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Oxidative Stress and Insulin Resistance Among Polycystic Ovary Syndrome Patients. A Cross-Sectional Study In Makassar, Indonesia

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ABSTRACT-- *This study aim to knowing the relationship between levels of oxidative stress on the incidence of insulin resistance in patients with Polycystic Ovary Syndrome (PCOS). This is Observational study with a cross-sectional approach, samples of 27 patients with insulin resistance confirmed by IR HOMA > 2 and 27 patients without insulin resistance confirmed by HOMA IR < 2, then all subjects were tested for Reactive Oxygen Species (ROS) levels using the Enzyme-Linked Immunosorbent Assay (ELISA) method from January to December 2019. The average level of Reactive Oxygen Species (ROS) was 1.58, the average value of HOMA-IR was 2.91. There was a significant relationship between the ROS levels to the value of HOMA IR in patients with Polycystic Ovary Syndrome (PCOS) (p = 0.001; p <0.05). This study concludes that significant relationship between ROS and insulin resistance (HOMA-IR) is based on the mechanism of hyperglycemia due to insulin resistance resulting in increasing in Reactive Oxygen Species (ROS) which results in hyperandrogenic conditions in patients with Polycystic Ovary Syndrome (PCOS).*

Keywords-- *polycystic ovary syndrome, oxidative stress, insulin resistance*

I. INTRODUCTION

Polycystic Ovary Syndrome is an endocrine and metabolic syndrome occurs in women manifest as a disturbance in menstruation cycle, hirsutism, acne, and obesity. It is a common endocrine disorder occurs in women at reproductive age where the prevalence ranging from 5-10% (O'Reilly et al., 2014). The onset may happen as early as 12 years old until 45 years old (Kabel, 2016). Other clinical manifestations that occur concurrently with PCOS are hyperandrogenism, hyperinsulinemia, and glucose intolerance. These conditions may affect fertility, recurrent spontaneous miscarriage, hyperlipidemia, type 2, diabetes mellitus, hypertension, atherosclerosis, and endometrial hyperplasia (Carmina, 2012, McCartney et al., 2016).

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The cause of PCOS remains unknown and there is no definite answer regarding this. But one assumption states that insulin resistance may play a role in PCOS. Some studies reveal a higher prevalence of insulin resistance among PCOS patients. Insulin resistance creates hyperinsulinemia as a compensated response to insulin insensitivity. This higher insulin level stimulates the production of androgen in ovarium through several mechanisms. Hyperinsulinemia will inhibit the secretion of Insulin-like Growth Factor Binding Protein-I (IGFBP-I) and increase the Insulin-like Growth Factor-I (IGF-I) in the liver. Excessive insulin will be bind by IGF-I which takes action in Teka cell to increase the LH level. Insulin will also activate the serin phosphorylase pathway which increases the cytochrome P450c17 in ovarium and adrenal gland and eventually stimulates androgen production. Insulin also suppresses the Sex Hormone Binding Globulin (SHBG) level and leads to an increased level of freeform androgen. This form of androgen will affect the aromatase pathway in granulose cells and induces an early atresia of the follicle. This process is responsible for the occurrence of the PCOS (Wahyuni et al., 2015, Lucidi, 2018).

Oxidative stress may play pivotal roles in endometriosis, infertility factor, regulation of ovulation, and affects the quality of oocyte in the human reproductive system (Schattman et al., 2015, Lu et al., 2018). Oxidative stress defined as the imbalance of antioxidant defense against the Reactive Oxygen Species (ROS). This imbalance induces an increase of ROS level which then leads to the destruction of the lipid membrane due to the peroxidation activity of lipid layer and *malonyl dialdehyde* (MDA) formation. An increasing ROS level creates an unsupportive environment for the viability of cells in reproductive tissue (Turan et al., 2013, Agarwal et al., 2014).

In diabetes mellitus, antioxidant defense and cellular regeneration system will be initiated when the oxidative reaction occurs. The source of oxidative stress comes from the formation of a free radical agent of glucose autooxidation, reduction of the low-weight antioxidant level in the tissue, and disturbance of enzymatic antioxidant defense system. The significant finding of stress oxidative is not constant but it is the belief that stress oxidative gives a contribution to the severity and complication of a disease (Setiawan et al., 2005).

From the explanation above, there is a plausible concept of stress oxidative and insulin resistance and this assumption might occur in patients with PCOS. Therefore, the investigators aimed to identify the association between stress oxidative and insulin resistance, particularly among PCOS patients. By identifying this factor, an effort to interfere with the PCOS can be done from the aspect of controlling the stress oxidative level.

II. METHODOLOGY

This cross-sectional study involved PCOS patients. The sampling in this study using purposive sampling technique.

Subjects

The population in this study were all PCOS patient recruited from the Obstetric and Gynecology clinic of Wahidin Sudirohusodo General Hospital Makassar Indonesia and other referral hospitals under the supervision of the Faculty of Medicine Hasanuddin University from January – December 2019. The inclusion criteria were PCOS patients diagnosed by the Rotterdam Criteria where at least two from three criteria are fulfilled (history of oligomenorrhea or amenorrhea, hyperandrogenism signs, and symptoms, clinical or Ferriman Gallwey score more

than 8 or through biomarker assessment (Luteinizing Hormone) and supportive ultrasound finding for PCOS), aged 18 – 40 years old, showing normal liver and kidney function on laboratory examination. Participants were excluded if pregnant or breastfeeding, diagnosed with endocrine diseases such as thyroid disease, Cushing syndrome, congenital adrenal hyperplasia, and ovarium tumor or adrenal tumor which leads to hyperandrogenism. Other participants were also excluded from having other chronic diseases such as diabetes mellitus, uncontrolled hypertension, cardiovascular disease, malignancy, and infection. People underwent laparoscopic ovarian diathermy (LOD) and in vitro fertilization (IVF) are not eligible. Patient under medication that affects the insulin and reproduction function during the observation period such as clomiphene citrate, an insulin sensitizer, aromatase inhibitor, glucocorticoid, gonadotropin-releasing hormone agonist (GnRHa), oral contraception pill, antiandrogen agent (spironolactone, cyproterone acetate, flutamide) and traditional medicine will not be included. The investigator also considered excluding patients with a sedentary lifestyle who consumed more than 3 times per week of fast food.

The number of participants determined from a formula of proportion based on a similar study by Jolanta et al in 2013. The investigator defined the proportion of insulin resistance in PCOS patients as 0,26%. With 5% type 1 error, 10 percent dropout, and 90% power, a total of 52 participants recruited in this study.

Experimental procedure

Data collection is done by observing first. Information regarding the characteristics and general condition of the respondent, including demographic data such as age which then discretized as under 30 and above 30, history of Oligomenorrhea and amenorrhea, hypoandrogenism clinically known by conducting direct interviews. Then obtained hypoandrogenism laboratory-confirmed, ultrasound imaging, medication, score, fast food intake, body mass index and modified Ferriman Gallwey. The modified Ferriman-Gallwey score evaluates nine different body areas with a Likert-scale response from 0-4 where the highest number indicates obvious hirsutism. A score of 8 defined as hirsutism (Brodell & Mercurio, 2010). where this can be classified into mild (8-15), moderate (16-25), and severe (>25) t (Yidiz et al., 2003). Ultrasound finding referred to an ASRM/ESHRE Rotterdam 2003 for PCOS where there should be 12 or more follicles with the diameter should be 2-9 mm in each ovarium or an increase of ovarium volume exceeding 10 cm3 (Fritz and Speroff, 2011).

The Human Reactive Oxygen Species modulator 1 level is the primary outcome and measured using blood serum underwent ELISA and categorized into two groups, more than 1 and lower than 1. The insulin resistance defined as the score of HOMA-IR more than 2 using ELISA.

Statistical Analysis

The data obtained were analyzed using SPSS 25 program and presented in the form of tables, narratives, and graphs. The statistical tests used were normality tests using the Kolmogorov-Smirnov test. If the data is normally distributed, a correlation Pearson test is do first and if the data is not normally distributed using spearman test.

A chi-square test was performed to see the relationship between categorical factors. The relationship between the reactive oxygen species and insulin resistance was performed using a correlation test with 95% Confidence Interval of odd ratio.

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This study is registered on Ethical Committee Faculty of Medicine Hasanuddin University number 1010/H4.8.5.31 and ensuring that the patient will be treated according to the Helsinki Declaration and when the participants develop unfavorable outcome, the treatment will be given accordingly.

III. RESULTS

A total of 54 participants involved in this study. The vast majority of the participants were under 30 years old (74,1%) while 14 people were above 30 years old. Around 43 people have a history of oligomenorrhea, 8 people show an absence of menstruation, and 3 people without these symptoms. Around 17,3% (9 people) have a family member with PCOS. No other medication affecting the outcome has been taken. The modified Ferriman Gallway assessments demonstrate that all participants belong to mild hirsutism. Furthermore, 33 participants (61,1%) consumed fast food once a week and 9 people eat at least twice a week. Regarding the BMI, 18 participants have normal BMI, 28 people (51,9%) with overweight, and 8 people (14,8%) with obesity.

Table 1 : Patient Characteristic

Variables	Number	Percentage
Age		
<30 years old	40	74.1%
≥30 years old	14	25.9%
History of disturbance		
Menstrual pattern		
Oligomenorrhea	43	79.6%
Amenorrhea	8	14.8%
Normal	3	5.6%
Family history of PCOS		
Present	9	16.7%
Absent	45	83.3%
Fast Food Intake		
Never	12	22.2%
Once a week	33	61.1%
Twice a week	9	16.7%
BMI		
Normal (18,5-24,9)	18	33.3%
Overweight (25-29,9)	28	51.9%
Obese (≥30)	8	14.8%
HOMA-IR		
High (≥2)	27	50%
Normal (<2)	27	50%
ROS		
High (≥1,0)	24	44.4%
Normal (Nilai <1,0)	30	55.6%

The number of people who have insulin resistance (HOMA IR>2) is similar to normal people (<2) 27 in each group. Meanwhile, the 24 people (44,4%) showed higher stress oxidative level according to the human ROS modulator 1 level (>1). These data can be seen in table 1. Some other information with zero percentage in one class is omitted.the Age group has no association with the ROS level (p=0.890) as well as the history of disturbance menstrual pattern (p=0.824), fast food intake (p=0.298). On the other hand, participants with a family history of

PCOS have an association with a higher ROS level (p=0.0414) the crude odds ratio for this is 5.76 (95% CI 1.07-31.02). Furthermore, body mass index is associated with the ROS level (p=0.0251) where the crude odd ratio of having normal body mass index to high ROS level is 0.23 (95% CI 0.06-0.83) which indicates that Normal body mass index is a protective factor from higher stress oxidative compare to overweight and obesity. This can be seen in table 2.The relationship between HOMA IR indicating insulin resistance and the ROS level was assessed using a spearman correlation. The mean of ROS in all participants is 1.58 ± 1.95 whereas the HOMA IR value is 2.91 ± 3.11. The spearman correlation shows r=0.441 indicating moderate correlation (p=0.001)

The investigators have successfully addressed the association between *Reactive Oxygen Species (ROS*) and *HOMA-IR* among PCOS patients as well as exploring other variables association. A study in Qatar shows that the mean age of PCOS patients is 26.88 which goes

Table 2 : Association of Variables

Variable		ROS		P
		High (≥1)	Low <1	
Age	<30 years old	18	22	0.890
	≥30 years old	14	8	
History of Menstrual disturbance	Normal	1	2	0.824
	Oligomenorhea	20	23	
	Amenorrhea	3	5	
Familial history of PCOS	Yes	7	2	0.041*
	No	17	28	
Fast Food Intake	Never	3	8	0.298
	Once A week	17	16	
	Twice a week or more	3	6	
BMI	Normal	4	14	0.0251*
	Overweight and Obese	20	16	
HOMA IR				0.001#

*significant with Chi Square

#significant with spearman correlation

Along with our result where the vast majority of the participants were under 30 years old and this also similar to a study in India (Soha *et al*, 2017; Nidhi *et al*, 2011). But there is no association between age and ROS level despite the aging is linear to stress oxidative event (Liguori *et al*., 2018). A study by Sulaiman in 2018 strengthen our finding (Sulaiman *et al*., 2018)

The PCOS patients are susceptible to pro-inflammatory condition (Palomba *et al*, 2014), Despite insulin resistance can occur at any level of body mass index (Li Q *et al*, 2013), the findings demonstrate the obvious protective effect of normal body mass index to ROS level and this study is linear with Moti et al(Moti M *et al*, 2015).

There is an association of family history of PCOS with ROS level (p=0.0414) similar to Yeon finding which emphasized the role of genetics in this situation particularly related to the mitochondrial function (Yeon Lee *et al*., 2010; Zhang *et al*., 2019). There are six variants of tRNA gene in mitochondria, the *tRNAGln, tRNACys, tRNAAsp, tRNALys, tRNAArg*, dan *tRNAGlu*, and seven variations of 12S gene of ribosomal *RNA (rRNA)* in PCOS which also linked to DM and hypertension, a common complication in PCOS patient (Zhang *et al*., 2019).

History of disturbance in the menstrual pattern also seen in the participants, similar to the finding in by Yeon Lee in 2010 where 87% of their respondents have oligomenorrhea (Yeon Lee *et al*., 2010) as well as in India (Nidhi *et al*, 2011), But there is no association between history of menstrual disturbance with ROS level which suggests the influence of other factors. Unfortunately, the investigator could not yield an association between the level of hirsutism and ROS level. This finding indicates that participants from our sample probably showing lower hirsutism levels compare to other countries (Karima, 2016).

Fast food intake has no relationship with *Reactive Oxygen Species* (*ROS*) (p= 0.298) and this finding is conflicting with the study in Oman where overnutrition due to fast food intake might be related to stress oxidative, particularly altering the cellular respiratory mechanism which stimulates the peroxide release and induces cellular damage (Sulaiman MA *et al*, 2018) and PCOS patients show the damage of DNA due to peroxide exposure which could arise from this mechanism (Zuo T *et al*, 2016).

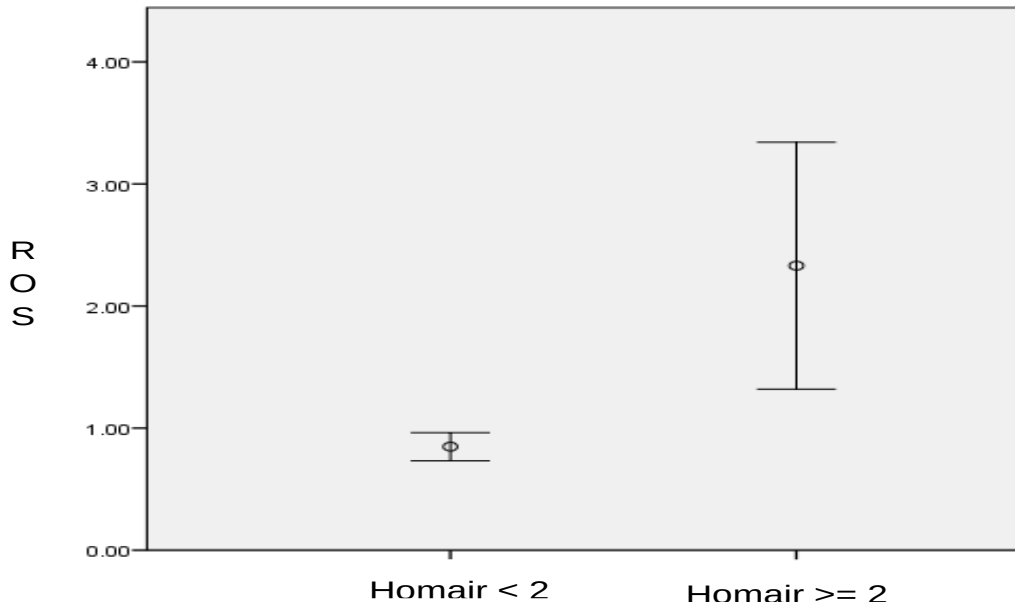

Figure 2 ROS level between HOMA IR Group

Overweight and Obesity can be seen in PCOS patients and a study of Sulaiman strengthens our finding (Sulaiman *et al*., 2018) the level of ROS also linear to the IMT in Zuo study which also similar to our finding (Zuo *et al*., 2016). Higher body mass index plays important roles in inducing stress oxidative and eventually leads to higher insulin resistance (Yeon Lee *et al*., 2010) but this finding can not be generalized as the stress oxidative even occur to PCOS patients who are underweight (Desai *et al*, 2014).

Insulin resistance occurs in 50 to 70% percent of PCOS patients (Zuo et al., 2016). Our significant finding of ROS level and insulin resistance is similar to the study by Victor and Pizzino (Pizzino G *et al*, 2017; Victor *et al*., 2016). The underlying mechanism perhaps due to a higher free fatty acid which induces the ROS production in the

patient with PCOS (Zuo *et al*., 2016). The oxidative stress arises from the free fatty acid metabolism (including pyruvic acid conversion and electron transfer chain reaction) induce the ROS as well as reuptake failure of intracellular ROS (Zuo *et al*., 2016). The increase of oxidative stress stimulates the phosphorylase of serin through activation of protein kinase which inhibits the IRS normal tyrosin, reduces the IRS-receptor contact, and suppresses the IRS-receptor to activate the enzyme *phosphatidylinositol 3- kinase (PI3K).* This alters the insulin signal to effector through (*InsR) / IRS / PI3K* (Zuo *et al*., 2016). And this condition is worsened by the mutation of allele regulating insulin in a patient with PCOS (Yeon Lee *et al*., 2010).

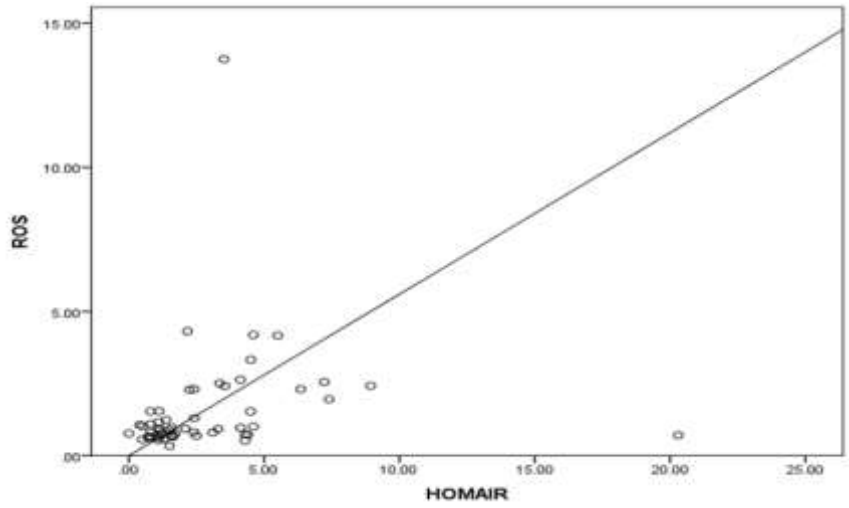

Figure 3 Scatter Plot of ROS level and HOMA IR Group

There is some limitation of this study. A higher number of sample and centers should be involved and some potential confounders were not addressed including the level of education which mainly affect the behavior that leads to higher stress oxidative. People with lower education may possess lower knowledge of insulin resistance prevention, including a healthy diet and avoid a sedentary lifestyle. At this point, lifestyle modification to reduce the stress oxidative should be offered to those patients with PCOS.

IV. CONCLUSION

The conclusion of this study is that there is significant relationship between increased levels of Reactive Oxygen Species (ROS) to the incidence of insulin resistance based on HOMA-IR levels in patients with polycystic ovary syndrome. For further research, it is expected to be able to control confounding variables.

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