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Immunohistochemical approach to obesity disease in terms of expression levels of glutathione s-transferase (sigma, zeta, theta) isozymes

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ABSTRACT

Objectives: Obesity is a complex multifactorial disease with recently increasing prevalence and incidence. Several studies have been conducted to explain the ethiology, pathophysiology, epidemiology, molecular and genetic mechanisms, and effective treatments of obesity. Glutathione S-transferase (GST) S1, GSTZ1, and GSTT1 are essential enzymes for oxidative stress and metabolism-related disorders. For this purpose, we aimed to reveal the role of GSTS1, GSTZ1, and GSTT1 in obesity.

Methods: The gastric tissue samples were taken from the patients diagnosed with obesity who underwent bariatric surgery in Ankara Keçiören Training and Research Hospital General Surgery Clinic between 2017 and 2019. Immunostaining was performed on paraffin-embedded tissues to evaluate GSTS1, GSTZ1, and GSTT1 expressions. Laboratory data of the patients were recorded. All the results were analyzed statistically.

Results: Weak GSTS1 expression was observed in 38.1% of tissues and moderate in 6.3%. 37.3% of the tissues presented weak GSTZ1 expression, and 11 (8.7%) displayed moderate. There were weak GSTT1 expressions in 7.1% of the tissues and moderate 0.8% of them. A positive and statistically significant correlation was observed between GSTS1 and GSTT1 expression levels ((r) = 0.028, $p = 0.010$; $p < 0.05$). There were no significant differences between expression levels and gender, age, comorbidities, and medication usage ($p > 0.05$).

Conclusions: GSTs, in particular GSTS1, GSTT1, and GSTZ1, might contribute to molecular mechanisms and the progression of obesity. In our study, GSTS1, GSTT1, and GSTZ1 were found to be moderately expressed in gastric tissues taken from obese patients. However, new studies using more samples and advanced techniques are needed to elucidate the relationship.

Keywords: Obesity, xenobiotics, phase II enzymes, GSTS1, GSTZ1, GSTT1, immunohistochemistry

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In addition to genetic predisposition, it is accepted that external factors such as lifestyle and cultural environment are the determining parameters in terms of obesity formation and susceptibility [1]. It is one of the most important steps towards the treatment of the disease to reveal the formation mechanism of this disease in a clearer and more understandable way. Due to these reasons, current research has gained importance in terms of revealing the functioning or anomaly of the genes responsible for these hormones, which are known to have hormonal and regulating effects on these hormones, together with habits such as human life style, nutrition and diet. In particular, studies related to the elimination of diseases such as type 2 diabetes and hypertension after surgical interventions related to obesity are seen in the literature. [2]. Adipose tissue constitutes 15-20% of the body weight of adult men and 25-30% of women. If this rate rises above 25% in men and 30% in women, obesity can be mentioned [3]. Following obesity; It has been determined that diseases such as hypertension, cardiovascular diseases, diabetes, degenerative arthritis, thrombophlebitis are seen directly or indirectly, and this is valid in all age groups [4]. Neuroendocrine system diseases such as Cushing's Syndrome, Hypothyroidism, Polycystic Ovary Disease are known to be factors in the formation of obesity [5, 6]. Along with parameters such as nutrition, lifestyle, genetic predisposition, and physical activity, drug use such as psychotropic drugs, steroids, and contraceptives has also been reported as another factor that can cause obesity [7, 8]. It has consequences in the form of various diseases on many systems such as the endocrine system, cardiovascular system, respiratory system, gastrointestinal system, nervous system. These diseases; hypertension, type 2 diabetes, coronary heart disease, acute myocardial infarction, cerebrovascular diseases, respiratory distress, obstructive sleep apnea, gallbladder disease, fatty liver, dyslipidemia, hyperuricemia, insulin resistance, breast cancer, osteoarthritis, nerve entrapment, proteinuria, lymphedema and in a psychological form [9]. In morbidly obese people, no results can be obtained with any non-surgical method. When morbidly obese people lose weight with non-surgical treatment methods, the frequency of weight regain is approximately 95%. Morbid obesity; The only treatment option that is effective, proven and can provide long-term results is surgical techniques [10]. In reactions catalyzed by metals, reactive oxygen compounds can be produced in two different ways. The first of these; It is produced endogenously in the cell by the catalytic effect of various enzymes such as peroxisomal oxidation, electron transport reactions catalyzed in mitochondria, microsomal cytochrome P450 metabolism, xanthine oxidase and aldehyde oxidase as a result of immune system activation. The second is; It can be produced exogenously as a result of radiation caused by X, gamma and UV rays. It can also be found in the atmosphere as pollution. Environmental pollutants, barbiturates, xenobiotics such as chlorinated compounds, some metal ions and smoking are other exogenous sources [11-13]. Beneficial and harmful effects of free radicals on the organism can be seen. It is known that they play a bidirectional role in biological systems. Examples of the beneficial effects of free radicals are the active roles they play in the immune system defense against infectious agents and in intracellular communication pathways. However, it also induces mitogenic response at low concentrations. Considering its negative effects, it causes damage to cell structures such as fats, proteins and nucleic acids at high concentrations [14, 15]. The harmful effects of Reactive Oxygen Compounds are kept under control by enzymatic and non-enzymatic antioxidants. Despite the antioxidant defense system, the excessive increase in the production of ROB or the decrease in the capacity of the antioxidant defense system and the accumulation of ROB in the body cause oxidative damage known as oxidative stress. Oxidative stress plays an important role in the emergence of cancer, atherosclerosis, arthritis, neurodegenerative disorders and many other diseases [16, 17]. Obesity-induced oxidative stress is thought to be the main cause of diseases that play a role in the pathogenesis of long-term complications such as cardiovascular diseases and Type 2 diabetes. Although body mass index, body fat percentage, low density lipoprotein oxidation and triglyceride levels are important indicators of oxidative stress, they are associated with obesity as well as parameters. A diet rich in fat and carbohydrates has been found to induce significant oxidative stress and inflammation in obesity [18]. It has been determined that reactive oxygen compounds may cause insulin resistance by negatively affecting the balance of insulin secretion in the pancreas and glucose transport system in skeletal tissue and adipose tissue [19, 20]. De-

creased insulin sensitivity is thought to be one of the main factors of the metabolic syndrome [21, 22]. Therefore, it can be said that there is a direct relationship between oxidative stress, obesity and metabolic syndrome [23]. Cardiovascular disease risk increases with metabolic syndrome [24]. There are many studies on the presence of obesity-induced oxidative stress [25-27].

Genetic variation can affect both toxic and xenobiotic pharmacokinetics and pharmacodynamics, and these consequences appear as phenotypes in pharmacogenomics. Pharmacokinetics refers to the body's actions on a drug, including the absorption, distribution, metabolism, and excretion (ADME) of an administered drug, performed by proteins such as metabolizing enzymes and membrane transporters involving the cytochrome P450 (CYP) system [28]. Through xenobiotic biotransformation, lipophilic (fat-soluble) chemicals are converted to hydrophobic (water-soluble) chemicals and excreted in urine or bile. As a result of xenobiotic biotransformation, it can change biological effects such as increase, decrease or loss in the pharmacological activity of the xenobiotic [29]. Examples of Phase I drug metabolizing enzymes localized in the endoplasmic reticulum are heme-containing CYP, flavin-containing monooxygenase, monoamine oxidase, and xanthine oxidase/aldehyde oxidases. CYP enzymes play the most prominent role in Phase I metabolism [30]. In cases where the polarity of the molecule formed as a result of Phase I reactions is not sufficient, Phase II reactions are carried out by attaching glucuronic acid, acetic acid, sulfuric acid, or an amino acid to the drug. Glucuronide in glucuronic acid conjugation; can be attached to oxygen, nitrogen and sulfur groups. Conjugation with glutathione is important for electrophilic compounds. Glutathione (GSH) is a tripeptide (γ-glutamylcysteinylglycine). Forms glutathione S-conjugates, which are excreted in the urine or bile. The reaction is catalyzed by glutathione S-transferases (GST). Acetyl-CoA is used as the acetyl donor in acetylation and the reaction is catalyzed by acetyltransferases. Xenobiotics are methylated by methyltransferases. S-adenosyl-methionine (SAM) is used as methyl donor [31]. GSH is an atypical tripeptide with gamma-L-glutamyl-L-cysteinyl glycine structure. It is atypical because of the gamma bond between the amine group of cysteine and the carboxyl group in the side chain of glutamate. GSH is synthesized in the body from L-cysteine, L-glutamic acid and glycine. It is a thiol that has a fundamental role in maintaining redox balance in the cell and responding to oxidative stress [32, 33]. GSH is found in high concentrations, especially in liver cells [34, 35]. GSH can reduce unstable molecules such as reactive oxygen compounds. GSH constitutes more than 90% of the GSH pool in healthy cells and tissues. GSH acts as a substrate in numerous conjugation and reduction reactions catalyzed by GST in the cytosol, microsomes, and mitochondria [36]. The aim of our study is contribute to the determination of therelationship of the detoxification mechanism with obesity in terms of GST- Sigma (S) 1, GST Zeta (Z) 1, and GST Theta (T) 1.The determination of the expressions of these isoenzymes in obesity patients was carried out in order to contribute to research on the prognostic, diagnosis and treatment of obesity.

Complete blood count, the most commonly used blood test in the whole world, is a blood test used to evaluate people's general health and detect a wide variety of disorders, as it provides information about the life cycle and physiology of the various cell components [37]. Especially the levels of white blood cell (WBC), hemoglobin (HGB), and platelet (PLT) give essential information to medical professionals about inflammatory conditions, metabolic diseases and obesity. There are several ways in which obesity increases cardiovascular and diabetes risk, including high low-density lipoprotein (LDL), total cholesterol, and triglyceride, low high-density lipoprotein (HDL), excessive fasting, postprandial blood glucose and hemoglobin A1c (HbA1c) and unsteady insulin levels [38, 39]. Several studies reported that there may be a relationship between obesity andhormone levels icluding free T3, and T4, thyroid-stimulating hormone (TSH), estradiol, follicle-stimulating hormone (FSH), luteinizing hormone (LH), C-peptide, cortisol, adrenocorticotropic hormone (ACTH) and sex hormone-binding globulin (SHBG) [40-43]. Therefore, this study also aimed to investigate the association of GSTS1, GSTZ1, and GSTT1 expressios and complete blood count, cardiovascular and diabetes and hormoneparameters. To the best of our knowledge our study is the first tho evaluate all this parameters simultaneously.

METHODS

In this study, 126 gastric tissues of obese patients who underwent bariatric surgery were received from the General Surgery Clinic of Keçiören Training and Research Hospital between 2017 and 2019. The gastric tissues were stained by the immunohistochemistry method to evaluate GSTS1, GSTZ1, and GSTT1 expressions.

Ethics committee approval of this study was provided by the decision of the Ethics Committee of Keçiören Training and Research Hospital with the decision number 2012-KAEK-15/2218.

Immunohistochemical (IHC) Staining

The tissue sections were peroxidase-incubated for 10 minutes using 3% hydrogen peroxide in methanol (v/v). After that, the sections were performed for 3 min using a 0.01M citrate buffer, pH 6.0 in a domestic pressure cooker. Sections were incubated at room temperature for 10 min with superblock (SHP125; Scy Tek laboratories, West Logan, UT). The sections were then covered with the primary antibodies diluted (1:250 for GSTS1; 1:250 for GSTZ1; 1:250 for GSTT1) in TBS at 4°C. AntiGST-Theta1 (PAA622Hu01) was obtained from Cloud Clone Corp. TX,USA; anti-GSTS1 (Sc-30,067) was obtained from Santa Cruz Biotechnology, Inc; anti-GSTZ1 (bs-13442R) was obtained from Bioss Antibodies Inc. Woburn, Massachusetts, USA.After washing for 15 minutes in TBS, the sections were incubated at room temperature with a biotinylated link antibody (SHP125; ScyTek Laboratories) followed by streptavidin/HRP complex (SHP125; ScyTek laboratories). The sections were incubated at room temperature with biotinylated link antibody (SHP125; ScyTek Laboratories) then diaminobenzidine was used to visualize peroxidase activity in tissues and they were counterstained with hematoxylin. Scoring of immunohistochemically stained sections were performed for each parameter was: 0 negative (no staining);1 weak staining, 2, moderate staining.

Laboratory Findings

The blood test results of the cases were obtained from the patients' documentation. The patients' WBC, HGB, and platelet PLT levels were recorded as complete blood count parameters. Cholesterol parameters were noted, including HDL, LDL, total cholesterol, and triglyceride. Diabetes parameters included fasting glucose, postprandial glucose, and HbA1c. The hormone parameters were assessed, including free T3, free T4, TSH, estradiol, FSH, LH, C-peptide, insulin, cortisol, ACTH, and SHBG.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Version 25.0 (Armonk, NY: IBM Corp). The data were presented as mean ± standard deviation (SD) and standard error of the mean (SEM). The categorized data were expressed as numbers of patients (n) and percentages (%). The Shapiro-Wilk test was used to evaluate the normality. The Levene test was performed to test the homogeneity of variances. The data was not normally distributed. Therefore, the Mann-Whitney U test was performed to compare differences between two independent groups, and The Kruskal-Wallis test was conducted to analyze three or more independent groups. Bonferroni correction was then applied. Spearman's rank correlation test was used to determine correlations. A p-value lower than 0.05 was considered statistically significant.

RESULTS

Our study group included 126 obese patients with a body mass index (BMI) equal to or greater than 40 kg/m². The mean weight was 125.10 kg, and the mean BMI was 46.77 kg/m². 111 female (88.1%) and 15 male (11.9%) patients were in our study group. Almost half of the patients (47.6%) were between the ages of 31 and 45. The mean age of the patients was 37.91 years. Obesity was the only disease in 75 patients, while 50 had an additional disease. 58.7% of the patients did not use medications, while 40.5% used.
The patients' whole blood parameters such as WBC, HGB, and PLT; glucose parameters such as fasting glucose, postprandial glucose, and HbA1c; cholesterol parameters such as HDL, LDL, total cholesterol, and triglyceride; hormone parameters such as free T3, and T4, TSH, estradiol, FSH, LH, C-peptide, insulin, cortisol, ACTH, and SHBG were recorded. The clinical and demographic characteristics of the patients are detailed in Table 1.

IHC staining levels for GSTS1, GSTZ1, and

Table 1. Clinical and demographic characteristics of the patients

Characteristics	Data Mean ± SD (Range)
Age (years) (n = 126)	37.91 ± 11.01 (16-63)
Height (cm) (n = 126)	163.48 ± 8.21 (148-189)
Weight (kg) (n = 126)	125.10 ± 19.26 (91-182)
BMI (kg/m²) (n = 126)	46.77 ± 6.18 (40-70.31)
WBC ($\times 10^9$/L) (n = 126)	8.96 ± 2.00 (5.27-14.90)
HGB (g/dL) (n = 126)	13.68 ± 1.55 (9.87-18.40)
PLT ($\times 10^3$/µL) (n = 126)	290.39 ± 80.71 (34.30-53.500)
Glucose (fasting) (mg/dL) (n = 126)	111.16 ± 54.50 (69.00-498.00)
Glucose (postprandial) (mg/dL) (n = 88)	139.35 ± 62.43 (71.00-394.00)
HbA1c (%) (n = 117)	6.06 ± 1.36 (4.10-13.10)
HDL (mg/dL) (n = 21)	43.19 ± 7.77 (32.00-60.00)
LDL (mg/dL) (n = 15)	124.60 ± 34.55 (55.00-168.00)
Triglyceride(mg/dL) (n = 118)	173.03 ± 85.86 (42.00-545.00)
Total cholesterol (mg/dL) (n = 118)	215.62 ± 170.75 (108.00-2016.00)
Total T3 (ng/dL) (n = 124)	3.01 ± 0.41 (1.84-4.08)
Free T4 (ng/dL) (n = 125)	1.03 ± 0.14 (0.61-1.55)
TSH (mlU/L) (n = 125)	2.58 ± 2.17 (0.05-16.28)
Estradiol (pg/mL) (n = 56)	78.45 ± 62.99 (9.00-247.00)
FSH (mIU/mL) (n = 75)	11.38 ± 15.91 (0.96-75.98)
LH (IU/mL) (n = 75)	10.54 ± 10.58 (1.07-45.09)
C peptide (ng/mL) (n = 117)	3.33 ± 1.09 (1.28-6.23)
Insulin (mIU/L) (n = 122)	19.60 ± 25.31 (1.70-275.60)
Cortisol (µg/dL) (n = 122)	10.17 ± 4.10 (0.8-20.00)
ACTH (pg/mL) (n = 87)	29.14 ± 19.10 (5.00-126.00)
SHBG (nmol/L) (n = 55)	37.80 ± 31.30 (7.51-226.00)

The data were presentsed as mean ± SD (minimum-maximum values). BMI = body-mass index, WBC = white blood cell, HGB = hemoglobin, PLT = platelet, HbA1c = hemoglobin A1c, HDL = high-density lipoprotein, LDL = low-density lipoprotein, TSH = thyroid-stimulating hormone, FSH = follicle stimulating hormone, LH = luteinizing hormone, ACTH = adrenocorticotropic hormone, SHBG = sex hormone-binding globülin

GSTT1 were determined in the tissues of the patients. The expression levels were evaluated as shown in Table 2. Weak GSTS1 expression was observed in 48 (38.1%) tissues and moderate in 8 (6.3%) ones. 47 (37.3%) of the tissues presented weak GSTZ1 expression, and 11 (8.7%) displayed moderate. Weak GSTT1 expression was noted in 9 (7.1%) tissues and moderate in 1 (0.8%).

Mean GSTS1, GSTZ1, and GSTT1 IHC scores of the tissues are presented in Fig. 1. The highest expression was observed in GSTZ1, followed by GSTS1 and GSTT1. IHC expression patterns of GSTS1, GSTZ1, and GSTT1 in gastric tissues of patients are shown in Figs. 2, 3, and 4, respectively.

IHC expression levels of GSTS1, GSTZ1, and GSTT1 were determined regarding the patients' clinical and demographic characteristics, including gender, age, comorbidities, medication, and operation status

Table 2. GSTS1, GSTZ1, and GSTT1 IHC stainingprofile

IHC Staining Scores	GSTS1	GSTZ1	GSTT1
0	70/126[a] (55.6%)	68/126[a] (54.0%)	116/126[a] (92.1%)
1	48/126[a] (38.1%)	47/126[a] (37.3%)	9/126[a] (7.1%)
2	8/126[a] (6.3%)	11/126[a] (8.7%)	1/126[a] (0.8%)
Mean	0.51 ± 0.05[b] (0-2)[c]	0.55 ± 0.06[b] (0-2)[c]	0.09 ± 0.03[b] (0-2)[c]

Staining scores were determined based on the staining intensity of the tissues. 0 = negative staining, 1 = weak staining, 2 = moderate staining. IHC = immunohistochemical, GST = glutathione S-transferases, GSTS1 =GST-Sigma 1, GSTZ1 = GST Zeta 1, GSTT1= GST Theta 1

[a]Number of samples stained at specified level / Total number of samples (percent:%),

[b]Mean ± SEM,

[c]Minimum-maximum

(Table 3). There were no significant differences between expression levels and these parameters ($p > 0.05$).

Correlation analysis was performed between the GSTS1, GSTZ1, and GSTT1 expressions, and the results are explained in Table 4. A positive and statistically significant correlation was observed between GSTS1 and GSTT1 expression levels ((r) = 0.028, $p = 0.010$; $p < 0.05$).

Correlation analyses were also carried out between the clinical and demographic characteristics of the patients and the expression levels. Table 5 is displayed the results of these analysis. There was no statistically significant correlation between the GSTS1 expression levels and the clinical and demographic characteristics examined ($p > 0.05$). GSTZ expression and PLT levels were positively correlated ($p < 0.05$). There was a positive and significant correlation between GSTZ1 expression and total cholesterol levels ($p < 0.05$). Free T4 levels of the patients were also significantly correlated with GSTZ1 expression ($p < 0.05$). A positive and significant correlation was noted between the GSTT1 expression and cortisol levels ($p < 0.05$).

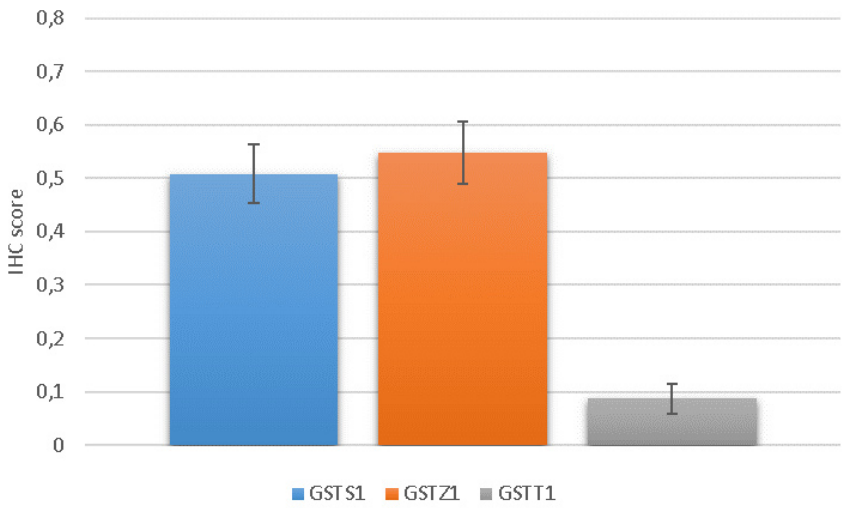

Fig. 1. Mean GSTS1, GSTZ1, and GSTT1 IHC scores of the tissues.

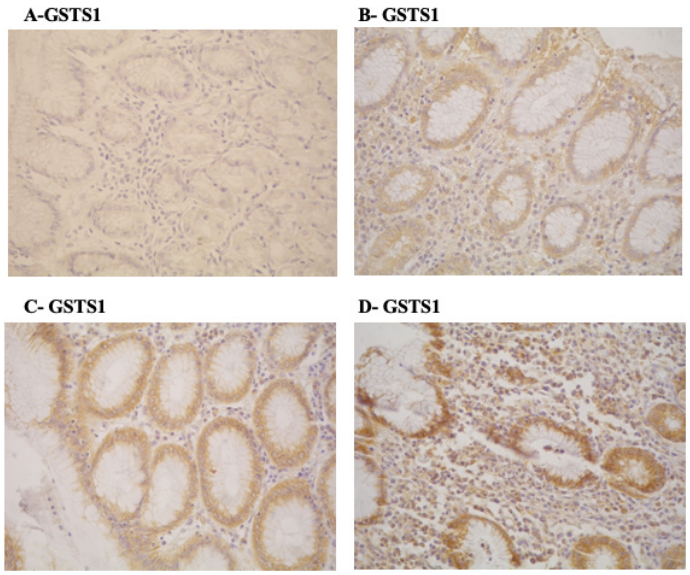

Fig. 2. Immunohistochemical expression of GSTS1 protein in stomach tissues (A: Expression of negative protein in stomach tissue, 40×; B: Weak (+1) protein expression in stomach tissue 20×; C: Weak (+2) protein expression in stomach tissue 20×; D: Weak expression in stomach tissue intense nuclear (+2) protein expression 40×).

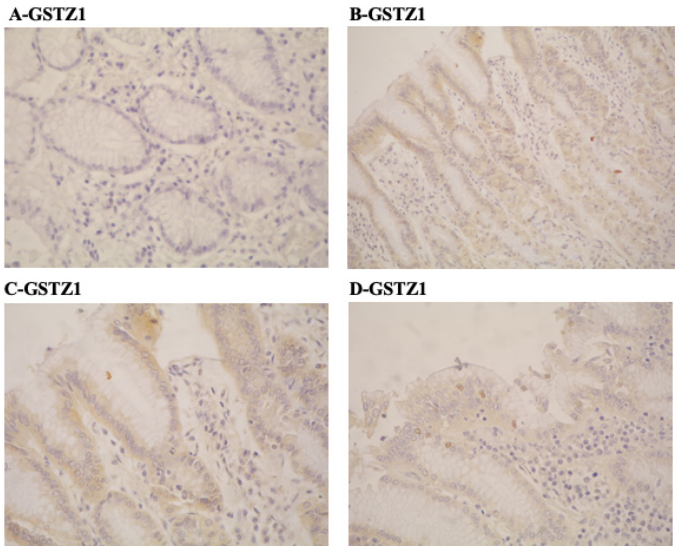

Fig. 3. Immunohistochemical expression of GSTZ1 protein in stomach tissues (A: Expression of negative protein in stomach tissue, 40×; B: Weak (+1) protein expression in stomach tissue 20×; C: Weak (+2) protein expression in stomach tissue 20×; D: Weak expression in stomach tissue intense nuclear (+2) protein expression 40×).

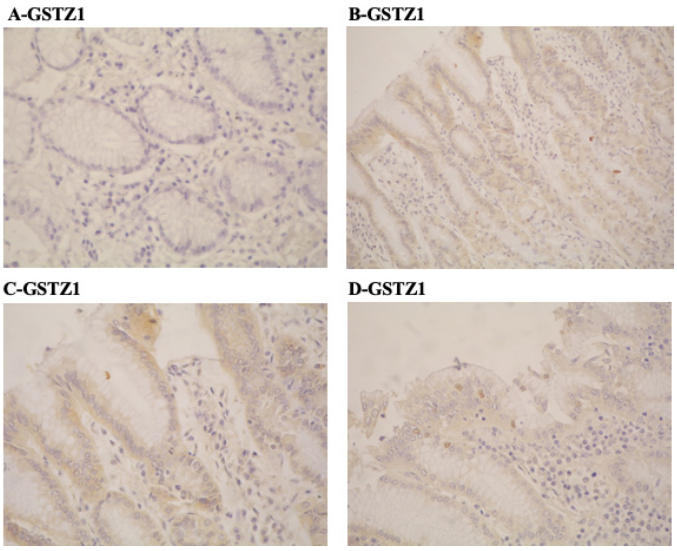

Fig. 4. Immunohistochemical expression of GSTT1 protein in stomach tissues (A: Expression of negative protein in stomach tissue, 40×; B: Weak (+1) protein expression in stomach tissue 20×; C: Weak (+2) protein expression in stomach tissue 40×; D: Weak expression in stomach tissue intense nuclear (+2) protein expression 40×).

DISCUSSION

Reactive oxygen species cause direct or indirect damage to different organs under many diseases and psychological conditions. Oxidative stress; includes pathological processes such as obesity, diabetes, cardiovascular disease. It has been reported that obesity can be stimulated by the oxidative stress system. Oxidative stress, on the other hand, is related to the irregular production of adipokines secreted from adipose

Table 3. IHC GSTS1, GSTZ1, and GSTT1 expressionsregarding the clinical and demographic characteristics of the patients

Variable	GSTS1	GSTZ1	GSTT1
Gender			
Female	0.51 ± 0.06[a] (0-2)[b]	0.56±0.06[a] (0-2)[b]	0.07 ± 0.02[a] (0-1)[b]
Male	0.47 ± 0.13[a] (0-1)[b]	0.47 ± 0.17[a] (0-2)[b]	0.20 ± 0.14[a] (0-2)[b]
p value	0.952	0,605	0,376
Age			
≤ 30	0.49 ± 0.10[a] (0-2)[b]	0.57 ± 0.12[a] (0-2)[b]	0.09 ± 0.05[a] (0-1)[b]
31-45	0.55 ± 0.09[a] (0-2)[b]	0.50 ± 0.08[a] (0-2)[b]	0.08 ± 0.04[a] (0-1)[b]
> 45	0.45 ± 0.09[a] (0-1)[b]	0.61 ± 0.11 [a] (0-2)[b]	0.10 ± 0.07[a] (0-2)[b]
p value	0.903	0.614	0.955
Comorbidities			
Yes	0.50 ± 0.08[a] (0-2)[b]	0.56 ± 0.09[a] (0-2)[b]	0.08 ± 0.05[a] (0-2)[b]
No	0.52 ± 0.07[a] (0-2)[b]	0.55 ± 0.07[a] (0-2)[b]	0.09 ± 0.03[a] (0-1)[b]
p value	0.993	0.973	0.527
Medication			
Yes	0.53 ± 0.08[a] (0-2)[b]	0.57 ± 0.09[a] (0-2)[b]	0.08 ± 0.05[a] (0-2)[b]
No	0.50 ± 0.08[a] (0-2)[b]	0.54 ± 0.08[a] (0-2)[b]	0.09 ± 0.03[a] (0-1)[b]
p value	0.606	0.726	0.494
Surgery			
Yes	0.54 ± 0.07[a] (0-2)[b]	0.49 ± 0.07[a] (0-2)[b]	0.11 ± 0.04[a] (0-2)[b]
No	0.46 ± 0.09[a] (0-2)[b]	0.63 ± 0.09[a] (0-2)[b]	0.06 ± 0.03 [a] (0-1)[b]
p value	0.367	0,227	0.385

GST = glutathione S-transferases, GSTS1 =GST-Sigma 1, GSTZ1 = GST Zeta 1, GSTT1= GST Theta 1
[a]Mean ± SEM,
[b]Minimum-maximum

Table 4. Correlation analyzes of GSTS1, GSTZ1, and GSTT1 expressions

	GSTS1		GSTZ1		GSTT1	
	correlation coefficient	*p value*	correlation coefficient	*p value*	correlation coefficient	*p value*
GSTS1	1.000	-	0,020	0.822	0.228	**0.010**
GSTZ1	0.020	0.822	1.000	-	-0.056	0.532
GSTT1	0.228	**0.010**	-0.056	0.532	1.000	-

GST = glutathione S-transferases, GSTS1 =GST-Sigma 1, GSTZ1 = GST Zeta 1, GSTT1= GST Theta 1

tissue, which contributes to the development of metabolic syndrome [44]. C-reactive protein and other oxidative damage biomarkers are high in obese individuals and are directly correlated with BMI and body lipid percentage, LDL oxidation and triglyceride level [45]. Antioxidant defense markers are lower than the amount of body fat in them [46]. Hartwich *et al.* determined that vitamin E, vitamin C, beta carotene and glutathione decreased in obesity [47]. Some research; showed that a high-fat and carbohydrate diet significantly stimulated the increase of oxidative stress and inflammation in obese individuals [48]. In literature studies on the detoxification mechanism, it has been shown that as a result of long-term obesity, a decrease in antioxidant sources and a decrease in the activities of super oxide dismutase and catalase enzymes [49]. In another study, it was shown that SOT and glutathione peroxidase activities were decreased in obese individuals compared to healthy individuals [50]. In a study, it was shown that oxidative stress in the cell increases as a result of the increase in CYP2E1 in mitochondria and has a role in liver diseases, obesity and type-2 diabetes [51].

In this study, it is known that oxidative stress is associated with the development of obesity, a multifactorial disease that is very common in all countries. II, which plays an important role in the xenobiotic mechanism. In this study, the relationship between the expressions of GSTT1, GSTS1 and GSTZ1 isozymes, which are members of the GST enzyme system that catalyzes the phase reactions, with obesity was investigated. In our findings, weak GSTS1 expression was observed 38.1% of the tissues and moderate in 6.3%. The GSTZ1 expression was weak in 37.3% of the tissues and moderate in 8.7%. Weak GSTT1 expression was noted in 7.1% of the tissues and moderate in 0.8%. Positive and statistically significant correlations were found between GSTZ1 expression and PLT, total cholesterol, and free T4 levels of the patients ($p < 0.05$). A positive and significant correlation was also found between GSTT1 expressionand the cortisol levels of the patients ($p < 0.05$).

It is seen that other studies in the literature indicate the relationship between obesity and oxidative stress. Apart from these studies, which also show their relationship with the concepts of obesity and oxidative stress separately, in this study, which we aim to contribute to revealing the relationship between obesity and Glutathione enzymes, which directly play a role in the xenobiotic mechanism, findings that overlap with other studies in the literature were obtained.

All these results indicate that GSTS1, GSTZ1, and GSTT1 enzyme amounts and oxidative stress increase in obesity. Significant changes occur in the amount GSTS1, GSTZ1, and GSTT1 enzymes in obese individuals. While these changes may occur as an adaptive response, the findings suggest that oxidative stress observed in obesity may be one of the possible mechanisms underlying this change. Findings that contribute to the definition of the physiopathology of obesity are important in the prevention of complications such as cardiovascular diseases that may develop due to obesity and in the development of preventive approaches.

CONCLUSION

In obese people, metabolic load due to overnutrition and, as a result, free radical formation due to overloading of metabolic pathways increase. Increased ROS in obese individuals cause oxidative stress. The negativities caused by free radicals are eliminated by the antioxidant defense system of the cell and the antioxidants taken through food. However, in the case

Table 5. Correlation analyses of IHC GSTS1, GSTZ1, and GSTT1 expression levels and the clinical and demographic characteristics

Data	GSTS1		GSTZ1		GSTT1	
	correlation coefficient	*p value*	correlation coefficient	*p value*	correlation coefficient	*p value*
Age	-0.002	0.979	0,042	0.640	-0.041	0.649
Height	-0.085	0.341	-0.017	0.852	0.177	**0.047**
Weight	-0.020	0.821	-0,063	0.481	0.171	0.056
BMI	0.122	0.172	-0.034	0.707	0.070	0.439
WBC	0.030	0.741	-0.023	0.797	-0.104	0.247
HGB	0.010	0.909	-0.081	0.365	-0.117	0.190
PLT	0.046	0.608	0.188	**0.035**	-0.058	0.520
Glucose (fasting)	0.052	0.566	0.115	0.201	0.071	0.431
Glucose (postprandial)	-0.002	0.988	0.007	0.952	0.105	0.330
HbA1c	0.052	0.578	0.180	0.052	-0.057	0.544
HDL	0.346	0.124	0.123	0.596	-	-
LDL	0.309	0.262	0.209	0.454	-	-
Triglyceride	0.067	0.469	0.022	0.816	0.088	0.345
Total cholesterol	-0.017	0.859	0.188	**0.041**	0.078	0.403
Free T3	-0.060	0.507	-0.017	0.855	0.162	0.072
Free T4	0.174	0.053	0.178	**0.046**	0.047	0.600
TSH	-0.118	0.188	0.090	0.318	0.029	0.746
Estradiol	0.053	0.699	0.212	0.117	-0.164	0.226
FSH	-0.016	0.891	0.042	0.718	-0.143	0.222
LH	0.024	0.840	0.009	0.940	-0.117	0.317
Insulin	-0.015	0.867	-0.043	0.636	-0.001	0.988
C Peptide	-0.011	0.910	-0.010	0.914	0.013	0.889
Cortisol	0.071	0.437	0.047	0.611	0.223	**0.014**
ACTH	0.058	0.592	0.069	0.524	-0.001	0.991
SHBG	0.037	0.789	0.112	0.415	0.062	0.655

IHC = immunohistochemical, GST = glutathione S-transferases, GSTS1 =GST-Sigma 1, GSTZ1 = GST Zeta 1, GSTT1= GST Theta 1, BMI = body-mass index, WBC = white blood cell, HGB = hemoglobin, PLT = platelet, HbA1c = hemoglobin A1c, HDL = high-density lipoprotein, LDL = low-density lipoprotein, TSH = thyroid-stimulating hormone, FSH = follicle stimulating hormone, LH = luteinizing hormone, ACTH = adrenocorticotropic hormone, SHBG =: sex hormone-binding globülin

of long-term obesity, antioxidant system enzymes are lower. Obesity causes cell damage and various diseases such as type 2 diabetes, cardiovascular diseases and cancer. GSTs, in particular GSTS1, GSTT1, and GSTZ1, might contribute to molecular mechanisms and the progression of obesity. GSTS1, GSTT1, and GSTZ1 were moderately expressed in gastric tissues taken from obese patients regarding to the resuls of study. However, new studies using more samples and advanced techniques are needed to elucidate the relationship.

Authors' Contribution

Study Conception: MD, HB; Study Design: HB,

OD, SO; Supervision: HB; Funding: SO; Materials: MD, HB, PK, SYS, SO; Data Collection and/or Processing: GGS, OD, PK, SYS, FNG, SO; Statistical Analysis and/or Data Interpretation: SYS, SO; Literature Review: OD, SYS, SO; Manuscript Preparation: OD and Critical Review: GGS, SO, HB.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999-2002. JAMA 2004;291:2847-50.
2. Buchwald H, Estok R, Fahrbach K, Banel D, Jensen MD, Pories WJ, et al. Weight and type 2 diabetes after bariatric surgery: systematic review and meta-analysis. Am J Med 2009;122:248-56.
3. Campfield LA, Smith FJ. The pathogenesis of obesity. Baillieres Best Pract Res Clin Endocrinol Metab 1999;13:13-30.
4. Toshima S, Hasegawa A, Kurabayashi M, Itabe H, Takano T, Sugano J, et al. Circulating oxidized low density lipoprotein levels. A biochemical risk marker for coronary heart disease. Arterioscler Thromb Vasc Biol 2000;20:2243-7.
5. Björntorp P. International Textbook of Obesity. John Wıley and Sons Ltd, 2001: pp. 305-14.
6. Goran MI. Energy Metabolism and obesity. Med Clin North Am 2000;84:333-45.
7. Hill JO, Peters JC. Environmental contributions to the obesity epidemic. Science 1998;280:1371-4.
8. Schrauwen P and Westerterp KR. The role of high-fat diets and physical activity in the regulation of body weigt. BJ Nutr 2000;84:417-27.
9. Fletcher GF, Grundy SM, Hayman LL. Obesity: Impact on cardiovascular disease. American Heart Association. Futura Publising Company, Armonk NY, 1999: pp. 3-46.
10. Steinbrook R. Surgery for severe obesity. N Eng J Med 2004;350:1075-9.
11. Seaver LC, Imlay JA. Are respiratory enzymes the primary sources of intracellular hydrogen peroxide? J Biol Chem 2004;279:48742-50.
12. Messner KR, Imlay A. Mechanism of superoxide and hydrogen peroxide formation by fumarate reductase, succinate dehydrogenase, and aspartate oxidase. J Biol Chem 2002;277:42563-71.
13. Imlay JA. Pathways of oxidative damage. Ann Rev Microbiol 2003;57:395-418.
14. Martemucci G, Costagliola C, Mariano M, D'andrea L, Napolitano P, D'Alessandro AG. Free radical properties, source and targets, antioxidant consumption and health. Oxygen 2022;2:48-78.
15. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006;160:1-40.
16. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44-84.
17. Djordjevic VB. Free radicals in cell biology. Int Rev Cytol 2004;237:57-89.
18. Wellman NS, Friedberg B. Causes and consequences of adult obesity: health, social and economic impacts in the United States. Asia Pac J Clin Nutr 2002;11:S705-S709.
19. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004;114:1752-61.
20. Maddux BA, See W, Lawrence JC Jr, Goldfine AL, Goldfine ID, Evans JL. Protection against oxidative stress-induced insulin resistance in rat L6 muscle cells by mircomolar concentrations of alpha-lipoic acid. Diabetes 2001;50:404-10.
21. Isomaa B, Almgren P, Tuomi T, Forsén B, Lahti K, Nissén M, et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 2001;24:683-9.
22. Grundy SM. Atherosclerosis imaging and the future of lipid management. Circulation 2004;110:3509-11.
23. Hansel B, Giral P, Nobecourt E, Chantepie S, Bruckert E, Chapman MJ, et al. Metabolic syndrome is associated with elevated oxidative stress and dysfunctional dense high-density lipoprotein particles displaying impaired antioxidative activity. J Clin Endocrinol Metab 2004;89:4963-71.
24. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA 2002;288:2709-16.
25. Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. Int J Obes (Lond) 2006;30:400-18.
26. Myara I, Alamowitch C, Michel O, Heudes D, Bariety J, Guy-Grand B, et al. Lipoprotein oxidation and plasma vitamin E in nondiabetic normotensive obese patients. Obes Res 2003;11:112-20.
27. RussellAP, Gastaldi G, Bobbioni-Harsch E, Arboit P, Gobelet C, Dériaz O, et al. Lipid peroxidation in skeletal muscle of obese as compared to endurance-trained humans: a case of good vs. bad lipids? FEBS Lett 2003;11:104-6.
28. Birdwell K. Role of pharmacogenomics in dialysis and transplantation. Curr Opin Nephrol Hypertens 2014;23:570-7.
29. Parkinson A, Ogilvie BW, Buckley DB, Kazmi F, Czerwinski M, Parkinson O. Biotransformation of Xenobiotics. In: Klaassen CD, Watkins III JB, eds. Casarett & Doull's Essentials of Toxicology. 3rd ed. United States, 2015: pp. 79-108.
30. Prakash C, Zuniga B, Song CS, Jiang S, Cropper J, Park S, et al. Nuclear receptors in drug metabolism, drug response and

drug interactions. Nucl Receptor Res 2015;2101178.
31. Haddad JJ, Land SC. O(2)-evoked regulation of HIF-1alpha and NF-kappaB in perinatal lung epithelium requires glutathione biosynthesis. Am J Physiol Lung Cell Mol Physiol 2000;278:L492-L503.
32. May JM. Ascorbate function and metabolism in the human erythrocyte. Front Biosci 1998;3:d1-10.
33. Powis G, Gasdaska JR, Gasdaska PY, Berggren M, Kirkpatrick DL, Engman L, et al. Selenium and the thioredoxin redox system: effects on cell growth and death. Oncol Res 1997;9:303-12.
34. Haddad JJ, Harb HL. L-gama-Glutamyl-L-cysteinyl-glycine (glutathione; GSH) and GSH-related enzymes in the regulation of pro- and antiinflammatory cytokines: a signaling transcriptional scenario for redox(y) immunologic sensor(s)? Mol Immunol 2005;42:987-1014.
35. Croom E. Metabolism of xenobiotics of human environments. Prog Mol BiolTransl Sci 2012;112:31-88.
36. Hoffmann MF, Preissner SC, Nickel J, Dunkel M, Preissner R, Preissner S. The transformer database: biotransformation of xenobiotics. Nucleic Acids Res 2014;42:1113-7.
37. Worku M, Aynalem M, Biset S, Woldu B, Adane T, Tigabu A. Role of complete blood cell count parameters in the diagnosis of neonatal sepsis. BMC Pediatr 2022;22:411.
38. Alston MC, Redman LM, Sones JL. An overview of obesity, cholesterol, and systemic inflammation in preeclampsia. Nutrients 2022;14:2087.
39. Klein S, Gastaldelli A, Yki-Järvinen H, Scherer PE. Why does obesity cause diabetes? Cell Metab 2022;34:11-20.
40. Lu D, Yuan Z, Gao Y, Liu W, Zhang J. Central obesity is associated with variations in TSH and ACTH levels among euthyroid obese individuals. Int J Endocrinol 2022;2022:3830380.
41. El-Zawawy HT, El-Aghoury AA, Katri KM, El-Sharkawy EM, Gad SMS. Cortisol/DHEA ratio in morbidly obese patients before and after bariatric surgery: relation to metabolic parameters and cardiovascular performance. Int J Obes 2022;46:381-92.
42. Sun Y, Teng D, Zhao L, Shi X, Li Y, Shan Z et al. Thyroid Disorders, Iodine Status and Diabetes Epidemiological Survey Group (TIDE) . Impaired Sensitivity to Thyroid Hormones Is Associated with Hyperuricemia, Obesity, and Cardiovascular Disease Risk in Subjects with Subclinical Hypothyroidism. Thyroid2022 32(4), 376-384.
43. Genchi VA, Rossi E, Lauriola C, D'Oria R, Palma G, Borrelli Aetal. Adipose tissue dysfunction and obesity-related male hypogonadism. Int J Mol Sci 2022;23:8194.
44. Esposito K, Ciotola M, Schisano B, Misso L, Giannetti G, Ceriello A, et al. Oxidative stress in the metabolic syndrome. J Endocrinol Invest 2006;29:791-5.
45. Pihl E, Zilmer K, Kullisaar T, Kairane C, Magi A, Zilmer M. Atherogenic inflammatory and oxidative stres markers in relation to overweight values in male former athletes. Int J Obes (Lond) 2006;30:141-6.
46. Chrysohoou C, Panagiotakos DB, Pitsavos C, SkoumasI, Papademetriou L, Economou M, et al. The implication of obesity on total antioxidant capacity apparently healthy men and women: The ATTICA study. Nutr Metab Cardiovasc Dis 2007;17:590-7.
47. Hartwich J, Goralska J, Siedlecka D, Gruca A, Trzos M, Dembinska-Kiec A. Effect of supplementation with vitamin E and C on plasma hsCPR level and cobalt-albumin binding score as markers of plasma oxidative stress in obesity. Genes Nutr 2007;2:151-4.
48. Vincent H, Vincent K, Vourguignon C, Braith R. Obesity and postexercise oxidative stress in older women. Med Sci Sports Exer 2005;37:213-9.
49. Raunio H, Pasanen M, Maenpaa J, Hakkola J, Pelkonen O. Expression of extrahepatic cytochrome P450 in humans. In: Pacifici GM and Fracchia GN (eds) Advances in Drug Metabolism in Man: 234-287. European Commission, Office for Official Publications of the European Communities, Luxembourg, 1995.
50. Imaoka S, Yamada T, Hiroi T, Hayashi K, Sakaki T, Yabusaki Y, et al. Multiple forms of human P450 expressed in Saccharomyces cerevisiae, systematic characterization and comparison with those of the rat. Biochem Pharmacol 1996;51:1041-50.
51. Butura A, Nilsson K, Morgan K, Morgan TR, French SW, Johansson I, et al. The impact of CYP2E1 on the development of alcoholic liver disease as studied in a transgenic mouse model. J Hepatol 2009;50:572-83.