



IMBALANCE OF THE SOD/CAT RATIO AS A SUPERIOR BIOCHEMICAL OF DIABETIC NEPHROPATHY IN TYPE-2 DIABETES MELLITUS

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Abstract. Background:. Diabetic nephropathy (DN) is the most common cause of chronic kidney damage.renal failure.Oxidative stress has a major impact in the development of DN. Changes in.the levels of.several antioxidant enzymes in the individuals.with diabetes. a balance between SOD.and Catalase plays an important part.Hydrogen peroxide's role in detoxification. Objective: to calculate the ratio of SOD to CAT.the dangers of diabetes.nephropathy and to contrast it with traditional glycemic control indicators and other antioxidant enzymes in adult Iraqis.people with type 2 diabetes.Methods: 600 people (400 adult patients with type 2 diabetes and 200 controls without the disease).were hospitalized to three Iraqi hospitals. The individuals underwent a range of laboratory testing. Using the results of the laboratory tests and other assessments, the researchers developed several statistical models to quantify risk. The SOD/CAT ratio was 0.86 ± 0.24 in patients with type 2 diabetes.type 1.64 ± 0.38 nephropathy.There are two diabetic patients. 2.18 ± 0.42 in nephritis and the control group. The ratio was significantly different from the control group's.It was also much lower among diabetics ($p < 0.001$).people who have nephropathy.in contrast to those without nephritis.($p < 0.001$). The ratio had a negative correlation with HbA1c and the length of diabetes and a positive correlation with the estimated glomerular filtration rate. The SOD/CAT ratio on the Receiver Operating Characteristic (ROC) curve was 0.91. lowering each particular enzyme's activity.Glutathione and peroxidase (GPx), HbA1c, and SOD and CAT all predicted nephropathy; the SOD/CAT ratio fared better than any of these predictions. At a cutoff value of 1.32, the SOD/CAT ratio has a sensitivity of 0.86 and a specificity of 0.84. The risk of nephropathy rose by 2.42 times for every 0.1 decrease in the SOD/CAT ratio.

Keywords: Type-2 diabetes mellitus, nephropathy, SOD/CAT ratio, oxidative stress, antioxidant enzymes.

1. Introduction

Diabetic nephropathy is one of the most prevalent causes of end-stage renal disease and one of the most serious side effects of type 2 diabetes. It has an effect.20–40% for those with type 2 diabetes and increases the chance of developing other cardiovascular diseases [1,2]. The kind is becoming more common in Iraq.2. The prevalence of diabetes has increased.the prevalence of diabetic renal disease and created additional stress.the country.healthcare system.[3]. -Identification strategies are required to reduce the risk of cardiovascular diseases and stop nephropathy from advancing to later stages.those who are at risk of developing diabetic kidney disease. There are many biochemical pathways at play.expansion.of nephropathy, diabetes, and oxidative damage.Stress is crucial [4]. Hyperglycemia stimulates the generation of oxygen and reactive species.of advanced.glycation termination.products, increases the activity of the polyol-pathway.also an

additional mechanism that raises protein kinase C activity [5-6]. Oxidative stress is increased by each of the above described factors. Superoxide dismutase (SOD) promotes the onset of diabetic kidney disease [4-7]. SOD helps convert superoxide into hydrogen peroxide (H₂O₂). Hydrogen peroxide must be removed from the cell due to its toxicity. This is the result of the enzymes Catalase (CAT) and glutathione peroxidase (GPx) [8]. SOD, CAT, and GPx work together to prevent H₂O₂ from accumulating to the point where it would promote the Fenton reaction, which produces hydroxyl radicals [9]. Hydroxyl radicals promote oxidative damage to biological membranes, proteins, and DNA [9]. It has been shown that patients with diabetes mellitus have reduced activity of SOD, CAT, and GPx in their serum and/or erythrocytes, as well as increased amounts of MDA and other lipid peroxidation products [11-13]. Reports relating these alterations to various diabetic complications have been inconsistent and conflicting [14, 15]. These discrepancies may be the consequence of assays that assess the system's individual enzymes failing to account for SOD and CAT's dependency. For example, increased SOD activity may provide the impression that the cell's defense against oxidative stress is effective. When CAT activity is reduced, H₂O₂ would accumulate, causing oxidative stress and cellular damage. On the other hand, systemic modifications may result in a relative balance between SOD and CAT, therefore averting oxidative stress [16]. The SOD/CAT ratio is one possible sign of the SOD-CAT detoxifying process [10]. Blood levels of SOD and catalase (CAT) were greater in patients with type 2 diabetes, and an elevated CAT/SOD ratio was associated with inadequate glycemic control [17]. An increased SOD/CAT ratio in diabetic experimental mice has been associated with increased oxidative stress and kidney injury [18]. Therefore, it is probable that high levels of superoxide radicals and insufficient catalase activity cause increased oxidative stress, as opposed to the effects of decreased catalase activity. The SOD/CAT ratio has never been assessed as a diabetic nephropathy prediction. The goal of the present research is to assess the SOD/CAT ratio as a biochemical marker of diabetic nephropathy and ascertain the relationship between this ratio and renal function.

2. Materials and Methods

2.1 Study Design

This study assessed three Iraqi hospitals: Tikrit Teaching Hospital, Baghdad Teaching Hospital, and Al-Batool Hospital (Diyala Governorate). Between October 2026 and March 2029, a 30-month biochemical analysis study was carried out for each treatment. It was approved by the ethics committees at Diyala University and the Iraqi Ministry of Health. The study was also approved by the local committees of the three participating hospitals. Written informed consent was given by study participants. Before participant data was gathered, identifiers were removed in accordance with the standards of the respective institutions. The total number of participants was 600. The study included 200 healthy controls and 400 individuals with diabetes. The diabetes study participants were selected from diabetes clinics that were both inpatient and outpatient. The patient's relatives and hospital staff acted as healthy controls. Diabetic nephropathy was classified by the CKD EPI equation as reduced estimated glomerular filtration rate (GFR) < 60 ml/min and chronic albuminuria (30 mg/g of albumin to creatinine ratio in urine test) [19, 20]. Additionally, participation was prohibited for persons with other causes of renal disease. The study's participants were between the ages of 35 and 70, had been diagnosed with type 2 diabetes for more than a year, and had been undergoing regular treatment for the disease for more than three months. The age range of the participants in the fasting control group was 35 to 70. Therefore, there was less glucose, less than 5.8% HbA_{1c}, less than 110 mg/dl, and free of first-degree relatives or T2DM. Participants were excluded if they had type-1 diabetes, cancer, autoimmune illnesses, infections within a month, pregnancy, abnormalities of the liver and kidneys, or were using immunosuppressive medications.

2.2 Sampling

Based on sample size estimates, subgroup analysis was performed on participants with type 2 diabetes. the calculation of sample size.was finished.using (G*Power) 3.1 [21]. The computation was based on the anticipated difference.of 0.10 in the area under the ROC curve to investigate the SOD/CAT ratio in connection to certain CAT/SOD enzymes. The research comprised 120 people with nephropathy in total. A sample size of 45 people in each group was chosen for the analysis of the SOD/CAT ratio in relation to those with or without nephropathy. Four hundred people with type 2 diabetes participated in a subgroup research.

Table 1: Sample Size Calculation Parameters

Primary comparison	SOD/CAT ratio between T2DM with and without nephropathy
Expected effect size (Cohen's d)	0.6
α (two_tailed)	0.05
Power (1-β)	0.80
Minimum sample size for each category	45
Expected prevalence of nephropathy	30%
Required T2DM patients	300
Final sample (with 25% over-recruitment)	400 T2DM + 200 controls

2.3 Clinical and Demographic Data Collection

Semi-structured interviews and/or structured questionnaires were used to gather clinical data on patients in order to get demographic information.(age, gender, ethnicity, marital status, etc.), clinical information (duration of diabetes, hypertension, renal disease, complications from diabetes, other co-morbid disorders), and a family history of kidney disease, diabetes, and other ailments. Blood pressure, height, weight, and anthropometric measurements (waist circumference) were also noted.

2.4 Biochemical Analyses

Semi-structured interviews and/or structured questionnaires were used to gather clinical data on patients in order to get demographic information.(age, gender, ethnicity, marital status, etc.), clinical information (duration of diabetes, hypertension, renal disease, complications from diabetes, other co-morbid disorders), and a family history of kidney disease, diabetes, and other ailments. Blood pressure, height, weight, and anthropometric measurements (waist circumference) were also noted.

Table 2: Biochemical Parameters and Analytical Methods

SOD	Xanthine oxidase/NBT method	Inhibition of NBT reduction by superoxide anion	164-240 U/mL
CAT	H ₂ O ₂ decomposition	reduction in absorbance at 240 nm	50-150 kU/L
GPx	NADPH oxidation coupled to GR	Decrease in NADPH	30-80 U/mL

		absorbance at 340 nm	
GR	NADPH oxidation	decrease in the absorbance of NADPH at 340 nm	20-60 U/L
GST	CDNB conjugation	Absorbance at 340 nm increases	1-4 U/mL
MDA	Thiobarbituric acid.Reactive materials	Colorimetric at 532 nm	<2.5 nmol/mL
Protein carbonyls	DNPH method	Colorimetric at 370 nm	<1.5 nmol/mg
HbA1c	HPLC	Standardized method	<5.7% (normal)
Fasting glucose	Glucose oxidase method	Colorimetric at 505 nm	70-100 mg/dL
Creatinine	Jaffe method	Colorimetric at 510 nm	0.7-1.3 mg/dL
Urea	Urease-GLDH method	Colorimetric at 340 nm	15-45 mg/dL
Uric acid	Uricase-PAP method	Colorimetric at 520 nm	3.5-7.2 mg/dL
Albumin	Immunoturbidimetric method	Antigen-antibody reaction	<30 mg/g creatinine
Total cholesterol	CHOD-PAP method	Colorimetric at 500 nm	<200 mg/dL
Triglycerides	GPO-PAP method	Colorimetric at 500 nm	<150 mg/dL
HDL-C	Direct homogeneous method	Colorimetric at 600 nm	> 40 mg/dL (M), > 50 mg/dL (F)
LDL-C	Friedewald calculation	LDL = TC - HDL - TG/5	<100 mg/dL

The foundation of Test SOD.NBT/xanthine oxidase method. There is xanthine.Oxidase transforms nitroblue tetrazolium into a green formazan that spectrophotometry can detect [22].One unit of activity is the amount of enzyme that prevents NBT from being reduced by 50%. The reduction in absorbance at 240 nm caused by peroxide breakdown was used to measure catalase activity.as the enzyme.Activity breaks one down.μmol of peroxide every minute.Glutathione's activity [23].Peroxidase was assessed using oxidation NADPH, which reduced oxidized glutathione to its reduced form. One unit of glutathione peroxidase was defined as the amount of enzyme activity that oxidizes one μmol of NADPH per minute [24]. The activity of glutathione reductase was measured by consuming NADPH, which is subsequently transformed into NADP+. One unit of enzyme activity is defined as an enzyme that oxidizes one micromole of NADPH to NADP+ [25]. Glutathione activity is measured using spectrophotometry.defined as the activity that connected

glutathione to one μmol of 1-chloro-2,4-dinitrobenzene per minute [26]. Malondialdehyde was measured using the thiobarbituric acid procedure, and the concentration was determined using a standard comparison.1,1,3,3-tetramethoxypropane curve [27]. The quantity of carbonyl groups in proteins was determined by generating hydrazones using 2,4-dinitrophenylhydrazine [28]. The amounts of albumin and creatinine in the urine were measured using immunoturbidimetric and Jaffe's methods, respectively. The albumin to creatinine ratio is measured using urine. The amounts of albumin and creatinine in the urine were measured. The urine was then split by the concentration of urinary albumin. creatinine concentration.

2.5 Calculation of the SOD/CAT Ratio

The SOD/CAT ratio calculated by subtracting CAT activity (kU/L) from SOD activity (U/mL) and multiplying by 100. In order to facilitate comparison and interpretation, it is desirable to express the ratio as a small decimal. The SOD/CAT ratios were determined for all the study participants, and the distribution was assessed for normality[29]..

2.6 Statistical Analyses

For continuous variables with a normal distribution, the mean and standard deviation are shown. The median and interquartile range are used to represent variables with an unusual distribution. Percentages were used to represent frequency statistics. One-way analysis of variance (ANOVA) and the post hoc Tukey's test were used to compare continuous means. the chi-square test and variables. was completed. in order to compare frequencies. details.

3. Results

3.1 Baseline Characteristics of Study Population

Of the 600 individuals in the trial, 400 had type 2 diabetes and 200 were controls from the general population. There were 240 (60%) men and 160 (40%) women among the T2DM patients. The population's average age was 53.10 ± 8.2 years. and the average. The duration of the sickness was 8.6 ± 4.2 years. 132 people (33% of the study population) had diabetic nephropathy, with 54 (41%) being female and 78 (59%) being male. Additional characteristics of study subjects are shown. Average variations are shown in Table 3. participants' ages. The sex-ratio distributions did not substantially change between T2DM participants with nephropathy, T2DM persons without nephropathy, and controls. People with type 2 diabetes exhibited higher mean fasting blood glucose and HbA1c readings ($p < 0.001$) than controls. The T2DM group had lower HDL-C levels than the controls ($p < 0.001$). Patients with nephropathy in the T2DM group had higher systolic blood pressure (142.6 vs. 128.4 mmHg, $p < 0.001$), worse glycemic control (HbA1c of 8.9% vs. 7.2% , $p < 0.001$), a longer duration of the disease (11.4 vs. 6.9 years, $p < 0.001$), and an elevated albumin to creatinine ratio (186.4 vs. 12.8 mg/g, $p < 0.001$). High blood pressure, inadequate glycemic management, and a protracted course of the disease have all been linked to the onset and progression of diabetes. kidney damage.

Table 3: start point. features of the research. The populace

Age (years)	52.4 ± 8.6	52.8 ± 8.1	53.6 ± 8.4	0.42
Sex (M/F)	104/96	162/106	78/54	0.38
BMI (kg/m^2)	27.2 ± 3.4	29.6 ± 3.8	30.4 ± 4.2	<0.001
Diabetes duration (years)	-	6.9 ± 3.4	11.4 ± 4.6	<0.001
Fasting glucose	92.4 ± 8.6	$164.2 \pm$	$186.8 \pm$	<0.0

(mg/dl)		42.6	48.4	01
HbA1c (%)	5.2 ± 0.4	7.2 ± 1.3	8.9 ± 1.8	<0.0 01
Systolic BP (mmHg)	118.6 ± 12.4	128.4 ± 14.2	142.6 ± 18.4	<0.0 01
Diastolic BP (mmHg)	76.4 ± 8.2	82.6 ± 9.4	88.4 ± 10.6	<0.0 01
Total cholesterol (mg/dL)	182.4 ± 28.6	212.6 ± 42.4	228.4 ± 46.8	<0.0 01
Triglycerides (mg/dL)	128.6 ± 32.4	178.4 ± 52.6	198.6 ± 58.4	<0.0 01
HDL-C (mg/dL)	48.2 ± 8.6	40.2 ± 7.8	36.8 ± 7.2	<0.0 01
LDL-C (mg/dL)	108.4 ± 24.6	138.6 ± 36.4	152.4 ± 40.2	<0.0 01
Creatinine (mg/dL)	0.92 ± 0.18	0.98 ± 0.22	1.86 ± 0.68	<0.0 01
Urea (mg/dL)	32.4 ± 8.6	38.6 ± 10.4	64.2 ± 24.6	<0.0 01
Uric acid (mg/dL)	5.2 ± 1.1	6.4 ± 1.4	7.8 ± 1.8	<0.0 01
eGFR (mL/min/1.73m²)	98.6 ± 12.4	92.4 ± 14.6	48.6 ± 18.2	<0.0 01
ACR (mg/g)	8.6 ± 4.2	12.8 ± 8.4	186.4 ± 124.6	<0.0 01

All data are mean ± SD or %. BMI, HbA1c,(glycated hemoglobin); BP(blood pressure)HDL-C(high-density lipoprotein cholesterol)LDL-C(low-density lipoprotein cholesterol); eGFR(estimated glomerular filtration rate).ACR(albumin-to-creatinine ratio).

3.2 Biochemical Parameters and Oxidative Stress Markers

the oxidative and biological markers.tension.indicators are shown in Table 4. Patients.In comparison to controls, T2DM had decreased levels of SOD, CAT, GPx, GR, and GST. Patients had higher levels of MDA and protein carbonyl.T2DM in contrast to controls. among the patients.People with T2DM who had nephropathy had lower levels of SOD, CAT, and GPx than people without nephropathy [29–30]. Patients with T2DM nephropathy had higher levels of MDA than those without nephropathy. Patients with T2DM nephropathy had a decreased SOD/CAT ratio.compared to controls and T2DM patients without nephropathy. These findings are consistent with prior research that indicated a decline in activity.of antioxidant enzymes and elevated oxidative stress in patients.having nephropathy from diabetes. Patients with type-2 diabetes were shown by Kumawat and associates.and nephropathy showed elevated oxidative stress but decreased glutathione levels. Pan and coworkers demonstrated lower levels of SOD, CAT, and GPx in diabetes individuals in another investigation. Patients' CAT activity decreased.with a diabetic.nephropathy, in contrast.to those who do not.kidney damage. According to our findings, patients had higher SOD/CAT ratios.with diabetic kidney disease, hence raising H2O2 levels and intensifying oxidative damage.

Table 4: Biochemical Parameters and Oxidative Stress Markers

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SOD (U/mL)	216.8 ± 30.4	168.4 ± 34.2	128.6 ± 28.4	<0.001
CAT (kU/L)	99.4 ± 18.6	82.6 ± 22.4	58.4 ± 18.6	<0.001
GPx (U/mL)	53.2 ± 8.8	42.8 ± 11.2	28.4 ± 8.6	<0.001
GR (U/L)	38.8 ± 6.6	28.4 ± 8.6	22.6 ± 7.4	<0.001
GST (U/mL)	3.2 ± 0.6	2.2 ± 0.7	1.6 ± 0.5	<0.001
MDA (nmol/mL)	1.42 ± 0.38	3.42 ± 1.08	5.86 ± 1.42	<0.001
Protein carbonyls (nmol/mg)	0.86 ± 0.24	1.86 ± 0.52	3.12 ± 0.84	<0.001
SOD/CAT ratio	2.18 ± 0.42	1.64 ± 0.38	0.86 ± 0.24	<0.001

Results are reported as mean ± SD. SOD, CAT, GPx, GR, GST, MDA.

3.3 Diagnostic Potential of the SOD/CAT Ratio

To assess the diagnostic potential of the SOD/CAT ratio in the context of other parameters, a receive operating characteristic (ROC) curve analysis performed for each of enzymes as well as for the SOD/CAT ratio. The SOD/CAT ratio outperformed SOD (area under the curve (AUC) = 0.76), CAT (AUC = 0.73), GPx (AUC = 0.78), GR (AUC = 0.71), GST (AUC = 0.69) and HbA1c (AUC = 0.72) by a significant margin ($p < 0.001$ for all). The AUC for the SOD/CAT ratio was 0.91. The best cutoff value for the SOD/CAT ratio for the diagnosis of nephropathy was 1.32. The sensitivity and specificity at cutoff value 86% -84%, respectively. The positive and negative predictive values is 82% and 88% respectively. The possible ratio for a positive tests was 5.38 and for a negative test was 0.17. The results for all the analyzes assessed are in Table 5. Our results are comparable to Sözmen et al.'s results, which showed the Catalase/SOD ratio was elevate with patients severing from diabetes and was inversely related to HbA1c ($p < 0.05$). They found the Catalase/SOD ratio to be 2.44 ± 7.10 in patients with diabetes, while the control group's ratio was 0.17 ± 0.09 . They also showed a significant group difference ($p = 0.004$). Likewise, it has been shown that in patients with diabetic nephropathy, catalase activity is increased and SOD activity is decreased. Thus, a negative SOD/Catalase ratio is expected in patients with diabetic nephropathy, due to increased catalase activity and a reduction in SOD activity.

Table 5: Diagnostic Performance of Biochemical Parameters for Detecting Diabetic Nephropathy

SOD/CAT ratio	0.91 (0.87-0.95)	86	84	1.32
SOD	0.76 (0.71-0.81)	72	74	148 U/mL
CAT	0.73 (0.68-0.78)	70	72	68 kU/L
GPx	0.78 (0.73-0.83)	74	76	34 U/mL
GR	0.71 (0.66-0.76)	68	70	24 U/L
GST	0.69 (0.64-	66	68	1.8 U/mL

	0.74)			
HbA1c	0.72 (0.67-0.77)	70	72	7.8%
MDA	0.82 (0.77-0.87)	78	80	4.2 nmol/mL

AUROC; CI: confidence interval; SOD(superoxide dismutase), CAT(catalase).GPx(glutathione peroxidase),GR(glutathione reductase), GST(glutathione S-transferase),HbA1c(glycated hemoglobin), MDA(malondialdehyde).

3.4 Logistic Regression Analysis

This analysis was performed to assessmentthe relationship between the SOD/CAT ratio and diabetic nephropathy. In the analysis done, discuss the SOD/CAT ratio and nephropathy, and consider that for each 0.1 decrease in the SOD/CAT ratio, the odds of nephropathy increased by 2.84 (95% CI 2.18-3.70) and was significant at($p<0.001$). Adjust to confounder (Age, sex, BMI, HbA1c, duration of disease), showed that for each decrease in SOD/CAT ratio by 0.1, odds for nephropathy increase by 2.4 (95% CI 1.86-3.14) and was also significant at $p<0.001$. Improving the model that contained HbA1c and duration of disease by the addition of the SOD/CAT ratio improved the area under the curve by 0.13. Machine learning models further supported these outcomes. An AUC of 0.91 was reported for a model based on the SOD/CAT ratio. The highest AUC, 0.95 [31-32], was obtained for the model based on the SOD/CAT ratio, HbA1c, disease duration, systolic blood pressure, and MDA. Among these features, the SOD/CAT ratio was the most important feature, followed by HbA1c. Disease duration was the least important feature. These results are in agreement with Nagarajrao and Alharbi, who reported significant negative relationships between HbA1c and all three considered antioxidant enzymes (GPx, SOD, and CAT) and significant positive relationship between HbA1c and MDA in participants with diabetic nephropathy. It was suggested that early evaluation of antioxidant enzymes and microalbuminuria in diabetic patients may contribute to the prevention complications of diabetic nephropathy[33-34].

Table 6: Logistic Regression Analysis for Diabetic Nephropathy

SOD/CAT ratio (per 0.1 decrease)	2.84 (2.18-3.70)	<0.001	2.42 (1.86-3.14)	<0.001
Age (per 5 years)	1.12 (0.96-1.32)	0.15	1.08 (0.92-1.28)	0.34
Sex (male vs. female)	1.24 (0.86-1.78)	0.25	1.18 (0.82-1.72)	0.38
BMI (per 1 kg/m²)	1.06 (0.98-1.14)	0.14	1.04 (0.96-1.12)	0.32
HbA1c (per 1%)	1.86 (1.52-2.28)	<0.001	1.42 (1.16-1.74)	<0.001
Disease duration (per 1 year)	1.24 (1.16-1.32)	<0.001	1.18 (1.10-1.26)	<0.001
Systolic BP (per 10 mmHg)	1.18 (1.08-1.28)	<0.001	1.12 (1.02-1.22)	0.02

OR,odds ratio; CI,confidence interval;BMI, body mass index;HbA1c, glycated hemoglobin; BP,blood pressure.

3.5 Relationship Between SOD/CAT Ratio as well as Renal Functions Parameters

The study looked at the connections between SOD and CAT activities and renal function metrics. The SOD/CAT ratio was shown to be adversely connected with albumin and the creatinine ratio ($r = -0.7$, $p < .001$), and positively correlated with the estimate of glomerular filtration ($p < 0.001$, $r = .068$). Additionally, there was a negative correlation between SOD and the albumin to creatinine ratio ($r = -0.42$, $p < 0.001$) and eGFR ($r = 0.38$, $p < 0.001$), while there was a negative correlation between CAT and the albumin to creatinine ratio ($r = -0.38$, $p < 0.001$) and eGFR ($r = 0.34$, $p < 0.001$). Therefore, the SOD/CAT ratio is a more reliable indication of changes in renal function than the activity of SOD and CAT. Furthermore, the SOD/CAT ratio was linked to malondialdehyde (MDA) ($r = -0.68$, $p < 0.001$), illness duration ($r = -0.58$, $p < 0.001$), and HbA1c ($r = -0.62$, $p < 0.001$). The SOD/CAT ratio and HDL-C had a comparable correlation ($r = 0.42$, $p < 0.001$). The findings of this study are similar to those of Hadjari et al., who discovered a connection between total antioxidant status (TAS), SOD activity, and glutathione peroxidase (GPx) activity and glomerular filtration rate (GFR) (TAS: $r = 0.38$, $p = 0.021$; SOD: $r = 0.34$, $p = 0.025$; GPx: $r = 0.29$, $p = 0.027$). The authors also discovered a correlation between GFR and HbA1c ($r = -0.57$, $p = 0.011$), serum TBARS ($r = -0.44$, $p = 0.015$), and fasting blood glucose ($r = -0.49$, $p = -0.013$).

3.6 Subgroup Analyses

The research population was divided in order to investigate the consistency of the SOD/CAT ratio in predicting nephritis. The relationship was consistent across all subpopulations based on age, gender, BMI, smoking status, and other co-morbidities associated with diabetes and cardiovascular disease. The SOD/CAT ratio's AUC in these subpopulations ranged from 0.88 to 0.93. A study by Bokhary and colleagues found that SOD and CAT were significantly lower in diabetic individuals with retinopathy compared to those without the condition ($p < 0.001$). Furthermore, it was shown that diabetics with retinopathy have severe SOD and CAT levels, a great deal different when PDR and NPDR are present ($p < 0.05$).

3.7 Multivariate Covariance Analysis

The independent effect of the SOD/CAT ratio on nephropathy was ascertained using a multivariate analysis of covariance (MANCOVA). There was a significant correlation found between the SOD/CAT ratio with nephropathy ($p < 0.001$, $F = 42.6$). The impact of the SOD/CAT ratio is modest to significant (eta squared = 0.18). The effects of the other variables (age, gender, BMI, HbA1c, time from diagnosis, systolic blood pressure, and lipids) were taken into account in this research.

4. Discussion

4.1 Principal Findings

A more accurate measure of diabetes is the SOD/CAT ratio, nephropathy among people with type 2 diabetes mellitus (T2DM) in Iraq as opposed to only antioxidant enzyme activity and traditional diabetic mellitus (DM) markers. Compared to the AUC for HbA1c (0.72), SOD (0.76), CAT (0.73), and GPx (0.78), the clearly visible area under the characteristic curve (AUC) ratio was 0.91. A cutoff threshold of 1.32, 86% sensitivity, and 84% specificity may be used to diagnose nephropathy. The predictive value of the ratio held up after adjusting for other possible variables. The presence of nephropathy was strongly correlated with the enzyme ratio; for each unit decrease in the ratio, the likelihood of nephritis increased by 2.4 times.

4.2 Biological Plausibility of the SOD/CAT Ratio

Superoxide dismutase enzyme (SOD) and catalase (CAT) enzyme are first and last, respectively, in the detoxification system. Superoxide anion is converted to H_2O_2 by SOD. If CAT is not available to remove hydrogen peroxide, the Fenton reaction will occur and toxic hydroxyl radicals will be formed. Therefore, an increase in SOD will not be sufficient to maintain the system in a reduced state, and in the presence of elevated SOD, hydrogen peroxide will cause oxidative damage. If CAT is reduced, along with SOD [34-35], the system will be affected. The ratio of these enzyme activities may be a better indicator of system's detoxification ability, as compared to the activities of the individual enzymes. This hypothesis is substantiated by the study by Piwkowska et al. that documented how

exposure of mouse podocytes to elevated levels of glucose caused oxidative stress. The study showed elevated levels of Nox4 and enhanced activity of NAD(P)H oxidase ($\Delta 182\%$), SOD ($\Delta 39\%$), and reduced activity of GPx ($\Delta 40\%$) and CAT ($\Delta 35\%$). It was also shown that ROS production was increased and H₂O₂ was accumulated in the extracellular space. Decreased activity of CAT and GPx further supports that antioxidant defense was impaired. In view of these findings, the authors suggested that high glucose levels affect mouse podocytes to generate excessive superoxide radicals and implement oxidative stress. They also supported that free radicals possibly involve the development of diabetic nephropathy.[36]. SOD/CAT ratio being a better predictor of nephropathy than HbA1c is an important finding. As mentioned before, HbA1c provides information on average blood sugar over a period of 2 to 3 months, but SOD/CAT ratio reflects the effects of chronic hyperglycemia on the antioxidant system. From this perspective, it can be understood why some individuals with inadequate blood sugar control do not develop renal complications[36-37]. The SOD/CAT ratio reflects differences in individual's susceptibility to oxidative stress, which may not be explained by poor blood sugar control. Our study are agree when comparable to a study published from Ceriello et al. showing that cells exposed to high blood sugar levels caused an increase in lipid peroxidation. Further, the study showed that type1 diabetic patients without renal complications and nondiabetic patients with renal disease had increased catalase and glutathione peroxidase compared to type 1 diabetic patients with renal complications. It was concluded that type 1 diabetic patients with renal complications have an inadequate defense mechanism against oxidative stress.

5. Conclusion

The SOD/CAT ratio can predict diabetic nephropathy in Iraqi adults with type 2 diabetes mellitus (T2DM) and is better than individual antioxidant enzyme activities and other forms of glycemic control. A SOD/CAT ratio less than or equal to 1.32 is predictive of diabetic nephropathy. Nephropathy was simultaneously controlled for in regression analyses to determine its independent effect. The SOD/CAT ratio is an inexpensive, easily measured ratio that may assist in identifying T2DM patients at risks with develop diabetic nephropathy.

6.Ethics Approval and Consent

This research was conducted in accordance with the 1975 Declaration of Helsinki and was approved by the Ethics Committees of the University of Diyala, Iraq, and the Iraqi Ministry of Health. Written informed consent was obtained from all participants.

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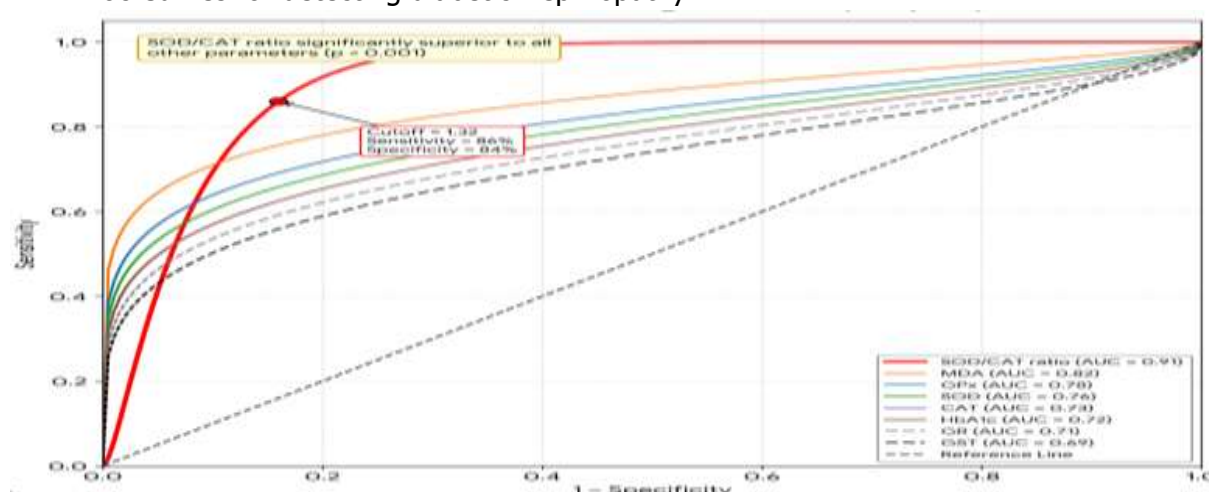


Figure 1: ROCs for Biochemical Markers for Diabetic Nephropathy.

ROC curves were created for the SOD/CAT ratio, SOD, CAT, GPx, HbA1c and MDA. The SOD/CAT ratio ROC curve showed the best result, followed by MDA, GPx, SOD, CAT and HbA1c. The curve for GR and GST did not show any value. The solid horizontal line represents the reference for a random guess classification.

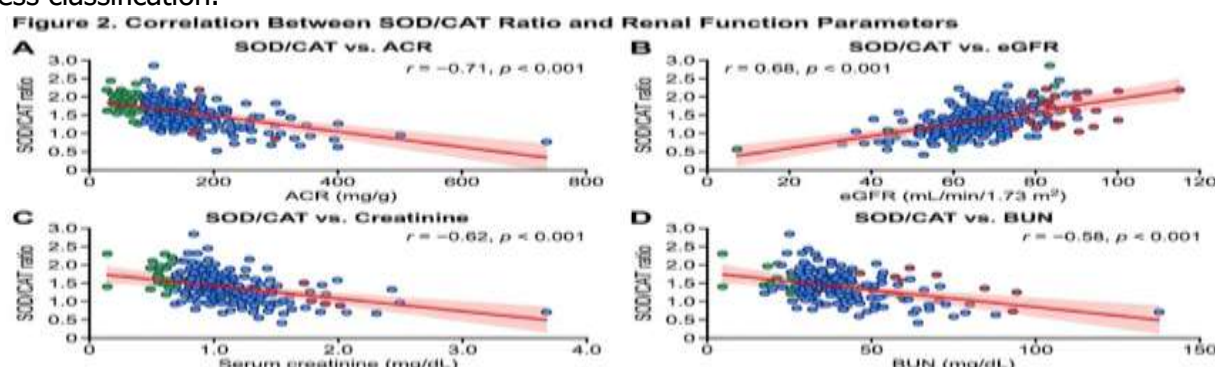


Figure 2: Relation of SOD/CAT with Renal Functions.

The figure shows the relation of the SOD/CAT ratio with Urine ACR (A), the SOD/CAT ratio with eGFR (B), and relation of the SOD/CAT ratio with serum Creatinine (C) and Serum BUN (D), respectively. Each data point represents a patient and the line represents the best fit line.

