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Analysis of JAM-A T>C (rs790056) and LFA-1 2120 G>C (rs2230433) gene variations in uterine leiomyoma: a pilot study

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ABSTRACT

Aim: We aimed to investigate the effect of junctional adhesion molecule-A (JAM-A) and lymphocyte function-associated antigen 1 (LFA-1) gene variants on the development of leiomyoma in Turkish women.

Material and Method: Retrospectively, leiomyoma tissues from 102 patients who were operated due to leiomyoma between May 2018 and April 2019 and healthy myometrium tissues from 70 control group patients without leiomyoma who underwent hysterectomy due to other reasons were included in the study. JAM-A rs790056 (T>C) and LFA-1 rs2230433 (G>C) gene variants in all tissues were examined by the quantitative real-time polymerase chain reaction (qRT-PCR) technique. Statistical analyses were performed using SPSS 16 software package.

Results: The frequency of JAM-A rs790056 CC genotype and C allele was significantly higher in the leiomyoma group compared to the control group (p=0.01, p=0.02), as well as the LFA-1 rs2230433 GG genotype which was also higher compared to the control group (p=0.01).

Conclusion: JAM-A rs790056 was found to be more effective than LFA-1 rs2230433 in determining the risk of uterine leiomyoma. According to the results, it has been determined that variations in JAM-A and LFA-1 genes may cause predisposition to uterine leiomyoma in Turkish women.

Keywords: Leiomyoma, uterus, gene variations, Turkish women

INTRODUCTION

Uterine leiomyomas, also known as fibroids, are the most common gynecological tumors in women of reproductive age with an incidence of 30-70% (1). Despite being benign, the fact that leiomyomas cause abnormal uterine bleeding, recurrent miscarriage, pelvic pain, premature birth and infertility in 10-30% of women and there is no definitive treatment other than surgery makes them an important problem for women's health (2).

Leiomyoma growth is known to be regulated, in particular, by steroid hormones such as estrogen and progesterone. These hormones are the key to regulating the genes that facilitate the development and function of uterine tissues. Leiomyoma has been reported to result from the growth and proliferation of a single smooth muscle cell. So, failed DNA repair can be a triggering factor in the development of myomas (3). For example, single nucleotide polymorphisms (SNPs) of DNA genes such as XRCC1, XRCC3 and XRCC4 have been associated with the risk of leiomyoma (4-6). By comparing uterine leiomyoma tissue and healthy uterine smooth muscle tissue, gene expression patterns of these two different cell lines have been investigated, and important results have been reported in various studies so far. Thus, important steps have been taken to understand which genes are involved in the development and growth of leiomyomas (7,8).

Tumor cells undergo multi-step adhesive reactions before settling in a distant organ. There are remarkable similarities between the proliferation of tumor cells and the migration of leukocytes to sites of inflammation and their localization therein (9). Therefore, changes in integrin receptors can lead to tumor invasion and metastasis. Following selectins, integrins provide strong adhesion to endothelial cells (10,11). The lymphocyte function-associated antigen 1 (LFA-1), an integrin from B2 subgroup, is a transmembrane glycoprotein expressed

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on the surface of leukocytes. LFA-1 I-domain has been shown to contain the binding domain for the junctional adhesion molecule-A (JAM-A)(12). During leukocyte migration, these receptors are known to allow leukocytes to bind through homophilic transendothelial interactions (13). Junctional adhesion molecules (JAM) are members of the immunoglobulin superfamily. In general, JAMs are expressed in cell-cell junctions in the endothelium and epithelium and exhibit various homotypic and heterotypic adhesion patterns (14). JAMs also play an important role in regulating leukocyte/endothelial cell interactions that control the monocyte, neutrophil or lymphocyte uptake in various inflammation models (13). In vivo studies have shown that, when JAM-A is blocked, inflammation and diapedesis are reduced (15).

In the literature, there are limited number of studies on LFA-1 and JAM-A variations. The effects of LFA-1 and JAM-A variations on breast cancer (16), central obesity (17) and Behçet's disease (18) have been investigated, but there are no studies that have investigated the effect of these gene variations on leiomyoma formation so far. Since JAM-A and LFA-1 have potential effects on migration, they may play a role in the development and growth of leiomyomas. The aim of the present study is to investigate the effect of JAM-A and LFA-1 gene variants on the development of leiomyomas in Turkish women.

MATERIAL AND METHOD

Ethics committee approval for the study (2017/360) was obtained from Gaziantep University Faculty of Medicine. All procedures were performed adhered to the ethical rules and the Helsinki Declaration of Principles. Written consent forms were obtained from all individuals included in the study.

Provision of Samples

Leiomyoma tissues from 102 patients who were operated in Gaziantep University Clinic of Obstetrics and Gynecology between May 2018 and April 2019 due to leiomyoma were determined as the leiomyoma group, whereas healthy myometrium tissues from 70 patients without leiomyoma who underwent hysterectomy due to other reasons were determined as the control group. The diagnosis of leiomyoma was made by histological examinations following immunohistochemical procedures. The inclusion criteria of the leiomyoma patient group included having undergone operation with pre-diagnosis of leiomyoma which was histopathologically confirmed in the postoperative period, histopathological absence of other pathology concomitant with leiomyoma, and absence of medical and/or surgical treatment history related to leiomyoma. Exclusion criteria included all conditions that did not conform to those stated above. The inclusion criteria of patients in the healthy control group were having undergone hysterectomy due to abnormal uterine bleeding, polyp, atony or uterine rupture, absence of malignancy, absence of leiomyoma or adenomyosis in the hysterectomy material in postoperative immunopathological examinations, and absence of previous history of uterine surgery. The exclusion criteria of this group were the conditions that did not conform to those stated above. Preoperative age, body mass index, presence of chronic disease (diabetes, chronic heart disease, chronic lung disease, thyroid disease, chronic liver disease, chronic kidney disease, metabolic diseases), median gravida, median parity, Ca 125 and hemoglobin values were recorded in all patients included in the study.

DNA isolation from tissue samples

DNA isolation from uterine leiomyoma tissue samples was performed using TRIzol according to the manufacturer's protocol. Nanodrop 2000 device was used to determine the purity and concentrations of the DNA samples obtained. Tissue samples were stored at –80°C until the study was conducted. Tissues from healthy female individuals were included in the study population as control samples.

SNP analysis with real-time PCR device

In the samples with complete isolation, probes were designed for LFA-1 and JAM-A polymorphism changes and procured from the manufacturer. Changes in the rs2230433 position (exon 21 2120 G>C) of LFA-1 gene and rs790056 (intron 6 T>C) position of JAM-A gene were displayed on the real-time PCR device. The primer and probe sequences used in the study are as given in **Table 1**. The real-time PCR mix was prepared on Biorad CFX Connect device in 2x iTaq Universal Probes Supermix (Applied Biosystems), 10 ng of purified DNA and RNase-free water conditions, each containing 10 µM FAM- and HEX-labeled TaqMan probes. Reaction conditions: 5 minutes at 95°C, and then 40 cycles of amplification (95°C for 1 minute, 58°C for 30 seconds).

Tablo 1. Demographic and clinical data of the patients

	Leiomyoma group	Control group	p value
Number (%)	102 (59.3%)	70 (40.7%)	0.11
Average age	41.2±3.4	40.6±5.6	0.08
BMI (kg/m^2)	26±4.7	24.3±4.5	0.07
Median gravida	2 (1-4)	2 (1-3)	0.07
Median parity	0 (0-3)	1 (1-3)	0.08
Ca 125 (U/ml)	28±2.5	29±1.9	0.27
Hemoglobin (g/dl)	11.1±3.1	11.5±2.6	0.07
Chronic disease *	4.4±1.8	4.2±1.9	0.23

Abbrevations: BMI: Body mass index
*: Diabetes mellitus, chronic heart disorders, chronic lung disorders, chronic liver disorders, chronic kidney disorders, thyroid disorders, metabolic disorders

Statistical Analysis

Statistical analyses were performed using SPSS 16 software package. p<0.05 was considered statistically significant. Allele and genotype frequencies were compared by Chi-squared test. In the evaluation of risk factors for leiomyoma, logistic regression was used. Differences between the leiomyoma and control groups were analyzed using Mann-Whitney U test or Chi-squared test.

RESULTS

Sixty two of 102 patients in the leiomyoma group underwent myomectomy with laparotomy (60.8%), 27 of them underwent abdominal total hysterectomy (26.5%), and 13 of them underwent laparoscopic total hysterectomy (12.7%). 12 of 70 patients in the control group underwent laparoscopic total hysterectomy (17.1%) and 58 of them underwent abdominal hysterectomy (82.8%). Postoperative histopathological diagnoses of the tissues in the leiomyoma group were as follows: 81 leiomyomas (79.4%), 9 atypical leiomyomas (8.8%), 8 cellular leiomyomas (7.8%), 5 leiomyomas with high mitotic activity (4.9%). The sociodemographic and clinical characteristics of the patients included in the study and the differences between the groups are summarized in **Table 1**, and odds ratio rates for the presence of leiomyoma are in **Table 2**.

Tablo 2. Odds ratio for leiomyoma

Variable	OR (95% Cl)	p value
Crude logistic regression model:	-	-
Family history of leiomyoma	3.60 (1.40-9.30)	0.004
BMI ≥30 (kg/m²)	3.51 (1.73-7.12)	0.009
DM	2.37 (0.55-10.31)	0.342
Hypertension	1.28 (0.51-3.20)	0.467
COC	0.91 (0.15-5.66)	0.824
Astım	1.01 (0.45-2.27)	0.896
Hipotiroidizm	2.02 (0.90-4.58)	0.085

Abbrevations: OR: odds ratio; BMI; body mass index; COC: combined oral contraception; CI: confidence interval

Within the scope of the present study, variation changes between the association of uterine leiomyoma with JAM-A rs790056 and with LFA-1 were analyzed. In line with the results, we determined that the JAM-A rs790056 minor allele frequency (C) was 27.9% in the control group and 38.2% in the leiomyoma group (p=0.02). The normal TT genotype and T allele were relatively low in the control group compared to the leiomyoma group (p>0.05), while the mutant CC genotype and the C allele were lower in the UL leiomyoma group than in the control group (p=0.001 and p=0.02, respectively). The frequency of GG and G allele in LFA-1 rs2230433 was found to be higher in leiomyoma group compared to the control group (p=0.037, p>0.05, respectively). No significant change was observed between the binary allele (TG haplotype, CG haplotype) frequencies of JAM-A rs790056 and LFA-1 rs2230433 polymorphisms. Primer probe sequences are shown in **Table 3**.

Tablo 3. Primary probe sequences

JAM-A (rs2230433)	5'-3'
Forward	ATCCTCCCTCCATCTT
Reverse	GGCTGGCAAAAGCAGTTAG
WT (HEX)	CAGGGCTCTGGAACTGTGG
MT (FAM)	CAGGGCTGTGGAACTGTGG
LFA-1 (rs790056)	**5'-3'**
Forward	GATTGGGGTCTGCTTTTCA
Reverse	ATCAGGGTTACAAGGACGG
WT (FAM)	TGATACTGTAGGTACCGTGTGTGAG
MT (HEX)	TGATACTGTAGGTGCCGTGTGTGAG

In the leiomyoma group, the frequency of the LFA-1 rs2230433 GG genotype was higher (p=0.037), and although the frequency of the CC genotype was lower than the control group, the change was not significant (p>0.05). In the leiomyoma group, LFA-1 rs2230433 G allele carriers have higher than the control group. Genotypes and alleles of JAM-A and LFA-1 are shown in **Table 4**.

Tablo 4. Genotypes and alleles in JAM-A and LFA-1 gene

Polymorphism	Control n=70	Leiomyoma n=102	p value	OR
JAMA (rs790056)				
TT, n (%)	34 (48.6)	52 (51)	-	2.98
CC, n (%)	7 (10)	18 (17.6)	0.001	1.27
TC, n (%)	29 (41.4)	32 (31.37)	-	0.86
Allele				
T, n (%)	101 (72,1)	126 (61.8)	-	1,3
C, n (%)	39 (27.9)	78 (38.2)	0.02	0.89
LFA-1 (rs2230433)				
GG, n (%)	3 (4.3)	9 (8,8)	0,037	1,02
CC, n (%)	18 (25.7)	26 (25.5)	-	2.45
GC, n (%)	49 (70)	67 (65.7)	-	2.32
Alleles				
C, n (%)	86 (61.4)	147 (72)	-	0.94
G, n (%)	54 (38.6)	57 (28)	-	1.7

Abbrevations: OR: Odds ratio

DISCUSSION

Although extensive research has been done to investigate the genetics of leiomyoma in recent years, genetic and epigenetic studies have been insufficient to elucidate the molecular mechanisms that directly cause the formation of leiomyoma. Polymorphism changes in the rs2230433 (exon 21 2120 G>C) position of LFA-1 gene and rs790056 (intron 6 T>C) position of JAM-A gene, which had previously been evaluated in some other types of patients, are shown for the first time in the

present study in individuals diagnosed with leiomyoma. LFA-1/JAM-A interaction plays an important role in the early steps of leukocyte transendothelial migration (13). LFA-1 allows the migrating leukocyte to be taken into endothelial cell junction and blocks the second domain of JAM-A, which is important for stabilizing the homophilic interaction of JAM-A. Thus, LFA-1 relaxes the contacts at endothelial junction and destabilizes homophilic interaction of JAM-A, facilitating leukocyte diapedesis. LFA-1/JAM-A interaction then allows the leukocyte to proceed beyond the endothelial junction (19). Like matrix metalloproteinases, cytokines and growth factors, these genes are involved in tissue regeneration (20). Considering the hypothesis that the LFA-1/JAM-A interaction plays a key role in paracellular leukocyte migration and tissue regeneration, we thought that variations of these genes may be involved in the formation of leiomyoma as a risk factor in leiomyoma pathogenesis (13). Therefore, we aimed to determine the distributions of JAM-A rs790056 and LFA-1 rs2230433 variations in patients with leiomyoma, as well as their individual and combined effects.

There are few studies investigating the effects of JAM-A and LFA-1 gene variations on the development of diseases. Ong et al. (17) investigated JAM-A rs790056 gene variation in 509 Chinese individuals, including hypertensive patients not taking anti-hypertensive drugs and normotensive healthy control group, and reported no significant difference between the study groups in terms of distribution of rs790056 genotype ($p>0.05$). Fu et al. (21) investigated LFA-1 rs8058823 variation in 537 female patients diagnosed with infiltrating ductal carcinoma and 577 healthy Chinese women, and reported significant difference between the study groups in terms of distribution of LFA-1 rs8058823 genotype and allele ($p=0.0397$).

So far, studies investigating JAM-A and LFA-1 have been related to melanoma, coronary artery diseases (22), atherosclerosis (23), breast cancer (16), cerebral ischemia-reperfusion injury (24), kidney cancer (25)and colorectal cancers (26). To our knowledge, JAM-A and LFA-1 gene variations in leiomyoma have been investigated for the first time in our study.

Based on the results, we found that the JAM-A rs790056 minor allele frequency (C) was 27.9% in the control group and 38.2% in the leiomyoma group ($p=0.02$), suggesting that individuals with minor allele frequency could be protective against uterine leiomyoma. Normal TT genotype and T allele were lower in the control group compared to the leiomyoma group ($p>0.05$), whereas the mutant CC genotype and the C allele were lower in the leiomyoma group than in the control group ($p=0.001$ and $p=0.02$). The frequency of GG and G allele in LFA-1 rs2230433 was found to be higher in leiomyoma group compared to the control group ($p=0.037$, $p>0.05$, respectively). No significant change was observed between the binary allele (TG haplotype, CG haplotype) frequencies of JAM-A rs790056 and LFA-1 rs2230433 polymorphisms.

In the leiomyoma group, the frequency of the LFA-1 rs2230433 GG genotype was higher ($p=0.037$), and although the frequency of the CC genotype was lower than the control group, the change was not significant ($p>0.05$). LFA-1 rs2230433 G allele carriers of leiomyoma group were observed to have higher than the control group.

As expected, family history ($p=0.004$) and obesity ($p=0.09$) were found to be associated with an increased risk of leiomyoma in our study, which is consistent with numerous studies that have previously demonstrated this condition (27,28). However, history of diabetes, hypertension, asthma, hypothyroidism, or use of combined oral contraceptive were not found to be associated with an increased risk of leiomyoma.

Blocking the interaction of adhesion molecules, which play a key role in the inflammation process and intercellular connectivity, can prevent leiomyoma formation at a very early stage. Therefore, it is important to better understand the role of JAM-A and LFA-1 in leiomyoma for new therapeutic interventions.

Although the present study population involved a relatively small study group, the results suggest that JAM-A rs790056 variation may be associated with the risk of leiomyoma. In sum, we can argue that normal genotypes and alleles of JAM-A rs790056 variation may be associated with an increased risk of leiomyoma, but small alleles may be protective.

CONCLUSION

The relationship between JAM-A and LFA-1 polymorphisms was analyzed in Turkish women diagnosed with uterine leiomyoma. In this regard, the JAM-A rs790056 variation was found to be significant in order to estimate the risk of leiomyoma.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Gaziantep University Faculty of Medicine Local Ethics Committee (Decision no: 2017/360).

Informed Consent: Because the study was designed retrospectively, no written informed consentform wasobtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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