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Netrin-1 and 8-Hydroxy-2-Deoxyguanosine as Predictor Biomarkers for Diabetic Nephropathy in Type 2 Diabetes Mellitus

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Abstract

Background: Diabetic nephropathy (DN) is a significant problem for medicine. The purpose of the study was to investigate whether or not high blood levels of 8-Hydroxy-2-Deoxyguanosine (8-OHdG) and Netrin-1 may accurately predict diabetic nephropathy.

Methods: In this research, there were a total of 60 patients with type 2 diabetes mellitus (T2DM), and they were divided into three groups based on the ratio of their urine albumin to creatinine: 30 patients with T2DM who did not have DN, 30 patients with T2DM who did have DN, and 30 healthy controls. All of the individuals had their serum levels of Netrin-1, 8-OHdG, creatinine, glycosylated hemoglobin, and eGFR tested. Additionally, their fasting blood glucose, lipid prolife, and glycosylated hemoglobin were evaluated.

Results: In patients with type 2 diabetes who also had DN, the level of Netrin-1 was found to be much lower than in patients with type 2 diabetes who did not have DN. However, there was no statistically significant difference between these two groups and the control group. There was no statistically significant difference in the levels of 8-OHdG between the control group and the T2DM patients with or without DN.

Conclusion: According to the findings of our research, neither blood levels of Netrin-1 nor serum levels of 8-OHdG were substantially related with diabetic nephropathy.

Keywords: Diabetic Nephropathy, Netrin1, 8Hydroxy2Deoxyguanosine, Biomarker, Type 2 Diabetes Mellitus

1. Introduction

A significant problem in healthcare nowadays is Diabetic Nephropathy (DN). It affects up to half of people with diabetes, is a leading cause of end-stage renal disease (ESRD) requiring dialysis or a kidney transplant, and is linked to dramatically increased cardiovascular morbidity and death [1].

Although albuminuria is widely accepted as a sensitive sign of DN, it has limitations in predicting the development of the disease [2, 13]. The scientific community is moving in a new path to improve the ability of biomarkers to identify patients who may develop DN or who are at risk of advancing to ESRD [3].

The mammary gland, along with the nervous, muscular, pancreatic, vascular, and pulmonary systems, all depend on Netrin-1 for proper embryonic development. Netrins are a family of laminin-like proteins, and Netrin-1 regulates cell-cell interactions, cell migration, and adhesion of cell-extracellular matrix [4].

Numerous studies have shown that Netrin has a role in a wide range of pathologies as cancer [5], cardiovascular disorders [6], neurological con-

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ditions [7] and diabetic nephropathy progression [8]. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a byproduct of oxidative DNA damage, which happens when oxidized guanosine is removed from mitochondrial and nuclear DNA by the base excision and repair system [9]. It has been said that oxidative stress is a frequent cause of consequences of diabetes, such as nephropathy [10].So, 8-OHdG is a measure for both oxidative DNA damage and diabetic nephropathy [9].

2. Materials and methods

The study here includes 30 healthy participants and 60 type 2 diabetes patients which were collected from the Family Medicine clinic and the diabetes clinic at the Suez Canal University Hospital in Egypt.

2.1. Study groups

Study population sample was split into three groups:

- In Group I, there were 30 healthy people who were the same age and gender as controls (without any chronic illness).

- Group II had 30 people with T2DM who didn't have DN (normoalbuminuric),

- Group III was made up of 30 people with T2DM and DN (microalbuminuric).

That is based on Albumin Creatinine ratio (ACR) where:

• Normoalbuminuira is (less 30 mg albumin / gm creatinine)

• Microalbuminuria is (30 – 300 mg albumin / gm creatinine)

• Macroalbuminuria is (more than 300 mg albumin / gm creatinine) [11]

According to the standards established by the American Diabetes Association (ADA), both the diagnosis of diabetes mellitus and the confirmation of its chronic complications have been established [12, 13]. Patients having autoimmune diseases causing secondary diabetes, Patients with other chronic disease, Patients affected by chronic nephrosis, excepting DN, History of cardiovascular diseases were excluded from participation.

2.2. Demographic and anthropometric analysis:

• Blood pressure measurement

• The body mass index (BMI) may be determined with the use of the following equation: BMI = (Weight/kg) / (Height/m) ^{2}

2.3. Collecting samples and performing biochemical analyses

Approximately 5 milliliters of blood was taken from each subject in the following manner: Following the collection of 2 milliliters of the blood sample in an EDTA tube for the HbA1C test, 3 milliliters of the blood were transferred into a plain tube, allowed to clot, and then centrifuged; the serum was then placed in a freezer at a temperature of -20° C to estimate Lipid profile: low density cholesterol (LDL), total cholesterol (TC), high density cholesterol (HDL), Fasting blood sugar (FBS), triglycerides (TG), and serum creatinine (by Jaffé rate blanking method) using fully automated autoanalyzer Cobas c 501 (Roche Diagnostics, Mannheim, Germany). eGFR (mL/min/1.73 m2) was determined using the MDRD equation = 186 X [serum creatinine (mg/dl)]-1.154 X [age]-0.203 X [0.742 if patient is female] X [1.212 if patient is African American] [14].

We used an enzyme-linked immunosorbent assay (ELISA) Kit from BT LAB (Bioassay Technology Laboratory) to quantify serum Netrin-1 Cat No E1277Hu and 8-OHdG Cat No E1436Hu.

Urine samples were collected randomly to calculate the ACR using fully automated auto-analyzer Cobas c 501 (Roche Diagnostics, Mannheim, Germany).

2.4. Statistical analysis

The Shapiro-Wilk test was utilized in order to ascertain whether or not the distribution was normal. The mean and standard deviation (SD) of the quantitative data were computed, and then the chi-square test was used to analyze the data's significance. ANOVA was used to make comparisons, and the Kruskal-Wallis test was used to determine whether or not there was statistical significance. The significance level for the correlation between two quantitative variables was set at P 0.05. It was determined using the Spearman coefficient.

The Receiver Operating Characteristic (ROC) curve was created by graphing the sensitivity on the Y-axis against the specificity on the X-axis at different estimated values. This was done in order to determine the accuracy of the estimations. The performance of the test was measured by its area under the ROC curve, which served as an indication.

3. Results

The results of our analysis indicate that there is no discernible difference in terms of age or gender among the three groups that were looked at in the study we performed. When compared to the group that served as the control, both Group II Type 2 Diabetes without DN and Group III Type 2 Diabetes with DN experienced substantial increases in BMI (Table 1). As can be shown in table 1, there was no statistically significant difference in the amount of time that has passed after the diagnosis between group II T2DM patients without ND and group III T2DM patients with DN. For the purposes of measuring blood pressure, group II T2DM without DN had statistically significant elevated systolic blood pressure in comparison to the control group, while group III T2DM with DN had statistically significant elevated systolic blood pressure in comparison to group II T2DM without DN and control group also had statistically significant elevated diastolic blood pressure in comparison to control group (Table 1).

For the renal function test, group II T2DM without DN and group III T2DM with DN both had statistically significant decreases in eGFR in comparison to the control group. Additionally, group II T2DM without DN and group III T2DM with DN both had statistically significant increases in creatinine in comparison to the control group. Finally, group III DM with DN had statistically significant elevated ACR in comparison to group II T2DM without DN and the control group (Table 2).

In terms of the diabetes profile tests, there was a statistically significant difference between the three groups that were investigated with regard to FBS and HbA1C (Table 2). In terms of the lipid profile, both group II T2DM without DN and group III T2DM with DN exhibited statistically significant differences in comparison to the control group in terms of Total Cholesterol, LDL, HDL, and TG, as shown in table 2.

As can be seen in figure 1, patients in group III with type 2 diabetes and DN had a significantly lower level of Netrin-1 compared to patients in group II without type 2 diabetes, but there was no statistically significant difference between them and the control group.

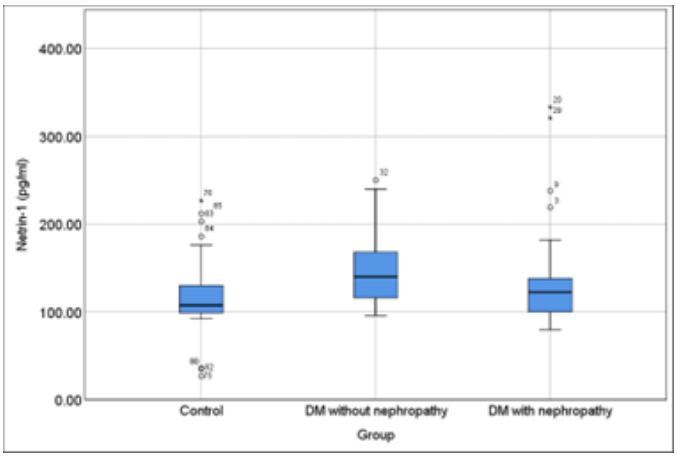

Figure 1: Figure 1: Serum levels of netrin-1 among study groups

In comparison to the control group, Group II T2DM patients without DN showed a statistically significant rise in their levels of Netrin-1. In contrast, neither group II T2DM without DN nor group III DM revealed any statistically significant change in 8-OHdG in comparison to the control group (Figure 2).

The diagnostic accuracy for the utility of 8-OHdG and Netrin-1 in the diagnosis of diabetic nephropathy are shown in figure 3. At a cut-off point of less than or equal 5.38 ng/ml for 8-OHdG, sensitivity was 53.33%, specificity was 77.97%, and accuracy was 40%. For Netrin-1, at a cut-off point of less than 103 ng/ml, sensitivity was 33.33%, specificity was 77.97%, and accuracy was 77.97%.

Table 3 displays a moderate positive statistically significant correlation between Netrin-1 and control group age, BMI, SBP, DBP, Creatinine, FBS, and TG.

Table 4 shows a moderate positive statistically significant link between 8-OHdG and Netrin-1 in

Table 1: Demographic and anthropometric data of controls and type II DM patients with and without DN

Group	Group I Control n=30	Group II T2DM without nephropathy n=30	Group III T2DM with nephropathy n=30
Variable	Mean ± SD	Mean ± SD	Mean ± SD
Age (years)	54.29 ± 10.64	53.23 ± 9.11	56.57 ± 11.04
BMI (kg/m2)	25.43 ± 2.61	31.53 ± 5.89*	29.77 ± 4.13*
Duration since diagnosis (years)	—	7.77 ± 6.88	12.23 ± 7.94
SBP (mmHg)	118 ± 4.07	126.5 ± 9.39*	134.33 ± 13.05#
DBP (mmHg)	78 ± 4.07	80.17 ± 5.17	82.67 ± 7.51*

T2DM: Type II diabetesmellitus, BMI: Body massindex, SD: standard deviation, SBP: Systolic blood pressure, DBP: Diastolicblood pressure
Data are expressed as mean ± standard deviation
(*) indicatesignificant P < 0.05 difference compared with group I control using theKruskal-Wallis test.
(#) indicate significant P < 0.05 difference compared with group II T2DMwithout nephropathy.

Table 2: Laboratory investigations of controlsand type II DM patients with or without nephropathy.

Group	Group I Control n=30	Group II T2DM without nephropathy n=30	Group III T2DM with nephropathy n=30
Variable	Mean ± SD	Mean ± SD	Mean ± SD
eGFR (ml/min/1.73 m2)	98.67 ± 6.22	75.23 ± 13.83*	66.37 ± 21.14*
ACR	7.83 ± 4.31	9.9 ± 5.05	84.87 ± 56.52*#
Creatinine (mg/dl)	0.75 ± 0.1	0.97 ± 0.2*	1.1 ± 0.25*
FBS (mg/dl)	90.47 ± 6.68	175.83 ± 89.44*	178.77 ± 62.02*
HbA1C (mg/dl)	5.28 ± 0.38	7.95 ± 1.76*	8.49 ± 1.83*
Cholesterol (mg/dl)	159.93 ± 28.56	215.03 ± 62.48*	196.9 ± 47.91*
LDL (mg/dl)	89.5 ± 25.58	137.43 ± 62.6$	121.17 ± 43.85$
HDL (mg/dl)	53.23 ± 8.08	44.13 ± 9.46*	44.67 ± 15.2*
Triglycerides (mg/dl)	83.9 ± 28	159.97 ± 77.25*	153.93 ± 39.91*

T2DM: Type II diabetesmellitus, eGFR: Estimated glomerularfiltration rate, ACR: Albumin / Creatinine ratio, FBS: Fasting blood sugar, HbA1C: Glycosylated haemoglobin,LDL: Low density cholesterol, HDL: Highdensity cholesterol.
($) indicate significant P < 0.05 differencecompared with group I control using ANOVA.
(*) indicate significant P < 0.05 differencecompared with group I control using the Kruskal-Wallis test.
(#) indicate significant P < 0.05 differencecompared with group II T2DM without nephropathy.

patients with Type 2 Diabetes and DN. In the control group, 8-OHdG was positively correlated with body mass index, eGFR, fasting blood sugar, and triglyceride levels.

4. Discussion

Microvascular complications of diabetes mellitus, such as DN, are a major cause of ESRD [15]. It involves a clinically persistent detection of proteinuria associated with increasing of blood pressure and decreased GFR [16] and stated first by the presence of microalbuminuria (30-300 mg albumin /gm creatinine) and then by macroalbuminuria (>300 mg albumin / gm creatinine) and eventually progression to ESRD [11].

It is necessary to develop a novel biomarker in

Table 3: Correlation between Netrin-1with different parameters in all studiedgroup

	Group I Control n=30		Group II T2DM without nephropathy n=30		Group III T2DM with nephropathy n=30	
	P	P value	P	P value	P	P value
Age (years)	0.346	0.061	0.157	0.407	0.352	0.057
BMI (kg/m^2)	0.402	0.028a	0.333	0.073	-0.186	0.325
Duration since diagnosis (years)	—	—	0.023	0.904	0.101	0.596
SBP (mmHg)	0.410	0.024a	-0.005	0.981	0.176	0.351
DBP (mmHg)	0.410	0.024a	-0.226	0.230	0.254	0.176
eGFR (ml/min/1.73 m^2)	-0.026	0.891	0.190	0.314	-0.017	0.927
ACR	0.166	0.382	0.110	0.564	-0.149	0.432
Creatinine (mg/dl)	0.366	0.046a	-0.335	0.070	0.055	0.771
FBS (mg/dl)	0.364	0.048a	0.051	0.791	-0.269	0.150
HbA1C (mg/dl)	0.136	0.474	0.080	0.676	-0.182	0.340
Cholesterol (mg/dl)	0.150	0.429	0.072	0.706	-0.048	0.800
LDL (mg/dl)	0.006	0.975	-0.026	0.890	-0.030	0.873
HDL (mg/dl)	-0.130	0.493	-0.049	0.795	-0.019	0.923
Triglycerides (mg/dl)	0.431	0.017^a	0.137	0.471	-0.066	0.728

BMI: Body mass index, SBP: Systolic blood pressure,DBP: Diastolic blood pressure, eGFR: Estimated glomerular filtration rate, ACR: Albumin/ Creatinine ratio, FBS: Fasting blood sugar, HbA1C: Glycosylated haemoglobin, LDL: Low density cholesterol, HDL: High density cholesterol.
$^{(a)}$ Statistically significant at P < 0.05.

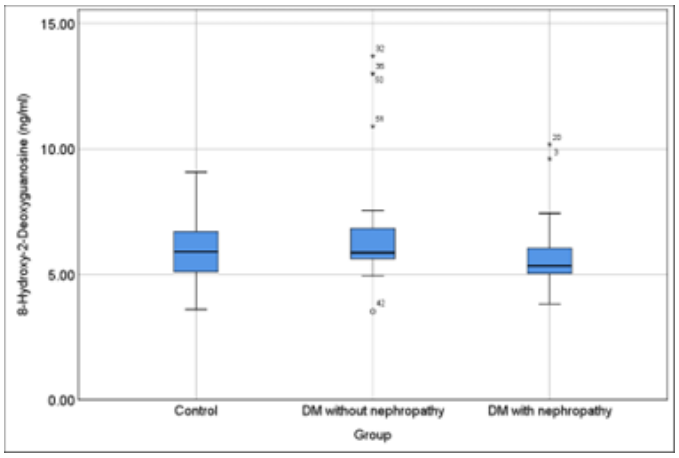

Figure 2: Serum levels of 8-OHdG among study groups

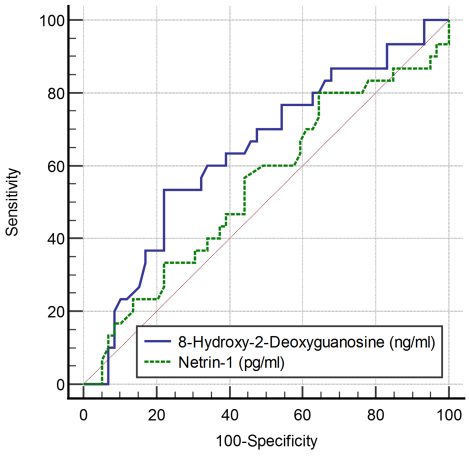

Figure 3: The diagnostic accuracy for the utility of 8-hydroxy-2-deoxyguanosineand netrin in the diagnosis of diabetic nephropathy

order to circumvent the constraints posed by both eGFR and albuminuria. The laminin-related proteins that belong to the family known as Netrin-1 are found extracellularly [17]. It is the axon-guidance molecule that transforms into an inves-

Table 4: Correlation between 8-OHdG1 with different parameters in all studied group

	Group I Control n=30		Group II T2DM without nephropathy n=30		Group III T2DM with nephropathy n=30	
	P	P value	P	P value	P	P value
Age (years)	0.153	0.419	0.023	0.904	-0.077	0.685
BMI (kg/m^2)	0.576	0.001^a	0.215	0.254	-0.033	0.864
Duration since diagnosis (years)	-	-	-0.124	0.514	0.000	0.998
SBP (mmHg)	0.029	0.879	-0.151	0.904	-0.172	0.363
DBP (mmHg)	0.029	0.879	-0.073	0.426	0.007	0.972
eGFR (ml/min/1.73 m^2)	-0.633	<0.001^a	0.196	0.702	0.022	0.908
ACR	0.077	0.687	-0.035	0.300	-0.076	0.691
Creatinine (mg/dl)	0.308	0.098	-0.112	0.853	-0.135	0.479
FBS (mg/dl)	0.475	0.008^a	-0.137	0.557	0.167	0.378
HbA1C (mg/dl)	0.034	0.857	-0.254	0.175	0.178	0.347
Cholesterol (mg/dl)	0.429	0.018^a	-0.281	0.133	0.002	0.993
LDL (mg/dl)	0.321	0.084	-0.352	0.057	0.061	0.749
HDL (mg/dl)	0.015	0.938	0.026	0.891	-0.158	0.405
Triglycerides (mg/dl)	0.260	0.164	-0.040	0.836	-0.045	0.814
Netrin-1	0.517	0.003^a	0.287	0.124	0.504	0.005^a

BMI: Body mass index, SBP: Systolic blood pressure,DBP: Diastolic blood pressure, eGFR: Estimated glomerular filtration rate, ACR: Albumin/ Creatinine ratio, FBS: Fasting blood sugar, HbA1C: Glycosylated haemoglobin, LDL: Low density cholesterol, HDL: High density cholesterol.
$^{(a)}$ Statistically significant at P <0.05.

tigative protein in the process of modifying inflammation, apoptosis, and a great number of other pathological modifications in renal tubular epithelial cells [18]. 8-OHdG is one of the main constituents of reactive oxygen species (ROS)-induced oxidative DNA damage [19]. The purpose of this study was to investigate the potential involvement of serum 8-OHdG and Netrin-1 as biomarkers for DN.

According to the findings of our research, there was no discernible difference in the sex and ages of the participants. The data were homogenous regarding the mentioned variables to eliminate bias in between the 3 groups which agrees with Parvanova et al. who found that no statistically difference between normoalbuminuric group and microalbuminuric group regarding age and sex [20].

In this study, both T2DM without DN and T2DM with DN had a significant decrease in eGFR compared to the control group. There was also a statistically significant difference between the T2DM without DN group and the T2DM with DN group and the control group when it came to serum creatinine. Kim et al. and Viswanathan et al. [21, 22].

In agreement with Li et al., the current study T2DM with DN had statistically significant elevated ACR compared to T2DM without DN and control group [23].

In our study, the T2DM group with DN had a higher HbA1C level than the T2DM group without DN and the control group, which was consistent with the study by Zeng et al. [24].

Idowu et al. discovered in 2017 that there was a statistically significant difference between ACR and HbA1C among the study groups [25]. The researchers hypothesized that the rise in serum glucose levels and HbA1C observed in this study could be explained by an increased risk of developing

DN, which contributes to beta cell destruction in type II diabetes, which in turn increases the risk of diabetic complications [26].

Consistent with the findings of Fadel et al., who discovered that patients had considerably greater Cholesterol, TG, and LDL than the control group [27], our results showed that there were statistically significant differences between patients and controls in Cholesterol, TG, LDL, and HDL.

In agreement with ACAR et al. who reported that Netrin was not significantly correlated with creatinine [28], and incompatible with Selim et al. and Jayakumar et al. respectively who found positive association between Netrin-1, creatinine, and ACR [29, 30], we found no statistically significant correlation between these three variables.

Concerning the relationship between the studied markers and DN, we found that the levels of Netrin-1 were lower in the group of diabetics with DN compared to the group of type 2 diabetics without DN. However, the diagnostic accuracy of Netrin for DN in the type 2 diabetics with DN group compared to the group of diabetics without nephropathy was 33.33% sensitive and 77.97% specific, indicating that there was no significant association with DN.

In our research, we found that patients with type 2 diabetes who received DN had significantly lower levels of Netrin-1 than patients with type 2 diabetes who did not get DN. Acute kidney injury (AKI) and chronic kidney disease (CKD) both have been shown to be associated with decreased levels of the protein Netrin-1, and we have a hypothesis on why this could be the case. Ziegon et al. found that Netrin-1 has the ability to influence immune cell motility as well as the generation of cytokines and the polarization of macrophages. Through the downregulation of inflammatory cytokines, the netrin-1 receptor UNC5B was able to mediate anti-inflammatory effects in acute kidney injury (AKI). The use of netrin-1 in treatment reduces the expression of inflammatory factors and boosts the production of anti-inflammatory molecules with beneficial effects. The reduction in inflammation, neutrophil invasion, and cell death that occurred as a result of treatment with netrin-1 led to an improvement in kidney function. As a potential therapeutic target for the development of novel markers to combat AKI and CKD, netrin-1 is being seriously explored [31].

In 2010, Pan et al. found that the amount of 8-OHdG in T2DM patients with or without microvascular problems was significantly higher than it was in normal people (P0.01). Also, patients with DN had much higher levels of 8-OHdG than diabetic patients without circulatory problems (P0.05) [32].

In agreement with our findings, Dai et al. [33] found no relationships between 8-OHdG and age, gender, DM, systolic blood pressure, or body mass index. Similarly, Leinonen et al. [34] found no associations between urine 8-OHdG and body mass index (BMI), blood pressure (BP), fasting insulin (FI), or lipid-related factors.

We found no significant differences between 8-OHdG, eGFR, creatinine, and HbA1c, which contradicts the findings of Xu et al. and Dai et al. [10, 33].

Similar to the findings of shin et al. [35], we found no statistically significant difference between 8-OHdG and FBS. Hyperglycemia has been linked to increased free radical production in diabetic patients, but these researchers found no correlation between hyperglycemia and serum 8-OHdG.

When comparing the DM with nephropathy group to the DM without nephropathy group, we found no statistically significant change in 8-OHdG. 8-OHdG had a sensitivity of 53.33 % and a specificity of 77.97 % for diagnosing diabetic nephropathy in the DM with nephropathy group compared to the DM without nephropathy group.

5. Conclusions

Our results reveal that neither Netrin-1 nor 8-OHdG in the blood are substantially linked to diabetic nephropathy.

6. Conflicts of interest

The authors report no conflicts of interest, either financial or otherwise.

7. Ethical approval

The protocol was approved by the Research Ethical Committee of faculty of science, Suez Canal University (approval no: REC30/2020).

8. Acknowledgements

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