The Difference of Endometrial Leptin Expression in Woman with Polycystic Ovarian Syndrome (PCOS) Compared to Normal

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ABSTRACT

Background: Decreased endometrial receptivity is suspected to be one of the factors causing infertility in women with Polycystic Ovary Syndrome (PCOS). Endometrial abnormalities in PCOS due to impaired balance include estrogen, progesterone, growth factors, cytokines and cell attachment modulators including leptin. This study aims to determine the expression of leptin in the endometrium which is suspected to be more common in PCOS patients and related factors.

Subjects and Method: A retrospective-case control study was conducted at Dr. Moewardi general hospital, Surakarta. A total of 60 women were involved; 30 infertile women with PCOS based on Rotterdam criteria (if found two of the three symptoms, including hyperandrogenism, ovulation dysfunction and/or polycystic ovaries from ultrasonography) and 30 normal women as control group. Demographic data such as age, family history of PCOS, menarche, BMI, occupation, and education were recorded. Leptin expression was acquired from endometrium biopsy with IHC examination on day 19-24 of menstrual cycle; under the condition that inclusion and ex-clusion criteria are fulfilled. The statistical analysis was performed using Mann-Whitney test.

Results: Mean of leptin expression was found significantly higher in PCOS group (Mean= 59.16; SD= 49.34) compared to control group (Mean= 6.00; SD= 22.98, with p= 0.001. PCOS (b= 0.42; 95% CI= 0.10 to 0.73; p= 0.010) and obesity (b= 0.31; 95% CI= 0.41 to 0.58; p= 0.025) increased leptin expression.

Conclusion: There is a difference in leptin expression in the endometrium of PCOS compared to normal. Leptin expression is higher in the endometrium of women with PCOS and obesity compared to normal.

Keywords: Leptin expressions, endometrium, polycystic ovarian syndrome.

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BACKGROUND

The decrease of endometrial receptivity may contribute to low fertility rate in women with Polycystic Ovarian Syndrome (PCOS). PCOS displays hormonal imbalance that will disrupt the mediator and modulator expression in endometrium, thus affecting endometrial receptivity and implantation process (Rarani et al, 2018). Several contributors to implant-tation that may altered in PCOS include:
1) estrogen, progesterone and its receptors;
2) growth factor, Insulin-like Growth Factor-1 (IGF-1), Vascular Endothelial Growth Factor (VEGF); 3) cytokines such as Leukemia Inhibitory Factor (LIF); MatrixMetalopro-tein-9 (MMP-9); Interleukin-11; Colony Stimulating Factor-1 (CFS-1); Cyclooxygena

se-2 (COX-2); and 4) modulator for cells attachments: Leptin, Integrin, Basigin (BSG) (Lessey and Young, 2019).

The choice of endometrial receptivity markers is still a challenge due to the complexity of its mediator and modulator expression. Therefore, further studies to understand the expression and its role to implantation in PCOS are required.

In endometrium, LH and GnRH receptors are expressed highest during luteal phase. The activation is accomplished through Adenylate cyclase and Phospholipase C signaling pathways and results in increased leptin concentration and its products (Dornbush and Aeddula, 2018). Normally, leptin is expressed in endometrium during apposition in endometrial stroma. However, the expression is believed to increase in woman with PCOS, especially in those with obesity. This study aims to further understand the expression of leptin and its correlation with implantation process in woman with PCOS.

SUBJECTS AND METHOD

1. Study Design
This was a case-control study conducted at Dr. Moewardi general hospital, Surakarta.

2. Population and Sample
A total of 60 out-patient was selected by consecutive sampling and was classified into two groups. The case group consisted of 30 infertile women with PCOS based on Rotterdam criteria. The control group consisted of 30 women who underwent laparoscopy or laparotomy and were not diagnosed with PCOS; underwent sterilization, underwent a routine IVA test; underwent history taking and gynecological examination and were not suspected of having PCOS (good fertility, no history of pelvic pain, no history of dysmenorrhea or dyspareunia, normal clinical gynecology examination). Women who were found to have a history or malignancy and refused as study subjects were not included in the study.

3. Study Variables
The dependent variable is leptin expression, Independent variables is PCOS woman with infertile and external variable age, family history of PCOS, menarche, body mass index (BMI), occupation, and education level.

4. Operational Definition of Variables
PCOS was heterogeneous abnormalities which characterized by two of the three symptoms from Roterdam criteria.

Leptin expression is stained brownish color in luminal and glandular epithelial cells from coloring in Immunohistochemistry (IHC) through microscope.

Age is the time of subject existence from the beginning of birth until this study conducted.

Family history of PCOS was information of PCOS diseases in family members.

Menarche was the first occurrence of menstruation.

Body mass index (BMI) is a person's weight in kilograms divided by the square of height in meters

Occupation was the formal job or profession to earn income.

Education level was the highest level of formal education achieved by the subject.

5. Study Instruments
The diagnosis of PCOS we gather from medical record. Leptin expression from endometrium biopsy was carried out on day 19−24 of menstrual cycle using Pipelle Curette, 2-3 cm from the fundus area, followed by one micro-curettage scraping. The sample was put into 10% buffered formalin and was sent to anatomical pathology department of Dr. Sardjito general hospital for immunohistochemistry (IHC) examination, using Rabbit Anti Leptin Polyclonal Antibody produced by BIOSS serial number bs-0409R which is specific for human antigens. While age, family history of PCOS, menarche, occupation, and education level are gather from quisionaire and body mass index (BMI) from measurment using scales.

6. Data Analysis

Univariate analysis to describe the frequency distribution and percentage characteristics of the subjects. Bivariate analysis study to identified correlation between variables, in this study we use chi-square test and Mann-Whitney test. Multivariate analysis using linear regression were performed using Statistical Product and Service Solution (SPSS) version 22.0 software program.

7. Research Ethics

Research ethics included inform consent, anonymity, confidentiality and ethical clearance. The eligibility in this study from Ethics Committee, Dr. Moewardi Regional Hospital. Ethic letter number 140/I/HREC/2020.

1. Univariate analysis

The subjects of this study were 60 people; comprised of 30 infertile women with PCOS and the rest30 subject were normal condition. In univariate analysis, showing data of characteristics in both group. The PCOS group as a case and the normal group as a control. For Age category, the subjects were classified into two age groups: <36 years old and > 37 years old. This was based on the data that in women aged >37 years old, there is a decrease in egg quality and anti Mullerian hormone.

Table 1. Sample Characteristics

Variable		n	(%)
Age	<36 years old	40	66.6
	≥37 years old	20	33.3
Employment	unemployed	34	56.6
	employed	26	43.3
Education	Primary school	24	40
	High school/ collage	36	60
Family history of PCOS	No	54	90
	Yes	6	10
Menarche	<13 years old	26	43.3
	≥14 years old	34	56.6
BMI	Normal	44	73.3
	Obese	16	26.6

Table 1 showed that age <36 years were 40 women (66.6%) and ≥37 years were 20 (33.3%). The subjects were also classified according to their education level; including 9 years of primary school (elementary school/ junior high school); 3 years of high school or college graduate. Distribution at the level of education for the two groups is elementary/ junior high as many as 24 (40%) and high school/ graduate as many as 36 (60%). There is a family history with PCOS of 54 (90%) and no family history of PCOS of 6 (10%). Menarche <13 years were 26 (43.3%) and menarche ≥14 years were 34 (56.6%). The normal BMI was 44 (73.3%) while the obese were 16 (26.6%). All demographics are further shown in Table 1.

2. The result of bivariate analysis

Chi-square was conducted to assess whether the variables are statistically equal in affecting endometrial receptivity, as shown in Table 2. From the data obtained, mean of age in PCOS group was 32.30 years old, while in the control group was 35.80 years old. In <36 age group, 24 of them (60%) were included in PCOS group, while 16 of them (40%) were included in control group. In >37 age group, there were 6 (30%) women included in PCOS group and 14 (70%) women included in control group.

Table 2. Bivariate analysis

Independent Variable		PCOS		Control		Total		OR	p
		n	%	n	%	n	%		
Age	<36 years old	24	60	16	40	40	100	0.28	0.028
	≥37 years old	6	30	14	70	20	100		
Employment	unemployed	7	20.6	27	79.4	34	100	29.57	0.001
	employed	23	88.5	3	11.5	26	100		
Education	Primary school	3	12.5	21	87.5	24	100	21.0	0.001
	High school/ collage	27	75	9	25	36	100		
Family history of PCOS	No	26	48.1	28	51.9	54	100	2.15	0.671
	Yes	4	66.6	2	33.3	6	100		
Menarche	<13	12	46.2	14	53.8	26	100	1.31	0.602
	>14	18	52.9	16	47.1	34	100		
BMI	Normal	17	38.6	27	61.4	44	100	6.88	0.040
	Obesity	13	81.2	3	18.8	16	100		

The homogeneity between PCOS group and control group in terms of family history and menarche is not statistically significant (p= 0.602). In contrast, there was a significant difference (p= 0.040) of BMI between the two groups. In PCOS group, 13 of them (81.2%) were obese and the rest have normal BMI; while in control group, 3 of them (18.8%) were obese.
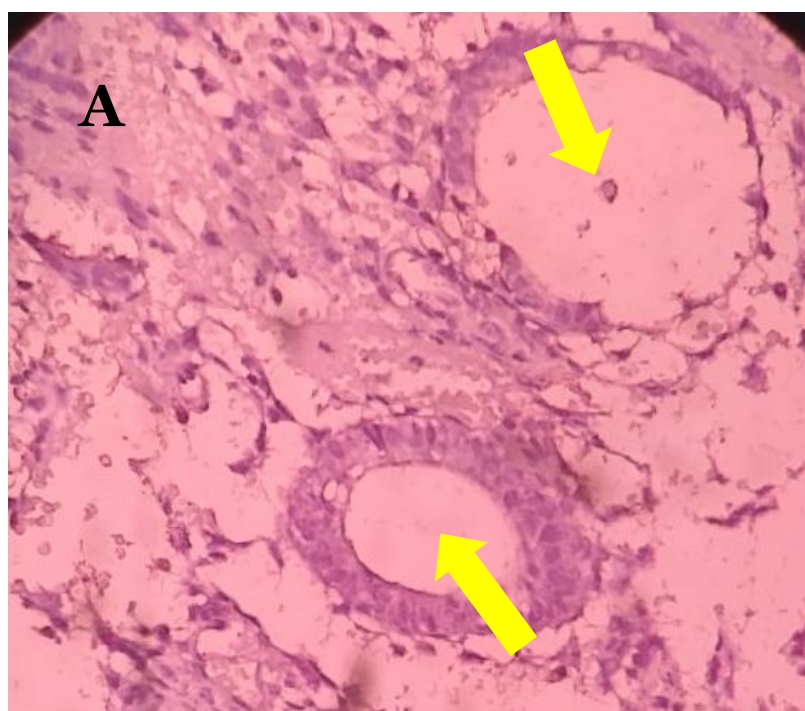

Figure 1. Leptin Expression in endometrium from Immunohistochemistry examination of control group (A)
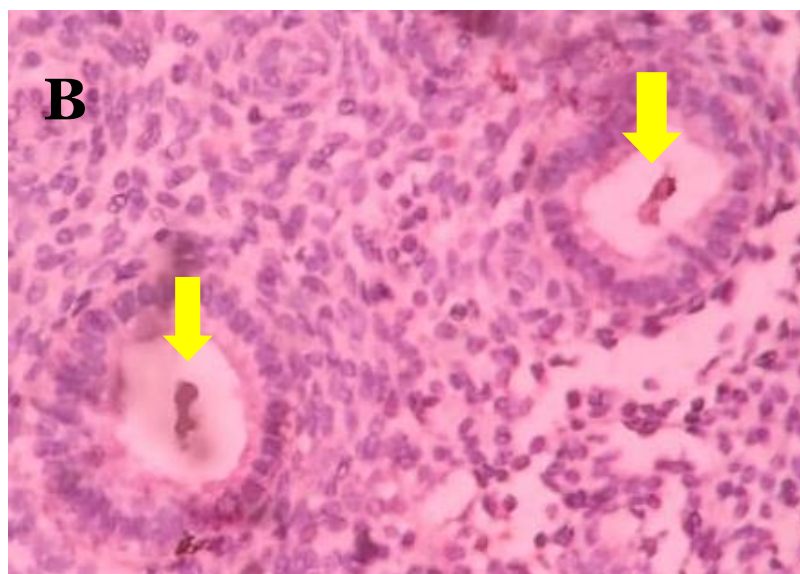
Figure 2. Leptin Expression in endometrium from Immunohistochemistry examination of PCOS group (B)

The difference of leptin expression among two control groups and its correlation with the occurrence of PCOS were assessed with bivariate analysis and correlation analysis. For that matter, endometrial tissue of the two groups were observed with immunohistochemistry technique and visually analyzed using microscope magnification 40x10. The results are shown in Figure 1.

In figure 1, the glandular cytoplasm and stroma are clear. While in figure 2, there is a brownish hue in glandular cytoplasm and stroma, indicative of increased leptin expression. Visually, it was clear that leptin expression on endometrial epithelia and glands during secretion phase were higher in PCOS group compared to control group.

Table 3. Differences in leptin expression in two groups

Groups	N	Mean	SD	p
Leptin with PCOS	30	59.16	49.34	0.001
Leptin with Normal	30	6.0	22.98	

Bivariate analysis with Mann-Whitney test was conducted to compare leptin expression among the two groups. The result is shown on Table 3 showed the average leptin expression was higher in PCOS group (Mean= 59.16; SD= 49.34) compared to control group (Mean= 6.0; SD= 22.98), with p= 0.001.

3. The result of multivariate analysis

Multivariate analysis was conducted to see correlation between variables with linear regression. The result is shown in Tables 4 and 5. Table 4 shows a statistically significant effect between PCOS with leptin expression.

PCOS affects the increase in leptin expression as much as 0.53 units (b= 0.53; 95% CI= 0.32 - 0.73; p= 0.001). Likewise, Table 5 shows the influence of PCOS on Leptin expression after considering all existing external variables. Statistically significant influence was obtained. SOPK gives an effect of increasing as much as 0.42 units of leptin expression (b= 0.42; 95% CI= 0.10 - 0.73; p= 0.01). Obesity also has effect of increasing as much as 0.31 units on leptin expression (b= 0.31; 95% CI=0.41 - 0.58; p= 0.025) and it was statistically significant.

Table 4. Leptin expression to PCOS

	Unstandardized coefficient (b)	Standardized coefficient (β)	95% CI	p
PCOS	0.53	0.56	0.32 – 0.73	0.001

Table 5. Leptin expression to PCOS and external variable

Independent variable	Unstandardized coefficient (b)	Standardized coefficient (β)	95% CI	p
PCOS	0.42	0.45	0.10 – 0.73	0.010
Age	< 0.01	< 0.01	-0.22 – 0.23	0.973
Employment	< 0.01	< 0.01	-0.28 – 0.30	0.962
Education	<-0.01	<-0.01	-0.28 – 0.27	0.970
Family history of PCOS	0.14	0.09	-0.23 – 0.51	0.446
Menarche	<-0.01	<-0.01	-0.22 – 0.21	0.952
BMI	0.31	0.29	0.04 – 0.05	0.025

DISCUSSION

Implantation process in endometrium is regulated with complex interaction between the embryo and endometrium. This dialogue is set to happen when a synchronization between oocytes, endometrial maturation, followed by blastocyst orientation to endometrial wall, along with apposition, adhesion, and invasion is accomplished. These simultaneous steps, from cellular adhesion, invasion, until endometrial decasualization, are a part of implantation process (Raheem, 2018)

During the majority of menstrual phase, the endometrium basically denies embryo. Thus, physiological attempts are required to restore endometrium condition and enable implantation. In the early phase of menstrual cycle, the level of estrogen is increased, augmenting the proliferation of endometrial cells. After ovulation, the luteinized follicles secrete progesterone that matures endometrial cells in favor of implantation (Wu et al, 2018). A receptive and fully functioned endometrium is crucial for implantation. During menstrual cycle, endometrium undergoes biological and morphological changes, preparing the endometrium for embryonal interaction and successful implantation. After the biological alteration is adequate, the embryo will have the capacity to adhere, invade, and finally implanted in endometrium (Craciunas et al., 2019).

Endometrial receptivity is morphologically characterized by the presence of endometrial pinopodes – rounded nodules found on endometrial lining. Pinopodes are expressed in short period of time, approximately 2 days on menstrual cycle, also known as window of implantation. Blastocyst implantation is shown to occur in the peak of pinopode (Altmäe et al, 2017).

Endometrial receptivity plays a key role in fertility. Any alteration in this factor will highly affect the success of assisted reproductive technique (ART) and contribute to infertility in PCOS. Endometrium of woman with PCOS is considered to be a model of 'dysfunctional' endometrium, with decreased expression of cytokines, immunomodulators, and the activity of steroid receptor; all of those lead to low fertility rate (Demiral et al, 2015). In PCOS, the disruption of follicle maturation and prolonged anovulation will cause chronic progesterone deficiency, thus affecting the whole endometrial environment. Moreover, in PCOS, alterations of expressed steroid receptors and glucose transporter 4 (GLUT 4) occurred due to progesterone resistance (Ruiz-Alonso et al, 2013). Women with PCOS may show irresponsive progesterone in vitro and altered production

of pro-implantation modulators including leptin (Altmäe and Aghajanova, 2015)

Leptin expression is higher in PCOS group compared to control group, as shown in Table 3. Shows the Mann-whitney leptin expression test results in PCOS compared to normal. The mean leptin expression in SOPK was higher than the normal group of 59.16 ± 49.34 and the normal group 6.0 ± 22.98 which were statistically significantly different with p= 0.001. It can be concluded that leptin does have effect on endometrial receptivity. Thus, the use of leptin for endometrial receptivity marker is encouraged.

Leptin itself is included in many factors that contribute to blastocyst and endometrium interaction, along with IGF-1, MUC-1, MMP-9, VEGF, and many other (Blesa et al., 2014). Leptin has a dualism of effect in reproduction. Positively, leptin has the capacity to induce puberty in hypothalamus-pituitary axis by secreting estrogen. But when the expression is exaggerated, ovary response will be inhibited (Daghestani et al, 2018).

Leptin expression is markedly reduced during the adhesion of conception product. During that time, because of the long duration, endometrium is exposed to progesterone stimulation (Miravet-Valenciano et al., 2015). After 8-10 days exposed to progesterone, the receptors in uterine lining will be down regulated, thus leads to the loss of its direct effect to endometrial cells. Leptin will be decreased and enabling receptive condition for conception products. The decrease of leptin is localized in particular receptor area (Méndez-López et al., 2017)

Leptin has an anti-adhesive characteristic that will lead to implantation failure if expressed excessively. The increase of leptin expression, as shown in PCOS, will disrupt embryo adhesion and increase the needs of progesterone receptors (Pérez-Pérezet al, 2017). Leptin is also proven as a regulator of angiogenesis by augmenting the expression

of vascular endothelial growth factor (VEGF) and its receptor, VEGF-R2; resulting in neo-vascularization. The altered leptin level and sOB-R will disrupt angiogenesis and re-modeling process during placenta formation, induce hyperactivity of angiogenic pathway, and ends with endothelial dysfunction. An observational study in 2014 by Perez et al stated that there is a correlation between excessive, deficient, and resistance of leptin with the abnormality of reproduction function (Pérez-Pérez et al., 2014)

In Table 4 model 2, it was found that in obese women, leptin expression increased 2.31-fold with p = 0.025 and 95% CI (0.04 - 058). It is believed that body mass index is directly correlated with fat deposition. Leptin itself is synthetized from white adipose tissue (WAT) as a response of fat deposition in the body. Leptin will induce a feedback to hypothalamus by suppressing appetite signal, such as AGRP, neuropeptide-Y (NP-Y), and galanin. In high adipose deposition, leptin is secreted in abundant amount, enabling the attachment of alpha-melanocyte stimulating hormone (α-MSH) to MCR-4 and suppressing appetite. Meanwhile, when the fat deposit is low, leptin is secreted in low amount, enabling AGRP to increase and attach to MCR-4, thus inducing appetite. In majority of obese individuals, however, the increase of leptin level is not followed by the use of energy or decrease in appetite (Sumadewi, 2017). In obese individuals, leptin is found in free-form as a result of sOB-R reduction; leptin function is also decreased. The resistance of leptin or inadequately functioned leptin is characterized with hiperleptinemia (Rizk and Sharif, 2015)

It is also believed that hyperinsulinemia in PCOS also plays a part in anovulation, as a result of inhibited proliferation of granulose cells (Daghestani et al, 2018). Hyperinsulinemia and insulin resistance will induce the regulation of leptin mRNA in adipose, and

conversely, leptin will inhibit the activation of gonadotropin in insulin-mediated steroid genesis (Rizk and Sharif, 2015). This condition will inhibit ovarian response and deliver negative effect to fertility.

Leptin expression is significantly higher in PCOS and obese women. A high level of leptin will disrupt adhesion process, thus affecting endometrial receptivity.

AUTHOR CONTRIBUTION

CONFLICT OF INTEREST

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