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ORIGINAL INVESTIGATION / ÖZGÜN ARAŞTIRMA

Relationship between MMP-9 Gene Expression in Adipose Tissue and Morbid Obesity

Adipoz Dokuda MMP-9 Gen Ekspresyonu ile Morbid Obezite Arasındaki İlişki

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ABSTRACT

Objective: Obesity requires a flexible extracellular matrix (ECM) as it is characterized by adipose tissue expansion. ECM plays an important role in adipocyte development and function, lipid metabolism, and obesity. One of the most important enzymes in the remodeling of ECM is matrix metalloproteinase-9 (MMP-9). In our study, we aimed to investigate MMP-9 gene expression in adipose tissue in morbidly obese patients and to reveal their relationship with obesity.

Method: This study comprised 44 people, 14 of whom were of normal weight and 30 were morbidly obese. MMP-9 gene expression in adipose tissue was evaluated by quantitative real time reverse transcription polymerase chain reaction (qRT PCR). The data were evaluated by statistical methods.

Results: MMP-9 mRNA levels were found to be slightly higher in obese patients (6.36±2.54) than healthy controls (4.41±1.03); however, this was not statistically significant (p=0.570). Furthermore, there was no difference in MMP-9 mRNA levels between men and women in both groups (p=0.925).

Conclusion: We showed that the MMP-9 mRNA level in visceral adipose tissue (VAT) was slightly higher in morbidly obese patients than in nonobese controls; however, the difference was not statistically significant. More research is needed to completely understand the role of MMP-9 in obesity.

Keywords: Adipose tissue, MMP-9, morbid obesity

ÖZ

Amaç: Obezite, yağ dokusu genişlemesi ile karakterize olduğu için esnek bir hücre dışı matriks (HDM) gerektirir. HDM, adiposit gelişimi ve fonksiyonu, lipid metabolizması ve obezitede önemli bir rol oynar. HDM 'nin yeniden şekillenmesinde en önemli enzimlerden biri matriks metalloproteinaz-9'dur (MMP-9). Çalışmamızda, morbid obez hastaları yağ dokusunda MMP-9 gen ekspresyonu araştırmak ve obezite ile ilişkisini ortaya çıkarmak amaçlanmıştır.

Yöntem: Çalışmaya 14'ü normal kilolu, 30'u morbid obez olmak üzere 44 kişi dahil edilmiştir. Adipoz dokuda MMP-9 gen ekspresyonu, Gerçek Zamanlı Ters Transkriptaz Polimeraz Zincir Reaksiyonu (qRT PCR) ile değerlendirilmiştir. Veriler istatistiksel yöntemlerle değerlendirilmiştir.

Bulgular: MMP-9 mRNA düzeyleri obez hastalarda (6,36±2,54) sağlıklı kontrollerden (4,41±1,03) biraz daha yüksek bulunmuştur, ancak istatistiksel olarak anlamlı değildir (p=0,570). Ayrıca, her iki grupta da kadın ve erkek arasında MMP-9 mRNA seviyelerinde fark yoktur (p=0.925).

Sonuç: Morbid obez hastaların visseral yağ dokusunda, MMP-9 mRNA seviyesinin obez olmayan kontrollere göre hafifçe yüksek olduğu gösterilmiştir, ancak aralarındaki fark istatistiksel olarak anlamlı bulunmamıştır. MMP-9'un obezitedeki rolünü tam olarak anlamak için daha fazla araştırmaya ihtiyaç vardır.

Anahtar kelimeler: Adipoz doku, MMP-9, morbid obezite

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INTRODUCTION

Obesity, defined as "an abnormal or excessive accumulation of fat that poses a health risk,"[1] has become a worldwide public health problem that causes metabolic syndrome, diabetes, cardiovascular diseases, and cancer among other life-threatening pathologies.[2] The increased accumulation of adipose tissue in obesity is strongly associated with a systemic proinflammatory and pro-oxidative state.[3,4] Adipocytes, endothelial cells, adipocyte precursors, and macrophages make up adipose tissue, which is an endocrine organ.[5] White adipose tissue (WAT) dysfunction, including adipocyte hypertrophy, adipocyte mortality, macrophage infiltration, and increased inflammatory cytokines, is linked to obesity.[6,7] The excessive accumulation of fat in adipose tissue recruits macrophages and leads to the increased production of proinflammatory cytokines and chemokines.[8] Obesity progression is linked to changes in adipose tissue such as adipogenesis, angiogenesis, and extracellular matrix (ECM) remodeling.[9]

Obesity requires a flexible ECM as it is characterized by adipose tissue expansion. Adipocytes need enough space to expand (hypertrophy) and create new ones (hyperplasia) from precursor cells through adipogenesis; therefore, ECM remodeling and rearrangement are required.[10,11] ECM plays an important role in adipocyte development and function, lipid metabolism, and obesity. ECM consists of highly conserved and insoluble proteins that are mostly collagens (I, II, III, and IV), fibronectin, and laminin.[12] The most important enzymes in the remodeling of ECM are matrix metalloproteinases (MMPs).[13]

MMPs are a large family of zinc-dependent endopeptidases that can proteolyze all components of the ECM. MMPs mediate ECM degradation and induce adipogenesis, angiogenesis, and adipose tissue expansion. Macrophages, neutrophils, endothelial cells, fibroblasts, vascular smooth muscle cells, T lymphocytes, platelets, chondrocytes, keratinocytes, epithelial cells, and mesenchymal cells are among the cells that secrete MMPs.[14]

The proteolytic activities of MMPs are regulated by specific inhibitors, which are alpha-2 macroglobulin and tissue inhibitor of metalloproteinase. In the maintenance of metabolic activities in the organism, there is a constant balance between the activities of MMPs and their specific endogenous inhibitors. Disruption of this balance causes the destruction of the matrix and hence the formation of pathophysiological conditions.[15] For ECM remodeling, changes in the expression patterns and activities of MMPs are critical.[14]

So far, 28 MMPs have been discovered and classified based on substrate specificity.[16] MMP-9, also known as Type IV collagenase or Gelatinase B, degrades collagen IV, a major component of the basement membrane.[17] Several studies suggest that MMP-9 plays an important role in obesity-mediated adipose tissue remodeling. One study showed increased levels of MMP-9 in the stromal vascular fraction of obese subjects. Increased MMP-9 levels have been linked to the development of a variety of inflammatory diseases, including obesity.[18] In another study, it was stated that higher MMP-9 levels can prevent and regulate the development of obesity in a high-fat-diet mouse model.[19] Both decreased and increased MMP-9 mRNA levels have been associated with obesity. This suggests a role for MMP-9 in the remodeling of obese adipose tissue but has not been fully elucidated. In our study, we aimed to investigate MMP-9 gene expression in adipose tissue in morbidly obese patients and reveal their relationship with obesity.

METHOD

Study Groups

The study included 30 (15 female, 15 male) morbidly obese patients who presented to a private hospital in Istanbul with a body mass index (BMI) of 35 kg/m^2 and were scheduled to undergo bariatric surgery, as well as 14 (7 female, 7 male) nonobese controls with normal weight (BMI=18.5–24.9 kg/m^2) who were scheduled to undergo abdominal surgery for pathologies other than obesity. BMI was calculated as body weight (kg) divided by the square of height (m^2). BMI, demographic data, and laboratory values were collected in accordance with standard procedures (Table 1). The mean age was obese patients was 35.28±10.54 years, while that of the nonobese control group was 40.31±11.56 years (range 18–65 years). Patients who had already undergone bariatric surgery or other major abdominal surgery, or who had psychiatric issues, were excluded from the study. All of the patients were treated surgically according to national and international standards. The study protocol complied with the ethical guideline for the 2013 Declaration of Helsinki and was approved by the Istanbul Aydin University Non-interventional Clinical Research Ethical Committee (Ethic No: B. 30.2.AYD.0.00.00-050.06.04/445). Informed consent was obtained from the participants of the study.

Sample Collection

Biopsies of visceral adipose tissue (VAT) were taken intraoperatively and laparoscopically. A biopsy of around 1 cm^3 of omental adipose tissue was obtained, and the samples were immediately put in RNAse-free buffer and kept at −80°C.

Table 1. Distribution of age, anthropometric, and biochemical measurements of subjects

	Obese (n=30) Mean±SD	Nonobese (n=14) Mean±SD	p
Gender (women/men)	1:1	1:1	
Age	35.28±10.54	40.31±11.56	0.239
BMI (kg/m^2)	40.11±16.14	23.17±13.17	0.021
Glucose (fasting) (mg/dL)	122.20±21.40	93.50±18.94	0.037
Insulin (fasting) (µIU/mL)	19.17±5.47	6.50±2.13	0.023
LDL (mg/dL)	95.80±22.71	87.83±26.22	0.257
HDL (mg/dL)	56.71±9.6	65.3±11.5	0.642
Total cholesterol (mg/dL)	180.6±32.5	165.7±28.4	0.763

Parametric data are presented as mean±standard deviation with p-values determined by t-test. BMI: Body mass index; kg: Kilogram; m: Meter; mg: Miligram; dL: Deciliter; µ: Micro; mL: Milliliter; IU: International Unit; LDL: Low-density lipoprotein; HDL: High-density lipoprotein

qRT PCR Analysis

The gene expressions were determined using the qRT-PCR technique. Total RNA was isolated from the adipose tissues using TRIzol (Invitrogen, Carlsbad, CA, USA) as directed by the manufacturer. The optical density at a wavelength of 260 nm was used to calculate the RNA concentration. Using 200 ng of RNA from each sample, cDNA was generated using the cDNA synthesis kit (Biomatik, Delaware, USA) technique. 5xfrst-strand buffer, dNTPs (10 mM), random hexamer primer, DEPC-treated water, RNasin (40 U/L), M-MLV, and template RNA were used to make the cDNA synthesis reaction mixture. In a thermal cycler (Techne® Prime Thermal Cycler, UK), the reaction mixture was incubated for cDNA synthesis for 60 min at 37°C and for 5 min at 70°C. The cDNAs were kept at −20°C until they were used. To evaluate the MMP-9 (Hs00957562 m1; Applied Biosystems, California, USA) gene expression, an RT-PCR reaction mixture containing cDNA (RT reaction product), primer-probe (Taqman® Gene Expression Assay 20X), PCR Master Mix (Taqman® Gene Expression Master Mix), and Nuclease-Free water was prepared. In qRT-PCR, reaction mixtures were incubated for 2 min at 50°C, 10 min at 95°C, then 40 cycles for 15 s at 95°C, 60 s at 60°C. MMP-9 (Hs00957562 m1; Applied Biosystems, California, USA) gene expression was evaluated using a qRT-PCR reaction mixture. cDNA (RT reaction product), primer-probe (Taqman® Gene Expression Assay 20X), PCR Master Mix (Taqman® Gene Expression Master Mix), and nuclease-free water were all included in this mix. In qRT-PCR, reaction mixtures were incubated for 2 min at 50°C, 10 min at 95°C, then 40 cycles for 15 s at 95°C, 60 s at 60°C. Under optimal PCR conditions, the samples were analyzed on PCR Thermal Cycler (Stratagene, Mx3000p, California, USA). The housekeeping gene 18S RNA (Hs99999901 s1; Applied Biosystems, California, USA) was used to normalize MMP-9 gene expression. The Ct values acquired from the RT-PCR were used to calculate gene expressions using the method $2-\Delta Ct=2-^{(Ct\ gene-Ct\ housekeeping\ gene)}$.[20]

Statistical Analysis

SPSS version 19 was used to conduct statistical analyses (IBM Co., Ltd., Chicago, IL, USA). The mean and standard deviation are used to express the data. After verifying for normality and homogeneity of variance (Shapiro-Wilk and Levene's t-tests), variables were examined. When comparing two groups, the independent samples t-test (Student's t-test) was used, and when the prerequisites were not met, the Mann-Whitney U test was utilized. The significance level of the tests was accepted at $p<0.05$.

RESULTS

The level of MMP-9 gene expression in VAT was measured using qRT-PCR. Figure 1 indicates that there was no significant difference in MMP-9 expression between obese and nonobese people (p=0.570). MMP-9 mRNA levels were found to be slightly higher in obese patients (6.36±2.54) than nonobese controls (4.41±1.03) (Fig. 1). Furthermore, there was no difference in MMP-9 mRNA levels between men and women

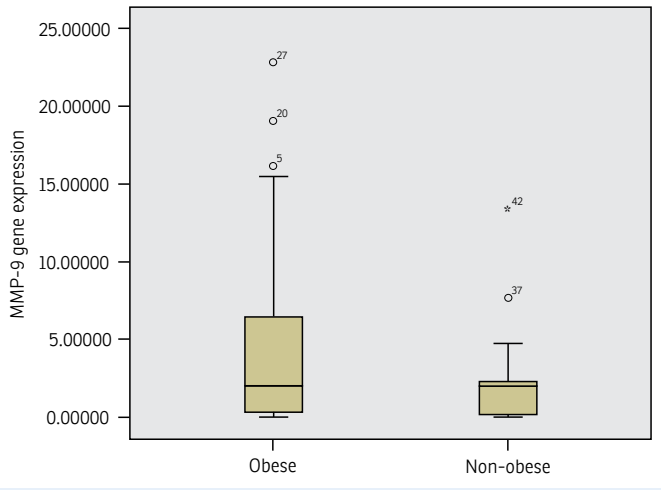

Figure 1. MMP-9 mRNA levels in obese (n=30) and nonobese group (n=14). Statistically, no differences between obese and nonobese subjects were found (p=0.570 and p>0.05, respectively; Mann-Whitney U test statistics)

MMP: Matrix metalloproteinases

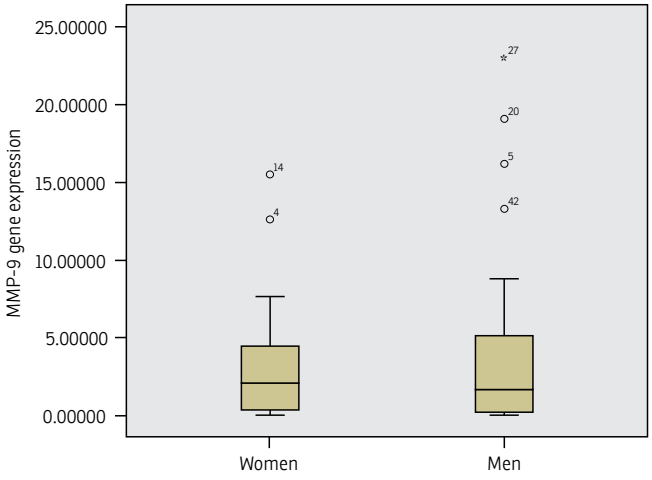

Figure 2. MMP-9 mRNA levels did not differ between men (n=22) and women (n=22) in both groups (p=0.925) (p=0.925 and p>0.05; Student's t-test statistics)

MMP: Matrix metalloproteinases

in both groups (p=0.925) (Fig 2).

DISCUSSION

ECM remodeling is involved in the process that starts with excessive energy intake and adipocyte hypertrophy. One of the enzymes that degrade the ECM is MMP-9. Tinahones et al.,[21] reported that adipose tissue expression of MMP-9 was positively correlated with the homeostasis model evaluation index of insulin resistance (HOMA-IR) in obese patients. In some studies performed on animal models, MMP-9 levels were found to be downregulated in abdominal WAT.[22]

Our findings revealed that the MMP-9 mRNA level in VAT was slightly higher in obese patients than in nonobese controls; however, the difference was not statistically significant (p=0.570). Similar results have been reported by several studies. MMP-9 levels in the WAT of patients with cardiovascular risk-related obesity have been found to be higher.[23] Catalán et al.,[24] showed that MMP-9 is expressed higher in VAT in obese individuals compared with lean individuals, but also decreased in subcutaneous adipose tissue after weight loss in obese patients with metabolic syndrome. Akra et al.,[25] observed that MMP-9 expression in adipose tissue was significantly correlated with adipose tissue amount, insulin sensitivity, insulin, C-peptide, waist circumference, and BMI. Another study discovered that MMP-9 expression was positively correlated with BMI and negatively correlated with insulin sensitivity.[26]

The relationship between the MMP-9 protein and obesity is still complicated. To prevent the progression of obesity, revealing the relationship of ECM remodeling will be the new determinant in therapeutic approaches.

This study has a limitation. Due to the difficulty of obtaining adipose tissue samples, the number of subjects who participated in the study was few. Further studies with larger sample sizes are needed.

CONCLUSION

In our study, we determined that the MMP-9 mRNA level in VAT in morbidly obese patients was slightly higher than in nonobese controls, but the difference was not statistically significant. More research is needed to completely understand the role of MMP-9 in obesity.

Disclosures

Ethics Committee Approval: The study was approved by the Istanbul Aydin University Non-interventional Clinical Research Ethics Committee (No: 445, Date: 11/04/2021).

Informed Consent: Written informed consent was obtained from all patients.

Peer-review: Externally peer reviewed.

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Conflict of Interest: No conflict of interest was declared by the authors.

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