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8- Hydroxy-Deoxyguanosine (8-OhDG) urine as a biomarker of oxidative damage in late elderly diabetes mellitus

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***Abstract*---**Diabetes Mellitus (DM) is a chronic metabolic disease characterized by high blood sugar levels that exceed normal limits. The DM pathomechanism involves oxidative stress, which has the potential to cause damage to cell components, including Deoxyribonucleic Acid (DNA). 8-hydroxy-deoxyguanosine (8-OhDG) is the most frequently detected oxidative DNA lesion. It is water-soluble and excreted in the urine. This study aimed to compare urine 8-OhDG levels in patients with controlled and uncontrolled blood sugar diabetes and healthy controls aged 56-65 years. Analytical descriptive research with a cross-sectional study design involved 80 participants determined with purposive sampling. The levels of 8-OhDG were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) method. The results showed that urine 8-OhDG levels in DM patients (1.426±0.204ng/mL) were higher than in healthy controls

(1.372±0.190ng/mL), although not statistically significant. Urine levels of 8-OhDG in DM patients aged 61-65 years (1.468±0.220ng/mL) were higher than in DM patients aged 56-60 years (1.394±0.190ng/mL), although not statistically significant. Urine levels of 8-Ohdg in controlled DM patient were lower (1.282±0.131 ng/mL) than in uncontrolled DM patient (1.569±0.159 ng/mL), were significantly different ($p<0.05$). 8-OhDG produced by the body is the final product of oxidative DNA damage. It is potentially a marker of oxidative stress related to aging in controlled and uncontrolled blood sugar DM patients. This research can still be developed by adding several variables supporting evidence that urine 8-OhDG levels can be used as a biomarker of oxidative damage in DM patients.

***Keywords*---**diabetes mellitus, aging, urine 8-OhDG, oxidative stress.

Introduction

Diabetes Mellitus (DM) is a chronic metabolic disease characterized by high blood sugar levels that exceed normal limits. In elderly, many cases of type 2 DM (DM-2) are caused by an increase in blood sugar due to decrease insulin due to damage of pancreatic gland, and also associated with an unhealthy lifestyle. Unhealthy eating patterns, irregular physical activity, not maintaining a normoweight, and tobacco use are one of the triggering factors for Type 2 DM. Type 2 DM can be treated and its consequences can be avoided or delayed with lifestyle modification such as diet, physical activity, screening, and routine medication to prevent complications (WHO Health Report, 2019). More than 90% of all DM population is type 2 DM (Eva, 2019). The International Diabetes Federation (IDF) in 2019 reported that DM cases in Indonesia ranked 7th in the world after China, India, the United States, Pakistan, Brazil and Mexico with 10.7 million people diagnosed with DM. Globally, the prevalence of DM in 20-79 years old population of the world is 8%. Countries in the Arab region – North Africa and Pakistan were ranked first and second, among the 7 countries in the world, the highest at 12.2% and 11.4%. The Southeast Asian region, including Indonesia ranks 3rd at 11.3% (Infodatin, Ministry of Health, 2019).

In line with this, The *Riset Kesehatan Dasar* (Riskesdas) 2018 showed that almost all provinces showed an increase prevalence in 2013-2018, including in South Sulawesi. The prevalence was 1.8% from the average prevalence in Indonesia, which was 2% (Badan Litbangkes, Ministry of Health, 2019). In type 2-DM, pancreatic beta cells exposed to hyperglycemia will produce Reactive Oxygen Species (ROS). Excessive increase in ROS will cause damage to pancreatic beta cell. Chronic hyperglycemia is a condition that can lead to reduced insulin synthesis and secretion and gradually damage to beta cells (Eva, 2019). In adults, beta cells have a life span of 60 days. In normal conditions, 0.5% of beta cells undergo apoptosis but are offset by replication and neogenesis. Normally, beta cell size is relatively constant so that the number of beta cells is maintained at optimal levels throughout adulthood. With age, the number of beta cells will decrease because apoptosis exceeds than replication and neogenesis. This explains the risk of type-2 DM is higher in elderly. (Eva, 2019).

The mechanism that is thought to underlie physiological aging is caused by tissue damage due to free radicals, namely oxygen, so it is called the "Free Radical Theory of Aging". (Gan Wei et al, 2018). Free radicals in the form of ROS species involved in the etiology and pathology of various health conditions, which has an important role as a major contributor to the aging process (Liochef SI, 2015). On aging process, oxidative stress produced by ROS. Oxidative stress causes oxidative damage to lipids cell membrane, proteins and DNA (Guo et al, 2017).

To assess oxidative stress in DM patients, the 8-OhDG marker can be used. 8-OhDG is a relatively plentiful and easily detectable product as a result of oxidative DNA damage. Cytosine (C) and Guanine (G) groups are the most plentiful in the body. DNA damage most often occurs in guanine (Aunan, J, R., et al 2016). Guanine is a free radical target that is very susceptible to oxidation, because it has a low redox potential (oxidation reduction reaction). The guanine in the double helix binds to a cytosine called guanosine, which when oxidized becomes 8-Hydroxy-Deoxy-Guanosine or 8-Hydroxy-2-Deoxy-Guanosine (8-OhDG). When deoxyguanosine (dG) undergoes an oxidation reaction, where the hydroxyl radical (OH•) formed can attack the position of the carbon-8 (C-8) base of guanine in DNA to form a DNA adduct which will become 8-hydroxy-2'-deoxyguanosine (8-OhDG) (Parwata., 2016, 2016). Guo, et al., 2017, Singh, et al., 2019). The interaction of HO- with the nucleic bases of the deoxyribonucleic acid (DNA) chain, such as guanine, results in the formation of C-8 hydroxyguanine (8-OHGua) or its nucleoside form deoxyguanosine (8-hydroxy-2-deoxyguanosine), with one electron reduction, 8- hydroxy-2-deoxyguanosine (8-OH-dG) is formed. The product level of 8-OhDG depends on the damage and repair level. Damaged DNA repair through Base Excision Repair (BER) mechanism can excrete the truncated damaged DNA. The damaged DNA releases 8-OhDG into the blood by the action of the DNA repair enzyme glycosylase, and then excreted in the urine (Parwata., 2016, Fenga, et al., 2017, Andarina, R and Djauhari, T., 2017, Singh, et al., 2019).

ROS are thought to be involved in the pathogenesis of DM. Increased ROS generation may be one of the factors that contribute to tissue damage in long-term DM. The damaged products resulting from DNA damage are eliminated by repair enzymes and detected as nucleoside derivatives. The levels of these products depend on the balance of damage and repair process, so these levels can be used as a relative amount of overall DNA damage by OH^- ions. A marker of oxidative DNA base damage is 8-OhDG, which occurs when OH^- oxidizes deoxyguanosine. The amount of product excreted through the urine reflects the magnitude of the damage (Indraprasta et al, 2016). Several studies have mentioned the involvement of oxidative stress in DM, because the ROS that produced such ashydroxyl radical (OH•), superoxide anion(O2-•), hydrogen peroxide (H2O2), singlet oxygen ($^1O2$) or RNS (nitric oxide) (NO) in excessive amounts can cause damage to cells and tissues in the body (Andarina, R and Djauhari, T., 2017, Singh, et al., 2019). So that 8-OhDG can be used as a marker of oxidative damage associated with the aging process (Transfo, et al., 2019, Indraprasta, et al., 2016).

To date, a number of studies have focused on 8-OhDG. 8-OhDG is a relatively plentiful and easily detectable product as a result of oxidative DNA damage after

ROS induces 8-hydroxylation of guanine bases in mitochondrial and nuclear DNA. When the damaged DNA is repaired, the 8-OhDG produced is excreted in the urine without further metabolism. Urinary 8-OhDG is more widely used as a useful and relevant marker for oxidative stress. An increase in body mass index has been shown to be associated with an increased risk of DNA damage from oxidative stress and increased risk of cardiovascular disease. Patients with type-2 DM showed significantly increased oxidative DNA damage, which was measured by 8-OhDG as the most sensitive and useful biomarker in the diagnosis of type-2 DM. Current evidence shows that 8-OhDG lesion can be found in DNA during cellular replication and produce somatic mutation. (Al-Aubaidy, 2011). The urine biomarker level shows an integrated redox balance index in a longer period of time compared to the blood level. (Il'yasova et al, 2012). Monitoring of oxidative stress in vivo is easier by the ability to use non-invasively samples, such as urine (Evans et al, 2010).

Research Methods

This study is a descriptive analytic study with a cross-sectional study approach involving 80 participants, 40 samples for the diabetes group and 40 samples for the non-diabetic group which were determined by purposive sampling. In this study, the research subjects were patients with diabetes mellitus and non-diabetes mellitus who were treated as outpatients at the Kalukubodoa Health Center and Mangasa Health Center Makassar City. Research subjects who agreed to participate in the study and signed the informed consent. The research sample was direct urine collected using the pot that had been provided. The direct urine sample then brought by the researcher to the Research Unit in the HUMRC laboratory, Hasanuddin University Hospital for examination of 8-OhDG levels using the Enzyme Linked Immunosorbent Assay (ELISA) method. The criteria for sample rejection are patients who taking antioxidant supplements or systemic anti-inflammatory drugs in the past 1 month or according to the half-life of each drug. And patients with condition of systemic disorders (malignancy, hypertension, liver disorders, kidney disorders, heart problems). Data analysis was performed in accordance with the aim and measuring scale of the data variables and then analyzed through a computer using Statistical Product and Service Solution (SPSS).

Results and Discussion

This study was performed on 40 samples with diabetes mellitus and 40 control samples that appropriate with the inclusion and exclusion criteria.

Table 1
Characteristics of Respondents

Parameter	DM N=40	Healthy control N=40
[a]Gender		
Man	14 (35.0)	14 (35.0)
Woman	26 (65.0)	26 (65.0)

[a]Age			
56-60 Years	23 (57.5)	28 (72.5)	
61-65 Years	17 (42.5)	12 (27.5)	
[a] DM			-
Controlled	20 (50.0)	-	
Uncontrolled	20 (50.0)		

n= Amount of sampel
[a]:Parameters for categorical data(n %)

In this study, the number of subjects with diabetes mellitus (DM) and healthy controls were 40 people each, consisting of 14 men (35%) and 26 women (65%), withcomparison of female subjects more than men. Of the 40 subjects with DM, 23 subjects (57.5%) were aged 56-60 years old and 17 subjects (42.5%) were aged 61-65 years old. Meanwhile, from 40 healthy control subjects, 28 subjects (72.5%) aged 56-60 years old and 12 subjects (27.5%) aged 61-65 years old. Of the 40 subjects with DM, 20 subjects with controlled DM (50%) and 20 subjects with uncontrolled DM (50%). The research results will be described as :

Differences of 8-OhDG levels between DM Group and Control Group urine

Table 2
Differences of 8-OhDG levels between DM Group and Control Group urine

Group	N	Average of 8-OhDG Urine Level (ng/ml) ± SD	*p
DM	40	1.426± 0.204	
			0.397
Healthy Control	40	1.372± 0.190	

**p: Mann-Whitney Test*

Based on the Mann-Whitney Test, showed that in the DM group, namely 1.426 ± 0.204 ng/ml, and the healthy control group, namely 1.372 ± 0.190 ng/ml, there was no significant difference in 8-OhDG urine levels in the DM group and healthy controls group (p> 0.05). 8-OhDG urine level in DM patients was higher than in healthy controls group. This condition reflects the aging process in DM patients caused by oxidative stress due to excessive ROS in the body (Goyal and Jiala, 2015). The same thing was also found in research by Tsukahara et al in Japan, 8-OhDG urine levels were higher in the case group than in the healthy control group. (Indraprasta, et al 2016). In DM condition, aging process occurs. Aging is characterized by periodic loss of physiological integrity, which can lead to impaired function and increased susceptibility to death. The aging process in DM condition is characterized by the occurrence of carbohydrate, lipid, and protein metabolism disorders associated with absolute or relative deficiency of insulin action and/or insulin secretion (Kharroubi, AT, and Darwish, HM., 2015).

Differences of 8-OhDG urine levels between age 56-60 Years and 61-65 Years in cases group.

Table 3
Differences of 8-OhDG urine levels between age 56-60 Years and 61-65 Years in cases group

Age	N	Average of 8-OhDG Urine Level (ng/ml) ± SD	*p
56-60	23	1.394± 0.190	0.268
61-65	17	1.468± 0.220	

**p: Independent Sample T-Test*

Based on the Independent Sample T-Test, the average of 8-OhDG urine level at the age of 61-65 years is 1.468 ± 0.220ng/ml, compared to the age of 56-60 years which is 1.394 ± 0.190 ng/ml, shows that there is no significant difference in 8-OhDG urine levels at the age of 56-60 years and the age of 61-65 years with DM ($p>0.05$). Age is closely related to an increase in blood sugar. With increasing age, so does the prevalence of DM especially type-2 DM. Research performed by Pasaribu in 2014 found that mostly DM patients were aged 40-60 years (Goyal and Jiala, 2015, Wardatu. A., et al, 2019). Type-2 DM mostly in middle-aged and older adults with prolonged hyperglycemia due to poor lifestyle and dietary choices. (Sapra A, Priyanka B, 2021).

The urine levels of 8-OhDG will increase with age. Apart from exogenous and endogenous factors, there are other factors that also play a major role in the occurrence of aging, namely telomeres. Telomere length decreases with age. Telomere shortening is progressive, causing the aging process of cells. Telomere length at each division will be fixed by the enzyme telomerase. In addition to telomere shortening, loss of proteostasis is also involved in the aging mechanism. Proteostasis involves protein folding stabilization mechanisms, heat-shock protein groups and protein degradation mechanisms. Changes and accumulation of misfolded proteins are factors in the aging process. There is a relation between increased protein aggregation with age, so the cell loses its ability to remove misfolded proteins over time. (Aunan, J,R., *et al* 2016).

The conditions mention above are suitable with the free radical aging theory or also known as the oxidative stress theory which states that the functional loss of the body is related to age, which is caused by the accumulation of oxidative damage by ROS to macromolecules such as DNA, lipids and proteins (Ligouri, et al., 2018). It can be concluded that over time and increasing age, the body will be exposed to more and more exogenous ROS, so that urine level of 8-OhDG will also be higher. (Indraprasta, et al 2016). Likewise, with age, the repair function becomes less efficient, DNA damage will increase due to the aging process in the body, so that the produced of urine level of 8-OhDG will also increase (Fenga, et al., 2017). So that the risk to get diabetes is strongly influenced by age that

involves ROS, so that the immune system decreases (Guo et al, 2017, Gan Wei et al, 2018).

Differences of 8-OhDG urine levels between men and women in control group

Table 4
Differences of 8-OhDG urine levels between men and women in control group

Gender	N	Average of 8-OhDG Urine Level (ng/ml) ± SD	*p
Men	14	1.343± 0.173	
			0.269
Women	26	1.387± 0.200	

**p: Maan-Whitney Test*

Based on the Mann-Whitney Test, it showed that urine levels of 8-OhDG in men were 1.343 ± 0.173 ng/ml and in women it was 1.387 ± 0.200 ng/ml, there was no significant difference in urine levels of 8-OhDG in men and women. healthy control women (p>0.05). This supported by Irwan's statement in 2010, women are more susceptible to DM than men because women have more Low Density Lipoprotein (LDL) (Wardatu, A, et al. 2019).

Differences of 8-OhDG urine levels between men and women in cases group

Table 5
Differences of 8-OhDG urine levels between men and women in cases group

Gender	N	Average of 8-OhDG Urine Level (ng/ml) ± SD	*p
Men	14	1.493± 0.225	
			0.129
Women	26	1.389± 0.187	

**p: Independent Sample T-Test*

Based on the Independent Sample T-Test, it showed that urine levels of 8-OhDG in men were 1.493 ± 0.225 ng/ml compared to women with DM, namely 1.389 ± 0.187 ng/ml, there was no significant difference in urine levels of 8-OhDG in men and women with DM (p>0.05). The results of research by Awad et al in 2011, at the Endocrine Polyclinic of RSU Prof. Dr. RD Kandou Manado, which also found more female patients, namely 78 patients (57%), while 60 men patients (43%). According on results of the research of Indraprasta et al, it was found that urine levels of 8-OhDG in the male case group were higher than in the female case group. This is because the basal metabolic rate in men is higher than in women, which produces more free radicals and causes greater DNA damage, resulting in oxidative stress that can damage the body's biocomponents (Minno, A., et al.,

2016). Oxidative stress is also more likely to occur in men, due to more activities, an unhealthy lifestyle including smoking, drinking alcohol and others. This condition can cause an imbalance between free radicals and antioxidants, which has an impact to the immune system so that the risk to get DM increase. (Altika, S., and Rahayu, RS. 2017).

Differences of 8-OhDG urine levels between controlled and uncontrolled in DM cases group

Researchers divided the controlled and uncontrolled DM to compare the level of oxidative damage.

Table 6
Differences of 8-OhDG urine levels between controlled and uncontrolled in DM cases group

DM patients	N	Average of 8-OhDG Urine Level (ng/ml) ± SD	*p
Controlled	20	1.282±0 .131	0.000
Uncontrolled	20	1.569± 0.159	

**p: Independent Sample T-test*

Based on the Independent Sample T-Test, it showed that urine levels of 8-OhDG in controlled DM were 1,282 ± 0.131ng/ml, compared to uncontrolled DM which was 1,569 ± 0.159 ng/ml, there was a significant difference in urine levels of 8-OhDG in controlled and uncontrolled DM ($p<0.05$). Controlled DM patients are who routinely seek treatment at the primary health care facility every month and check their blood sugarwhich are recorded in the medical record within normal limits. This DM patient is registered as a Referral Patient or *"Pasien Rujuk Balik"* (PRB). This is a service program for chronic disease in controlled conditions on Primary Health Care Facility and still requires long-term treatment. Patients receiving regular treatment are taking injections of rapid-acting insulin, medium-acting insulin, fast-acting insulin, or taking biguanides (metformin), sulfonylureas (glimepiride), thiazolidinediones (pioglitazone) or gliclazide (diamicron). There are DM patients who are given therapy using a combination of insulin with anti diabetics drugs, a combination of two types anti diabetic drugs, some are given with single therapy, namely insulin injection therapy alone or only one type of anti diabetic drugs. Controlling blood sugar levels is accompanied by education on changing diet and regular exercise or physical activity at least once a week.

In uncontrolled DM, there is relation between hyperglycemia, insulin resistance and vascular endothelial disorders that can cause damage to various organ systems, leading to complications. This happens because chronic hyperglycemia that synergizes with metabolic disorders can cause damage to various organ systems, leading to complications that can be life-threatening. This condition reflects the aging process in DM patients caused by oxidative stress due to

excessive ROS in the body (Goyal and Jiala, 2015). In DM condition, there is an aging process. Aging is characterized by a progressive loss of physiological integrity, leading to impaired function and an increased susceptibility to death. There are nine signs of aging namely genomic instability, telomere friction, epigenetic changes, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cell aging, stem cell exhaustion, and altered cell-to-cell communication (Aunan, J, R., et al 2016, Marques, A, R., et al 2018). The aging process in DM is characterized by disturbances of carbohydrate, lipid, and protein metabolism associated with absolute or relative deficiency of insulin action and/or secretion (Kharroubi, AT, and Darwish, HM., 2015)

Conclusion

Levels of 8-OhDG produced by the body is the end product of DNA oxidative damage, there is a tendency to be used as a marker of oxidative stress related to the aging process in controlled and uncontrolled Diabetes Mellitus (DM) patients.

Suggestion

This research can still be developed by adding several variables that support the evidence of urine levels of 8-OhDG can be used as a biomarker of oxidative damage in DM patients.

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