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Investigation of the Role of CYP1A1 and CYP1B1 Expressions in Obesity Susceptibility

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Abstract: Obesity, a chronic inflammatory disease, needs to be understood with a more multidisciplinary perspective based on metabolic functioning. The rapid increase in the incidence and prevalence of the disease in recent years has caused it to become a global health problem. CYP1A1 is a key enzyme in the metabolization of several exogenous and endogenous substrates including polyunsaturated fatty acids. CYP1B1 plays a regulatory role in metabolic pathways, such as the metabolism of steroid hormones and fatty acids. Therefore, the purpose of our study is to reveal the role of cytochrome p450 (CYP1A1 and CYP1B1) isozymes in obese patients. In addition, this is the first study to examine the effects of CYP1A1 and CYP1B1 in obese patients concerning various hormones, lipids, and also diabetic and whole blood parameters. Patients with obesity who underwent bariatric surgery were included in our study and the expression of CYP1A1 and CYP1B1 in surgery tissues was determined by immunohistochemistry. CYP1A1 was weakly expressed in 33.3%, moderately in 32.5%, and strongly in 21.4% of tissues. Weak CYP1B1 expression was observed only in 28.6% of the tissues while moderate or strong expression was not observed in any of the tissues. No statistically significant differences were found between expression levels of CYP1A1 and CYP1B1 isozymes in obese patients and demographic characteristics ($p>0.05$).Correlation analyses were also performed between the expression levels and the clinical data of the patients. There was a significant correlation between CYP1A1 expression and hemoglobin levels ($p<0.05$). A significant correlation was found between CYP1B1 expression and insulin as well as HDL levels ($p<0.05$). These detoxification enzymes could be considered one of the key targets in obesity. However, higher expressions should be elucidated with other studies, especially in the molecular pathway. Moreover, new approaches are needed to evaluate susceptibility of the disease and obtain effective treatments.

Obezite Duyarlılığında CYP1A1 Ve CYP1B1 Ekspresyonlarının Rolünün Araştırılması

Anahtar Kelimeler
Obezite, Oksidatif stres, CYP1A1, CYP1B1, Ksenobiyotikler

Öz: Metabolik işleyiş, etkileri ve neden olduğu hastalıklar açısından daha multidisipliner bir bakış açısıyla anlaşılması gereken obezite, son yıllarda prevalansı ve insidansı artan hastalıklardan biri olarak görülmektedir. Bu hastalığın metabolic yolunda önemli enzim gruplarından biri olan sitokrom p450 (CYP1A1 ve CYP1B1) izozimlerinin rolünün ortaya konulması amaçlanmıştır. 2017-2019 yılları arasında Ankara Keçiören Eğitim ve Araştırma Hastanesi Genel Cerrahi Kliniği'nde obezite tanısı konulan ve bariatric cerrahi, ksenobiyotik metabolizması uygulanan 152 hastaya immünohistokimya yöntemiyle CYP1A1 ve CYPB1 izoenzimlerinin ekspresyonu araştırılmıştır. CYP1A1 açısından elde edilen bulgular; 152 kişide CYP1A1 ve CYP1B1 immünohistokimya boyama düzeyleri incelenen dokuların %12.7'sinde CYP1A1 ekspresyonu gözlenmezken; %33.3, %32.5 ve %21.4'te zayıf bir CYP1A1 ifadesi gözlenmiştir. Dokuların %71.4'ünde CYP1B1 ekspresyonu görülmezken, dokuların %28.6'sında zayıf ekspresyon izlenmiştir. Hiçbir dokuda orta veya güçlü CYP1B1 ekspresyonu gözlenmemiştir. Kadın hastalardan alınan dokuların ortalama CYP1A1 ve CYP1B1 boyama seviyeleri erkek hastalardan daha yüksek bulunmuştur. Klinik verilerden diyabet parametresi ($p<0.05$) ile anlamlı olduğu gözlenmiştir. Çalışmamızda elde edilen veriler ışığında obez hastalarda anlamlı CYP1A ve CYP1B1 ekspresyonları gözlenmiş ve detoksifikasyon mekanizması nedeniyle antioksidan metabolizma ve işlevsellik mevcuttur. Obez hastalarda bu enzim düzeyindeki artışın özellikle moleküler yolakta başka çalışmalarla aydınlatılması gerektiği ve benzer çalışmalara yol göstereceği düşünülmektedir.

1. INTRODUCTION

Obesity, whose prevalence and incidence is increasing rapidly, is one of the biggest health problems encountered in recent years [1]. Oxidative stress plays an important role in the emergence of cancer, atherosclerosis, arthritis, neurodegenerative diseases, cardiovascular diseases, diabetes and also metabolic disorders including obesity [2-4]. In obesity, oxidative stress biomarkers are associated with body mass index (BMI), body fat percentage, low-density lipoprotein (LDL) and triglyceride levels. The capacity of the antioxidant defense system decreases according to the level of fat percentage in the body. A diet high in fat and carbohydrates has been shown to induce oxidative stress and inflammation in obese subjects significantly [5]. It has been stated that oxidative stress may cause insulin resistance, which is a common condition in obesity, by disrupting insulin secretion from β-cells in the pancreas and glucose transport in skeletal and adipose tissue [6]. Independent of obesity, the metabolic syndrome also induces oxidative stress alone. Uncontrolled adipokine production induced by oxidative stress is one of the mechanisms contributing to the development of the metabolic syndrome [7]. Obesity appears to cause the presence of oxidative stress [8]. The wide variety of detoxification processes that make these xenobiotics that reach the body less toxic, more polar and easily excreted is called xenobiotic biotransformation or xenobiotic metabolism [9]. Cytochrome P450 (CYP) enzymes play the most prominent role in Phase I xenobiotic metabolism[10]. CYP1A1 is the biomarker that metabolizes polycyclic aromatic hydrocarbons inducible by smoking. CYP1B1 is involved in the estrogen metabolic pathway. Especially, this role is realized by the regulation of 4-hydroxy estradiol in the pathogenesis of breast and endometrial cancer [11]. Investigation of the functionality of metabolic components such as glutathione, superoxide dismutase against oxidative stress and reduction of toxic substances is continued in order to prevent metabolic exposure from causing cellular and tissue damage [12].

In our study, it was aimed to contribute to the understanding of the development of the obesity from an epidemiological point of view by examining the expression status of CYP1A1 and CYP1B1 proteins, which are important ondeoxification mechanisms, based on patients' demographic and clinical characteristics.

2. MATERIAL AND METHOD

2.1.Immunohistochemical Staining

In this study, 149 paraffin tissue blocks of obesity patients were received from the Keçiören Research and Education Hospital. They were stained by immunohistochemistry (IHC) method for CYP1A1 and CYP1B1 isoenzymes.

For immunohistochemistry, 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 (1:750 for

CYP1A1; 1:400 for CYP1B1) in TBS at 4 °C. anti-CYP1A1 (sc-20,772) and Anti-CYP1B1 (sc-32,882) were obtained from Santa Cruz Biotechnology Inc., Dallas, TX. 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 enzyme was: (-), negative (no staining);1, weak staining; 2, moderate staining; 3, strong staining.

2.2.Statistical Analysis

IBM SPSS Version 25.0 (Armonk, NY: IBM Corp) was used for statistical analysis. The numerical variables describing the clinical and demographic characteristics of the patients were expressed as mean±standard deviation or standard error of mean. The categorized variables were described as numbers of patients (n) and percentages (%) based on descriptive statistics. The Shapiro-Wilk test was performed to assess the distribution profiles of the numerical variables. The Levene test was used to assess the homogeneity of variances. The Mann-Whitney U test was used to compare differences between two independent and The Kruskal-Wallis test was three or more independent groups for non-normally distributed variables. Bonferonni correction was performed. The correlation analysis was evaluated by Spearman's rank correlation test. Differences at the $p<0.05$ level were considered statistically significant.

3. RESULTS

The demographic and clinical characteristics of the patients were categorized and explained in Table 1. A total of 126 obese patients were included in our study. The study group consisted of 111 females (88,1%) and 15 males (11,9%). 35 of the patients (27,8%) were 30 or younger than 30 years old, 60 patients (47,6%) were between 31 and 45 years old, and 31 patients (24,6%) were older than 45 years. All of the patients were obese, so there were no patients with a BMI below 40. Only 3 of the patients (2.4%) had a BMI of 40, and 123 patients (97,6%) had a BMI above 40. 59.5% (75 patients) of the patients were only obese, while 39.7% (50 patients) had additional diseases (comorbidity). 74 of the patients (58.7%) did not use any medication, while 51 patients (40.5%) used some. 72 of the patients (57.1%) underwent surgery, while 54 patients (42.9%) did not undergo surgery. Additionally, a number of other measurements were recorded and classified including patients' white blood cell (WBC), hemoglobin (HGB), platelet (PLT), fasting and postprandial glucose, high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol, triglyceride, free T3 and T4, thyroid-stimulating hormone (TSH), estradiol, follicle-stimulating hormone (FSH), luteinizing hormone (LH), C-peptide, insulin, cortisol, adrenocorticotropic hormone (ACTH), sex hormone-binding globulin (SHBG) and hemoglobin A1c (HbA1c) levels.

Table 1. Categorized demographic and clinical characteristics of patients

Variables	Category	Number (%)
Gender	Female	111 (%88,1)
	Male	15 (%11,9)
Age	≤30	35 (%27,8)
	31-45	60 (%47,6)
	>45	31 (%24,6)
BMI	≤40	3 (%2,4)
	>40	123 (%97,6)
Comorbidities	Yes	50 (%39,7)
	No	75 (%59,5)
	Missing (unknown)	1 (%0,8)
Medication usage status	Yes	51 (%40,5)
	No	74 (%58,7)
	Missing (unknown)	1 (%0,8)
Surgery status	Yes	72 (%57,1)
	No	54 (%42,9)
WBC	<4,5	0 (%0)
	4,5-11,0	106 (%84,1)
	>11,0	20 (%15,9)

HGB	<11,0	7 (%5,6)
	11-16	110 (%87,3)
	>16	9 (%7,1)
PLT	<130	3 (%2,4)
	130-400	111 (%88,1)
	>400	12 (%9,5)
Glucose (fasting)	<70	1 (%0,8)
	70-105	84 (%66,7)
	>105	41 (%32,5)
Glucose (postprandial)	<140	58 (%46,0)
	140-200	21 (%16,7)
	>200	10 (%7,9)
	Missing (unknown)	37 (%29,4)
Triglyceride	≤150	57 (%45,2)
	>150	61 (%48,4)
	Missing (unknown)	8 (%6,3)
Total cholesterol	≤200	67 (%53,2)
	>200	57 (%45,2)
	Missing (unknown)	2 (%1,6)
Free T3	<1,8	0 (%0)
	1,8-4,6	124 (%98,4)
	>4,6	0 (%0)
	Missing (unknown)	2 (%1,6)
Free T4	<0,9	17 (%13,5)
	0,9-1,7	108 (%85,7)
	>1,7	0 (%0)
	Missing (unknown)	1 (%0,8)
TSH	<0,27	4 (%3,2)
	0,27-4,2	105 (%83,3)
	>4,2	16 (%12,7)
	Missing (unknown)	1 (%0,8)
HbA1C	≤6	80 (%63,5)
	>6	38 (%30,2)
	Missing (unknown)	8 (%6,3)

The results were expressed as n (%).BMI: body-mass index, WBC: white blood cell, HGB: hemoglobin, PLT: platelet, TSH: thyroid-stimulating hormone, HbA1c: hemoglobin A1c.

The patients' demographic and clinical characteristics were shown in Table 2. Patients' age, height, weight and BMI were recorded as demographic data. Patients' WBC, HGB, PLT, fasting and postprandial glucose, HDL, LDL, total cholesterol, triglyceride, free T3 and T4, TSH, estradiol, FSH, LH, C-peptide, insulin, cortisol, ACTH, SHBG, HbA1c levels were recorded as clinical data. Patients' mean age was 37,91 years (16-63), mean weight was 125,10 kg (91-182), and mean BMI was 46,77 kg/m² (40-70.31).

Table 2. The patients'demographic and clinical characteristics

Variable (unit)	Number	Mean±SD
Age (year)	126	37,91 ± 11,01 (16-63)
Height (cm)	126	163,48 ± 8,21 (148-189)
Weight (kg)	126	125,10 ± 19,26 (91-182)
BMI (kg/m²)	126	46,77 ± 6,18 (40-70,31)
WBC	126	8,96 ± 2,00 (5,27-14,90)
HGB	126	13,68 ± 1,55 (9,87-18,40)
PLT	126	290,39 ± 80,71 (34,30-535,00)
Glucose (fasting)	126	111,16 ± 54,50 (69,00-498,00)
Glucose (postprandial)	88	139,35 ± 62,43 (71,00-394,00)
HDL	21	43,19 ± 7,77

		(32,00-60,00)
LDL	15	124,60 ± 34,55 (55,00-168,00)
Triglyceride	118	173,03 ± 85,86 (42,00-545,00)
Total cholesterol	118	215,62 ± 170,75 (108,00-2016,00)
Free T3	124	3,01 ± 0,41 (1,84-4,08)
Free T4	125	1,03 ± 0,14 (0,61-1,55)
TSH	125	2,58 ± 2,17 (0,05-16,28)
Estradiol	56	78,45 ± 62,99 (9,00-247,00)
FSH	75	11,38 ± 15,91 (0,96-75,98)
LH	75	10,54 ± 10,58 (1,07-45,09)
C peptide	117	3,33 ± 1,09 (1,28-6,23)
Insulin	122	19,60 ± 25,31 (1,70-275,60)
Cortisol	122	10,17 ± 4,10 (0,8-20,00)
ACTH	87	29,14 ± 19,10 (5,00-126,00)
SHBG	55	37,80 ± 31,30 (7,51-226,00)
HbA1C	117	6,06 ± 1,36 (4,10-13,10)

The results were expressed as mean±SD (minimum-maximum values). BMI: body-mass index, WBC: white blood cell, HGB: hemoglobin, PLT: platelet, 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 globulin, HbA1c: hemoglobin A1c.

IHC staining was performed on the tissues as explained in the material and method section. CYP1A1 and CYP1B1 expression levels were evaluated and explained in Table 3. Weak CYP1A1 expression was observed in 33.3% of the tissues. Moderate expression of CYP1A1 was detected in 32.5% of tissues, and strong expression was in 21.4%. Weak CYP1B1 expression was observed in 28.6% of the tissues. There was no moderate or strong expression of CYP1B1 in any of the tissues.

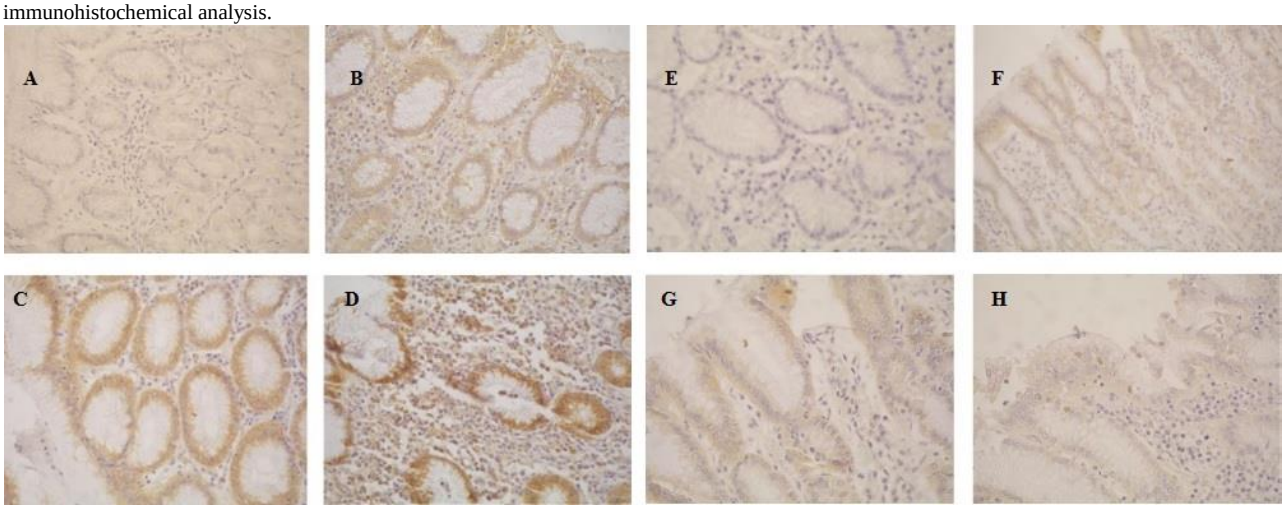
Table 3. CYP1A1 and CYP1B1 expression levels of the tissues

Expression Level	CYP1A1	CYP1B1
0	16/126[a] (%12,7)	90/126[a] (%71,4)
1	42/126[a] (%33,3)	36/126[a] (%28,6)
2	41/126[a] (%32,5)	0/126[a] (%0)
3	27/126[a] (%21,4)	0/126[a] (%0)
Mean	1,63 ± 0,09[b] (0-3)[c]	0,29 ± 0,04[b] (0-1)[c]

Staining scores were determined according to the staining intensity of the tissues. 0: negative staining, 1: weak staining, 2: moderate staining, 3: strong staining.
a: Number of samples stained at specified level / Total number of samples (percent),
b: Mean staining level ± Standard Error of Mean (SEM)
c: The lowest staining level – The highest staining level

Microscopic images of CYP1A1 and CYP1B1 protein expressions in tissues obtained surgically from obesity patients after immunohistochemical analysis are shown in Figure 1.

Figure 1. Microscopic images of CYP1A1 and CYP1B1 protein expressions in tissues obtained surgically from obesity patients after immunohistochemical analysis.



CYP1A1 protein; A: Expression of negative protein in stomach tissue, 40X; **B**: Weak (+1) protein expression in stomach tissue 40X; **C**: Moderate (+2) protein expression in stomach tissue 40X; **D**: Strong (+2) protein expression in stomach tissue protein expression 40X. **CYP1B1 protein;E**: Expression of negative protein in stomach tissue, 40X; **F**: Weak (+1) protein expression in stomach tissue, 20X; **G**: Weak (+1) protein expression in stomach tissue, 40X; **H**: Weak nuclear in stomach tissue (+1) protein expression 40X)

The expression levels of CYP1A1 and CYP1B1 were analyzed based on the patients' demographic characteristics such as gender, age, comorbidities, medication use, and operation status in Table 4.

CYP1A1 and CYP1B1 expression levels were determined based on the patients' age, as shown in Figure 3. The 31-45 age group had the highest CYP1A1 expression levels. However, there were no statistically significant differences in CYP1A1 staining levels between patients of different age groups ($p>0.05$). The patients over 45 years of age expressed the highest levels of CYP1B1. However, there were no statistically significant differences in CYP1B1 expression levels among patients in different age groups ($p>0.05$).

Table 4. CYP1A1 and CYP1B1 expression levels based on patients' demographic characteristics

Variable	CYP1A1	CYP1B1
Gender		
Female	1,65±0,09 [a] (0-3)[b]	0,31±0,04 [a] (0-1)[b]
Male	1,47±0,26 [a] (0-3)[b]	0,13±0,09 [a] (0-1)[b]
p-value	0,545	0,166
Age		
≤30	1,59±0,18 [a] (0-3)[b]	0,29±0,08 [a] (0-1)[b]
31-45	1,78±0,12 [a] (0-3)[b]	0,25±0,06 [a] (0-1)[b]
>45	1,35±0,16 [a] (0-3)[b]	0,35±0,09 [a] (0-1)[b]
p-value	0,154	0,579
Comorbidity		
Yes	1,50±0,12 [a] (0-3)[b]	0,26±0,06 [a] (0-1)[b]
No	1,71±0,12 [a] (0-3)[b]	0,31±0,05 [a] (0-1)[b]
p-value	0,241	0,574
Medication usage		
Yes	1,49±0,12 [a] (0-3)[b]	0,25±0,06 [a] (0-1)[b]
No	1,72±0,12 [a] (0-3)[b]	0,31±0,05 [a] (0-1)[b]
p-value	0,215	0,499
Surgery status		
Yes	1,62±0,11 [a] (0-3)[b]	0,28±0,05 [a] (0-1)[b]
No	1,63±0,14 [a] (0-3)[b]	0,30±0,06 [a] (0-1)[b]
p-value	0,961	0,821

a: Mean ± SEM, b: The minimum-the maximum

CYP1A1 and CYP1B1 expression levels were evaluated in terms of the patients' gender, as shown in Figure 2.Mean CYP1A1 and CYP1B1 expression levels of tissues taken from female patients were higher than those of male patients. However, the difference between the groups was not statistically significant (p>0.05).

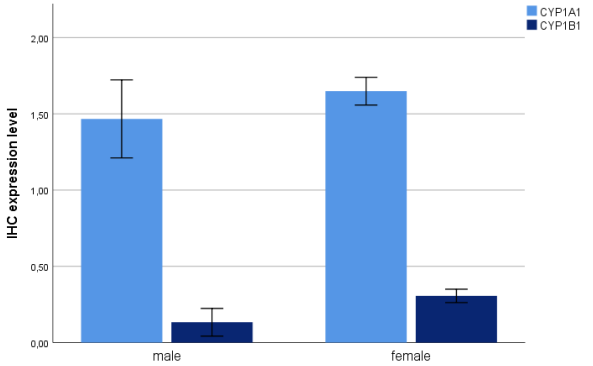


Figure 2. CYP1A1 and CYP1B1 expression levels based on patients' gender

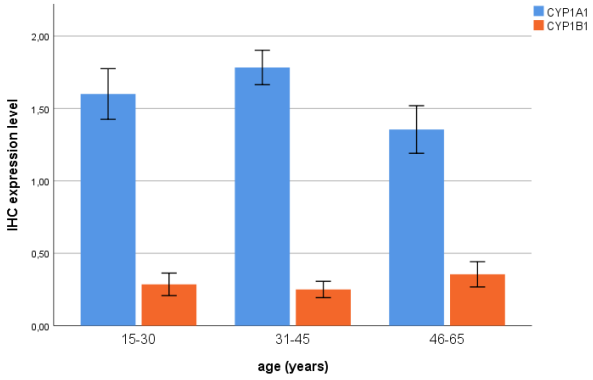


Figure 3. CYP1A1 and CYP1B1 expression levels in terms of patients' age

CYP1A1 and CYP1B1 expression levels were determined in terms of the patients' comorbidities as seen in Figure 4. CYP1A1 and CYP1B1 expression levels of patients without comorbidities were higher than those with comorbidities. However, this difference was not statistically significant in both expressions (p>0.05).

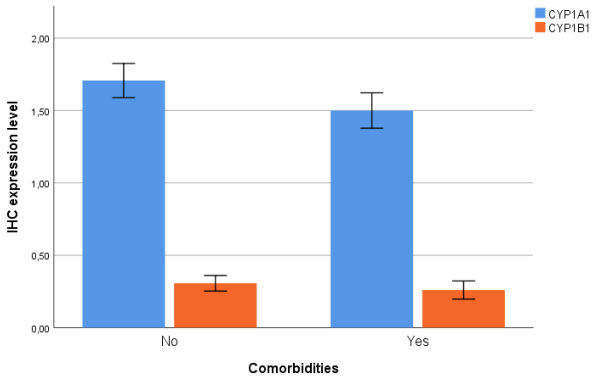


Figure 4. CYP1A1 and CYP1B1 expression levels based on patients' comorbidities

CYP1A1 and CYP1B1 expression levels were assessed with respect to the patients' medication usage status, as illustrated in Figure 5. CYP1A1 and CYP1B1 expression levels were higher in tissues taken from patients not using medications than those using medications. However, the difference was not statistically significant in both expressions (p>0.05).

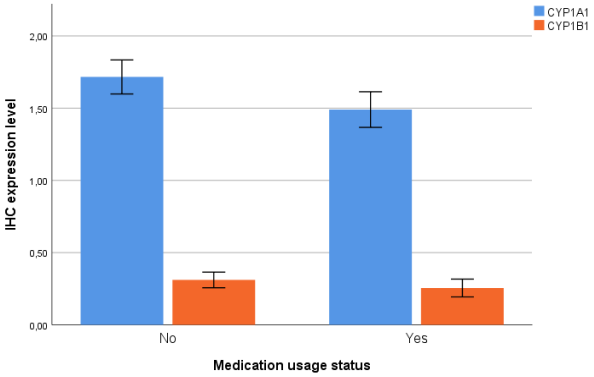


Figure 5. CYP1A1 and CYP1B1 expression levels in terms of patients' medication usage

The expression levels of CYP1A1 and CYP1B1 were analyzed concerning operation status as displayed in Figure 6. There was no statistically significant difference between the patients' operation status and CYP1A1 and CYP1B1 expression levels (p>0.05).

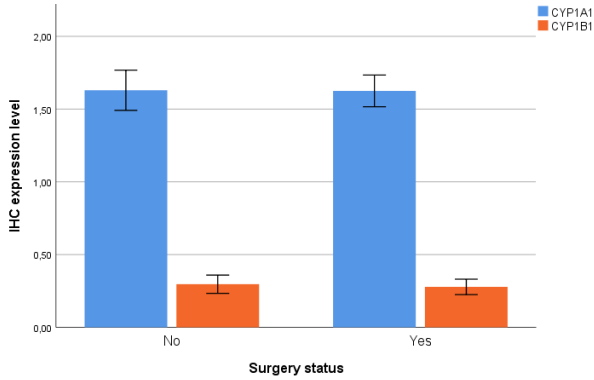


Figure 6. CYP1A1 and CYP1B1 expression levels in terms of patients' surgery status

Correlation analysis was performed between the CYP1A1 and CYP1B1 expression levels and the patients' clinical and demographic characteristics (Table 5). A negative and significant correlation was found between CYP1A1 expression and HGB levels (p<0.05). A negative and significant correlation was observed between CYP1B1 expression and HDL levels (p<0.05). A negative and significant correlation was noted between CYP1B1 expression and insulin levels (p<0.05)

Table 5. Correlation analyzes of patients' clinical and demographic characteristics and CYP1A1 and CYP1B1 expression levels

	CYP1A1		CYP1B1	
Variable	**Correlation coefficient**	**p-value**	**Correlation coefficient**	**p-value**
Age	-0,125	0,162	0,019	0,830
Height	-0,012	0,891	-0,093	0,301
Weight	-0,038	0,672	-0,059	0,512
BMI	0,020	0,826	-0,024	0,788
WBC	0,087	0,334	-0,134	0,135
HGB	-0,215	0,016*	0,106	0,238
PLT	0,167	0,062	0,019	0,832
Glucose (fasting)	0,136	0,128	-0,010	0,910
Glucose (postprandial)	-0,020	0,850	0,120	0,267
HDL	0,280	0,219	-0,484	0,026*
LDL	0,139	0,622	0,164	0,560
Triglyceride	-0,017	0,856	-0,026	0,779
Total cholesterol	0,128	0,166	0,179	0,053
Free T3	0,104	0,248	-0,005	0,960
Free T4	0,130	0,150	0,095	0,293
TSH	0,148	0,099	0,033	0,712
Estradiol	0,128	0,347	-0,118	0,387
FSH	-0,174	0,136	-0,015	0,900
LH	-0,167	0,153	0,019	0,869
C Peptid	-0,076	0,418	-0,115	0,218
Insulin	-0,005	0,954	-0,228	0,011*
Cortisol	0,129	0,157	0,047	0,607
ACTH	0,094	0,388	-0,077	0,479
SHBG	-0,117	0,394	0,084	0,541
HBA1C	0,086	0,358	0,151	0,104
CYP1A1	1,000	-	0,116	0,196
CYP1B1	0,116	0,196	1,000	-

(*) There is a statistically significant correlation ($p<0.05$). BMI: body-mass index, WBC: white blood cell, HGB: hemoglobin, PLT: platelet, 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 globulin, HbA1c: hemoglobin A1c.

4. DISCUSSION AND CONCLUSION

Oxidative stress is associated with the development of obesity, a multifactorial disease that is very common in all over the world. Oxidative stress has been observed to occur with the random production of adipokines from adipose tissue, which usually results in the development of metabolic syndrome [13]. Parameters such as oxidative damage and C-reactive protein (CRP) are higher in obese individuals. Depending on this situation, BMI has a direct correlation withLDL levels and triglyceride ratio [14]. In some obese individuals, antioxidant defense parameters can belower than their fat ratios. The levels of vitamin E, vitamin C, beta carotene and glutathione have been shown to decrease in obese individuals [15]. It was also determined that it resulted in the induction of significant oxidative stress and inflammation in individuals on a carbohydrate and fat diet [16].The published studies showed that long-term obesity cause decreasing in antioxidant sources and in the activities of antioxidant enzymes including super oxide dismutase (SOD) and catalase [17].SOD and glutathione peroxidase activities were decreased in obese individuals compared to healthy individuals [18]. Some endogenous and exogenous sources of obesity are also causes metabolic and hereditary disorders such as DNA damage, tissue damage, mutation, cell aging and even cancer.

Xenobiotic metabolism is classified in three main groups as phase I,phase II and also phase III. Carcinogens are either broken down into non-reactive products and excreted directly from the body or converted to reactive metabolites by Phase I reactions.

In this study, the relationship between obesity and the expression of CYP1A1 and CYP1B1 isozymes, which are important members of the cytochrome P450 (CYP) enzyme system was investigated.Considering that no diet is applied, especially in the development of obesity, significant increases of CYP1A1 and CYP1B1 enzymes in the metabolic pathway to provide reducing roles and removal from the metabolic pathway were observed, as expected. This situation coincides with the epidemiologically guiding confirmed data in terms of causing a metabolic correlation with similar and current studies in the literature and preparing the ground for the formation of other diseases.

Knowing that CYP1A1 and CYP1B1 are among the most important Phase I enzymes with their effects on reactive oxygen compounds in the continuity of the anti-cancer mechanism and with their flovanoid metabolizer role, it is possible to investigate the effects on some types of cancer by inducing cytochrome p450 on cell lines with these compounds. In the study of Surichan et al., 2018, the role of CYP1A1/CYP1B1-mediated enzyme induction in human breast cancer in reducing the 4-hydroxy tangeretin product was revealed [19].

It is encountered in studies that try to reveal the role and functioning principle of CYP1A1 and CYP1B1 enzyme expressions in the metabolic pathway. In the study conducted by Androutsopoulos et al.,2011, the status of cytochrome p450 compounds in the diet-treated samples in terms of flavonoid compounds were investigated comparatively in terms of CYP1A1 and CYP1B1. CYP1A1 and CYP1B1 are two extrahepatic enzymes involved in carcinogenesis and cancer progression. Selective inhibition of CYP1A1 and CYP1B1 by dietary

components, particularly the flavonoids class, has been characterized as a paradigm corresponding to the concept of dietary chemoprevention. The ability of CYP1 enzymes to selectively metabolize dietary flavonoids into transformation products that inhibit cancer cell proliferation has been highlighted. In the study conducted with 14 different flovonoid markers, the status of CYP1A1 and CYP1B1 components was analyzed by HPLC and tried to be rationalized. And the results obtained overlap with the role of these phase I enzymes in the literature, and the states of flovonoids showed homology [20].

All these results indicated that CYP1A1 and CYP1B1 expression was increased in obesity. Significant changes occur in the amount of CYP1A1 and CYP1B1 enzymes in obese people. 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 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. Thus the results of our study can be in the development of preventive approaches.

The results of our study will contribute to the epidemiological examination of obesity and to reveal new solutions. Clarifing the role of enzymes involved in detoxification in the development and progression of the disease could be a key target to cope with this disease. New approaches are needed to evaluate obesity susceptibility and to find effective treatment.

Conflicts of interest

The authors declare no conflicts of interest

Ethical statement

All procedures were conducted in accordance with the Declaration of Helsinki. This study was approved by the Research Ethics Committee of the Keçiören Education and Research Hospital (2012-KAEK-15/2073). Written informed consent was obtained from all parents or guardians.

REFERENCES

[1] Yach D., Stuckler D., Brownell K.D. Epidemiologic and economic consequences of the global epidemics of obesity and diabetes. *Nat. Med.*2006;12:62–66.

[2] Manna, P., & Jain, S. K. Obesity, oxidative stress, adipose tissue dysfunction, and the associated health risks: causes and therapeutic strategies. *Metabolic syndrome and related disorders*, 2015; 13(10): 423-444.

[3] Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., &Telser, J. Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology*, 2007; 39(1): 44–84. https://doi.org/10.1016/j.biocel.2006.07.001

[4] Djordjević V. B. Free radicals in cell biology. *International review of cytology*, 2004; 237:57–89. https://doi.org/10.1016/S0074-7696(04)37002-6

[5] Wellman, N.S., Friedberg, B. Causes and consequences of adult obesity: health, social and economic impacts in the United States. *Asia Pacific Journal of Clinical Nutrition*, 2002;11, S705–S709. https://doi.org/10.1046/j.1440-6047.11.s8.6.x

[6] Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., Nakayama, O., Makishima, M., Matsuda, M., & Shimomura, I. Increased oxidative stress in obesity and its impact on metabolic syndrome. *The Journal of clinical investigation*, 2004; 114(12): 1752–1761. https://doi.org/10.1172/JCI21625

[7] Hansel, B., Giral, P., Nobecourt, E., Chantepie, S., Bruckert, E., Chapman, M. J., &Kontush, A. Metabolic syndrome is associated with elevated oxidative stress and dysfunctional dense high-density lipoprotein particles displaying impaired antioxidative activity. *The Journal of clinical endocrinology and metabolism*, 2004; 89(10): 4963–4971. https://doi.org/10.1210/jc.2004-0305

[8] Russell, A. P., Gastaldi, G., Bobbioni-Harsch, E., Arboit, P., Gobelet, C., Dériaz, O., Golay, A., Witztum, J. L., &Giacobino, J. P. Lipid peroxidation in skeletal muscle of obese as compared to endurance-trained humans: a case of good vs. bad lipids? *FEBS letters*, 2003; 551(1-3): 104–106. https://doi.org/10.1016/s0014-5793(03)00875-5

[9] Patterson, A. D., Gonzalez, F. J., & Idle, J. R. Xenobiotic metabolism: a view through the metabolometer. *Chemical research in toxicology*, 2010; 23(5): 851–860. https://doi.org/10.1021/tx100020p

[10] Prakash, C., Zuniga, B., Song, C. S., Jiang, S., Cropper, J., Park, S., & Chatterjee, B. Nuclear Receptors in Drug Metabolism, Drug Response and Drug Interactions. *Nuclear receptor research, 2015*; 2: 101-178. https://doi.org/10.11131/2015/101178

[11] Anttila, S., Hakkola, J., Tuominen, P., Elovaara, E., Husgafvel-Pursiainen, K., Karjalainen, A., Hirvonen, A., &Nurminen, T. Methylation of cytochrome P4501A1 promoter in the lung is associated with tobacco smoking. *Cancer research*, 2003; 63(24): 8623–8628.

[12] Paluzar, H., &Sagiroglu, A. Effects of Organophosphorus and Pyrethroid Pesticides on Antioxidant Enzymes and Reactivation Effects of Pralidoxime: In vitro Studies. *Kuwait Journal of Science*,2021;https://doi.org/10.48129/kjs.11847

[13] Esposito, K., Ciotola, M., Schisano, B., Misso, L., Giannetti, G., Ceriello, A., &Giugliano, D. Oxidative stress in the metabolic syndrome. *Journal of endocrinological investigation*, 2006; 29(9): 791–795. https://doi.org/10.1007/BF03347372

[14] Pihl, E., Zilmer, K., Kullisaar, T., Kairane, C., Mägi, A., &Zilmer, M. (2006). Atherogenic inflammatory and oxidative stress markers in relation to overweight values in male former athletes. *International journal of obesity*

(2005), 30(1), 141–146. https://doi.org/10.1038/sj.ijo.0803068

[15] Hartwich, J., Góralska, J., Siedlecka, D., Gruca, A., Trzos, M., &Dembinska-Kiec, A. Effect of supplementation with vitamin E and C on plasma hsCRP level and cobalt-albumin binding score as markers of plasma oxidative stress in obesity. *Genes & nutrition*, 2007; 2(1): 151–154. https://doi.org/10.1007/s12263-007-0041-6

[16] Patel, C., Ghanim, H., Ravishankar, S., Sia, C. L., Viswanathan, P., Mohanty, P., &Dandona, P. Prolonged reactive oxygen species generation and nuclear factor-kappaB activation after a high-fat, high-carbohydrate meal in the obese. *The Journal of clinical endocrinology and metabolism*, 2007; 92(11): 4476–4479. https://doi.org/10.1210/jc.2007-0778

[17] Amirkhizi, F., Siassi, F., Minaie, S., Djalali, M., Rahimi, A., &Chamari, M. Is obesity associated with increased plasma lipid peroxidation and oxidative stress in women? *Arya Atherosclerosis*, 2010; 2(4).

[18] Ozata, M., Mergen, M., Oktenli, C., Aydin, A., Sanisoglu, S. Y., Bolu, E., Yilmaz, M. I., Sayal, A., Isimer, A., &Ozdemir, I. C. Increased oxidative stress and hypozincemia in male obesity. *Clinical biochemistry*, 2002; 35(8): 627–631. https://doi.org/10.1016/s0009-9120(02)00363-6

[19] Surichan, S., Arroo, R. R., Tsatsakis, A. M., &Androutsopoulos, V. P. Tangeretin inhibits the proliferation of human breast cancer cells via CYP1A1/CYP1B1 enzyme induction and CYP1A1/CYP1B1-mediated metabolism to the product 4' hydroxy tangeretin. *Toxicology in vitro : an international journal published in association with BIBRA*, 2018; 50: 274–284. https://doi.org/10.1016/j.tiv.2018.04.001

[20] Androutsopoulos, V. P., Papakyriakou, A., Vourloumis, D., &Spandidos, D. A. Comparative CYP1A1 and CYP1B1 substrate and inhibitor profile of dietary flavonoids. *Bioorganic & medicinal chemistry*, 2011;19(9): 2842–2849. https://doi.org/10.1016/j.bmc.2011.03.042