ANALYSIS OF 8-HYDROXY-DEOXYGUANOSINE (8-OHdG) LEVELS IN THE URINE AS A SIGN OF OXIDATIVE DAMAGE AND ITS RELATIONSHIP WITH METABOLIC SYNDROME IN OBESITY SUBJECTS

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ABSTRACT

Obesity has been defined as a global epidemic with a prevalence reaching 600million people worldwide. Obesity is also a risk factor for metabolic syndrome. Increased adipose tissue in obese individuals can cause systemic dysregulation resulting in inflammation and increased reactive oxygen species (ROS). This can cause oxidative stress. One of the markers of oxidative damage to DNA is 8-Hydroxy-Deoxyguanosine (8-OHdG). This study aims to analyze 8-OHdG levels in obese subjects and their relationship with Metabolic Syndrome. This research is analytical research using a cross-sectional research design involving 60 subjects. Each participant was examined for fasting blood sugar, HDL, and TG using blood samples and examination of 8-OHdG levels using urine samples with the ELISA method. The research results show that urine 8-OHdG levels in obese subjects had a higher value than non-obese subjects (p=0.308), urine 8-OHdG levels in obese subjects had relatively higher levels than non-metabolic syndrome subjects, although it did not have a significant value statistically (p=0.667), obesity status has a significant relationship with the incidence of metabolic syndrome where in obese people, there is a 25 times higher risk of developing metabolic syndrome than subjects without obesity (p<0.001). Based on these results, it can be concluded that there is no significant difference in urine 8-OHdG levels in obese compared to non-obese. The same is true for 8-OHdG levels in urine in obese subjects with and without metabolic syndrome.

Keywords: 8-Hydroxy-Deoxyguanosine (8-OHdG), Oxidative Damage, Metabolic Syndrome

INTRODUCTION

Currently, obesity has become a world problem. Even the World Health Organization (WHO) has declared obesity a global epidemic. Globally, more than 1.9 billion adults are overweight, while more than 600 million are obese.[1]Obesity is the occurrence of excessive accumulation of fat tissue, which results in a health risk. Excessive fat accumulation in obesity is due to an imbalance between energy intake and energy expenditure for a long time.[2]

Obesity is a form of malnutrition characterized by excessive fat accumulation, either by increasing size (adipocyte hypertrophy) and/or by increasing the number of cells (hyperplasia).[3]Excessive adipose tissue, especially those located in the viscera, not only functions as a tissue for storing energy reserves but also as an endocrine organ that can release certain bioactive substances known as adipocytokines or adipokines.[4]Based on the complex interactions between these adipokines, obesity is characterized by a chronic low-level inflammation condition where this condition contributes to an increase in pro-inflammatory status and oxidative stress.[5]Oxidative stress is the result of an imbalance between the production of *reactive Oxygen Species*(ROS) and antioxidant defenses, this can cause oxidative damage to lipids, proteins, and DNA.[6]

Obesity can cause systemic oxidative stress, which is associated with adipokine production and contributes to metabolic syndrome.[7] 7 Increased oxidative stress causes dysregulation of adipose tissue and is the initial pathophysiology of the metabolic syndrome. A metabolic syndrome is a group of metabolic disorders that occur together. These metabolic disorders are obesity, insulin resistance, dyslipidemia, and hypertension.[8,9]

One widely used oxidative damage marker in clinical studies is *8-hydroxy-deoxyguanosine* (8-OHdG). 8-OHdG is a marker of DNA oxidative damage.[10,11]This study aimed to analyze 8-OHdG levels in obese subjects and their relationship with metabolic syndrome.

METHOD

Study Design and Subjects

This research is analytical research using a cross-sectional research design. The subjects of this study were recruited by *purposive sampling* technique. The research subjects were adults aged 19-24 years. The inclusion criteria in this study were nutritional status based on BMI ≥ 27 kg/m2 and not smoking. The subjects of this study were divided into two groups, namely the case group and the control group. The case group was adults aged 19 -24 years with obesity (BMI≥ 27 kg/m2), and the control group was non-obese adults aged 19-24 years (BMI < 27). This study used the NCEP ATP III criteria to establish a diagnosis of metabolic syndrome.

This research was approved by the Ethics Commission of the Faculty of Medicine, University of Hasanuddin Makassar. All subjects of this study agreed and signed an informed consent form for all procedures to be carried out, such as taking and examining capillary blood and urine.

Sample Collection and Measurement

The research subjects collected the urine sample used in this study in the morning. Urine will be put into a urine pot and handed to the researcher. Urine will be stored at -20oC until assay*8-Hydroxy-deoxyguanosine* (8-OHdG) is carried out. Examination of 8-OHdG levels used the 8-Hydroxydeoxyguanosine ELISA Kit from MyBioSource, which was examined at the HUMRC Laboratory at Hasanuddin University Hospital.

The fasting blood sugar, HDL, and TG were examined using capillary blood samples taken after the study subjects had fasted. The examination uses the Autocheck tool to check Fasting Blood Sugar (GDP) and LipidPro to check HDL and Triglycerides.

Statistical Analyses

In this study, statistical analysis used SPSS 24.0 (IBM Inc., USA). The univariate test describes the characteristics of the research subjects, while the bivariate test is carried out

using the Independent T-test, the Mann Whitney U test, and the Chi-Square test, which are based on the type of data being tested. Data displayed with the mean± and p<0.05 are values that are considered statistically significant.

RESULTS

Table 1. Characteristics of Research Subjects

	Non-Obesity	**Obesity**	P-values
Gender, n			
Man (%)	23 (38.3)	21 (35.0)	0.559*
Woman (%)	7 (11.7)	9 (15.0)	
Weight (kg)	65.51 ± 11.65	90.16 ± 12.18	**<0.001****
Height(cm)	167.07 ± 8.21	164.97 ± 6.72	0.361**
BMI (kg/m2)	23.28 ± 2.59	33.14 ± 4.27	**<0.001****
Abdominal Circumference (cm)	81.83 ± 12.64	106.13 ± 14.01	**<0.001****
Blood Pressure (mmHg)			
systole	116.27 ± 6.36	120.20 ± 8.21	**0.025*****
diastole	78.80 ± 6.32	80.17 ± 6.08	0.796***
Fasting Blood Sugar (GDP)	89.70 ± 8.66	92.23 ± 18.19	0.900***
HDL (mg/dl)	39.50 ± 9.68	38.03 ± 8.73	0.541**
Triglycerides (mg/dl)	106.63 ±27.56	141.80 ± 77.04	0.222***

**chi-square test, **Independent t-test, ***Mann-Whitney U test, sig. p<0.05*

Table 1 above shows the characteristic data on each observed variable. The value of sex, weight, height, and BMI are the characteristics of the respondents who are the research subjects. In this indicator, the variables of sex and height do not show a statistically significant relationship, while the variables of weight and BMI show significant values because they are associated with clinical obesity status. In addition, the criteria for metabolic syndrome were assessed using the NCEP ATP III criteria. In this criterion, an assessment was made of five indicators where only abdominal Circumference and systolic blood pressure variables showed significant values (p <0.05)

Table 2. Comparison of urine 8-OHdG levels based on obesity status

	Non-Obesity		**Obesity**		P-values
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	
8-OHdG	1,553± 0.127	1.550(0.116)	1,587± 0.337	1.530(0.337)	0.308*

**Mann-Whitney U test*

Based on Table 2, it is known that the value of urine 8-OHdG levels in obese subjects has a higher value compared to non-obese subjects, although it does not show a statistically significant value (p=0.308)

Table 3. Relationship between obesity status and metabolic syndrome

STATUS	**METABOLIC SYNDROME**		P-values	OR (95% CI)
	No n(%)	Yes n(%)		

Obesity Status	No	29(48.33)	1(1.67)	**<0.001***	25,375
	Yes	16(26.67)	14(23.33)		(3.050-211.104)
TOTAL		45(75)	15(25)		

**chi-square test*

Table 3 shows the relationship between obesity status and the incidence of metabolic syndrome. The results above show that obesity status has a significant relationship with the incidence of metabolic syndrome. In obese people, there is a 25 times higher risk of developing metabolic syndrome than in subjects without obesity. In this study, NCEP ATP III was used as a criterion for determining metabolic syndrome status in research subjects. NCEP ATP III has five criteria, central obesity (waist circumference >40 inches for males and >35 for females), hypertension (>130 mmHg for systolic blood pressure and >85 mmHg for diastolic blood pressure), dyslipidemia (≥150 mg /dL triglyceride levels and HDL levels <40 mg/dL for males and <50 mg/dL for females) and hyperglycemia (fasting blood sugar > 100 mg/dL). Determination of metabolic syndrome status if three of the five criteria are met. Interestingly, in this study, one non-obese person had metabolic syndrome. The study subjects found that although this subject did not have abdominal circumferences that met the criteria, three NCEP ATP III criteria were met (blood pressure, fasting blood sugar, and HDL). For subjects with obesity, the criteria met varied, but the most common were low levels of HDL and abdominal circumference, while the least was blood pressure criteria.

Table 4. Relationship between urine 8-OHdG levels in obese subjects with and without metabolic syndrome

	OBESITY				
	Non-metabolic syndrome	Meds (IQR)	Metabolic syndrome	Meds (IQR)	P-values
8-OHdG	1,585±0.292	1,531±0.069	1,589±0.394	1,506±0.157	0.667*

*Mann-Whitney U test

Table 4 shows the relationship between urinary 8-OHdG levels in obese subjects with and without metabolic syndrome. The examination results showed that urine 8-OHdG levels in obese subjects had relatively higher levels than in non-metabolic syndrome, although it had no statistical significance (p=0.667)

DISCUSSION

Obesity is a health problem with increasing numbers worldwide. Obesity has a greater risk of several diseases, such as diabetes, high blood pressure, cardiovascular disease, and stroke.[12]Obesity causes a physiological imbalance in adipose tissue and its normal function, resulting in hyperglycemia, dyslipidemia, and inflammation. These conditions lead to oxidative stress, which is exacerbated because obesity also decreases the antioxidant defense system.[13]In obesity, adipose tissue dysfunction contributes to oxidative stress by increasing the expression of adipokines (MCP-1, -2, -4, and macrophage inflammatory protein (MIP) -1α, -1β, -2α) which trigger macrophage infiltration and further increase production of ROS and inflammatory cytokines.[14]ROS can bind to guanine bases on DNA which are prone to oxidation. Guanine oxidation most often forms 8-hydroxy-deoxyguanosine (8-OHdG), and therefore 8-OHdG is widely used as a biomarker for oxidative stress.[15]

In this study, measurements of 8-OHdG levels in the urine were carried out where the 8-OHdG levels in the urine of obese subjects had a higher value than non-obese subjects.

However, these results did not show a statistically significant value (p=0.308). In accordance with the results of this study, research conducted by Vanja et al. also reported no significant difference in the value of 8-OHdG in urine in obese subjects and subjects with normal weight.[16]Research conducted by Selvaraju et al. (2019) also reported no increase in 8-OHdG levels in the urine in children with obesity.[17]

Obesity is a risk factor for metabolic syndrome. ROS levels increase in obese individuals, while oxidative stress occurs due to a redox imbalance in which there is excess ROS/free radicals or a reduction in the ability of endogenous antioxidants. ROS are thought to contribute to the occurrence of insulin resistance, which is an essential precursor of metabolic syndrome.[18]In this study, it can also be seen that obesity is related to metabolic syndrome, where the odds ratio (OR) was 25. Meanwhile, the results from the comparison of 8-OHdG levels in urine in obese subjects with and without metabolic syndrome also did not show a significant value (p=0.667). Agreeing with the results of this study, Iwanaga et al., who measured urine 8-OHdG levels in subjects with and without the metabolic syndrome showed differences in urine 8-OHdG levels, but not significant.[19]In addition, Black et al. also reported the same thing.[20]

Obesity is associated with oxidative stress, which can cause DNA damage. Oxidative DNA lesions that cause modifications and breaks in DNA base sequences can be repaired with a DNA repair mechanism, namely Base Excision Repair (BER). This DNA repair mechanism is to repair DNA damage and maintain the structure of DNA.[21,22]Likewise with 8-OHdG, which is the result of oxidative DNA. The enzyme 8-oxo guanine DNA glycosylase (OGG1) is an enzyme that plays a vital role in the repair process of oxidized guanine base products, including 8-OHdG. OGG1 acts as a guanine nucleotide exchange factor, catalyzing the release of oxidized purines from DNA by cutting damaged nucleotides and replacing them with new nucleotides via the BER mechanism.[22,23]

DNA repair mechanism, especially Base Excision Repair (BER), is a factor considered influential in this study's results. BER, which plays an important role and is responsible for repairing damage to DNA bases, decreases its ability to perform DNA repair with age.[24,25,26]In line with this, it can be seen from the research conducted by Abdelmarouf et al. (2020) in subjects who were older than this study (> 40 years) and found that the results of 8-OHdG levels were significantly different in obese and non-obese subjects.[27]

The limitation of this study is that this study only assessed one biomarker (8-OHdG) and only examined the urine so that the relationship between DNA damage and obesity and metabolic syndrome was accurate. Therefore, further research is still needed by investigating more than one biomarker and/or different types of samples, such as examining blood serum.

CONCLUSION

This study showed no significant difference in urine 8-OHdG levels in obese compared to non-obese. The same is true for 8-OHdG levels in urine in obese subjects with and without metabolic syndrome. However, in this study, it can be seen that subjects with obesity are more atrisk metabolic syndrome.

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