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GENE EXPRESSION OF HOXA10 IN ENDOMETRIAL TISSUE OF UTERINE FIBROID PATIENTS

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Abstract : Fibroids, benign tumors in the uterus, affect the expression of the HOXA10 gene and growth factors involved in implantation. Disruption of HOXA10 gene expression can lead to implantation failure and changes in endometrial function. This study aims to evaluate the gene expression of HOXA10 in endometrial tissue in patients with uterine fibroids. This study was conducted as an observational analytical case-control study at Adam Malik General Hospital and affiliated hospital from November to December 2022, involving 22 women in each group. Ultrasound examination was performed to classify the uterine fibroids. Endometrial tissue samples were collected to assess the expression of the HOXA10 gene using immunohistochemical staining method. This study found that in the abnormal uterine fibroid group, 55% of cases showed HOXA10 expression at level +1, and only 5% had expression at level +3. In submucosal and intramural uterine fibroids, the majority (76.48%) exhibited HOXA10 gene expression at level +1. Statistical analysis indicated a significant difference in the expression pattern of HOXA10 in uterine fibroids between the normal and abnormal uterine fibroid groups ($p<0.05$). Furthermore, a significant association was observed between uterine fibroid classification and the level of HOXA10 expression ($p<0.05$). These findings indicate a decrease in HOXA10 expression in uterine fibroids, particularly in the submucous and intramural types.

Keywords **- HOXA10, endometrium, uterine fibroids**

I. INTRODUCTION

Uterine fibroids are the most common benign tumors in reproductive-aged women, occurring in 20-50% of the female population in this age group. Fibroids are found in approximately 5-10% of women with infertility, and they are the sole identified factor in 1-2.4% of cases.[1] Fibroids can occur in 60% of women before the age of 40 and 80% before the age of 50. Fibroids may be the sole cause of infertility in 2-3% of women.[2]

Homeotic genes, particularly the HOXA/Hoxa group, play an important role in the organogenesis and functional processes of the adult female uterus. Uterine fibroids synthesize molecules that support their growth and can affect the myometrium and other endometrial tissues through paracrine pathways. Some molecules secreted by fibroids reach the endometrium and disrupt its biosensor function. Transforming growth factor-beta 3 (TGF-β3) produced by fibroids interferes with the receptive function of the endometrium by blocking the expression of the bone morphogenetic protein 2 (BMP-2) receptor and inhibiting the release of HOXA10 and LIF, leading to disturbances in decidualization and implantation failure.[3,4]

Good uterine receptivity is characterized by high expression of the HOXA/Hoxa10 gene. In patients with fibroid uterus, decreased expression of endometrial HOXA10 is associated with infertility. Studies on the examination of HOXA-10 gene expression in patients with uterine fibroids are still very limited. This research aims to provide up-to-date information on the potential biomarker of HOXA-10 gene expression to plan optimal management for patients with uterine fibroids and predict the prognosis of patients with uterine fibroids.

II. METHODS

2.1 Study Design and Sample Population

This study was conducted as an observational analytical case-control study at Adam Malik General Hospital and affiliated hospital from November to December 2022. The study sample consisted of patients with uterine fibroids who sought treatment at Adam Malik General Hospital and affiliated hospital, meeting the inclusion and exclusion criteria. The sample size was calculated using the formula for the difference test for 2 populations, resulting in a minimum required sample size of 22 individuals for each group.

2.2 Inclusion and Exclusion Criteria

In this study, the inclusion criteria were as follows: (1) Cases comprised women diagnosed with uterine fibroids based on histopathological examination conducted at the Department of Anatomic Pathology, Faculty of Medicine, University of Sumatera Utara, Adam Malik General Hospital in Medan, University of Sumatera Utara hospital, Pringadi hospital, and Stella Maris hospital, (2) Controls were healthy and fertile women without uterine fibroids, selected from Adam Malik General Hospital in

Medan, University of Sumatera Utara hospital, Pringadi hospital, and Stella Maris hospital, and (3) Patients who had undergone hysterectomy as a management procedure for uterine fibroids. Patients who had received pharmacological treatment for uterine fibroids, had concomitant gynecological diseases, a history of contraceptive use, findings suggestive of malignancy, or damaged tissue samples were excluded from this study.

2.3 Patient Recruitment and Data Collection

Patient recruitment for uterine fibroids was done using consecutive sampling technique. Patient data, such as age and parity, were documented through interviews, physical examinations, and medical records. The patients were then divided into control and case groups. In the case group, ultrasound examination was performed to classify the uterine fibroids. Subsequently, endometrial tissue samples were collected to assess the expression of the HOXA10 gene using immunohistochemical staining method. The expression of the HOXA10 protein in the endometrium was evaluated through immunohistochemical examination. The endometrial tissue was fixed with formalin, embedded in paraffin, cut into 5 µm thickness, and placed on slides. Endometrial dating was confirmed through histological examination.

2.4 Immunohistochemical Staining Process

The immunohistochemical process involved several steps. Slides were deparaffinized and dehydrated through washing with xylene and ethanol, followed by permeabilization with cold 95% ethanol. After being washed with distilled water for 5 minutes, the antigen was retrieved by immersing the slides in 0.01M sodium citrate buffer solution for 20 minutes, then cooled for 20 minutes. The slides were washed for 5 minutes in PBS solution with 0.1% Tween 20 (PBST), and unwanted areas were delineated using a hydrophobic pen. Endogenous peroxidase enzyme was inhibited with 3% hydrogen peroxide for 5 minutes, then washed with PBST for 5 minutes. Non-specific binding was blocked with 1.5% normal horse serum in PBST for 1 hour at room temperature. The slides were incubated with the primary antibody overnight at 4ºC. HOXA10 antibody (sc 17159) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Normal goat IgG (Santa Cruz Biotechnology) was used as a negative control for the HOXA10 antibody. The results were interpreted using the H-SCORE, calculated with the following equation: H-SCORE = Pi (i + 1), where the nuclear staining intensity of HOXA10 had a scale of 0: None, 1: Weak, 2: Moderate, 3: Strong. Pi represented the percentage of nuclei stained for each intensity, ranging from 0 to 100%

2.4 Data Analysis and Statistical Methods

The data were analyzed descriptively to observe the frequency distribution of study subjects based on the characteristics of the research sample. Normality test was performed using the Shapiro-Wilk test. The level of HOXA gene expression was presented as Mean + SD if the data were normally distributed, but if the data were not normally distributed, it was expressed as Median (Min-Max) for each group. Bivariate analysis was conducted using the Independent T-test if the data were normally distributed, or the Mann-Whitney U test if the data were not normally distributed. The results of the analysis were considered significant if $p < 0.05$, with a confidence level of 95%.

III. RESULTS AND DISCUSSION

3.1 Results

This study used a sample of 49 cases of uterine fibroids. Table 3.1 shows that in the normal group, there were 18 patients (75%) in the <30 years age group and 9 patients (25%) in the 31-40 years age group. Meanwhile, in the uterine fibroid group, the highest number of cases was found in the >40 years age group, with 10 patients (65%), and the lowest number was in the <30 years age group, with 1 patient (15%).

Table 3.1: Sample Characteristics

Characteristics	Normal		Uterine Fibroids		Total
	n	%	n	%	n
Age (years)					
<30	18	75	1	15	28
31-40	9	25	3	20	11
>40	0	0	10	65	10
Parity					
0	18	95	8	45	26
1-3	8	5	15	55	23
≥4	0	0	0	0	0

Based on Table 3.2, there were 12 patients (25%) with intramural uterine fibroids, 8 patients (17%) with submucosal uterine fibroids, and 2 patients (3%) with subserosal uterine fibroids.

Table 3.2: Distribution of Uterine Fibroid Classification

Uterine Fibroid	n (Patients)	%
Normal	27	55
Intramural	12	25
Submucosal	8	17
Subserosal	2	3
Total	49	100

According to Table 3.3, it can be seen that among patients with abnormal uterine fibroids, the intensity of HOXA 10 expression was +1 in 11 cases (55%), followed by +3 in 1 case (5%). In contrast, in the normal uterine fibroid group, the most

common HOXA expression was +3 in 16 cases (60%), and +1 was found in 1 case (5%). Statistical analysis using Chi-square test showed a significant difference in the expression pattern of HOXA 10 in uterine fibroids between the normal and abnormal uterine fibroid groups ($p<0.05$).

Table 3.3: Differential Expression of HOXA10 Gene in the Endometrium Between Uterine Fibroids and Non-Fibroids Group

HOXA-10	Uterine Fibroids		Normal		P value
	n	%	n	%	
Negative	6	25	0	0	0.0000*
+1	11	55	1	5	
+2	4	15	10	35	
+3	1	5	16	60	
Total	22	100%	27	100%	

Table 3.4 shows that in subserosal and normal uterine fibroids, a larger percentage had HOXA10 expression with a value of +3 (88.24%) and no negative expression, while in submucosal and intramural uterine fibroids, the majority had HOXA 10 expression with +1 in 12 cases (76.48%) followed by HOXA 10 expression with 0 in 6 cases (100%). The chi-square statistical test also yielded a p-value < 0.05, indicating a significant association between the classification of uterine fibroids and the expression value of HOXA 10.

Table 3.4. Relationship between Uterine Fibroid Classification and HOXA10 Gene Expression

Uterine Fibroid Stage	HOXA10 Expression				Total	p-value
	Negative	+1	+2	+3		
Subserosal and Normal	0 (0%)	1 (23.52%)	10 (82,36%)	16 (88,24%)	27 (100%)	0.000*
Submucosal and Intramural	6 (100%)	12 (76,48%)	4 (17.64%)	1 (11.76%)	22 (100%)	

3.2 Discussion

Uterine fibroids can be categorized into three main types based on anatomical location: subserosal, intramural, and submucosal. Subserosal fibroids are the least common type, growing outward from the uterus with little impact on fertility. Intramural fibroids are the most common type, growing within the muscular layer of the uterus and can have a negative impact on fertility. Submucosal fibroids generally protrude into the uterine cavity and have a more significant impact on fertility due to their proximity to the endometrium.[5,6]

The HOXA10 gene plays a crucial role in uterine embryogenesis and implantation processes. The expression of this gene is dependent on the menstrual cycle and is primarily expressed during uterine development. HOXA10 deficiency can cause structural changes in the uterus and affect endometrial receptivity. Genes regulated by HOXA10 include the homeotic gene EMX2, β2-integrin, insulin-like growth factor binding protein-1 (IGFBP-1), cyclin-dependent kinase inhibitor, Wnt family genes, FK506 binding protein 4, prostaglandin EP-3 and EP-4 receptors. EMX2 plays a role in the differentiation of the female reproductive tract, IGFBP1 is involved in the decidualization process, p/CAF acts as a transcription co-factor for p53 involved in proliferation, and β3 Integrin subunit is known to have a function in cell-cell interactions. Additionally, the HOXA11 gene also plays a role in implantation. During the secretory phase of the menstrual cycle, HOXA11 gene expression increases in the endometrium, particularly during implantation. Based on Zehrah et al.'s research, it was observed that the expression of HOXA10 and HOXA11 decreases in the presence of fibroids.[6,9]

The expression of HOXA10 and HOXA11 genes decreases in women with uterine fibroids, especially in submucosal fibroids. They did not find significant changes in the expression of these genes in the presence of intramural fibroids. However, a significant decrease in HOXA10 levels was observed based on the location of leiomyoma. Salinah et al. conducted examinations on patients with intramural leiomyoma, and the results were consistent with Rackow and Taylor's study. Rackow and Taylor also stated that submucosal leiomyoma causes overall changes in the endometrial surface compared to intramural and subserosal fibroid locations. In Shamilah et al.'s study, random biopsies of endometrial tissue were performed through the endometrium. Although intramural fibroids (without changes in the endometrial cavity) were not associated with significant changes in HOXA10 and HOXA11 gene expression, a decrease in HOXA10 mRNA in the endometrium and stromal protein expression was noted in the submucosal uterine fibroid group compared to the control group (subserosal fibroids). The overall effect of submucosal fibroids on endometrial receptivity is said to be mediated by diffusible signaling molecules originating from fibroids. The researchers suggest that the same signaling pathway may also exist from intramural leiomyoma to the endometrium. However, due to the greater distance and therefore lower concentration, these signaling molecules have less pronounced effects on endometrial receptivity compared to what is observed with submucosal fibroids.[6,7,10]

Intramural fibroids can cause molecular abnormalities in the endometrium, although histologically the endometrium may appear normal. In Bruce et al.'s study, the pathophysiological cause of implantation failure in patients with non-cavityntramural uterine fibroids that distort the endometrial cavity is still unclear. In Christopher et al.'s study, it has been shown that HOXA10 is reduced in submucosal uterine fibroids, but findings regarding intramural fibroids are less clear. While one previous study showed no difference in HOXA10 between control endometrium (without uterine fibroids) and patients with intramural fibroids, another study showed significant differences in HOXA10 in that cohort study. The main difference in these studies is the different menstrual cycle phases at which the samples were obtained. In addition to the HOXA10 and HOXA11 genes, other factors such as leukemia inhibitory factor (LIF) also play an important role in the implantation process. LIF enhances embryo apposition and attachment to the endometrium and influences trophoblast growth and function.[10,11.]

Further research is needed to investigate the significance of HOXA10 in infertility cases associated with uterine myoma, particularly based on the location of the uterine myoma, with larger sample sizes and divided groups. Additional studies should compare the levels of HOXA10 in patients with uterine myoma and endometriosis as predictors of endometrial fertility and explore meaningful management strategies for patients with uterine myoma and endometriosis.

III. CONCLUSION

Based on this study, it was found that there were differences in the expression of the HOXA10 gene between the normal uterine myoma group and the abnormal uterine myoma group. Statistical analysis showed significant differences in the expression of the HOXA10 gene between the normal and abnormal uterine myoma groups, as well as a significant relationship between the classification of uterine myoma and the level of gene expression

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