



## Assessment of Urinary 8-Hydroxy-2-Deoxyguanosine as Oxidative DNA Damage for Diabetic Nephropathy

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### Abstract

Diabetic nephropathy (DN) is the main cause of chronic kidney disease (CKD), and represents the most common and serious complication of diabetes. 8-hydroxy-2'-deoxyguanosine (8-OHdG) emerged as a marker of DNA damage. Therefore, the study assessed association between urinary 8-OHdG and DN as early biomarker for DN. The study group included 100 volunteers, 50 cases with DN and 50 healthy control subjects matched in age with cases. The glycated hemoglobin (HbA1c), high sensitive C reactive protein (hsCRP), erythrocyte sedimentation rate (ESR), 25 OH vitD3 and urinary 8-OHdG levels were measured. Anthropometric measures comprised body weight, height, waist circumference (WC) was measured. Significant increase in levels of HbA1c, urinary 8-OHdG, BMI and WC in DN group compared to control. Significant positive linear relation was observed between WC and urinary 8-OHdG. Moreover, significant decline of vitamin D3 and significant increase of ESR and CRP was observed in DN cases. Oxidative damage had a role in the development of DN and increased levels of 8-Hydroxy-2-Deoxyguanosine is independently associated with WC. Our study found that, both ESR and hs-CRP were increased in DN that may be independent risk factors correlated with the severity of disease.

Keywords: Diabetic nephropathy, urinary 8-OHdG, HbA1c, CRP, ESR

### 1. Introduction

One of the complications of diabetes is DN, which increases the risk of death in diabetic individuals and can cause renal failure (1). Uncontrolled blood sugar (chronic hyperglycemia) is a factor in podocyte damage and vascular problems. Recent studies concentrate on determining how glomerular endothelial damage, hypoxia and reactive oxygen species (ROS) contribute to the development of DN (2). Various markers of oxidative damage have been identified. F2-isoprostane, malondialdehyde (MDA), oxidized low density lipoprotein (LDL), MDA-modified LDL, auto-antibodies against oxidized LDL, and conjugated diene were formerly the most widely used lipid peroxidation markers. It has been determined that protein oxidation is indicated by finding novel carbonyl group, dityrosine, and oxidized histidine. There were few markers for DNA oxidation. 8-OHdG, or 8-oxodG has become recognized as a sign of oxidative stress (3). Because it is noninvasive and technically simpler, in particular urinary 8-OHdG has been evaluated most commonly to determine the degree of oxidative damage. In addition, there is a relation between elevated quantities of 8-OHdG in urine and leukocyte DNA,

which they linked to the severity of diabetic retinopathy and nephropathy (4). Patients with a higher excretion of 8-OHdG in urine compared to those with a lower or moderate excretion of 8-OHdG revealed a substantial advancement of diabetic nephropathy. The leukocyte 8-OHdG contents and the onset of nephropathy, however, were not found to be significantly correlated. Their multivariate logistic regression study indicated that among several well-known risk factors, urine 8-OHdG was the best predictor of nephropathy. Evidently, the pathophysiology of diabetic nephropathy is mostly influenced by the elevated oxidative stress. The clinical marker 8-OHdG found in urine can help diabetic patients in prediction the onset of diabetic nephropathy. Oxidative stress might accelerate the development of tubulointerstitial damage in people with diabetic nephropathy (5), ESR and hs-CRP were the most frequently utilized laboratory tests for detecting systematic inflammation among all plasma inflammatory indicators (4). ESR and hs-CRP are both significant indicators in many disorders associated with inflammation. The elevated urine albumin excretion was linked to elevated ESR and CRP (5). Beyond its function in bone/calcium metabolism, vitamin D

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also plays a significant role in type 2 diabetes (T2DM) and insulin resistance. According to research, there is a significant positive correlation between serum 1,25-OH-vitamin D and insulin secretion and sensitivity, a negative correlation between vitamin D deficiency and glycemic control, and a positive correlation between type 2 diabetes and vitamin D deficiency (6). Serum 25OHD is a measure of overall health as well as vitamin D status, which makes interpretation difficult(6,7).

2. It is well known that diabetes mellitus causes an oxidative stress state and oxidative damage, which directly contribute to the onset and progression of diabetic complications. This state is caused by an increase in reactive oxygen species production and the failure of endogenous antioxidant defenses against them. The oxidized derivative of deoxyguanosine, one of the most prevalent oxidative products of cellular DNA, is a helpful indicator of oxidative DNA damage. However, prior investigations found no correlation between spot urine levels of 8-hydroxy-2'-deoxyguanosine at diagnosis and future mortality in patients with type 2 diabetes mellitus, despite the well-known role of oxidative stress in diabetic complications. This unexpected result might be a reflection of the difficulties in doing quantitative urinalysis on type 2 diabetes mellitus patients in the community, particularly when such patients have renal disease or are poorly managed (have polyuria and hyperfiltration). 8-OHdG can also be evaluated in stored plasma samples to reduce these difficulties, but since it is a renally cleared metabolite, it must be corrected for the concurrent estimated glomerular filtration rate (eGFR).

## 2. Subjects and methods

The study group included 100 volunteers, 50 cases with DN and 50 healthy control subjects matched in age with cases. 5 ml blood were taken, divided into 2 ml on EDTA for HbA1c and 3 ml was put in a plain tube, left to clot then centrifuged and serum was separated and kept at -80 till analyzed. Also urine samples were collected.

The Helsinki Declaration on Human Experiments' rules were followed in every way by the methods employed in this investigation. The local ethics committee of the National Research Centre (No: 13176) approved the study; prior to starting the study, the control women and patients were informed of the protocol's purpose and their written informed consent was obtained.

### Anthropometric measures:

Measurements of body weight, height, waist, and hip circumferences were all part of the anthropometric assessment. On the left side of the body, each measurement was made three times, and the average of the three results was used. Measurements of height and body weight were taken to the closest 0.1 cm and 0.1 kg, respectively. The patients were measured for height while standing with their backs resting on the same scale stadiometer. Meters Square (kg/m<sup>2</sup>) was used to calculate body mass index (BMI). A plastic, non-stretchable tailor's tape was used to measure WC in centimeters.

### Biochemical measurements:

#### Estimation of hemoglobin A1c (%)

A method for determining HbA1c using high-performance liquid chromatography (HPLC) is presented (8).

### Estimation of high sensitive C reactive protein

Hs-CRP was measured using enzyme-linked immune-sorbent assay (ELISA). According SinogeneClon Biotech Co. Catalog No. SL - 0881 Hu.

### Estimation of 25- OH vit D<sub>3</sub>

Vitamin D direct ELISA Kit (EIA-4696) (DRG® International, Inc. USA) was used to measure serum 25 hydroxy vitamin D (25 (OH) vit D<sub>3</sub>) (9) .

### Estimation of urinary 8-OHdG levels

Urine Collection: The collected urine samples were centrifuged at 2000 rpm for 10 min and supernatants were withdrawn. Sample was adjusted at PH 4.5 and stored at - 20°C until assayed for the analysis of 8-OHdG by ELISA technique.

Using ELISA kits, the urinary concentrations of 8-OHdG were measured twice according SinogeneClon Biotech Co. Catalog No. SL- 2044 Hu (10).

### Estimation of the erythrocyte sedimentation rate

The Westergren method was used to measure the ESR. In order to use the Westergren method, 2 ml of venous blood must be drawn into a tube with 0.5 ml of sodium citrate. Store it for no more than two hours at room temperature or six hours at four degrees Celsius. A Westergren-Katz tube is filled with blood up to the 200 mm mark. After an hour at room temperature, the tube is positioned strictly vertically in a rack, and the distance between the upper limit of the red cell sediment and the lowest point of the surface meniscus is measured. The ESR is the erythrocyte fall distance, measured in millimeters per hour (11) .

### Statistical Analysis

The mean ± SD was used to express all values. The Shapiro-Wilk test was used to determine whether the data were normal. A non-parametric test or an independent samples t-test (two-tailed) was used to compare the DN. To assess the relationship between the parameters, regression analysis was performed.

## 3. Results

Table 1 shows means of studied variables in DN and control group. Comparison revealed significant increase in mean values of urinary 8-OHdG, waist circumference, BMI and HbA1c in DN patients compared to controls.

Figure 1 shows positive correlation between waist circumference and urinary 8-OHdG in DN patients

Table 1: Mean of age, anthropometry, HbA1C-peptide, CRP and urinary 8-hydroxy-2'-deoxyguanosine in DN cases and controls

Variables	DN	controls	P
Age (year)	37.42±9.25	37.26±8.54	0.78
Urinary 8-OHdG (pg/ml)	1063.03±86.05	993.63±14.24	0.01
Waist circumference (cm)	102.79±11.02074	82.39±7.89	0.04
BMI (kg/m <sup>2</sup> )	35.16±6.69	26.77±3.09	0.01
HbA1C %	7.83±2.12	5.53±0.44	0.01

Table 2: Mean values of serum 25(OH)D<sub>3</sub>, ESR and CRP levels in DN and controls

	DN	Control	P
25(OH)D <sub>3</sub> (ng/ml)	14.97±7.64	25.36±5.69	0.001
hsCRP (ng/ml)	9.34±5.37	5.21±4.05	0.001
ESR (mm/hr)	44.59±25.79	29.73±19.73	0.01

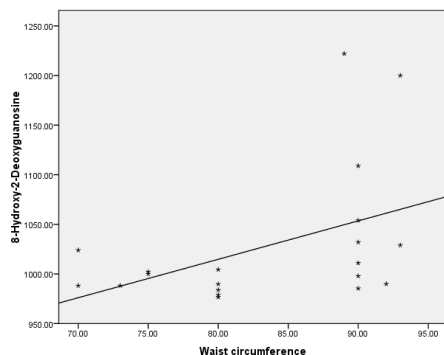


Figure 1: Linear regression between waist circumference and urinary 8-OHdG in DN cases

In DN patients, there is a positive correlation between urinary 8-OHdG and waist circumference.

#### 4. Discussion

As oxidized DNA lesion excretion in the urine (8-OHdG) was significantly higher in patients with diabetic nephropathy progression, it was discovered that endothelial cells in the glomerulus of patients with diabetic kidney disease (DKD) had increased oxidative damage. This is contrary to our findings, as they claimed that this increase in 8-OHdG is only due to progression of diabetic nephropathy (12,13). Uncontrolled blood sugar is indicated by an increase in fasting blood glucose and glycated haemoglobin in all diabetic categories (14). Results with higher urea and creatinine indicate impaired kidney function. The OHdG has been extensively utilized in numerous studies as a biomarker for the assessment of endogenous oxidative DNA damage as well as a risk factor for various disorders, comprising cancer (15,16). To the best of our knowledge, this is the first study to discuss the link between urine oxidative stress markers and deteriorating renal function. Prior studies on the relationship between renal function and indicators of oxidative stress tended to be cross-sectional and concentrated on individuals with CKD who already had the condition (17). The levels of eGFR and albumin-creatinine ratio (ACR) are linked with the oxidative stress indicators 8-OHdG, symmetric dimethylarginine (SDMA), and asymmetric dimethylarginine (ADMA) in CKD patients in Indonesia. The levels of 8-OHdG, SDMA, and ADMA significantly varied depending on the stage of CKD, whether a patient was receiving dialysis or not, and whether they had diabetes or not (18). In conclusion, 8-OHdG represents DNA oxidation and generalized cellular oxidative stress. In patients with DN, the content of 8-OHdG can be utilized as a marker for DNA damage in kidney cells and can foretell nephropathy before albuminuria develops. Further studies that looked into how vitamin D supplementation affected patients of different patient demographics' glucose metabolism (19). The findings of this population-based case-control study support the inverse correlation between serum 25(OH)D<sub>3</sub>, triglycerides (TGs), and HbA1c found in earlier research. Results from recent trials support a substantial body of data from observational studies showing vitamin D

plays a role in modifying the risk of diabetes (20). Hs-CRP levels indicate inflammation, which could be connected to incident DN (21).

The rate and severity of DKD in patients with T2DM were independently correlated with urine 8-OHdG, ESR, but not hsCRP (22). Anti-neutrophil cytoplasmic antibody-associated vasculitis is one of the many chronic diseases for which ESR is frequently utilized as a prognostic biomarker (4,23). As a result, there was a correlation between ESR and hsCRP and DN, with ESR acting as a separate risk factor for DKD and being positively connected with DKD severity. The findings back up the need for more precisely targeted antioxidant treatments to lower the risk of DNA damage and, consequently, the risk factor for diabetic kidney disease.

#### Conflict of interest

None

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