Original Article

Evaluation of Endometrial Receptivity by Measuring HOXA-10, HOXA-11, and Leukemia Inhibitory Factor Expression in Patients with Polycystic Ovary Syndrome

Mustafa Kara[1]*, Seda Sabah Ozcan[2], Turhan Aran[3], Ozlem Kara[4], Neziha Yilmaz[5]

[1]Department of Gynecology and Obstetrics, Bozok University School of Medicine, [2]Department of Medical Biology, Bozok University School of Medicine, Yozgat, [3]Department of Gynecology and Obstetrics, Karadeniz Technical University School of Medicine, Trabzon, [4]Department of Hystology and Embryology, Institute of Health Sciences, Erciyes University, Kayseri, [5]Department of Infection Disease and Medical Microbiology, Bozok University School of Medicine, Yozgat, Turkey

Abstract

Context: Polycystic ovary syndrome (PCOS) is an important cause of infertility. In women with PCOS have increased rate of spontaneous abortion and reduced rate of conception. HOXA–10 and HOXA–11 are proteinous products of homeobox gene group and play an important role during implantation.
Aims: The aim of this study was to evaluate endometrial receptivity by measuring HOXA–10, HOXA–11, and leukemia inhibitory factor (LIF) gene expressions in women with PCOS.
Settings and Design: A tertiary referral center.
Materials and Methods: This study was conducted on reproductive age women with abnormal uterine bleeding without sonographically proven anatomical reason. Endometrial sampling procedures were done in proliferative phase using low-pressure endometrial suction device to exclude endometrial pathology. HOXA–10, HOXA–11, and LIF gene expressions were measured from endometrial sampling material. Blood sample was taken to measure serum estradiol level on the day of endometrial sampling.
Statistical Analysis Used: Statistical analysis was performed using SPSS software version 17 (SPSS Inc., Chicago, IL, USA). Mann–Whitney U-test was used to compare the variables.
Results: A total of 53 patients were included in this study. Study group consisted of 33 patients with PCOS. Gene expressions of HOXA–10, HOXA–11, and LIF were significantly lower in patients with PCOS ($P < 0.05$).
Conclusions: This study results showed that in patients with PCOS have decreased gene expression of HOXA-10, HOXA-11, and LIF which might contribute PCOS-related infertility.

Keywords: Leukemia inhibitory factor, HOXA-10, HOXA-11, infertility, polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinopathy affecting 5%–10% of women in the reproductive age.[1] The syndrome is surrounded by controversies regarding both its diagnosis and treatment. Because of its multifactorial nature and individual differences in clinical presentation, the diagnosis of the disease is difficult. PCOS is characterized by oligo-anovulation, hyperandrogenism, and infertility.[2] The prevalence of infertility in women with PCOS varies between 70% and 80%.[3] It causes infertility primarily due to ovulatory dysfunction.[4] However, the underlying mechanisms of

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Address for correspondence: Dr. Mustafa Kara, Bozok University Medical Faculty, Adnan Menderes Boulevard No 44, 66200 Yozgat, Turkey.
E-mail: mustafa.kara@bozok.edu.tr

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PCOS-associated infertility remain unclear. Many factors may lead to PCOS-associated infertility, and an impaired endometrial implantation could be responsible for this situation.[5]

Unfortunately, there is still little information about the molecular mechanisms regarding implantation of an embryo.[6] HOXA-10 and HOXA-11 are homeobox genes and play an important role in implantation. In menstrual cycle, HOXA-10 and HOXA-11 expression start to increase during the luteal phase and reach to the peak level during the implantation window.[7] Benson *et al*. observed peri-implantation defects in HOXA-10 mutant mice. They concluded that HOXA-10 was required for a healthy endometrial receptivity.[8] There are conflicting data about HOXA-10 and HOXA-11 genes and their probable role for implantation. Some authors reported that HOXA-10 and HOXA-11 expressions resulted in an increase during implantation period.[9,10] On the contrary, Kao *et al*. detected no change.[11]

Leukemia inhibitory factor (LIF) gene is released from the endometrial tissue and required for endometrial receptivity and early embryonic development.[12] LIF beta (β) receptors were shown to regulate endometrial sensitivity end embryo development before implantation.[13] The aim of this prospective randomized study was to assess the effects of HOXA-10, HOXA-11, and LIF gene expressions on endometrial receptivity in women with PCOS.

Materials and Methods

This study was conducted prospectively in the reproductive age women with abnormal uterine bleeding in a tertiary referral center. The time period for the study was 18 months. The study protocol was in compliance with the Declaration of Helsinki and approved by the Local Ethics Committee (no: 17522305/478/2013). This study was supported by the Bozok University Scientific Research Projects' Unit with the number of 2013TF/A-74.

Patients referred to our gynecology outpatient clinic with the complaint of heavy menstrual bleeding were included in the study. Transvaginal ultrasonographic scan and sonohysterography were performed to rule out myoma uteri- and endometrial polyp-related abnormal uterine bleeding. Endometrial sampling was planned in patients with persistent (6 cycles) abnormal uterine bleeding in the setting of failed medical management, or followed by prolonged periods of amenorrhea (6 or more). Endometrial sampling procedure was performed in proliferative phase of the following hormone free cycle using low-pressure endometrial suction device to exclude endometrial cancer. Blood sample was taken to measure serum estradiol level on the day of endometrial sampling.

Endometrial tissue samples

Endometrial tissue samples, immediately after surgery, were collected in RNA storage reagent (RNAlater® Stabilization Solution, Ambion) processed and stored at −80°C for RNA isolation. Study group consisted of patients with PCOS and control group consisted of patients with non-PCOS. The diagnosis of PCOS was based on the 2003 Rotterdam criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group, 2004; NIH, 2013).

Real-time quantitative polymerase chain reaction

Total RNA was isolated from tissue samples using the total RNA isolation kit (High Pure RNA Tissue Kit, Roche), and the RNA was used as a template for the synthesis of complementary DNA (cDNA) using a commercial kit (Transcriptor High Fidelity cDNA Synthesis Kit, Roche). The cDNAs were stored at −20°C until they were used as a template in real-time polymerase chain reaction (RT-PCR). Relative quantitative analysis was performed by RT-PCR (Light Cycler 480 Real Time PCR System, Roche Diagnostics, Mannheim, Germany) for HOXA-10 (target gene), HOXA-11 (target gene), LIF (target gene) and beta-actin (reference gene). Primers and probes were designed for target and reference genes (Real Time ready assay, Roche). For the genes, the PCR mixture included 1 μL for a single assay, 10 μL of LightCycler 480 Probes Master (Roche), 4 μL of PCR-grade water, and 5 μL cDNA samples and the same protocol was performed. Thermal cycling conditions included an initial activation step at 95 C for 10 min, followed by 45 cycles of the amplification phase consisting of denaturation, annealing, and extension phases (95°C for 10 s, 60°C for 30 s, and 72°C for 1 s, respectively). At the end of the cycles, a cooling step at 40 C was performed for 30 s for each reaction. All runs were included one negative cDNA control consisting of DNase- and RNase-free water. RT-PCR of the samples was completed using optimized protocols, and the relative expression levels were quantified. Expression of HOXA-10, HOXA-11, and LIF was analyzed using housekeeping beta actin gene in each run and final results were performed with LightCycler 480 software.

Statistics

Statistical analysis was performed using SPSS software version 17 (SPSS Inc., Chicago, IL, USA). Mann–Whitney U-test was used to compare the variables. A value of $P < 0.05$ was considered to indicate statistical significance. The results are expressed as a mean ± standard deviation.

Results

A total of 53 patients with abnormal uterine bleeding were included in the study. Study group consisted of 33 patients with PCOS and control group consisted of 20 patients without PCOS. The characteristics of the patients are summarized

in Table 1. There was no significant difference in age, BMI, serum estradiol levels, TSH, PRL, and day of sampling between the study and control groups.

The two groups were also compared in terms of HOXA-10, HOXA-11, and LIF gene expressions. HOXA-10 mRNA expression levels in endometrial glandular epithelial cells were significantly lower in patients with PCOS when compared to the control group (0.60 ± 0.14 vs. 1.23 ± 0.21) ($P < 0.05$). Gene expressions of HOXA–11 and LIF were also significantly lower in patients with PCOS than the control group ($P < 0.05$) [Table 2].

The mRNA expression level of HOXA-10, HOXA-11, and LIF genes are depicted in Figure 1.

Discussion

In this clinical study, endometrial receptivity was assessed by measuring HOXA-10, HOXA-11, and LIF mRNA expressions in women who had PCOS. There is only one study demonstrating the association between PCOS and HOXA-10 gene. Our findings suggested that HOXA-10, HOXA-11, and LIF levels were significantly lower in patients with PCOS.

There is a conflict regarding the mechanisms between PCOS and infertility. The probable factors are ovulatory disturbances, obesity, inflammation, insulin resistance, and implantation failure. Women with PCOS might have a deteriorated endometrial receptivity.[5,6,14,15] Moreover, Balen *et al*. reported that miscarriage rates were found to be higher in women with polycystic ovaries.[16]

HOXA-10 and HOXA-11 are transcription factors that are required for embryo implantation. Satokata *et al*. reported that mutant mice in relation with HOXA-10 exhibited implantation failure.[17] The embryos who had deficiency of HOXA-10 gene were normal morphology, but when these embryos were transferred into the uterus mice failed to implant. Bagot *et al*. found that the lack of HOXA-10 gene led to an implantation insufficiency in murine endometrium.[18]

HOXA-10, HOXA-11, and LIF mRNA expressions reach the peak level in the mid luteal phase, coincident with the time of implantation.[19] In the present study, we evaluated the association among HOXA-10 and HOXA-11, and endometrial receptivity in women with PCOS. We found a statistically significant lower HOXA-10 and HOXA-11 mRNA expression levels in PCOS group compared to the control group. Our results were similar to those reported by Cermik *et al*.[20] These authors investigated only HOXA-10 expression in patients with PCOS, and they showed significantly decreased HOXA-10 mRNA expression patients with PCOS compared with the control group. This article is the only study investigating the relationship between HOXA-10 and PCOS.

Changes in HOXA gene expression are associated with decreased implantation rates. These genes are thought to be receptivity markers and their altered expressions may help to identify women with implantation failure.[21] Szczepańska *et al*. showed that the expression of the HOXA-10 and HOXA-11 transcript levels were lower in infertile patients compared to the controls.[22] Similarly, Matsuzaki *et al*. have reported that significantly lower level of HOXA-10 mRNA in endometrial stromal cells in infertile patients compared to that of normal fertile women.[23]

Table 1: Characteristics of the study population

	PCOS group ($n=33$)	Control group ($n=20$)	*P*
Age (year)	31.27±8.42	38.17±10.36	0.06
Gravida	2.40±1.13	2.97±1.41	0.12
Parity	1.91±0.76	2.05±1.10	0.89
BMI	26.18±4.76	28.01±5.06	0.38
Day of menstruation	9.07±3.21	8.92±4.03	0.26
Estrogen (pg/ml)	154.72±48.64	149.14±54.72	0.77
TSH	3.41±0.86	3.93±0.90	0.34
PRL	14.04±2.70	12.21±2.46	0.61

BMI: Body mass index, TSH: Thyroid-stimulating hormone, PRL: Prolactin, PCOS: Polycystic ovary syndrome

Table 2: Comparison of HOXA-10, HOXA-11, and leukemia inhibitory factor gene expressions between the study group and control group

Genes	PCOS group ($n=33$)	Control group ($n=20$)	*P*
HOXA-10	0.60±0.14	1.23±0.21	0.01
HOXA-11	0.89±0.23	1.34±0.35	0.03
LIF	0.42±0.17	0.61±0.22	0.04

PCOS: Polycystic ovary syndrome, LIF: Leukemia inhibitory factor

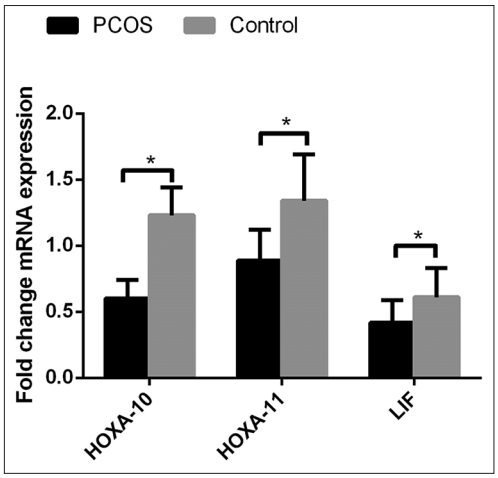

Figure 1: The mRNA expression level of HOXA-10, HOXA-11, and leukemia inhibitory factor in tissue samples

LIF is essential for embryo implantation in the mouse and evidence suggests it has a role in implantation in humans. LIF protein is maximal in the mid-late secretory phase of the menstrual cycle and detected in uterine flushings. A decrease in the amount of LIF protein was observed in the endometrium flushings from women with unexplained fertility compared with normal fertile women.[24-26] However, evidence for the role of LIF in uterine receptivity is conflicting. Interestingly, in some studies, endometrial LIF mRNA levels did not differ between fertile and infertile women.[27,28] Contrary, other studies reported lower LIF levels in women having uterine flushings and in women with primary unexplained infertility compared to that of fertile women. Furthermore, lower LIF secretion was yielded from endometrial explants of infertile women compared to the fertile women during the implantation window.[24,29,30] Dimitriadis *et al*. reported that decreased levels of LIF in luminal epithelium could be responsible from the poor implantation.[31]

In other studies with human tissues confirm that LIF mRNA and protein levels increase during ovulation and on the 4th day of pregnancy.[32,33] Ropka-Molik *et al*. indicated that there could be an association between increased LIF levels and high progesterone. They concluded that this relationship was the strongest during luteal phase.[34]

Despite all the previous studies reported that HOXA-10, HOXA-11, and LIF levels were important for implantation, there is still debate about the gene levels in women with PCOS. The limitations of the prior studies were small number of patients and heterogeneity of the population. Therefore, we planned to assess the effects of HOXA-10, HOXA-11, and LIF gene expressions on endometrial receptivity in women with PCOS. Our results indicated that the levels of these genes were found to be lower in PCOS group than the control group ($P < 0.05$). Our findings were similar with the most of the previous studies.

The limitations of this study were the timing of endometrial biopsy and small number of groups. In fact, HOXA-10, HOXA-11, and LIF expressions were found to be increase during the luteal phase of the cycle. The most serious issue is that the introduction and discussion discuss the gene expression is maximal in the luteal phase and yet the study was done in the proliferative phase. The gene expression may be lower in PCOS patients because of the lack of ovulation. The levels in the luteal phase may be fine if you get someone to ovulate. However, we measured these hormone levels during proliferative phase because of a pregnancy probability.

Conclusions

An implantation failure could be responsible from the infertility in patients with PCOS. The decreased expression level may be the results of PCOS or associated with the factors that contribute to PCOS. However, these findings should be supported with clinical pregnancy rates and live birth rates. Therefore, large prospective and randomized clinical trials are required.

We are aware of the fact that this is just a pioneer study. The expression changes in HOXA-10, HOXA-11, and LIF genes can be caused by mutations in these genes. A complete understanding of the complex regulatory mechanism of these genes may provide new therapeutic targets in female with PCOS.

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Conflicts of interest

There are no conflicts of interest.

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