RESEARCH ARTICLE

Correlation of anti-oxidant and pro-oxidant enzymes with insulin resistance in diabetic nephropathy

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Abstract

Objective: To assess the changes in the expression of anti-oxidant and pro-oxidant enzymes in patients with type 2 diabetes and diabetic nephropathy, and to examine their correlation with insulin resistance.

Method: The case-control study was conducted from March to November 2021 at the King Abdulaziz University Hospital, Jeddah, Saudi Arabia, and comprised adult patients of either gender with diabetic nephropathy in DN group, patients with type 2 diabetes but without diabetic nephropathy in T2D group, and non-diabetic individuals in the control group. Serum insulin levels were measured using a modular analyser, while nicotinamide adenine dinucleotide phosphate oxidase, glutathione S-transferase and superoxide dismutase 3 levels were measured using enzyme-linked immunosorbent assay. Data was analysed using SPSS 29.0.1.

Results: Of the 74 subjects, 45(60.8%) were females and 29(39.2%) were males. The overall mean age was 53±14 years. There were 20(27%) patients in DN group with mean age 60±11 years, 29(39.2%) patients in T2D group with mean age 56±12 years, and 25(33.8%) in the control group with mean age 43±11 years. Nicotinamide adenine dinucleotide phosphate oxidase levels were significantly lower in T2D and DN groups than in the control group ($p<0.05$). Glutathione S-transferase levels were significantly lower in the DN group than in the control group ($p<0.05$). T2D and DN groups had significantly lower superoxide dismutase 3 levels than the control group ($p<0.05$). Glutathione S-transferase levels correlated positively with glycated haemoglobin levels in the DN group, and negatively with fasting blood glucose levels in the T2D group ($p<0.05$). Superoxide dismutase 3 levels were negatively correlated with insulin and homeostatic model assessment of insulin in T2D group ($p<0.05$).

Conclusion: Type 2 diabetes and diabetic nephropathy induced changes in the levels of superoxide dismutase 3, glutathione S-transferase and nicotinamide adenine dinucleotide phosphate oxidase. Low levels of superoxide dismutase 3 in type 2 diabetes correlated with insulin resistance, suggesting the need for anti-oxidant replacement therapy as part of diabetes control measures to prevent diabetic nephropathy.

Key Words: Type 2 diabetes mellitus, Diabetic nephropathy, Insulin resistance, Oxidative stress, Anti-oxidant.

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Introduction

Type 2 diabetes (T2D) is a group of metabolic diseases caused by insulin resistance (IR), inhibiting the cell's glucose uptake.[1] Insulin is a polypeptide hormone secreted by beta cells of pancreatic islets (islets of Langerhans) in response to elevated plasma glucose levels. It decreases plasma glucose levels by inducing its entry into cells. This process starts with insulin to its receptor on the target cell membrane, which initiates a cascade of enzymatic reactions, resulting in glucose uptake by the cell. Hyperglycaemia associated with diabetes induces the generation of reactive oxygen species (ROS), which play causal roles in the development of secondary complications, such as diabetic nephropathy (DN).[2] Under normal conditions, the body neutralises ROS produced by pro-oxidant nicotinamide adenine dinucleotide phosphate oxidase (NOX) through the enzymatic activities of antioxidant enzymes, such as superoxide dismutase 3 (SOD3) and glutathione S-transferase (GST). However, the relationship of these anti-oxidant and pro-oxidant enzymes with IR in diabetes, with or without DN, is unclear.

GST is a phase II family isozyme that is essential for the detoxification of by-products of oxidative stress (OS).[3] It catalyses the conjugation between glutathione and various endogenous and exogenous electrophilic compounds. A significant increase in GST activity in patients with T2D and DN has been suggested as a compensatory mechanism against OS.[4]

SOD is an anti-oxidant enzyme that catalyses the dismutation of superoxide anions into hydrogen peroxide

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and molecular oxygen. It occurs in three isoforms in humans: SOD1 in the cytoplasm, SOD2 in the mitochondria, and SOD3 in the extracellular matrix.[5] SOD3, found in the extracellular matrix of various tissues, including the pancreas, skeletal muscle and blood vessels, is the major extracellular scavenger of superoxide radicals.[6] Patients with T2D exhibit a decrease in serum and urinary SOD3 levels, reflecting the early signs of damage to the vascular endothelium and susceptibility to DN.[7]

NOX catalyses the production of superoxide via the one-electron reduction of oxygen (O_2) using nicotinamide adenine dinucleotide (NAD) + hydrogen (H) (NADH) or NADPH as an electron donor. It is a potent stimulator of ROS in many tissues, particularly under hyperglycaemic conditions.[8] Interestingly, NOX also acts as an indirect anti-oxidant by reducing oxidised antioxidant enzymes.[9] The inhibition of NOX in vivo improves the IR-induced impairment of endothelial cell function.[10]

Uncontrolled blood glucose levels and IR-induced OS in diabetes may lead to early chronic kidney disease (CKD).[11] Enhancement of insulin sensitivity and anti-oxidant enzyme activity with flavonoid extract treatment in T2D mice was reported to reduce kidney dysfunction.[12] This indicates that IR and impaired anti-oxidant defence may be the leading causes of diabetic kidney complications.
The current study was planned to investigate the changes in the expression of anti-oxidant and pro-oxidant enzymes in patients with T2D, with or without DN, and to assess their correlation with IR.

Subjects and Methods

The case-control study was conducted from March to November 2021 at the King Abdulaziz University Hospital, Jeddah, Saudi Arabia. After approval from the institutional ethics review committee, medical records of patients scheduled for sample collection for routine tests were reviewed to raise the sample. The sample size was determined based on previous studies.[13-15] Patients with glycated haemoglobin (HbA1c) levels ≥6.5% and fasting blood glucose (FBG) levels ≥126mg/dL were considered diabetic.[16] Diabetic patients with estimated glomerular filtration rate (eGFR) <60mL/min/1.73m^2 and urine albumin-creatinine ratio >30mg/g were considered diabetic with DN.[16] Diabetic patients with heart or liver problems and those having type 1 diabetes were excluded. Adult patients of either gender with diabetic nephropathy were placed in DN group, patients with T2D without DN were placed in T2D group, and non-diabetic individuals were placed in the control group. T2D and DN patients followed their respective treatment plans, while those in the control group were healthy individuals with no diabetes, kidney disease or other conditions that could need medication.

After taking informed consent from all the subjects, blood samples were collected in serum-separating and ethylenediaminetetraacetic acid (EDTA) tubes. Whole blood serum 2ml and whole blood aliquots were kept at -20°C until further analysis. FBG was measured using the hexokinase (HK) method[17] with a glucose HK kit (Sigma, Poole, United Kingdom). HbA1c levels in the serum were measured using the turbidimetric inhibition immunoassay method[18] with the A1c-3 kit (Roche Diagnostics GmbH, Mannheim, Germany). The Elecsys Insulin kit (Roche Diagnostics GmbH, Mannheim, Germany) was used to measure insulin levels following a previously described protocol.[19] All immunoassays were performed on a Cobas e601 Modular Analyser (Roche Diagnostics GmbH, Mannheim, Germany), following the manufacturer's instructions.

NOX, GST, and SOD3 levels were measured using an anzyme-linked immunosorbent assay (ELISA) (MyBioSource, San Diego, CA, United States), following the manufacturer's instructions. Homeostatic model assessment of IR (HOMA-IR) and homeostatic model assessment to quantify beta-cell function (HOMA-β) were calculated using the following equations according to Matthews et al.[20]:

"HOMA-IR = " "FBG " ("mmol/L")" × Fasting Insulin (mU/L)" /22.5

"HOMA-β = " "20 × Fasting Insulin (mU/L)" /("FBG " ("mmol/L")-3.5)

Data was analysed using SPSS 29.0.1. Data normality was assessed using the Shapiro-Wilk test. The student's t-test was employed to compare groups, and data was presented as mean ± standard error of the mean (SEM). Correlation analyses were performed using the Pearson correlation coefficient (r). $P<0.05$ was considered statistically significant.

Results

Of the 74 subjects, 45(60.8%) were females and 29(39.2%) were males. The overall mean age was 53±14 years. There were 20(27%) patients in DN group with mean age 60±11 years, 29(39.2%) patients in T2D group with mean age 56±12 years, and 25(33.8%) in the control group with mean age 43±11 years.

FBG and HbA1c levels were significantly higher in T2D and DN groups compared to the control group ($p<0.001$). Insulin levels were lower in the DN group relative to the

Table-1: Intergroup comparison of patient characteristics.

	Control (n = 25)	T2D Patients (n = 29)	DN Patients (n = 20)	P-Value (T2D vs Control / DN vs. Control)
FBG (mmol/L)	5.40 ± 0.51	8.83 ± 2.81	8.77 ± 2.31	<0.001/ <0.001
HbA1c (%)	5.59 ± 0.33	7.64 ± 1.29 (n = 26)	8.18 ± 1.26 (n = 10)	<0.001/ <0.001
Insulin (pmol/L)	74.54 ± 33.41	55.25 ± 31.93	52.16 ± 61.13	0.02/0.08
HOMA-IR	2.53 ± 1.38	3.11 ± 1.96	2.90 ± 4.00	0.10/0.30
HOMA-β	110.94 ± 53.06	34.89 ± 23.51	28.47 ± 27.19	<0.001/ <0.001
NOX (ng/mL)	4.65 ± 1.34	4.07 ± 0.98	3.25 ± 1.7	0.03/0.002
GST (ng/mL)	0.70 ± 0.18	0.72 ± 0.20	0.56 ± 0.09	0.3/<0.0006
SOD3 (ng/mL)	18.77 ± 1.34	17.50 ± 3.23	17.74 ± 2.35	0.02/0.03

T2D: Type 2 diabetes, DN: Diabetic nephropathy, FBG: Fasting blood glucose, HbA1c: Glycated haemoglobin, HOMA-IR: Homeostatic model assessment of insulin resistance, HOMA-β: Homeostatic model assessment to quantify beta-cell function, NOX: Nicotinamide adenine dinucleotide phosphate oxidase, GST: Glutathione S-transferase, SOD3: Superoxide dismutase. 3..

control group (p=0.08). The HOMA-IR index was slightly elevated in T2D group compared to controls (p=0.10). The HOMA-β index was significantly reduced in both T2D and DN groups compared to the control group (p<0.001) (Table 1).

NOX levels were significantly lower in T2D and DN groups than in the control group (p<0.05). GST levels were significantly lower in the DN group than in the control group (p<0.05). T2D and DN groups had significantly lower SOD3 levels than the control group (p<0.05). GST levels correlated positively with HbA1c levels in the DN group, and negatively with FBG levels in the T2D group (p<0.05). SOD3 levels were negatively correlated with IR and HOMA-IR in T2D group (p<0.05) (Table 2).

Table-2: Correlation of NOX, GST and SOD3 levels with clinical parameters and insulin resistance in patients with T2D and DN.

		T2D			DN		
		NOX	GST	SOD3	NOX	GST	SOD3
FBG (mmol/L)	r	−.015	−.369	−.113	.046	−.128	.182
	P	.469	.024*	.280	.847	.592	.444
HbA1c (%)	r	0.214	−.189	−.035	.146	−.077	.712
	P	.147	.178	.432	.688	.832	.021*
Insulin (pmol/L)	r	−.068	.011	−.426	.136	.027	.054
	P	.363	.476	.011*	.578	.914	.826
HOMA-IR	r	−.031	−.197	−.393	.155	.007	.023
	P	.437	.153	.017*	.527	.977	.923
HOMA-β	r	−.094	.210	−.280	.035	.188	−.090
	P	.314	.137	.070	.888	.428	.706

T2D: Type 2 diabetes, DN: Diabetic nephropathy, FBG: Fasting blood glucose, HbA1c: Glycated haemoglobin, HOMA-IR: Homeostatic model assessment of insulin resistance, HOMA-β: Homeostatic model assessment to quantify beta-cell function, NOX: Nicotinamide adenine dinucleotide phosphate oxidase, GST: Glutathione S-transferase, SOD3: Superoxide dismutase, *: significant correlation (p<0.05).

Discussion

To our knowledge, the current study is the first to report a strong association between decreased levels of anti-oxidant enzymes and IR. Consistent with previous studies,[7] the current study demonstrated significantly lower SOD3 levels in patients with T2D, with or without DN, than those in healthy controls. The reduction in SOD3 levels could be explained by its high consumption under OS resulting from prolonged hyperglycaemia. The increased FBG and HbA1c levels indicated poor control of blood glucose levels in these groups, demonstrating their exposure to high blood glucose levels for long periods. This also corresponded with the DN group's positive correlation between SOD3 and HbA1c. IR, HOMA-IR, and HOMA-β results suggested an increase in IR, beta cell dysfunction, and low insulin secretion in T2D and DN groups compared to those in the control group. The negative correlation between SOD3 and insulin levels and HOMA-IR indicated an inverse relationship between enzyme levels and IR in T2D group.

Although a negative correlation was observed between GST and FBG levels in T2D group, the difference in their levels between T2D and control groups was non-significant. Concordant with these findings, a previous study showed GST levels in the DN group to be lower than those in the T2D group.[21] Sharma et al. speculated that the lower GST levels in the DN group than those in the T2D group could be explained by the lower GST activity in the DN group attributed to certain substances in the uraemic plasma[21], but the exact mechanism of low GST levels in patients with DN is unknown. Variation in GST levels in subjects with chronic kidney disease (CKD) depends on GST polymorphism,[22] which may explain the lower GST levels observed in the DN group in the present study.

NOX produces superoxide free radicals by transferring one electron to oxygen. Inhibition of NOX reduces ROS levels in patients with DN.[23] Furthermore, hyperglycaemia increases NOX levels.[24] Contrary to these findings, the current study revealed a significant decrease in NOX levels in T2D and DN groups. These conflicting results could be explained by patient heterogeneity or the medication used. However, the current results are consistent with those of a recent study in diabetic rats that showed lower NOX levels in the diabetic group than those in the control group due to reduced insulin levels.[25] Significantly lower levels of insulin in T2D group, along with high HOMA-β levels in T2D and DN groups, indicated beta cell dysfunction and low insulin secretion, which corresponded with the higher FBG and HbA1c levels in both the groups compared to the control group. Another

supporting study using a cancer model characterised by increased ROS levels showed similarly low levels of NOX, which the authors suggested could be a compensatory response to OS, or had been caused by the oxidation of proteins by ROS.[26] However, the mechanisms underlying the low levels of NOX in both groups remain undefined. On the basis of the findings, the current study suggests that anti-oxidant drugs, along with diabetes control drugs, should be prescribed as prophylaxis to prevent diabetic complications in newly-diagnosed patients with T2D.

The current study has limitations. The patients in T2D and DN groups were older than those in the control group, which could have affected the disease progression, but it has been shown using models of diabetic retinopathy (DR) that hyperglycaemia causes alterations in anti-oxidant enzyme levels regardless of age.[27] Moreover, antioxidant enzyme levels were reported to increase with age in a previous study.[28] However, the current study found decreased levels of anti-oxidant enzymes in diabetic patients with or without nephropathy, which is in agreement with previous studies.[27] Therefore, alterations in the anti-oxidant enzyme levels in the current study were related to the observed increase in blood glucose levels. Lack of information regarding the duration of diabetes and the stage of DN were the other limitations of the present study. Future studies should target larger a sample size, and examine whether the changes in the levels of these anti-oxidant enzymes in T2D and DN are due to gene polymorphisms.

Despite the limitations, the current study would be clinically useful and help design therapeutic approaches for anti-oxidant replenishment along with diabetes control drugs to manage the complications associated with diabetes.

Conclusion

The levels of anti-oxidant enzymes SOD3 and GST were decreased in patients with T2D and DN, indicating reduced protection against OS. In addition, the levels of NOX were reduced in both groups. A negative correlation between SOD3 levels and IR was observed in patients with T2D.

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TA & SS: Concept, design, data acquisition, analysis, interpretation, drafting and final approval.
HK: Concept, design and final approval.
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