Hyperbaric Oxygen Therapy Effect on Androgen Receptor and Superoxide Dismutase in Insulin-Resistant Polycystic Ovary Syndrome

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Abstract

It has been found that insulin resistance (IR) affects majority of the women with polycystic ovarian syndrome (PCOS) regardless its correlation with obesity. Hyperinsulinemia leads to hyperandrogenemia state, showed by an over-expression in androgen receptor (AR). It has also been discovered that oxidative stress level is significantly increased in patients with PCOS, showed by an increase in superoxide dismutase (SOD) serum level. Hyperbaric oxygen therapy (HBOT) is proved to be able to reduce IR and oxidative stress level in other cases. This study aims to evaluate the effect of HBOT on AR expression and SOD serum level in insulin-resistant PCOS rat model.

This study was an experimental study with randomized posttest only control group design. Twenty female Rattus norvegicus strain Wistar as insulin-resistant PCOS rat model was used in this study, randomly divided into control group and treatment group. HBOT was given at 2.4 ATA pressure with 100% oxygen. AR expression was measured semi-quantitatively with Immunoreactive Score (IRS), while SOD serum level was measured with ELISA method.

Mean IRS score between control group and treatment group were significantly different (1.64 ± 1.38 vs 0.8 ± 0.6, p = 0.025). Mean SOD serum level between control group and treatment group was significantly different (0.13 ± 0.03ng/ml vs 0.2 ± 0.06ng/ml, p = 0.011).

HBOT could reduce the AR expression and increase the SOD serum level in insulin-resistant PCOS rat model. HBOT therapy could be taken into consideration for treating insulin-resistant PCOS patients.

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Introduction

Polycystic ovarian syndrome (PCOS) is a common health problem among women in their reproductive age due to an imbalance of reproductive hormones. PCOS was first described in 1935 as the combination of hirsutism, amenorrhea, chronic anovulation, infertility, obesity, and cystic ovaries [1]. Currently, there are 3 diagnosis criteria for PCOS, which are NIH criteria (1990), Rotterdam Criteria (2003), and Androgen Excess and PCOS Society Criteria (2006)[2-4]. According to latest diagnosis criteria, PCOS is a heterogenous disorder defined by a combination of sign and symptoms of androgen excess and ovarian dysfunction in the absence of other specific diagnoses[4].

It has been found that insulin resistance (IR) affects majority of the women with PCOS. Regardless the correlation between IR and obesity, part of the IR appears to be independent of obesity and related specifically to PCOS because of the abnormalities of insulin action and receptor function. Moreover, hyperinsulinemia also act as co-gonadotropin and mimic the effect of the increased LH stimulus, as seen in majority of PCOS patients. Hyperinsulinemia that occurs because of the IR, lead the body to hyperandrogenemia state and maintaining it by acting directly to induce excess androgen production by theca cells. Besides that, hyperandrogenemia may also be caused by the abnormal ovaries, adrenal glands, peripheral fat,

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and hypothalamus-pituitary compartment [5-7]. Chronic elevation of androgen in women with PCOS will lead to increase in androgen receptor (AR) expression. It has been found that there is an over-expression of AR in the endometrium of PCOS patients [8].

The increase of IR in women with PCOS is significantly correlated with oxidative stress (OS), independent of obesity. OS is defined as an imbalance between excessive formation of oxidants in the presence of limited antioxidant defenses [9]. IR encourages OS because hyperglycemia and higher levels of free fatty acid lead to reactive oxygen species (ROS) production. Vice versa, OS has been demonstrated to play crucial roles in pathogenesis of IR by impairing glucose uptake in muscle and adipose tissue, and it reduces insulin secretion from pancreatic b cells[10,11].

Other than its correlation with IR, OS is also positively correlated with androgen levels in PCOS patients [12]. Previous in vitro study reported that OS enhance the activities of ovarian steroidogenesis enzymes, which could stimulate androgen generation [13]. Previous meta-analysis study found that Superoxide dismutase (SOD), one of the circulating markers for oxidative stress and a potent antioxidant enzyme, is significantly higher in PCOS patients compare to normal patients. Increase in SOD serum level in PCOS patients is a compensatory mechanism in response to the increased production of oxidant molecules [11].

Hyperbaric Oxygen Therapy (HBOT) is a therapy where patients breath with 100% oxygen in high pressurized cabin with increased atmospheric pressure. At first this therapy is designated to treat decompression sickness and arterial gas embolization, but now this therapy has been used in many other cases. Although the mechanism is not fully known, the use of this therapy has been widely accepted. Previous studies showed that HBOT improves peripheral insulin sensitivity in type 2 diabetes mellitus and it also has a significant effect on oxidative stress alteration [14-17]. Another study found that it could be a possible treatment option for infertility by improving endometrial receptivity through blood vessel resistance alteration [18].

However, until now there is no study that analyze the effect of HBOT towards insulin-resistant PCOS. This study aims to evaluate the effect of HBOT on AR expression and SOD serum level in insulin-resistant PCOS rat model.

Materials and methods

This study was an experimental analytic study with randomized post-test only control group design conducted on July – August 2015. HBOT was done in Navy Hospital Dr. Ramelan Surabaya. Histopathologic evaluation was done in Anatomical Pathology Laboratory Faculty of Veterinary Medicine Universitas Airlangga. Samples in this study was female Wistar-strain *Rattus norvegicus*. Inclusion criterias were rats aged 3 - 4 months old, weigh 150 - 200 gram, and healthy. Exclusion criteria in this study were rats with congenital defect or had an aggressive behavior. Dropout criteria for this study were rats which were wounded or died during the study period.

There were 20 Wistar rats used in this study. All rats were injected with 1 mg / 100 gr Bodyweight testosterone propionate subcutaneously for 28 days to obtain insulin-resistant PCOS rat model [19]. After the rat model was obtained, samples were divided into 2 groups (control group and treatment group) using simple random sampling technique. Control group was sacrificed and evaluated for SOD serum level and AR expression without receiving any treatment. Treatment group was placed in the animal chamber and was given 2.4 ATA pressure with 100% oxygen. Treatment group underwent 2 cycles in the animal chamber with 1-day break between cycle. Every cycle consisted of 5 session, where every session was done in 90 minutes period with 5 minutes air break every 30 minutes. After 2 cycles, treatment group were sacrificed and evaluated for AR expression and SOD serum level.

SOD serum level was measured with ELISA method using blood plasma. AR expression was evaluated using immunohistochemistry (IHC) method with monoclonal antibody staining. AR expression was measured semi-quantitatively with Immunoreactive Score (IRS) by evaluating the visualized grade of color intensity (staining) and fraction of cells in each intensity category [20]. The score was averaged from the measurement of 10 different visual field with 400x magnification. Light microscope Nikon Eclipse Ci equipped with calibrated Digital Camera Optilab Plus 12 Megapixel and image processing software Image

Roaster 3 was used for this evaluation.

Acquired data was analyzed using SPSS version 17.0 (SPSS, Inc., Chicago IL). Shappiro-Wilk test was used to analyze the normality of the data. Mann-Whitney test was used to analyze the bodyweight difference between groups. Independent T-test was used to analyze the AR expression difference and SOD serum level between groups. A $p < 0.05$ was considered statistically significant.

This study was ethically approved by Ethics Committee of Faculty of Veterinary Medicine, Universitas Airlangga (Ethical Clearance Number 480-KE) before conducting the study. All experiments were performed in accordance with relevant regulations.

Results

There were 20 Female Wistar rats included in this study. There were no dropout samples in this study. Bodyweight of Wistar rats used in this study ranged from 100 to 120 gram. After the rats had been randomly grouped into 2 groups, normality test showed that the bodyweight in control group and treatment group was not normally distributed ($p = 0.08$; 0.36, respectively). There was no significant difference of mean bodyweight between control group and treatment group (108.5 ± 8.83 gram vs 109 ± 7.38 gram, $p = 0.808$).

IRS of AR expression in control group and treatment group in this study were normally distributed ($p = 0.313$; 0.474, respectively). Mean IRS score between control group and treatment group were significantly different (1.64 ± 1.38 vs 0.8 ± 0.6, $p = 0.025$) (Table 1). SOD serum level in control group and treatment group were normally distributed ($p = 0.599$; 0.114, respectively). Mean SOD serum level between control group and treatment group were significantly different (0.13 ± 0.03ng/ml vs 0.2 ± 0.06ng/mg, $p = 0.011$) (Table 1).

	Control group	Treatment group	*p* value
IRS	2.19 ± 1.09	0.8 ± 0.6	0.025*^
SOD Serum Level(ng/ml)	0.13 ± 0.03	0.2 ± 0.06	0.011*^

Table 1. IRS and SOD serum level between groups
*A $p < 0.05$ was considered statistically significant.
^Independent T-test was used.
IRS= Immunoreactive score, SOD= Superoxide dismutase.

Discussion

To our knowledge, this is the first study that evaluates the effect of HBOT towards AR expression and SOD serum level in insulin-resistant PCOS rat model. In this study, we found that HBOT significantly decreased the AR expression. Since the elevated AR expression in PCOS was caused by the elevated androgen serum level, while the elevated androgen serum level was because of the hyperinsulinemia, and the hyperinsulinemia was due to IR, we argue that the decrease of AR expression in treatment group in present study was because of the effect of HBOT towards IR.

It is speculated that HBOT improves insulin sensitivity by reducing adipose tissue hypoxia and subsequently inflammation [15]. Other than that, it may also act by stimulating mitochondrial biogenesis through the increase of expression in peroxisome proliferator activated receptor-1 alpha (PGC1-α), a master regulator of mitochondrial biogenesis [21]. Novel study which shows that insulin sensitivity is increased during hyperbaric oxygen therapy in obese individuals with type 2 diabetes mellitus (T2DM) individuals, also found an improvement in insulin sensitivity and reduction in HbA_{1c} in non-obese individuals without T2DM, suggesting this insulin-sensitizing effect was not confined to individuals with T2DM only [15].

In this study, we found that HBOT significantly increase the SOD serum level in insulin-resistant PCOS rats model. Previous experimental study in acute necrotizing pancreatitis rat model showed that HBOT was significantly increase SOD serum level [14]. Experimental study that evaluates the effect of HBOT during liver regeneration in rats also found that SOD level were significantly increase in HBOT-treated rats compare to untreated and control rats [16]. Other experimental study which evaluates the antioxidant activity in muscles found that acute exposure to HBOT did not significantly increase the SOD serum level, however chronic exposure did [17].

SOD is the first line of defense in antioxidant reactions against ROS. At low to moderate concentrations, ROS is involved in physiological roles including defense against infectious agents and several cellular signaling systems. But when present in excess, ROS may damage DNA, cellular lipids and proteins,

interfering with their normal function [22]. It removes ROS from the cellular environment by catalyzing the dismutation of O_2^- to H_2O_2 and O_2, which will be scavenged by peroxisomal catalase. It requires copper (Cu) and zinc (Zn) for their optimal function, and deficiency of these elements may cause a decrease in the enzyme activities. Cu plays a role in the redox reaction which promotes cellular instability, while Zn plays a role in protecting tissues from the damage caused by free radicals [14, 16].

HBOT triggers and upregulates the antioxidant enzyme as a defense mechanism against OS. Increased tissue oxygenation may activates other endogenous factors that prevent hazardous effects of the disease itself [23]. There is concern that HBOT might increase oxidative stress via the production of ROS. However, this treatment is considered safe since the activity of free radical scavenger is increased. In long term and repeated administration, HBOT can decrease oxidative stress by reducing lipid peroxidation and by up-regulating the activity of antioxidant enzymes [24]. It is suggested that HBOT reduces lipid peroxidation through its inhibition of neutrophil adhesion [25]. However, in this current study, short term HBOT administration had already showed a significant increase in SOD serum level. For safety reason, HBOT pressure should never excess 3 atm and should not last longer than 90 minutes per therapy session. If these safety guidelines are not followed, free radicals may accumulate and may cause oxygen toxicity in the central nervous system and in the lungs [16].

Conclusions

HBOT could reduce the AR expression and increase the SOD serum level in insulin-resistant PCOS rat model. These findings have suggested that HBOT could be taken into consideration as a treatment option in insulin-resistant PCOS patients.

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Declaration of Interest

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