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Role of acetyl-CoA acetyltransferase 1 expression in the molecular mechanism of adenomyosis

Asetil-KoA asetiltransferaz 1 ekspresyonunun adenomyozis moleküler mekanizmasınaki rolü

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Abstract

Objective: Adenomyosis is a benign uterine illness characterized by endometrial gland and stromal invasion into the myometrium. Acetyl-CoA acetyltransferase 1 (ACAT1) is an enzyme localized in mitochondria that is involved in ketogenesis and ketolysis processes by reversibly catalyzing the formation of acetoacetyl-CoA from two acetyl-CoA molecules. The current study investigated the expression of the ACAT1 molecule in tissue samples of patients diagnosed with adenomyosis and healthy endometrial tissues. It is aimed to determine the differences in *ACAT1* gene expression and in this way to discover the first information about the role of ACAT1 in the development and molecular mechanism of adenomyosis.

Materials and Methods: In the current retrospective study, formalin-fixed paraffin-embedded archival tissues were employed. A total of 76 patient samples were included in the study. Of these samples, 28 are adenomyotic tissue (Group I), 30 are eutopic endometrial tissue (Group II), and 18 are the Control Group. In these groups, the expression levels of the *ACAT1* gene were determined by the reverse transcription-polymerase chain reaction method.

Results: When the expression results of the *ACAT1* gene were evaluated, statistically significant differences were found between the groups ($p<0.05$). There was a difference between Group I-Group II and Group I-Control Group regarding the *ACAT1* gene. No statistically significant change was observed between Group II and Control Group. It is a remarkable finding that the expression of ACAT1 in adenomyosis tissue is decreased compared with both eutopic endometrium and control groups tissues.

Conclusion: The results suggest that ACAT1 may be associated with the molecular pathogenesis of adenomyosis.

Keywords: Adenomyosis, ketogenesis, gene expression

Öz

Amaç: Adenomyozis, endometriyal bez ve stromanın miyometriyuma invazyonu ile karakterize iyi huylu bir rahim hastalığıdır. Asetil-KoA asetiltransferaz 1 (ACAT1) iki asetil-KoA molekülünden asetoasetil-KoA oluşumunu geri dönüşümlü katalize ederek ketogenez ve ketoliz süreçlerinde yer alan mitokondride lokalize bir enzimdir. Mevcut çalışmada, adenomyozis tanısı konmuş hastaların doku örnekleri ve sağlıklı endometrial dokularda ACAT1 molekülünün ekspresyonu incelenmiştir. *ACAT1* gen ekspresyonundaki farklılıklarının belirlenmesi ve buna bağlı olarak adenomyozis gelişimi ve moleküler mekanizmasında ACAT1'in rolü ile ilgili ilk bilgilerin keşfedilmesi amaçlanmıştır.

Gereç ve Yöntemler: Gerçekleştirdiğimiz retrospektif çalışmada, formalinle fikse edilmiş parafine gömülü arşiv dokuları kullanılmıştır. Çalışmaya toplam 76 hasta örneği dahil edildi. Bunların 28'i adenomyotik doku (Group I), 30'u ötopik endometrium dokusu (Group II) ve 18'i Kontrol Grubundan oluşmaktadır. Bu gruplarda, *ACAT1* geninin ekspresyon düzeyi reverse transkripsiyon-polimeraz zincir reaksiyonu yöntemiyle belirlenmiştir.

PRECIS: Low expression of Acetyl-CoA Acetyltransferase 1 (ACAT1) was observed in adenomyotic tissues. Decreased expression of ACAT1 may be involved in the molecular mechanism of adenomyosis development.

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Bulgular: *ACAT1* geninin ekspresyon sonuçları değerlendirildiğinde gruplar arasında istatistiksel olarak anlamlı farklılıklar tespit edilmiştir (p<0,05). Grup I-Grup II ile Grup I-Kontrol Grubu arasında *ACAT1* geni açısından farklılık bulunmuştur. Grup II ve Kontrol Grubu arasında yapılan incelemelerde ise istatistiksel açıdan anlamlı bir değişim gözlenmemiştir. Adenmyozis dokusunda ACAT1 ekspresyonunun hem ötopik endometriyum hem de kontrol grubu dokularına göre azalmış olması dikkat çekici bir bulgudur.

Sonuç: Elde edilen bulgular, ACAT1'in adenomyozisin moleküler patogenezi ile ilişkili olabileceğini düşündürmektedir.

Anahtar Kelimeler: Adenomyozis, ACAT1, ketogenez, gen ekspresyonu

Introduction

Adenomyosis is described as the aberrant implantation of endometrial tissue into the myometrium associated with uterine enlargement[1].

It is a common gynecological disorder that affects the reproductive period of women. Menorrhagia, dysmenorrhea, pelvic pain, dyspareunia, and abnormal uterine bleeding are the most common symptoms in adenomyosis patients and may also be asymptomatic in some women with adenomyotic lesions[2]. Endometriosis, leiomyomas, endometrial hyperplasia, and endometrial polyps are frequently related to adenomyosis[3]. Molecular studies on adenomyosis have revealed differences in the expression of genes involved in different metabolic pathways. In recent years, evidence has been reported showing that genetic mutations, gene expression, and epigenetic differences are associated with clinical findings. However, it is stated that more molecular studies are needed[4]. Although it is a disease with a high prevalence, its molecular pathogenesis remains unclear.

Acetyl-CoA acetyltransferase (ACAT) refers to two enzymes called ACAT1 and ACAT2 located in the mitochondria and cytoplasm, respectively[5]. ACAT1 encodes a mitochondrial enzyme that catalyzes the reversible synthesis of acetoacetyl-CoA from two acetyl-CoA molecules. Cells can use acetyl-CoA to produce the energy needed. The ACAT1 enzyme is responsible for the final step in ketolysis (convert acetoacetyl-CoA into two molecules of acetyl-CoA) during fat metabolism. The enzyme also performs the reverse reaction of this step called ketogenesis. Ketogenesis is a biochemical process in the liver that produces ketone bodies by breaking down fatty acids[6,7]. Studies have shown that ACAT1 and ACAT2 are potential markers and therapeutic targets in neoplastic tissues and may be associated with prognosis in cancer[8].

The molecular development mechanism of adenomyosis has not been fully explained. This indicates that different cellular metabolic pathways and several genes involved in these pathways are effective in the formation of the disease. Based on this, we determined expression levels of ACAT1 in different experimental groups to define the role of ACAT1 and ketone metabolism in the molecular mechanism of the disease, which has not been studied before in adenomyosis. In this way, we aimed to present a marker that can be diagnostic, prognostic, and therapeutic for adenomyosis patients whose diagnostic methods and pathognomonic molecular markers are limited.

Materials and Methods

Collection of Tissue Samples

In the current study, 76 paraffin-embedded archival tissues were used. The tissues were collected in three groups as follows: Group I (n=28); adenomyotic tissues (ectopic endometrial tissues) and Group II (n=30); normally located endometrial tissues (eutopic endometrial tissues) of adenomyosis patients. Control group: Endometrial tissues of individuals without adenomyosis (n=18). Tissues were collected surgically from 30 adenomyosis patients. The patients diagnosed with adenomyosis were selected carefully after clinical and histopathological examinations. Tissue collection was carried out in the Department of Obstetrics and Gynecology and the Department of Pathology, Hospital of Mersin University (Mersin/Turkey). The women in groups I and II were of reproductive age and had been diagnosed with adenomyosis. The control group was made up of women without adenomyosis and were likewise of reproductive age.

This study was approved by the ethics review board of Mersin University (approval number: 450, date: 24.06.2020).

Reagents

To isolate RNA from FFPE tissues, the innuPREP FFPE total RNA kit (Analytikjena PN: 845-KS-2050050) was utilized. RT-PCR was used to generate cDNA from the acquired RNAs. For cDNA synthesis, High-Capacity cDNA Reverse Transcription Kit (Thermo Cat. No. 4368814) was used. For the gene expression procedure, TaqMan® Gene Expression Master Mix (Appliedbiosystems PN: 4371135) was used.

RNA Extraction, cDNA Synthesis, and the Rt-qPCR Expression Method

The cDNA synthesis step was performed after the RNA extraction stage which employed the innuPREP FFPE total RNA kit. High-Capacity cDNA Reverse Transcription Kit methodology was used to create cDNA from RNA extracted from FFPE tissue samples. The following is the Thermal Cycler technique for converting RNA to cDNA: 10 min at 25°C, 120 min at 37°C, 5 s at 85°C, and 1 min at 4°C. Following this, the gene expression method was used. The gene expression method employed TaqMan® Gene Expression Master Mix, cDNAs, and primers (forward and reverse) designed for the genes under investigation.

The following stages were carried out with the Roche LightCycler 480 II: 50°C for 2 minutes (incubation phase), 95°C for 10 min (activation phase), amplification step (95°C for 15 seconds then 60°C for 60 seconds, 40 cycles), and 40°C for 30 seconds (cooling phase). The housekeeping gene ACTB (beta-actin) was employed as the control gene in this investigation. Following the completion of the experimental stages, Delta Ct (ΔCt) and $2^{-\Delta\Delta Ct}$ values were computed and employed in statistical analysis.

Statistical Analysis

Statistical analysis was performed using the IBM SPSS Statistics program. Data were expressed as the mean and standard deviation. In the comparison of the expression levels of the genes for the three groups in the study, the ANOVA and the Kruskal-Wallis tests were used for the normal and non-normal data distributions, respectively. P value of <0.05 was considered statistically significant.

Results

Although 90 paraffin-embedded archival tissues were collected, some samples were not used in statistical analysis due to RNA quantity and quality. After removing these samples, 76 patient samples were used. The samples were divided into three groups: adenomyotic tissues (Group I, n=28), endometrial tissues of adenomyosis patients (Group II, n=30), and endometrial tissues of individuals without adenomyosis (Control Group, n=18).

Ectopic and eutopic endometrial tissues of patients diagnosed with adenomyosis were used in Group I and Group II, respectively. Ectopic endometrial tissue samples include stroma and glands found in the myometrium of patients with adenomyosis. Eutopic endometrial tissue is a noninvasive, typically situated endometrial tissue of adenomyosis patients. The control group was formed from the endometrial tissues of individuals who were not diagnosed with adenomyosis and related gynecological diseases such as endometriosis, leiomyomas, endometrial polyps, and endometrial cancer. Samples of women in the reproductive period were used in all three groups.

In the evaluation made between the three experimental groups, a statistically significant difference was found in terms of the *ACAT1* gene (p<0.05) (Table 1).

Expression of ACAT1

Regarding *ACAT1* gene expression levels, significant differences were found between Group I-Group II (p=0.0001) and Group I-Control Group (p=0.025). No significant difference was found between the Group II and Control groups (p=0.261) (Figure 1).

These results show a statistically significant decrease in ACAT1 expression in adenomyotic tissues (Grup I) compared with eutopic endometrial tissues of adenomyosis patients (Grup II) and endometrial tissues of individuals without adenomyosis (Control Group).

Discussion

Tumor biusedy be utilized to investigate the metabolic processes of adenomyosis, which is also characterized as a tumor-like lesion in various studies, although it is a non-neoplastic lesion. Changes in energy metabolism due to glucose and fatty acids used by cells are highly determinative in tumor development. Therefore, similar metabolic changes may be effective in the molecular mechanism of adenomyosis, which is a benign lesion. Based on this, ACAT1, one of the enzymes involved in the ketogenic pathway, was examined in our study.

Decreased ACAT1 expression was observed in adenomyotic lesions compared with the eutopic endometrium and control groups. In contrast, no significant difference in the enzyme expression was found between the eutopic endometrium and control group. Our results suggest that the decrease in ACAT1 expression in adenomyotic tissue may be effective in the molecular mechanism of adenomyosis.

It has been shown that ACAT1 is associated with many aggressive cancer types, is expressed higher in malignant tissues compared to normal tissues and benign lesions, and is associated with poor prognosis[9,10]. One study with enzymes involved in the ketogenic pathway found that ACAT1 exhibited a higher expression profile in high-grade prostate cancer tissues compared to low-grade tissues[11]. In addition,

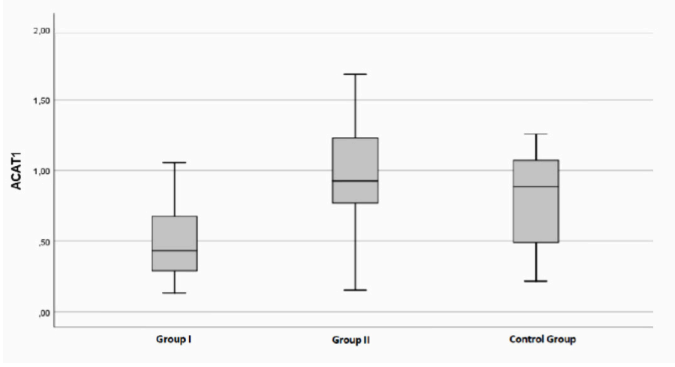

Figure 1. Comparison of $2^{-\Delta\Delta Ct}$ values of ACAT1 and 95% confidence intervals

ACAT1: Acetyl-CoA acetyltransferase 1

Table 1. Mean value and p-value of *ACAT1* gene for each group

Gene	Group I (n=28) Mean ± SD	Group II (n=30) Mean ± SD	Control (n=18) Mean ± SD	p-value[a]
ACAT1	0.535±0.361	1.044±0.504	0.809±0.350	0.0001*

[a]P-value shows the result of the ANOVA test for the normal distribution and the Kruskal-Wallis test for the non-normal distribution of the data.
SD: Standard deviation, ACAT1: Acetyl-CoA acetyltransferase 1, *p<0.05, statistically significant

the overexpression of ACAT1 in breast cancer cells has been noted to increase tumor growth and metastasis[12]. The decrease in ACAT1 activity by various inhibitors has a reducing effect on cell proliferation and tumor growth, and ACAT1 has been proposed as a pharmacologically potential anticancer target[13,14]. Similar results have been reported in many studies. It is stated that tumor cells use ketone bodies as an energy source and the increase in the expression of enzymes in the ketogenesis pathway, such as the ACAT1 contributes to carcinogenesis.

Contrary to the hypothesis supported by the previously mentioned research, some studies indicate that the ketogenesis pathway is limited in tumor cells with access to glucose because fatty acid degradation is restricted[15]. Fatty acids and cholesterol are needed for the production of membranes of new cells in uncontrolled proliferation. Therefore, it is expected that ketogenesis is suppressed in tumor cells, considering that anabolic pathways are more active in terms of lipid metabolism. According to this idea, a decrease in the expression of enzymes related to ketogenesis can be detected in tumor tissues. In a study that can provide proof of this idea, it has been reported that suppressing ketogenesis via ACAT1 increases the proliferation and metastasis of cancer cells, and overexpression of ACAT1 reduces tumor growth[16]. In another study with triplenegative breast cancer, an aggressive, malignant, and poor prognostic cancer type, it was reported that ACAT1 inhibited cell migration and invasion and suppressed cancer progression through different molecules[17]. Although different results regarding ACAT1 have been detected in different cancer types, it is emphasized that ACAT1 is a marker with therapeutic, diagnostic, and prognostic potential as a common opinion in studies. It is also pointed out that more molecular and pharmacological studies are needed.

Various investigations specify that adenomyosis is similar to tumor tissues in several metabolic pathways[18]. However, the role of ACAT1 and ketone metabolism in the development of the disease, which is the subject of our study, is unknown. The results of our study are similar to those of articles reporting lower ACAT1 expression in normal tissues and benign lesions. Considering that adenomyosis is a benign disease with low malignant transformation, it can be thought that ACAT1 may play a role in the molecular pathogenesis of the disease. In addition, it is noteworthy that we detected lower levels of ACAT1 expression in adenomyosis samples than in normal tissues. For this reason, it is another important finding for the disease that ketogenesis may also be suppressed.

This study is the first to investigate ACAT1 in adenomyosis samples. Since the molecular pathogenesis of adenomyosis has not been fully understood, each of the molecular studies will play an important role in illuminating the molecular mechanism of the disease. Although we have detected low ACAT1 expression in adenomyosis tissue suggesting that ketogenesis may be suppressed, investigation of different genes involved in ketone metabolism will provide a better understanding of this issue.

Study Limitations

Although more patient samples were examined, 76 samples whose RNA quantity and quality were suitable for the study could be used. More samples could not be included in the study due to the loss of quality and quantity of nucleic acids in formalin-fixed paraffin-embedded archive tissues.

Conclusion

In conclusion, the fact that there are still many unanswered questions about the development of the disease and the limited preoperative diagnosis and treatment options increase the importance of research on this subject. Investigating the genes involved in different metabolic pathways will provide a better understanding of the mechanism of adenomyosis development. We think that our research is a reasonable start to explain how ACAT1 and ketogenesis play a role in the pathogenesis of adenomyosis.

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Ethics

Ethics Committee Approval: This study was approved by the ethics review board of Mersin University (approval number: 450, date: 24.06.2020).
Informed Consent: Retrospective study.
Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.A., Concept: C.Y., E.A., Design: C.Y., Data Collection or Processing: E.A., F.T.D., Analysis or Interpretation: N.C., S.E., Literature Search: C.Y., H.Ö., Writing: C.Y.
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