ORIGINAL RESEARCH:

Changes in LIF expression on PCOS as biomarker implantation

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ABSTRACT

Objectives: This study aimed to compare the endometrial expression of LIF PCOS compared to normal and determine the effect of PCOS and external variables that affect LIF expression.
Materials and Methods: This retrospective case control study with a correlational approach was conducted at Sekar Clinic, General Hospital Dr. Moewardi Surakarta. Subject were taken by consecutive sampling starting from September 2018 –. Februari 2019. External variable: age, occupation, family history PCOS, menarche, and BMI were recorded. The research samples were 60 subjects consisting of 30 PCOS patients based on Rotterdam criteria and 30 fertile women. In the luteinizing hormone (LH) secretion phase at LH + 5 days - LH + 10 days, an endometrial biopsy is performed with pipelle curettage, then it is examined by immunohistochemistry. Statistical analysis was performed using the Mann-Whitney, linier regression test.
Results: Mean of LIF expression was found significantly lower in PCOS group (1.53±3.65) compared to control group (35.33±21.04, with p=<0.001). Multivariate analysis linear regression in the effect of PCOS and external variables to endometrial LIF expression models showed PCOS (b=-1.14; 95% CI=-1.56 – -0.72; p=<0.001) and occupation (b = 0.32; 95% CI=0.14 – 0.52; p=0.001) significantly decreases LIF expression. PCOS (B=-1.14) is more important than Occupation (B=0.33) in decreasing LIF expression.
Conclusion: LIF expression decreased in the endometrium of PCOS patients and occupations compared to normal group, with considering all existing variables.

Keywords: Endometrial Receptivity, Leukemia Inhibitory Factor, Polycystic Ovary Syndrome

ABSTRAK

Tujuan: Penelitian ini bertujuan untuk membandingkan ekspresi endometrium LIF PCOS dibanding normal dan mengetahui pengaruh PCOS dan variabel luar yang mempengaruhi ekspresi LIF.
Bahan dan Metode: Studi kontrol kasus retrospektif ini dengan pendekatan korelasional dilakukan di Klinik Sekar, Rumah Sakit Umum Dr. Moewardi Surakarta. Subjek diambil secara consecutive sampling mulai dari September 2018 –. Februari 2019. Variabel eksternal: usia, pekerjaan, riwayat keluarga PCOS, menarche, dan BMI dicatat. Subjek penelitian adalah 60 subjek yang terdiri dari 30 pasien PCOS berdasarkan kriteria Rotterdam dan 30 wanita subur. Pada fase sekresi hormon luteinizing (LH) LH + 5 hari hingga LH + 10 hari dilakukan biopsi endometrium dengan kuret pipelle, kemudian diperiksa dengan imuno-histokimia. Analisis statistik dilakukan menggunakan Mann-Whitney, uji regresi linier.
Hasil: Rata-rata ekspresi LIF ditemukan secara signifikan lebih rendah pada kelompok PCOS (1.53±3.65) dibandingkan dengan kelompok kontrol (35.33±21.04, dengan p=<0.001). Analisis multivariat linear regression pengaruh PCOS dan variabel eksternal terhadap ekspresi LIF endometrium menunjukkan PCOS (b=-1.14; 95% CI=-1.56 – -0.72; p=<0.001) dan pekerjaan (b=0.32; 95% CI=0.14 – 0.52; p=0.001) secara signifikan menurunkan ekspresi LIF. PCOS (B=-1.14) lebih penting dibandingkan Pekerjaan (B=0.33) dalam penurunan ekspresi LIF.
Simpulan: Ekspresi LIF menurun pada endometrium pasien PCOS dan pekerjaan dibandingkan dengan kelompok normal, dengan mempertimbangkan variabel yang ada.

Kata kunci: Reseptivitas endometrium, LIF, SOPK

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INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is one of the most common gynecological problems in reproductive age women that contributes to 15-20% infertility cases.[1,2] PCOS is known as an endocrine disorder that often occurs in women of childbearing age throughout the world.[3] Based on the Rotterdam ESHRE/ASRM, criteria of PCOS diagnosis must include at least two of the following three criteria: oligo or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovary features on ultrasound.[4]

The etiology of infertility in patients with PCOS is unknown. In many cases, PCOS infertility is often caused by anovulation. Some researchers think that endometrial receptivity has no effect on implantation. Meanwhile, other researchers also state that endometrial receptivity has an effect on implantation. Endometrial dysfunction is characterized by histomorphological disorders and disturbances in endometrial receptivity during the implantation phase.[5] Endometrial receptivity is a requirement for embryo implantation, starting from apposition, adhesion, and invasion. One embryo has been implanted, then endometrium is transformed into decidual tissues until placenta formation is complete.[6] Endometrial receptivity disorders in patients with PCOS are associated with the regulation of cytokine expression, resulting in down regulation of growth factors for embryo implantation.[7] Furthermore, the relationship between PCOS infertility and endometrial receptivity disorders are complex.[8]

Leukemia Inhibitory Factor (LIF) is a member of the cytokine IL-6 family this is produced and secreted by epithelial cells and endometrial stromal cells during implantation. This cytokine plays a vital role in endometrial receptivity during the invasion process by modulating trophoblast differentiation. The trophoblasts have two layers (syncytiotrophoblast and inner cytotrophoblast) of placenta formation, and before increasing maternal blood flow in the apposition to develop blood vessels in embryonic villi, induction of uterine spiral arteries is required.[9] Therefore, this study aimed to investigate LIF expression in endometrial receptivity of patients with PCOS affecting a uterine dysfunction and adverse reproductive outcomes.

MATERIALS AND METHODS

Research design

This cross-sectional study was conducted using PCOS patients and fertile women at the Sekar Clinic, General Hospital Dr. Moewardi Surakarta from September 2018 until Februari 2019.

Research subject

Research subjects consisted of ages 23-40 years, subjects were divided into 2 groups: 30 PCOS patients who are in accordance with Rotterdam criteria such as menstrual disorders, clinical hyperandrogen and polycystic ovary features on ultrasound and 30 fertile women undergoing sterilization, history and gynecological examination not suspected of having PCOS (fertility and clinical examination of normal gynecology) as control group. Exclusion criteria were normal women who have malignancy, use of contraception hormones and refuse to be research subjects.

Variables

The dependent variable was LIF expression. The independent variable was endometrial receptivity in PCOS and fertile women, while the external variables were age, cater occupation, family history PCOS, menarche, and body mass index (BMI).

Immunohistochemistry

Immunohistochemical examination for LIF expression using Anti Leukemia Inhibitory Factor (LIF, Cholinergic Differentiation Factor, CDF, DIA, Differentiation-stimulating Factor, D Factor, HILDA, Melanoma-derived LPL Inhibitor, MLPLI) with number L2024-01D, produced by United States Biological. All groups who met the inclusion and exclusion criteria signed the informed consent. In the luteinizing hormone (LH) secretion phase at LH + 5 days - LH + 10 days, PCOS patients undergo an endometrial biopsy with pipelle curettage about 2-3 cm below the uterine fundus. In fertile women the same procedure is performed, but previously the patient underwent female surgery method (MOW), permission to biopsy with pipelle curettage which did not cause bleeding. After that, the endometrium from the biopsy is put into a bottle with formalin buffer. The bottle label is written number, name, age, date of birth, sex and address of the patient. The biopsy results were sent to the Pathological Anatomy department of Dr. Sardjito Yogyakarta for immunohistochemical examination (IHC) to examine the expression of LIF. Calculation of LIF expression is through observing a number of 200 cytoplasmic epithelial cells, luminal epithelium and glandular epithelium using a microscope at 40x10 magnification, then counting positive cells that are brown in color. The measurement results are expressed as a percentage.

Data analysis

Bivariate analysis to identify the correlation between variables using chi-square test and Mann-whitney test. Multivariat analysis using linear regression. Statistical analysis was performed using SPSS 22.0.

Ethical clearance

Ethical clearance was obtained from the the commission of ethical health research of Dr. Moewardi General Hospital in Central Java, and the medical faculty of Sebelas Maret University, Surakarta, Central Java, Indonesia, Number: 650/VIII/HREC/2018.

RESULTS AND DISCUSSION

Table 1 showed that the percentage of PCOS is higher in patients aged <37, occupation status (no), without family history of PCOS, menarche ≥14, and normal BMI.

Table 1. Subjects Characteristics

Variable		N	(%)
Age	<37 years old	39	65
	≥37 years old	21	35
Occupation	No	36	60
	Yes	24	40
Family history PCOS	No	48	80
	Yes	12	20
Menarche	<14	20	33.3
	≥14	40	66.6
BMI	Normal	48	80
	Obesity	12	20

In table 2 of the age group, the highest percentage of PCOS sufferers are those aged <37 years with the number of PCOS patients at 58.9%. It was also shown in the table that the majority of those in the PCOS group were women employed 21 (87.5%). Job differences, family history of PCOS, and menarche in the PCOS group were compared with the normal group which was statistically significant with p <0.05. Homogeneity between the PCOS group and the control group in age and BMI was not statistically significant, with p> 0.05.

Both images were immunohistochemical results of LIF expression in the luminal epithelium and the glandular endometrial secretion phase and then analyzed visually using 40x10 magnification microscope. In Figure 1 (A) the glandular cytoplasm and brownish stroma show more LIF expression. Whereas in figure 1 (B) the LIF expression is limpid and only a few brownish LIF expressions are found.

Comparison of LIF expression between the two groups using bivariate analysis with the Mann-Whitney Test. The comparison results are shown in the table 3, where the mean LIF expression is lower on PCOS compared to the normal group. This difference was statistically significant with p ≤0.001.

Table 2. Bivariate analysis

Variables	Groups				Total		OR	p
	PCOS		Control					
	n	%	n	%	n	%		
Age								
<37 years old	23	58.9	16	41.1	39	100	0.35	0.058
≥37 years old	7	33.3	14	66.7	21	100		
Occupation								
No	9	25.0	27	75.0	36	100	21.00	<0.001*
Yes	21	87.5	3	12.5	24	100		
Family History of PCOS								
No	20	41.7	28	58.3	48	100	7.00	0.010*
Yes	10	83.3	2	16.7	12	100		
Menarche (year)								
<14	6	30.0	14	70.0	20	100	3.50	0.028*
≥14	24	60.0	16	40.0	40	100		
BMI								
Normal	21	43.8	27	56.2	48	100	3.86	0.053
Obesity	9	75.0	3	25.0	12	100		

*Significant : p<0.05

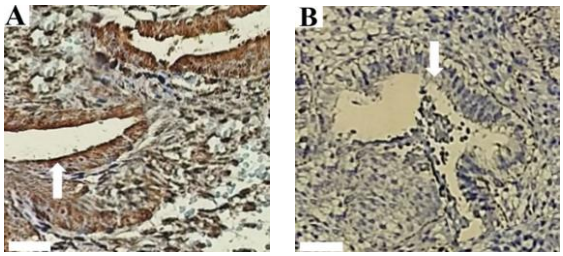

Figure 1. LIF expression in endometrium. The white arrows indicate endometrium in fertile women (A); and endometrium in PCOS patients (B). White bar: 10 µm
⇧: LIF Expression

Table 3. Difference in LIF expression in two groups

Groups	N	Mean	SD	p
LIF with PCOS	30	1.53	3.65	<0.001*
LIF with Normal	30	35.33	21.04	

*Significant : p<0.05

Table 4. Multivariate Analysis Linear Regression in The Effect of PCOS and External Variables to Endometrial LIF Expression

Variable	LIF expression to PCOS				LIF expression to PCOS and external variable			
	Unstand. Coeff. B	Stand. Coeff. b	95%CI	p	Unstand. Coeff. B	Stand. Coeff. b	95%CI	p
PCOS	-1.67	-0.75	-2.06 – -1.28	<0.001*	-1.14	-0.51	-1.56 – -0.72	<0.001*
Age					-0.13	-0.13	-0.31 – 0.04	0.123
Occupation					0.33	0.32	0.14 – 0.52	0.001*
Family history PCOS					0.16	0.13	-0.04 – 0.36	0.112
Menarche					-0.07	-0.07	-0.26 – 0.12	0.444
BMI					0.17	0.14	-0.03 – 0.38	0.090

* Significant : p<0.05

Multivariate analysis in table 4 was performed to see the correlation between variables with linear regression. In table 4 model 1 shows a statistically significant effect between PCOS and LIF expression. LIF expression on PCOS decreased as much as -1.67 times with p <0.001 and 95% CI (-2.06 - -1.28). Likewise, in PCOS table 4 model 2 also shows the effect of PCOS on LIF expressions after considering all external variables (age, occupation, family history PCOS, menarche, BMI), where LIF expression on PCOS decreased as much as -1.14 times with p values <0.001 and 95% CI (-1.56 - 0.72). The most common cause of infertility in women with PCOS is anovulation or ovarian dysfunction. Although it can still be cured by inducing ovulation, the rate of miscarriage and implantation failure is higher in women with PCOS.[10,11] Previous studies have shown that LIF decreases in serum and follicular fluid in women with PCOS compared to women without PCOS.[12] This is an important factor that disrupts foliculogenesis.[13] LIF is expressed in many endometrial glands at the time of blastocyst formation and before implantation, and this is most likely the result of increasing estrogen levels during the menstrual cycle.[14]

The effect of increasing levels of estrogen in the proliferative phase (follicles) due to the increase of ovarian follicles leads to blood vessel endothelium, epithelial proliferation and stroma for endometrioma regeneration.[7,15] The success of pregnancy depends on endometrial epithelium and blastocyst trophoblasts synchronized temporally and spatially. Here, LIF has an important role in the process of trophoblast invasion.[16,17] The shift from the proliferative status of the luminial epithelium to be differentiated is mediated by LIF through regulation of cell molecules that act as barriers to embryo invasion.[7] In the epithelial immunostaining, LIF is detected maximally in the middle and end of the secretory phase, but it is also detected with lower levels in other phases.

Meanwhile, stromal immunostaining is detected in all cycles.[18] The mechanism of LIF in regulating the function of uterine implantation is uncertain.[19] In this study LIF in PCOS patients decreased if it was compared to fertile women. This was in line with previous studies which reported that the level of LIF secretion in the endometrium decreased significantly under conditions of infertility as in PCOS patients.[9] This condition showed that LIF could be used for infertility therapy in women and, conversely, LIF antagonists could be used as a contraception. In addition, HOXA-10, HOXA-11, and LIF levels were reported to be significantly lower in PCOS patients who might contribute to PCOS related to infertility.[20] However, it is different from the results of studies that LIF expression increases significantly in the endometrium three months after laparoscopic ovarian dirlling (LOD).[21] Possibly one of the mechanisms underlying increased LIF expression after LOD is progesterone resistance, because LOD in the ovaries of PCOS can restore progesterone resistance so that it can increase LIF expression.[21,22]

The results showed that there was a decrease of LIF expression in women with PCOS compared to fertile women. Thus, this could be concluded that LIF played a role on endometrial receptivity. Therefore, it was necessary to do an early detection of LIF since a biomarker of endometrial reception was also necessary to avoid a uterine dysfunction and adverse reproductive outcomes.

However, the limitation in this study was the size of the uterine cavity, the elongated luteal phase, confirmation of ovulation, estrogen and progesterone levels were not measured. This is a limitation of research.

CONCLUSION

There are differences of LIF expression in PCOS patients and fertile women. LIF expression in PCOS patients decreases significantly compared to fertile women.

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CONFLICT OF INTEREST

Authors declare that there were no financial conflicts of interest existing in this study.

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