FABAD J. Pharm. Sci., 46, 2, 139-146, 2021

RESEARCH ARTICLE

Investigation of GSTM1 and GSTT1 Polymorphisms in Obesity Patients Under Bariatric Surgery

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SUMMARY

Obesity is a chronic disorder with increasing prevalence worldwide and occurs when energy intake is more than energy expenditure. Obesity is one of the factors that cause oxidative stress and arises from an imbalance between the reactive oxygen species ROS and the cell's antioxidant defense system. Increasing ROS in obesity, influencing the hypothalamic neurons, affect hunger and satiety control, so correspondingly on body weight control. When ROS amount increases, through DNA, protein, and lipid oxidation, cell damage, necrosis, and apoptosis take place. Oxidative stress increment in adipose tissue causes the development of metabolic syndrome in obese people. Also, weight loss due to calorie restriction or exercise reduces oxidative stress. Mitochondria is the essential source for ROS formation. In the electron transfer system, reactive oxygen species forming due to oxidative phosphorylation reactions are involved in various physiological processes such as cell proliferation and differentiation. Glutathione S-transferase M1 and T1 genes encode enzymes that have oxidant-scavenging activities. Deletion polymorphisms in these genes cause the absence of their corresponding enzymes. In this study, we investigated the parameters associated with obesity such as body mass index (BMI), TSH, glucose, satiety blood glucose, triglyceride, and cholesterol levels, and deletion polymorphisms of GSTM1 and GSTT1 genes in 152 patients diagnosed with obesity in a Turkish population. No statistically significant relationship was found between the parameters studied in obese patients and GSTM1 and GSTT1 polymorphisms. More studies are needed to elucidate the relationship of GSTM1 and GSTT1 polymorphisms with obesity.

Key Words: *Obesity, GSTM1, GSTT1, Oxidative stress, Polymorphism, Multiplex PCR.*

Bariatrik Cerrahi Olmuş Obezite Hastalarında GSTM1 ve GSTT1 Polimorfizmlerinin Araştırılması

ÖZ

Obezite, alınan enerjinin, harcanan enerjiden fazla olmasından kaynaklanan, tüm dünyada prevalansı endişe verici şekilde artan kronik bir hastalıktır. Obezite, oksidatif strese neden olan faktörlerden biridir ve oksidatif stres, reaktif oksijen türleri (ROT) ile hücrenin antioksidan savunma sistemi arasındaki dengesizlikten kaynaklanır. Obezitede artış gösteren ROT hipotalamik nöronlar üzerinde etkili olarak, açlık ve tokluğun kontrolünde ve buna bağlı olarak vücut ağırlığının kontrolünde etkili olurlar. ROT arttığında, DNA, protein ve lipitlerin oksidasyonu yoluyla hücre zedelenmesi, nekroz ve apopitoz oluşur. Adipoz dokuda oksidatif stresin artışı obez kişilerde metabolik sendrom gelişmesine neden olur. Diğer yandan kalori kısıtlaması veya egzersiz nedeniyle kilo kaybı oksidatif stresi azaltır. Mitokondri ROT oluşumunun en önemli kaynağıdır. Elektron transfer sisteminde, oksidatif fosforilasyon tepkimeleri sonucu oluşan ROT'lar, hücre sinyal mekanizması, hücre çoğalması ve farklılaşması gibi çeşitli fizyolojik olaylarda rol alırlar. Glutatyon S transferaz M1 ve T1 genleri, oksidan süpürücü aktivitelere sahip enzimleri kodlar. Bu genlerdeki delesyon polimorfizmleri, karşılık gelen enzimlerinin olmamasına neden olur. Bu çalışmada, Türk toplumunda obezite tanısı almış 152 hastada vücut kitle indeksi (VKİ), TSH, glikoz, tokluk kan şekeri, trigliserit ve kolesterol düzeyleri gibi obezite ile ilişki parametreler ile GSTM1 ve GSTT1 polimorfizmleri arasındaki ilişki araştırılmıştır. Obezite hastalarında çalışılan parametreler ile GSTM1 ve GSTT1 polimorfizmleri arasında istatistiksel olarak anlamlı ilişki bulunamamıştır. GSTM1 ve GSTT1 polimorfizimlerinin obezite ile ilişkisinin aydınlatılması açısından daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: *Obezite, GSTM1, GSTT1, Oksidatif stres, Polimorfizm, Multipleks PCR.*

Received: 29.07.2020
Revised: 27.09.2020
Accepted: 14.03.2021

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INTRODUCTION

A complex condition that is closely associated with many diseases such as obesity, hypertension, cardiovascular disease, diabetes, degenerative arthritis, thrombophlebitis, which are defined by the World Health Organization (WHO) as "abnormal or excessive fat accumulation in the body to the extent that it impairs health." It is considered a disease that concerns all age and socioeconomic groups with its severe social and psychological effects (Hofbauer, 2007). Obesity; both reduce the quality of life and shortens its duration. Overweight and obesity rank 5th among the causes of death worldwide. Obesity is reported that at least 2.8 million people die every year due to overweight or obesity. The prevalence of obesity has been increasing in almost all countries in the last 20-30 years. According to the WHO data, in 2016, there are over 1.9 billion overweight individuals aged 18 and over in the world. In 2016, 39% of adults aged 18 years and over (39% of men and 40% of women) were overweight. Overall, about 13% of the world's adult population (11% of men and 15% of women) were obese in 2016. Obesity, which was originally defined as the problem of high-income countries, is increasing in low and middle-income countries, especially in urban areas. Diet and exercise and medical treatments have been applied, and the lack of success has become the focus of researches on hormones and mediators, and genes that may be the source of obesity with surgical interventions. The need to apply surgical options has become inevitable in morbidly obese patients who do not have any additional diseases that can cause obesity and cannot lose weight with other methods. Although surgical procedures for obesity have been investigated in terms of their benefits and harms for years, unity has not yet been established on a globally accepted gold standard surgical procedure. There is a lot of evidence that many comorbid conditions such as hypertension, dyslipidemia, and type 2 diabetes can improve after obesity surgery (Buchwald, 2009). Bariatric surgery, also known as metabolic surgery, is a long-term, successful, and recently popular surgical method in obesity patients (Schernthaner and Morton, 2008). Although there are many bariatric surgery procedures, Laparoscopic Sleeve Gastrectomy LSG and Gastric Bypass (GBP) are the most preferred methods today. In some studies, LSG is advantageous in that it is less invasive than other operations, and weight loss is as successful as other procedures (Daskalakis and Weiner, 2009). Studies show that which of the 244 candidate genes were reported for obesity to obesity because a large number of genes leading to multifactorial characteristics are associated with different biological functions (nutrient intake regulation, energy expenditure, lipid, and sugar metabolism) (Rankinen, 2006). While multiple genes may affect the formation of a single disease, polymorphic variants of a single gene can also be effective in disease development. These genetic polymorphisms occur over time and are largely responsible for the diversity of the population. Some single nucleotide polymorphisms may have significant biological consequences, although they appear to be largely harmless (De Iuliis, 2015).

Oxidative stress occurs as a result of disruption between the formation of reactive oxygen species (ROS) and the inactivation of these products by the antioxidant defense system and plays an important role in the pathogenesis of many diseases, including inflammation, endothelial dysfunction, and atherosclerotic vascular diseases (Finaud 2006; Bhattacharyya, 2014). Adipose tissue is one of the main sources for ROS formation, and fat accumulation is closely related to increased oxidative stress through nicotinamide adenine dinucleotide phosphate (NADPH), reduced form oxidase activation. In obese people, the metabolic load increases due to overfeeding, and as a result, the free radical formation increases due to the overload of metabolic pathways (Roskams, 2003; Ceconi C., 2003; Qatanani M. and Lazar M.A., 2007; Manna, 2015). Increasing ROS in obese individuals causes oxidative stress (Fernandez-Sanchez, 2011).

The adverse effects of free radicals are eliminated with the cell's antioxidant defense system and antioxidants taken through the food. However, in long-term obesity, antioxidant system enzymes are lower (Lobo, 2010). Obesity causes cell injury and various diseases such as type 2 diabetes, cardiovascular diseases, and cancer (Senoner, 2019; Barone, 2020). Oxidative stress, which causes many diseases, can cause significant damage to the organism with its cellular effects (Uttara, 2009).

The antioxidant defense system, used by the organism to overcome oxidative stress caused by increased ROS, consists of enzymes and small molecules. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) play a direct role in detoxifying ROS (Valko, 2006; Valko, 2007). In general, aerobic organisms constantly produce ROS (Perez Campo, 1993; Halliwell and Gutteridge, 1999). The most important source of ROS production in mitochondria is the cell content (Boveris and Cadenas, 1982; Chance, 1979; Turrens, 1985), microsomes (Staats, 1988), and peroxisomes (Dhaunsi, 2004). ROS causes many cellular disorders with the depolymerization of polysaccharides and nucleic acids. They are involved in the oxidation and peroxidation of protein sulfhydryl groups. (Stohs, 2000). Known as classical, the antioxidant defense system is enzymatically composed of the following components; SOD, CAT, GSH-Px, glutathione reductase (GR), and glutathione-S-transferase (GST). Non-enzymatic components such as reduced glutathione (GSH) are also involved in detoxification in the process (Cadenas, 1989). One of the antioxidant defense mechanism components, GST, serves as a phase II enzyme in biotransformation. Besides, phase II enzymes used in the detoxification mechanism are thought to be important factors in some types of cancer (Oguztuzun, 2016). For example, in the thyroid tissue, which plays a key role in ensuring redox balance in the cell and responding to oxidative stress, all this is affected by the disruption of metabolic balance in possible cancer development (Haddad and Land, 2000; Haddad, 2000). Obesity is also associated with oxidative stress. In obese individuals, oxidative stress appears to be increased. GST enzymes are synthesized enzymes for protection against oxidative stress in the body. In the absence of these enzymes, the cell is exposed to oxidative stress. Genotypes of these enzymes may play a role in the formation of obesity. This study aims to investigate the relationship between the genetic polymorphisms of *GSTM1* and *GSTT1*, body mass index (BMI), TSH, blood glucose, satiety blood glucose, triglyceride, and cholesterol levels in obese patients who have undergone bariatric surgery.

MATERIALS AND METHODS

Our study group was 152 obese patients (24 men and 128 women) operated in Keçiören Training and Research Hospital General Surgery Clinic in 2017-2018. Each individual's peripheral blood for DNA extraction was 5-6 mL into EDTA-containing tubes. Individuals were informed, and their consent was obtained before participating in the study. This study's ethics committee approval was provided from the Ethics Committee of Keçiören Education and Research Hospital, with the decision numbered 2012-KA-15/1135.

DNA isolation from blood samples was performed according to the PROMEGA® DNA isolation kit protocol. *GSTM1* and *GSTT1* polymorphisms were determined by the multiplex PCR method described by Abdel Rahman et al. (1996). The primers and their sequences used in multiplex PCR are shown in Table 1. For the optimization and standardization of PCR conditions, the AptaTaqFast PCR master ROCHE® mixture containing all these components except primers and template DNA was used. The pre-PCR step was carried out using the recommended optimization rates found in the AptaTaqFast PCR master ROCHE® mixture protocol. While preparing the PCR mixture, the following ratios were used; Template DNA 3uL, AptaTaqFast PCR master ROCHE® mix 10uL, *GSTM1* primer (forward) 0.5uL, *GSTM1* primer (reverse)

0.5uL, internal control primer (forward) 0.5uL, *CYP1* internal control primer (reverse) 0, 5 µL, *GSTT1* primer (forward) 0.5 µL, *GSTT1* primer (reverse) 0.5 µL in a total volume of 25 µL final PCR mixture. PCR steps were performed as follows; Initial Denaturation at 95 °C for 2 minutes (for 1 cycle); the initialing is 2 minutes at 94 °C, 1 minute at 59 °C, 2 minutes at 72 °C (for 5 cycles); the annealing is 30 seconds at 94 °C, 45 seconds at 59 °C, 90 seconds at 72 °C (for 30 cycles); The final extension is 10 minutes at 72 °C (for 1 cycle).

Table 1. Primers used in the study

Primer	Sequence
GSTM1 (Forward)	GAACTCCCTGAAAAGCTAAAGC
GSTM1 (Reverse)	GTTGGGCTCAAATATACGGTGG
Internal control (Forward)	GAACTGCCACTTCAGCTGTCT
Internal control (Reverse)	CAGCTGCATTTGGAAGTGCTC
GSTT1 (Forward)	TTCCTTACTGGTCCTCACATCTC
GSTT1 (Reverse)	TCACCGGATCATGGCCAGCA

Gel electrophoresis was used to visualize and analysis of PCR products. TBE (Tris-Borate-EDTA) was used as the electrolyte solution. The solution was prepared as a 10-fold concentrate, and this stock solution was diluted with 10-fold distilled water and used as an electrolyte solution. In our study, 1.7% agarose gel was used. 0.5 µg / mL ethidium bromide was added into the gel during preparation. The samples were tested in the applied electrophoresis technique and carried out at 110 volts for 60 minutes. The gels were observed on the SYNGENE (Gene Genius Bio Imaging System) UV device stand. Images obtained with the gel imaging system were transferred to the computer and interpreted and photographed in black and white. The bands belonging to a single sample and base sizes formed in the gel image were observed as *GSTM1,* internal control, and *GSTT1*. The base sizes of the PCR products were shown in Figure 1. In the gel image, the relevant sample bands were evaluated as positive (+), and the bands that did not appear were evaluated as negative (-) for their corresponding genes. Based on this situation, the values given in the excel file format were given by giving "1" (one) value to the bands and "0" (zero) value to the absent bands. By using this table, the results were evaluated in terms of statistical analysis. The *GSTM1* and *GSTT1* genotypes and all other data of the patients from which the sample was taken into consideration for statistical analysis. One-way ANOVA analysis was used to test the significance of the data. The significance level is accepted as <0.05.

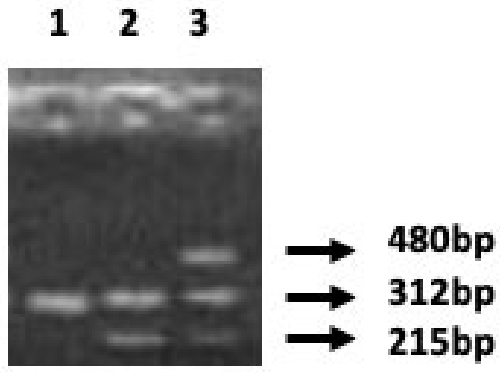

Figure 1. Base sizes of the PCR products in the electrophoresis gel image. Bands at 215bp and 480bp show *GSTM1* and *GSTT1* respectively and the band at 312bp should be always present as internal control that indicates the success of PCR. The sample in Lane 1 is both *GSTM1* and *GSTT1* negative. The sample in Lane 2 is *GSTM1* positive and *GSTT1* negative. The sample in Lane 3 is both *GSTM1* and *GSTT1* positive.

ETHICS COMMITTEE APPROVAL

Ethics committee approval of this study was provided by the decision of the Ethics Committee of Keçiören Education and Research Hospital with the decision numbered 2012-KA-15/1135.

RESULTS AND DISCUSSION

The distributions of the *GSTT1* and *GSTM1* polymorphisms are shown in Table 2. The frequency of *GSTM1*– and *GSTT1*– null genotypes were 72.37% and 53.29%, respectively. The average age of the patients is 40, the BMI of the patients is 46.5 kg/m^2, the average of TSH levels is 2.5, the average of insulin levels is 19.5, the average of satiety blood glucose levels is 111, the average of postprandial blood glucose levels is 137.6, the mean of triglycerides is 176, and the

cholesterol level averages are 215.6 (data not shown). When the correlation between *GSTT1* and *GSTM1* polymorphisms and BMI, TSH, satiety blood glucose, postprandial blood glucose, triglyceride, and cholesterol levels of the patients was examined statistically by variance analysis and ANOVA, it was observed that the results were not significant with the clinical data ($p>0.05$) (Table 3).

Table 2. Distribution of polymorphic status related to *GSTM1* and *GSTT1* genes in obesity patients.

Genotype	***GSTM1*** n (%)	***GSTT1*** n (%)
Positive	42 (27.63%)	71 (46.71%)
Negative	110 (72.37%)	81 (53.29%)

Table 3. Correlation between blood parameters and *GSTT1* and *GSTM1* polymorphisms.

	Variance Analysis		ANOVA	
GSTT1	Levene Statistic	Significance	F	Significance
Body mass index	3.694	0.057	0.472	0.494
TSH	0.662	0.418	0.034	0.855
Insulin	1.096	0. 298	0.373	0.543
Satiety blood glucose	2.213	0.14	1.79	0.184
Postprandial blood glucose	0.508	0.478	0.457	0.501
Trigliseride	0.007	0.936	0.01	0.922
Cholesterol	1.953	0.165	1.122	0.292
GSTM1	Levene Statistic	Significance	F	Significance
Body mass index	1.748	0.189	0.022	0.883
TSH	0.492	0.485	0.018	0.895
Insulin	0.924	0.339	0.436	0.51
Satiety blood glucose	0.08	0.778	0.052	0.821
Postprandial blood glucose	0.775	0.382	1.231	0.271
Trigliseride	0.417	0.52	0.316	0.575
Cholesterol	4. 212	0.043	1.645	0.203

Although there were no statistically significant relationships between *GSTM1* and *GSTT1* mutations and obesity parameters, the *GSTM1*– and *GSTT1*– null genotypes in our study were very high (72.37% and 53.29% respectively) compared with previous studies performed in control populations. For example, Garte et al. (2001) reported *GSTM1* and *GSTT1* deletion frequencies ranging 42%-60% and 13%-26%, respectively, in healthy Caucasians. Likewise, Ada et al. (2012) reported *GSTT1* and *GSTM1* null frequencies 53.68% and 18.61%, respectively in a healthy Turkish population. Since *GSTT1* and *GSTM1* are deleted from the gene region due to mutation, their corresponding enzyme activities would be absent. Oxidative stress increases in the absence of these enzymes. (Da Fonseca, 2010). In obese individuals, oxidant/antioxidant balance is observed to be impaired, and the presence of oxidative stress appears (Savini, 2013). The absence of these enzymes supports the formation of obesity and the susceptibility to obesity (Manna, 2015). The results seen in all these studies indicate that oxidative stress increases with *GSTM1* and *GSTT1* mutations in obesity. Significant changes occur in *GSTM1* and *GSTT1* polymorphisms in obese individuals.

According to our results, *GSTM1* and *GSTT1* deletions might be associated with obesity. Further studies are needed to clarify the possible association between *GST* polymorphisms and obesity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

Designed the concept and drafted the manuscript (Ünsal, A., Buluş, H., Oğuztüzün, S.), Prepared the figures (Oğuztüzün, S., Öztürk D.), held ethical approval and collected relevant samples and clinical data (Ü, HB, DÖ, and MC), carried out the laboratory applications of this study (AÜ and OD), reviewed the existing journal policy (Ada, A. O., Dirican,O.), contributed to the writing of the final version of the manuscript (Dirican, O., Ünsal, A., Buluş, H., Oğuztüzün, S., Öztürk, D., Cihan, Ada, O. A., İşcan, M.).

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