ORIGINAL ARTICLE

The differences of glycodelin and uterus NK cell expression in obese and non-obese rats (*Rattus norvegicus*)

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ABSTRACT

Objectives: To prove the existence of differences in glycodelin levels and uterine NK cell expression in obese and non-obese female white rats of Wistar strain (*Rattus norvegicus*).
Materials and Methods: . This study used a randomized post-test only controlled group design. This in vivo study used two groups of female rats (*Rattus norvegicus*). Group 1 was treated with the high obese diet for eight weeks, and group 2 was not treated with the high obese diet. After eight weeks, the rats were weighed, the proestrus phase was synchronized, and then the rats were terminated.
Results: In this study, there was no significant difference in glycodelin levels between the obese and non-obese groups with a p= 0.821 (p >0.05). Significant differences were found in uterine NK cell expression between obese dan non-obese groups with p=0.001 (p <0.05). The correlation test of glycodelin levels and uterine NK cell expression showed insignificant results with a correlation coefficient of 0.120 and p=0.513. This proved that there was no significant correlation between glycodelin levels and uterine NK cell expression.
Conclusion: There was no significant difference between glycodelin levels and uterine NK cell expression in obese and non-obese female white rats of Wistar strain (*Rattus norvegicus*).

Keywords: Obesity; recurrent miscarriage; glycodelin; uterine NK cells

ABSTRAK

Tujuan: Untuk membuktikan adanya perbedaan kadar glikodelin dan ekspresi sel NK uterus pada tikus putih betina (*Rattus norvegicus*) galur Wistar dengan obesitas dan tidak dengan obesitas.
Bahan dan Metode: Penelitian ini menggunakan rancangan *randomized post-test only controlled group design*. Penelitian in vivo ini menggunakan dua kelompok tikus betina (*Rattus norvegicus*). Kelompok 1 diberi diet tinggi lemak selama delapan minggu, dan kelompok 2 tidak diberi diet tinggi lemak. Setelah delapan minggu tikus ditimbang dan fase proestrus disinkronisasi. Kemudian tikus diterminasi.
Hasil: Dalam penelitian ini, tidak terdapat perbedaan kadar glikodelin yang signifikan antara kelompok dengan obesitas dan tidak dengan obesitas dengan p=0,821 (p>0,05). Perbedaan yang signifikan ditemukan pada ekspresi sel NK rahim antara kelompok dengan obesitas dan tidak dengan obesitas dengan p=0.001 (p <0,05). Uji korelasi kadar glikodelin dengan ekspresi sel NK uteri menunjukkan hasil yang tidak signifikan dengan koefisien korelasi 0,120 dan p=0,513. Hal ini membuktikan bahwa tidak ada hubungan yang signifikan antara kadar glikodelin dengan ekspresi sel NK uteri.
Simpulan: Tidak terdapat perbedaan bermakna kadar glikodelin dengan ekspresi sel NK uteri pada tikus putih betina galur Wistar (*Rattus norvegicus*) yang dengan obesitas dan tidak dengan obesitas.

Kata kunci: Obesitas; keguguran berulang; glikodelin; sel NK rahim

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● pISSN:0854-0381 ● eISSN: 2598-1013 ● doi: http://dx.doi.org/10.20473/mog.V29I22021.63-67
● Maj Obs Gin. 2021;29:63-67 ● Received 10 Nov 2020 ● Revised 15 Mar 2021 ● Accepted 30 Mar 2021

INTRODUCTION

Obesity increases the risk of comorbidities such as diabetes mellitus, high blood pressure, dyslipidemia, cardiovascular disease, obstructive sleep apnea, various types of cancer and death. A study found that obesity was responsible for causing infertility in women by 25% to 50%.[1] The increase in obesity incidence correlates with an increase in the incidence of recurrent pregnancy loss (RPL) or recurrent miscarriage. At least one factor identified recurrent miscarriage in more than half of cases, but no definite cause still unknown. Several conditions are associated with idiopathic recurrent miscarriage, a suspected abnormality including immune system disorders, behavioral and environmental conditions, and obesity. Recent statistics show that two-thirds of women aged >20 years in the United States have a body mass index (BMI) of $\geq$25 kg/m^2, indicating that they are overweight or obese, and 36% of people in the United States are classified as overweight (BMI) $\geq$30 kg/m^2). Thus, being overweight is a concern for their health because they have a higher risk of causing complications in pregestational period. These complications include miscarriage, fetal death, congenital abnormalities, fetal macrosomia, gestational diabetes, preeclampsia, complications during vaginal delivery, thromboembolism, post-partum infection and difficulty breastfeeding.[1]

The mechanism that causes miscarriage in women who are overweight or obese with a history of recurrent miscarriage and the pathophysiology is still unclear. The high number of women of childbearing age who are overweight and the cause of many cases of recurrent miscarriage is unclear. So this research studied female Wistar strain (*Rattus norvegicus*) white rats in order to determine differences in glycodelin levels and uNK cell expression in obese and non-obese rats.

MATERIALS AND METHODS

Study design and subjects

This study used a true experimental design in the laboratory in vivo using a post-test only controlled group design to identify the differences in glycodelin levels and uNK cell expression in obese and non-obese female white Wistar strain rats (*Rattus norvegicus*). This research was conducted at the Parasitology Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia, from September 2019 to October 2020. The variables used in this study were obesity as independent variable and glycodelin levels and uNK cell expression as dependent variables.

Obesity

Obesity, which has a nomical scale, was measured with Lee Index. The Lee Index consists of the division of the cubicle root of the weight in grams by the nasoanal length in millimeters multiplied by 1000.

Glycodelin examination

Glycodelin was taken from blood in the rat's heart, then centrifuged to obtain pure serum. The serum was then inserted into the Eppendorf tube and then immediately checked by using the ELISA method. Glycodelin has a ratio scale.

Uterine Natural Killer Cells (uNK cells) examination

uNK cells was evaluated using monoclonal antibodies by immunohistochemical staining of the endometrial tissues. uNK cell expression was measured in the endometrial tissue with total magnification of 400x. uNK cell expression has a ratio scale.

Data analysis

Data from the calculation of glycodelin levels and uNK cells' expression for each group were processed by tabulation. Based on these tabulations, statistical test was carried out using SPSS 25.0. Before statistical analysis, the existing data were tests for normality using the Sapphiro-Wilk method. If the results were normal, it was continued with the independent t-test. A correlation test was performed to determine the relationship between glycodelin levels and uNK cell expression in obese rats. The statistical test was carried out with 95% degree of confidence with α =0.05. The statistical test results were declared significant if $p < 0.05$.

RESULTS AND DISCUSSION

Weight profile and overweight index (Lee Index)

Characteristics of the observed Lee index of the rats indicated that the mean Lee index of the obese group was 313.7 ± 10.8, and the mean Lee index of the non-obese group was 271.6 ± 12.3. Descriptively, the Lee index of the obese rats group was higher than the non-obese group.

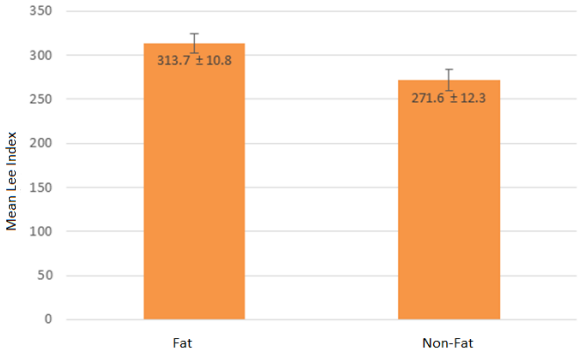

Figure 1. Lee Index characteristics

Glycodelin profile

The glycodelin level was examined after the rats, whose oestrous phase had been synchronized, were terminated. According to Lee's index, all obese rats with oestrous phase were synchronized with the oestrous phase. If the rats were in proestrus phase, termination and blood samples were taken from the rat's cardiac and uterine organs. The blood sample taken was then put into an EDTA tube and processed to obtain blood serum. The blood serum was examined for glycodelin levels by ELISA using MyBioSource Rat Progestagen-Associated Endometrial Protein (PAEP) ELISA KIT. The absorbance obtained was then converted to determine the glycodelin levels.

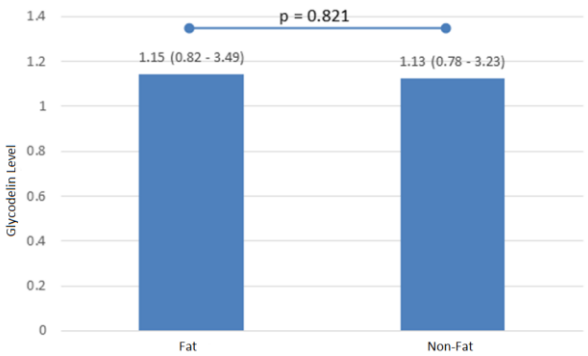

Figure 2. Glycodelin level measurement

uNK cell expression profiles

The uterus taken at termination was inserted into formalin then processed by cutting and fixation and staining with immunohistochemistry in Anatomic Pathology Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia. The finished slides were examined under a microscope at 400x magnification.

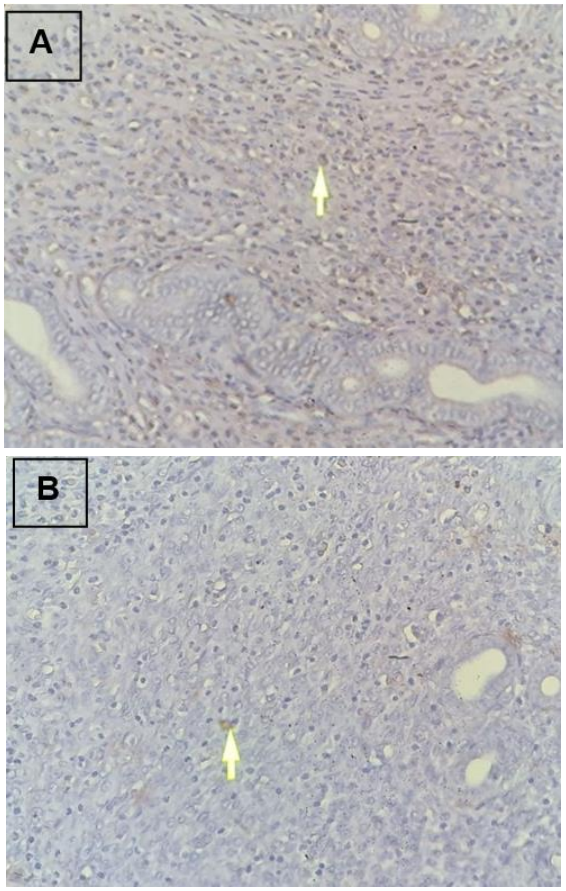

Figure 3. Histopathological profile of uNK cells in obese rat (A) and non-obese rat (B) at 400x magnification

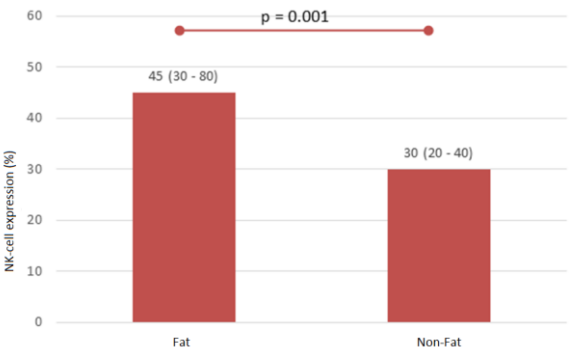

Figure 3. Comparison of uNK cells in obese rats and non-obese rats

Differences in glycodelin levels in obese and non-obese rats

This study aimed to determine whether there was a difference in glycodelin levels in obese and non-obese female rats. From the research results, the median value

of glycodelin content in the obese group was 1.15 (0.82 - 3.49), and the non-obese group was 1.13 (0.78 - 3.23), indicating that glycodelin content in the obese group of rats was higher than the non-obese group. Based on the Mann-Whitney test analysis results, the p-value was 0.821, more significant than $\alpha = 0.05$ ($p > 0.05$). So from this test, it could be concluded that there was no significant difference in glycodelin levels between the obese and non-obese groups. Overall, it could also be concluded that there was no significant difference in glycodelin levels in obese rats compared to non-obese rats with the median value of glycodelin levels in obese rats being higher than non-obese rats, but it was not statistically significant.

Overweight women, especially with upper-body obesity, have insulin resistance and hyperinsulinaemia, hyperandrogenaemia, increased peripheral aromatization of androgens to estrogen, increased gonadotrophin secretion, decreased SHBG, decreased growth hormone (GH) and (IGFBPs), increased leptin levels and neuroregulatory changes the hypothalamus-pituitary-gonadal axis. This causes ovulation disorders.[2,3]

The difference uNK cell expression in obese and non-obese rats

The study results and statistical testing showed significant differences in the expression of uNK cells between the obese and non-obese groups of rats. The median value of uNK cell expression in the obese group was 45 (30-80) and in the non-obese group was 30 (20-40). Based on the Mann-Whitney test analysis, the p-value was 0.001, smaller than $\alpha=0.05$ ($p <0.05$). So it was concluded that there was a significant difference in the expression of uNK cells between the obese and non-obese groups of rats. This test proved that the uNK cell expression of the obese rat's group was significantly higher than the non-obese group.

Obesity is a chronic subclinical inflammatory condition. Hypertrophy of obese tissue, causing hypoperfusion, hypoxia and stress on the endoplasmic reticulum are some of the mechanisms by which obesity results in increased obese tissue inflammation.[4-6] The increased release of fatty acids, hormones, and pro-inflammatory molecules in obesity is caused by changes in adipose tissue and peripheral endocrine's metabolic function. Increased peripheral leukocytes and high inflammatory conditions occur in women with obesity.[7,8]

Overweight women were found to have increased systemic inflammation, placental and endocrine function. Failure of decidualization is associated with a high rate of uNK cells, which are nearly 5% in the peri-implantation endometrium.[9] uNK cells in the endometrium are found in the endometrial tissue of women who are not pregnant with more than 30% of the total leukocytes, in contrast to the number in the blood, which is around 5-15% and in conditions of miscarriage, the cells are about 70-80% of lymphocytes.[10]

Correlation between glycodelin levels and uNK cell expression in obese rats

The results showed that there was no significant relationship between glycodelin levels and uNK cell expression. The correlation coefficient is 0.120, and the p-value is 0.513. The p-value of more than 0.05 ($p> 0.05$) proved no significant relationship between glycodelin levels and uNK cell expression due to the high glycodelin levels in obese rats, although statistically not significant, which did not affect the expression of uNK cells in the obese rats.

Obesity is associated with insulin resistance and hyperinsulinemia. Elevated insulin levels are associated with decreased glycodelin and insulin-like growth factor binding protein (IGFBP1). Low glycodelin levels are associated with recurrent miscarriage. IGFBP1 is an integral molecule involved in adhesion during implantation. Changes in these molecules can decrease endometrial receptivity in obese women.[11-15]

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Limitations

This study had limitations where the dietary treatment in rats was carried out within eight weeks so that the obesity that occurs was not chronic so that insulin resistance had not occurred, which affected the glycodelin levels.

CONCLUSION

There was no significant difference in glycodelin levels in obese and non-obese female white rats Wistar (*Rattus norvegicus*) in the study. UNK cell expression in obese female Wistar strain rats (*Rattus norvegicus*) was higher than non-obese rats. In the study, there was no correlation between glycodelin levels and uNK cell

expression in obese Wistar (*Rattus norvegicus*) female rats. Further research is needed in this study.

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