Contents lists available at IOCS

Science Midwifery

journal homepage: www.midwifery.iocspublisher.org

Atherosclerosis in rat PCOS model induced by testosterone propionate combined with high fat high fructose diet

Dirwan Suryo Sularto[1], Alfaina Wahyuni[2]

[1]Department of Anatomy, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Bantul, Indonesia

[2]Department of Obstetrict and Gynecology, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Bantul, Indonesia

ARTICLE INFO

Article history:

Received Dec 5, 2023
Revised Dec 6, 2023
Accepted Dec 18,, 2023

Keywords:

Atherosclerosis
High Fat Diet High Fructose Diet
PCOS
Testosterone Propionate

ABSTRACT

Atherosclerosis is closely related to the metabolic syndrome like what occurs to Polycystic Ovary Syndrome (PCOS). The recent increase in prevalence due to high calories diet and high carbohydrate, infrequent exercise, and the escalating obesity trend increased insulin resistance. PCOS is linked to many long term health problems, such as cardiovascular disease, atherosclerosis, and diabetes. How is the potential of atherosclerosis for PCOS model mice induced with testosterone propionate and high fat diet and high fructose? This research is important to conduct in order to determine the potential of atherosclerosis for PCOS through the observation on mice model of PCOS. This research employed Postest Only Control Group Design method. Twenty female Rattus norvegicus strain Wistar mice weighing between 100 and 130 grams, aged 3 months, served as the samples. After observing estrous cycle, 15 mice were randomly chosen to be made PCOS model induced with testosterone propionate 1mg/100 g BW/day intramuscularly combined with high fat diet and high fructose. The treatment was given on day 18, 21, and 24. Afterwards, the mice were sacrificed on day 19, 22, and 25. After 12 hours of fasting the mice, the mice were sacrificed and the coronary arteries attached to the heart were taken to be made histological preparations. Then, the degree of atherosclerosis potential was observed. Statistical analysis used One Way Anova test with significance level of α=0,05. Data analysis was performed using Statistical Product and Servive Solutions (SPSS) program version 24.0 for Windows.

Corresponding Author:

Alfaina Wahyuni,
Department of Obstetrict and Gynecology,
Faculty of Medicine and Health Sciences,
Universitas Muhammadiyah Yogyakarta,
Jl. Brawijaya, Geblagan, Tamantirto, Kecamatan Kasihan, Kabupaten Bantul, Daerah Istimewa Yogyakarta 55183 Indonesia
Email: alfaina.wahyuni@umy.ac.id

INTRODUCTION

With a prevalence of 5-10%, polycystic ovarian syndrome (PCOS) is one of the leading causes of chronic anovulation. Anovulatory infertility is a long-term result of PCOS (Begum et al., 2017; Kort, D.H., Lobo, R.A., 2014). Several criteria are used to diagnose PCOS, one of which is the Rotterdam criteria. PCOS is diagnosed if two of three indicators, anovulation, clinical and/or laboratory hyperandrogenemia, and polycystic ovaries with ultrasound examination, are present, according to the Rotterdam criteria (Abbara et al., 2019; Themes, 2016). PCOS has a long-term impact on degenerative disorders like cardiovascular disease, atherosclerosis, and diabetes, in addition to infertility (Teede et al., 2013). PCOS pathogenesis is still being investigated. Lipid metabolism abnormalities, hereditary factors, poor insulin sensitivity, and oxidative stress are all frequently linked to the pathophysiology of PCOS [(Bitla, 2014; Ebrahimi-Mamaghani et al., 2015; Zuo et al., 2016). The study's objective was to determine whether PCOS might be a factor in atherosclerosis. Testosterone propionate was used as an induction drug for PCOS in this trial, combined with a high-fat, high-fructose diet. This research contributes to public awareness of the fact that difficulties associated with polycystic ovary syndrome (PCOS) extend beyond reproductive health and may manifest systemically, including atherosclerosis.

RESEARCH METHOD

20 female Rattus norvegicus Wistar strains were used, aged 3 months and weighing 100-130 grams. Before the study began, an adaptation period was carried out for a week. Used female rats in healthy condition, in normal behavior and normal vaginal swab results and are not pregnant and have no anatomical abnormalities. All mice were kept in the same environment in plastic cages measuring 40x30x10cm covered with woven wire. Mice were kept under the same conditions of feed, water and lighting. On day 8, 20 animals were randomly selected for the treatment group. Then the groups were divided randomly as follows (n = 5 animals): Normal group and 3 treatment groups. Rat in the treatment group received testosterone propionate combained with High Fat Diet and High Fructose group orally for 18, 21, and 24 days.

Beginning on day 8, testosterone propionate treatment in 0.5% CMC was administered intramuscularly at a dose of 1 mg/100 g BW while a high-fat, high-fructose diet was also given as needed. Following treatment, daily vaginal smear tests were performed and assessed using Giemsa staining. When the estrus cycle undergoes a series of changes until persistent vaginal cornification is reached, PCOS is said to have been established. Following group-based treatment, the rats were given a 12-hour fast before having their blood pressure checked, having their hearts removed, and having histological preparations made using hematoxylen-eosin. Additionally, images of atherosclerosis in the aorta and coronary arteries were observed under a microscope with a magnification of 40 x. Macrophages, foam cells, intracellular and extracellular lipid accumulation, and atheroma images are histopathological indicators of atherosclerosis. Scores for atherosclerosis are determined by how many signs of the disease are present, with criteria of "1" for barely perceptible, "2" for moderately perceptible, and "3" for numerous in five fields of vision. For each mouse, five slides were observed.

RESULTS AND DISCUSSIONS

Table 1. The Atherosclerosis scores in the control group and testosterone propionate - high-fat, high-fructose diet (TP-HFHFD) treatment group

Group	The aortic atherosclerosis score	The coronary artery atherosclerosis score	Body Weight after treatment (g)	Weight Gain (g)
Control	0,00±0,00	0,00±0,00	173,00±34,76	6,25±1,89

TP+HFHFD18 days	0,60±0,55	1,00±1,00	214,40 ±3,04[a]	32,60±1,14[a,b,c,]
TP+HFHFD 21 days	1,20±0,48[a]	2,00±1,22[a]	221,20±2,38[a]	39,60±1,6 [a,b,d]
TP+HFHFD 24 days	1,40±0,89[a]	2,40±1,51[a]	226,60±2,88[a]	45,40 4,27[a,c,d]

Note: a: p<0,05 vs control; b: p<0,05 vs 24 days; c: p<0,05 vs 21 days; d: p<0,05 vs 18 days.

Table 1 displays the aortic atherosclerosis score, coronary artery atherosclerosis score, final body weight, and weight gain. There appears to be a link between aortic atherosclerosis score and both body weight and weight gain (r = 1, p<0.05). Body weight (r = 0.603; p< 0.05) and weight gain are related to the score of a. coronary atherosclerosis (r = 0.532; p<0.05). The histology of the aortic wall is shown in Figure 1, and the histology of the coronary arteries is shown in Figure 2.

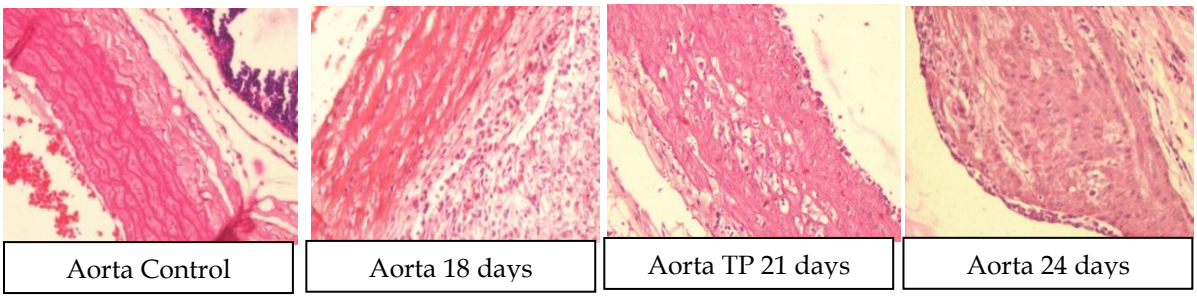

Figure 1. Features of atherosclerosis of the aortic wallAt 40x magnification, 5 histological slices of aorta with HE staining were examined. Histopathological hallmarks of atherosclerosis include macrophages, foam cells, intracellular and extracellular lipid accumulation, and atheroma images. The score is calculated by counting the number of slices that match the atherosclerosis image.

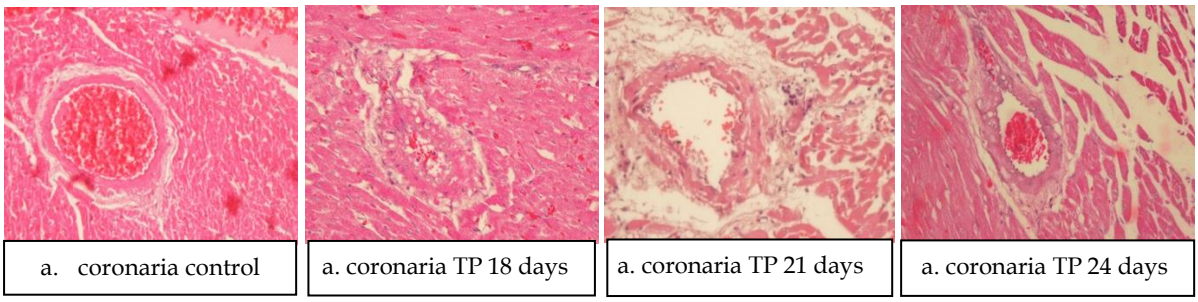

Figure 2. Hitology of artery coronary. At 40x magnification, 5 histological slices of aorta with HE staining were examined. Histopathological hallmarks of atherosclerosis include macrophages, foam cells, intracellular and extracellular lipid accumulation, and atheroma images. The score is calculated by counting the number of slices that match the atherosclerosis image.

Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disorder characterized by obesity, hyperandrogenism, and insulin resistance (Al-Mahmood et al., 2014; Altaş et al., 2010; Davinelli et al., 2020). Adverse lipid profiles have also been observed in women with PCOS, suggesting that these individuals may be at increased risk of coronary heart disease at a young age (Atasayan & Yoldemir, 2021; Diamanti-Kandarakis & Dunaif, 2012). Intercellular adhesion molecule-1 (ICAM-1) is a proinflammatory and proatherogenic cytokine associated with atherosclerosis, insulin resistance, and cardiovascular disease (CVD). PCOS patients had higher ICAM-1 expression values and serum levels. Among PCOS patients, T2DM patients had the highest ICAM-1 expression values and serum levels compared to the IGT and NGT subgroups. ICAM-1 expression and serum levels were significantly positively correlated with cardiovascular risk and PCOS phenotype. Linear regression test showed that HOMA-IR was the main predictor of serum ICAM-1 levels in PCOS (Andrade et al., 2016; Rashad et al., 2019).

Insulin resistance and hyperandrogenemia are linked to the metabolic syndrome in PCOS, which increases the risk of atherosclerosis developing early (Ebrahimi-Mamaghani et al., 2015; Escobar-Morreale et al., 2011). Compared to typical obese women, obese PCOS women have a greater chance of developing early asymptomatic coronary atherosclerosis (Mousa et al., 2023). Insulin resistance is linked to an increase in the flow of free fatty acids (FFA), which contribute to triglyceride accumulation. In addition, an increase in VLDL in hepatocytes causes an increase in oxidative stress and lipid peroxidation (Choi, 2019). This will increase proinflammatory cytokine release, blood sugar levels, and HDL levels, all of which will increase the risk of atherosclerosis and diabetes (Namavar Jahromi et al., 2017). The proinflammatory cytokine intercellular adhesion molecule-1 (ICAM-1) has been linked to atherosclerosis, insulin resistance, and cardiovascular disease. ICAM-1 expression and serum levels were greater in PCOS patients. In PCOS, HOMA-IR is a strong predictor of serum ICAM-1 levels (González, 2012; Rashad et al., 2019). The early predictor of atherosclerosis, CIMT (Carotid intima medium thickness), was found to be high in PCOS and was connected with age, BMI, waist circumference, and HOMA-IR (Kovačević et al., 2021; Mousa et al., 2023). Mean fasting insulin, homeostatic model assessment of insulin resistance index (HOMA-IR), triglycerides, total cholesterol, low density lipoprotein cholesterol, free testosterone levels, total testosterone, carotid intima media thickness (CIMT) were significantly higher in PCOS patients. Cardiac-type free fatty acid binding protein appears to have an important role in the metabolic response and subsequent development of atherosclerosis in insulin-resistant hyperandrogenemic PCOS patients (Buyukkaya et al., 2014; Furuhashi et al., 2017).

Long-term exposure to PCOS risk factors certainly has a negative impact on the health of PCOS women including the emergence of atherosclerosis at an early age. The risk increases with the increase in BMI which is closely related to insulin resistance. Young obese women with PCOS have a high prevalence of early asymptomatic coronary atherosclerosis, compared with obese controls (Patel & Shah, 2018). Based on the main PCOS pathophysiology involving insulin resistance, dyslipidemia, hyperandrogenemia, all of which make PCOS patients susceptible to future cardiovascular events (Mousa et al., 2023).

Adolescence may be a more appropriate time for intervention for PCOS patients, as many cardiovascular risks are already present during early adulthood. As far as BMI is concerned, mechanisms other than hyperandrogenemia and obesity may act as causative factors. Screening during adolescence and control of BMI and PCOS with exercise or medical therapy may alter and modify risk factors for cardiovascular and cerebrovascular disease in the future (Atasayan & Yoldemir, 2021). Weight control and physical activity can play an important role in PCOS risk management. However, there may also be a PCOS independent effect on BMI which may be mediated by low peak estradiol levels or hormonally regulated. Given the marked increase in subclinical disease even in thinner cases of PCOS, the use of insulin-lowering drugs in young women with PCOS, which was reported in short-term studies to have beneficial effects on endocrine parameters and lipid levels, should be investigated as long-term way to reduce CVD risk in later life (Kiranmayee et al., 2017; Kort, D.H., Lobo, R.A., 2014; Teede et al., 2018).

CONCLUSION

Atherosclerosis occurred in PCOS rat model induced by testosterone propionate combine with a high fat high fructose diet starting on day 21 of treatment. There is a correlation between the severity of atherosclerosis and body weight and weight increase on PCOS rat models. Polycystic ovary syndrome (PCOS) is a risk factor for atheroscerosis, as demonstrated by the findings of this study. Gain of body mass elevates the level of risk. Multiple risk factors for cardiovascular disease and diabetes mellitus are accelerated by insulin resistance, including obesity, hyperandrogenism, and oxidative stress, all of which contribute to the development of PCOS. To mitigate the risk of PCOS and its associated problems, PCOS screening and weight loss among PCOS patients via dietary and activity modifications are critical. The small sample size and the fact that the diagnosis

of atherosclerosis is only based on the aortic and coronary atherosclerosis hiystological scores are the study's limitations. More research with more samples and an examination of different atherosclerosis criteria, such as biomolecular parameters in blood and endothelial tissue using immunohistochemistry observations, can be done to support the findings of this study.

ACKNOWLEDGEMENTS

The researcher would like to thank the Universitas Muhammadiyah Yogyakarta as the funder for the research.

References

Abbara, A., Eng, P. C., Phylactou, M., Clarke, S. A., Hunjan, T., Roberts, R., Vimalesvaran, S., Christopoulos, G., Islam, R., Purugganan, K., Comninos, A. N., Trew, G. H., Salim, R., Hramyka, A., Owens, L., Kelsey, T., & Dhillo, W. S. (2019). Anti-Müllerian hormone (AMH) in the Diagnosis of Menstrual Disturbance Due to Polycystic Ovarian Syndrome. *Frontiers in Endocrinology*, *10*, 656. https://doi.org/10.3389/fendo.2019.00656

Al-Mahmood, A., Afrin, S., & Hoque, N. (2014). Dyslipidemia in Insulin Resistance: Cause or Effect. *Bangladesh Journal of Medical Biochemistry*, *7*(1), 27–31. https://doi.org/10.3329/bjmb.v7i1.18576

Altaş, M., Var, A., Köse, C., Özbilgin, K., & Arı, Z. (2010). *Endothelial dysfunction in high fructose containing diet fed rats: Increased nitric oxide and decreased endothelin-1 levels in liver tissue*. *37*(3), 7.

Andrade, M. I. S. de, Oliveira, J. S., Leal, V. S., Lima, N. M. S. da, Costa, E. C., Aquino, N. B. de, & Lira, P. I. C. de. (2016). Identification of cutoff points for Homeostatic Model Assessment for Insulin Resistance index in adolescents: Systematic review. *Revista Paulista de Pediatria (English Edition)*, *34*(2), 234–242. https://doi.org/10.1016/j.rppede.2016.01.004

Atasayan, K., & Yoldemir, T. (2021). The effect of PCOS status on atherosclerosis markers and cardiovascular disease risk factors in young women with vitamin D deficiency. *Gynecological Endocrinology*, *37*(3), 225–229. https://doi.org/10.1080/09513590.2020.1826428

Begum, G. S., Shariff, A., Ayman, G., Mohammad, B., Housam, R., & Khaled, N. (2017). *Assessment of Risk Factors for development of Polycystic Ovarian Syndrome*. *4*(1), 4.

Bitla, A. R. (2014). Oxidative Stress in Non-Obese Women with Polycystic Ovarian Syndrome. *JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH*. https://doi.org/10.7860/JCDR/2014/8125.4530

Buyukkaya, R., Besir, F. H., & Yazgan, S. (2014). The evaluation of carotid intima–media thickness and visceral obesity as an atherosclerosis predictor in newly-diagnosed polycystic ovary syndrome. *La Clinica Terapeutica*, *1*, e6–e11. https://doi.org/10.7417/CT.2014.1664

Choi, C.-I. (2019). Astaxanthin as a Peroxisome Proliferator-Activated Receptor (PPAR) Modulator: Its Therapeutic Implications. *Marine Drugs*, *17*(4). https://doi.org/10.3390/md17040242

Davinelli, S., Nicolosi, D., Di Cesare, C., Scapagnini, G., & Di Marco, R. (2020). Targeting Metabolic Consequences of Insulin Resistance in Polycystic Ovary Syndrome by D-chiro-inositol and Emerging Nutraceuticals: A Focused Review. *Journal of Clinical Medicine*, *9*(4), 987. https://doi.org/10.3390/jcm9040987

Diamanti-Kandarakis, E., & Dunaif, A. (2012). Insulin Resistance and the Polycystic Ovary Syndrome Revisited: An Update on Mechanisms and Implications. *Endocrine Reviews*, *33*(6), 981–1030. https://doi.org/10.1210/er.2011-1034

Ebrahimi-Mamaghani, M., Saghafi-Asl, M., Pirouzpanah, S., Aliasgharzadeh, A., Aliashrafi, S., Rezayi, N., & Mehrzad-Sadaghiani, M. (2015). *Association of Insulin Resistance with Lipid Profile, Metabolic Syndrome, and Hormonal Aberrations in Overweight or Obese Women with Polycystic Ovary Syndrome*. *33*(1), 12.

Escobar-Morreale, H. F., Luque-Ramírez, M., & González, F. (2011). Circulating inflammatory markers in polycystic ovary syndrome: A systematic review and metaanalysis. *Fertility and Sterility*, *95*(3), 1048-1058.e2. https://doi.org/10.1016/j.fertnstert.2010.11.036

Furuhashi, M., Ogura, M., Matsumoto, M., Yuda, S., Muranaka, A., Kawamukai, M., Omori, A., Tanaka, M., Moniwa, N., Ohnishi, H., Saitoh, S., Harada-Shiba, M., Shimamoto, K., & Miura, T. (2017). Serum FABP5 concentration is a potential biomarker for residual risk of atherosclerosis in relation to cholesterol efflux from macrophages. *Scientific Reports*, *7*(1), 217. https://doi.org/10.1038/s41598-017-00177-w

González, F. (2012). Inflammation in Polycystic Ovary Syndrome: Underpinning of insulin resistance and ovarian dysfunction. *Steroids*, *77*(4), 300–305. https://doi.org/10.1016/j.steroids.2011.12.003

Kiranmayee, D., Kavya, K., Himabindu, Y., Sriharibabu, M., Madhuri, G. J., & Venu, S. (2017). Correlations between anthropometry and lipid profile in women with PCOS. *Journal of Human Reproductive Sciences*, *10*(3), 167. https://doi.org/10.4103/jhrs.JHRS_108_16

Kort, D.H., Lobo, R.A.,. (2014). Preliminary evidence that cinnamon improves menstrual cyclicity in women withpolycystic ovary syndrome: A randomized controlled trial. *American Journal of Obstetrics and Gynecology 211*, *487.e1-487.e6*. https://doi.org/10.1016/j.ajog.2014.05.009

Kovačević, S., Brkljačić, J., Vojnović Milutinović, D., Gligorovska, L., Bursać, B., Elaković, I., & Djordjevic, A. (2021). Fructose Induces Visceral Adipose Tissue Inflammation and Insulin Resistance Even Without Development of Obesity in Adult Female but Not in Male Rats. *Frontiers in Nutrition*, *8*, 749328. https://doi.org/10.3389/fnut.2021.749328

Mousa, S., Saif, A., Fathy, M., Mansour, M., Abd Elhamid, A. M., Atef, A., Galal, M., Saad, S., & Aboulsoud, S. (2023). Assessment of early vascular changes in adult females with polycystic ovary syndrome: Correlation with insulin resistance. *Gynecological Endocrinology*, *39*(1), 2210226. https://doi.org/10.1080/09513590.2023.2210226

Namavar Jahromi, B., Dabaghmanesh, M. H., Parsanezhad, M. E., & Fatehpoor, F. (2017). Association of leptin and insulin resistance in PCOS: A case-controlled study. *International Journal of Reproductive BioMedicine*, *15*(7), 423–428. https://doi.org/10.29252/ijrm.15.7.423

Patel, R., & Shah, G. (2018). Insulin Sensitizers Modulate GnRH Receptor Expression in PCOS Rats. *Archives of Medical Research*, *49*(3), 154–163. https://doi.org/10.1016/j.arcmed.2018.08.001

Rashad, N. M., El-Shal, A. S., Abomandour, H. G., Aboelfath, A. M. K., Rafeek, M. el sayed, Badr, M. S., Ali, A. E., Yousef, M. S., Fathy, M. A., & Sharaf el din, M. taha A. (2019). Intercellular adhesion molecule-1 expression and serum levels as markers of pre-clinical atherosclerosis in polycystic ovary syndrome. *Journal of Ovarian Research*, *12*(1), 97. https://doi.org/10.1186/s13048-019-0566-5

Teede, H. J., Joham, A. E., Paul, E., Moran, L. J., Loxton, D., Jolley, D., & Lombard, C. (2013). Longitudinal weight gain in women identified With polycystic ovary syndrome: Results of an observational study in young women: Weight Gain and BMI in PCOS. *Obesity*, *21*(8), 1526–1532. https://doi.org/10.1002/oby.20213

Teede, H. J., Misso, M. L., Costello, M. F., Dokras, A., Laven, J., Moran, L., Piltonen, T., Norman, R. J., International PCOS Network, Andersen, M., Azziz, R., Balen, A., Baye, E., Boyle, J., Brennan, L., Broekmans, F., Dabadghao, P., Devoto, L., Dewailly, D., … Yildiz, B. O. (2018). Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome†‡. *Human Reproduction*, *33*(9), 1602–1618. https://doi.org/10.1093/humrep/dey256

Themes, U. (2016). Chronic Anovulation and the Polycystic Ovary Syndrome. *Obgyn Key*. https://obgynkey.com/chronic-anovulation-and-the-polycystic-ovary-syndrome/

Zuo, T., Zhu, M., & Xu, W. (2016). Roles of Oxidative Stress in Polycystic Ovary Syndrome and Cancers. *Oxidative Medicine and Cellular Longevity*, *2016*, 1–14. https://doi.org/10.1155/2016/8589318