	6	5.36 ± 0.36	4.6	6	5.33 ± 0.30	**< 0.001**
BMI	30.04	45.79	35.17 ± 3.99	30.08	65.33	37.91 ± 8.08	18.34	29.97	25.01 ± 3.03	**< 0.001**

Bold font indicates *p-values* of significance

Mean ± SD: Numerical data mean ± standard deviation

ANOVA test was used for inter-group statistical analysis of the parameters used in the study

Table 2
TRAIL gene expression levels

TRAIL	Diabetic Obese			Non-diabetic Obese			Control			*p*
	Min	Max	Mean ± SD*	Min	Max	Mean ± SD*	Min	Max	Mean ± SD*	
ΔCT	3.84	11.6	9.13 ± 1.57	-2.1	12.3	6.32 ± 3.17	-1.8	12.66	6.24 ± 3.12	**< 0.001**
ΔΔCT	-2.2	5.58	3.14 ± 1.57	-5.6	12.3	3.21 ± 4.45	-7.8	6.67	0.25 ± 3.12	**< 0.001**
$2^{-\Delta\Delta CT}$	0.02	4.44	0.25 ± 0.56	0.02	46.9	1.68 ± 5.68	0.01	216.8	10.95 ± 31.46	**< 0.001**

Bold font indicates *p-values* of significance

Mean ± SD: Numerical data Mean ± Standard Deviation

The Kruskal-Wallis test was used for inter-group statistical analysis of the parameters used in the study.

Table 3. Mutation identified by sanger sequencing

Mutation	Group
rs781673405 (ATGAA[C/G]AGCCC)	Diabetic obese group
rs143353036 (TGGCAAG[T/C])	Non-diabetic obese group
rs1244378045 (TTAGAAAG[A/T])	Diabetic obese group
rs767450259 (ATTTTG[A/G])	Diabetic obese group
rs759369504 (TTTTGAGAA[C/A])	Non-diabetic obese group
rs750556128 (TGAGAACCTC[T/C])	Diabetic obese group
rs369143448 (AAGAGGTC[C/T])	Diabetic obese group

Figures

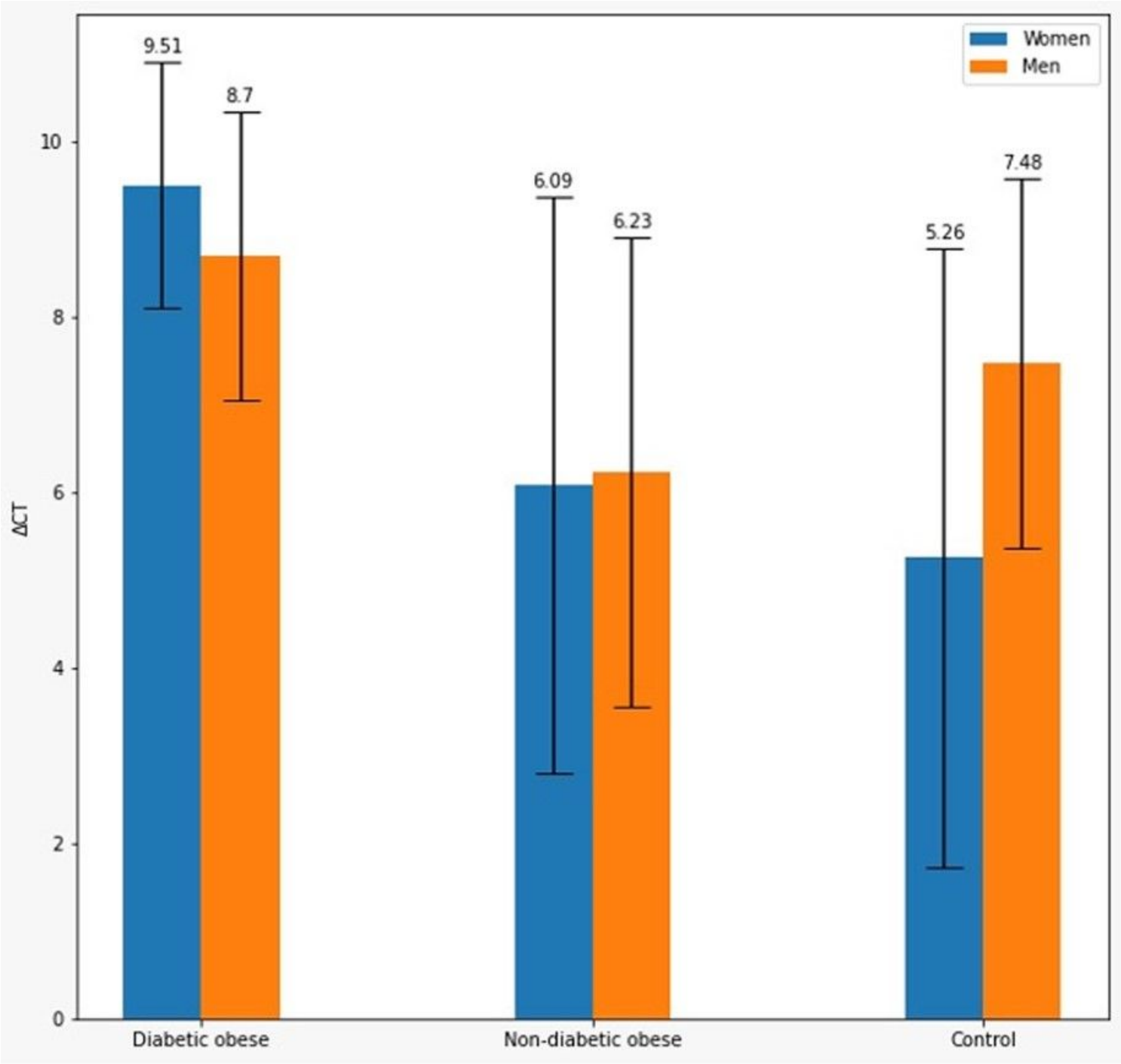

Figure 1

TRAIL gene expression by gender

TRAIL gene expression was compared according to gender in the diabetic obese, non-diabetic obese, and control groups as male and female. It was shown that ΔCT expression level in women in the diabetic obese group was higher and more significant than in men (p=0.015). TRAIL ΔCT expression level was higher in men in the non-diabetic obese group than in women, statistically significant differences could not be detected. TRAIL ΔCT expression level was found to be higher and more significant in men than women in the control group (p=0.015). Student's t and Mann-Whitney U tests were used for inter-group statistical analysis of the parameters used in the study.

Student's t and Mann-Whitney U tests were used for inter-group statistical analysis of the parameters used in the study.