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Determination of hypoxia-inducible factor-1 alpha, superoxide dismutase and galectin-3 levels in patients with type 2 diabetes

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Abstract

Background

The aim of this study was to evaluate the effect of hypoxia-inducible factor-1 alpha (HIF-1α), superoxide dismutase (SOD), and galectin-3 (Gal-3) as a new marker, and to investigate their correlation with micro- and macro- complications of type 2 diabetes (T2D).

Methods

The present study was conducted in 50 T2D patients (male/female: 24/26; age:18–75) and 30 healthy individuals (male/female: 10/20) matched for age, gender, and body mass index. The patients underwent complete blood count, routine biochemical tests, and urine tests. Serum HIF-1α, SOD, and Gal-3 levels were measured using an enzyme-linked immunosorbent assay.

Results

Serum HIF-1α, SOD, and Galectin-3 levels in the patient group were significantly higher than those in the control group ($p = 0.001$ for all parameters). According to the ROC analysis, all parameters demonstrated significant sensitivity and specificity for the patient groups (% sensitivity/% specificity: 80/76.7 for SOD; 44/100 for Gal-3; 72/96.7 for HIF-1α, respectively).

Conclusions

Our findings indicated that elevated serum levels of HIF-1α, SOD, and Gal-3 may be associated with endothelial dysfunction in T2D patients. Furthermore, measuring serum levels of HIF-1α, SOD, and Gal-3 levels could serve as a potential biomarker for complications in T2D patients.

Introduction

Type 2 diabetes is a chronic, widespread metabolic disorder in which the body cannot adequately utilize carbohydrates, fats and proteins due to defects in insulin function. The disease has acute and chronic complications [1]. Acute complications can be life-threatening, while chronic complications can lead to slow, long-term damage. The etiology indicates that both genetic and environmental factors influence the disease. In particular, patients over 30 years of age with metabolic syndrome and a body mass index over 25 often suffer due to dietary habits and a sedentary lifestyle. However, in recent years, there has been an increase in the rate of Type 2 diabetes diagnoses among 15- to 30- year-olds, due to genetic and environmental factors. The rise in complication rates may be influenced by patients' dietary habits, sociocultural factors, and a more active lifestyle [2]. The HIF-1α protein plays a crucial role in hypoxia,

angiogenesis, and cardiac development. It is a heterodimeric transcription factor that is activated as a normal response to hypoxia to regulate the body's ischemic reperfusion. When tissue oxygen levels drop, HIF-1α and HIF-1β react with the help of prolyl hydroxylase in the nucleus and activate the hypoxia response element (HRE) to reduce or prevent the damage [3]. Additionally, it is an important molecule regulated by insulin regarding intracellular glucose uptake in normoxic conditions. Superoxide dismutase (SOD) is an important antioxidant that converts metabolic superoxide anion radicals, which are dependent on NADPH oxidase, into a less reactive substance, hydrogen peroxide [4]. Deficiency of this substance can lead to chronic complications and conditons such as type 2 diabetes. Its deficiency readily leads to aging, tumor formation, and chronic diseases in the body. Gal-3, a member of the lectin family, is a soluble β-galactoside-binding lectin, ubiquitously distributed in various tissues, located in the cytoplasm and the extracellular space; its expression is upregulated in injured tissues undergoing remodeling processes. It is involved in many pathways, such as extracellular matrix interactions, macrophage activation, antimicrobial activity, apoptosis, metastasis, and angiogenesis. The presence of galectin is associated with the development of fibrosis and endothelial dysfunction, making it one of the key factors in chronic diseases such as T2DM. Galectin inhibitors play a role in tissue repair mechanisms aimed at preventing the development of fibrosis and endothelial dysfunction in the body [3,4].

According to the results of literature review, serum HIF-1α, SOD, and Gal-3 levels can serve as specific biomarkers for diagnosing of T2D patients and assessing their response to treatment. To the best of our knowledge, no studies have investigated HIF-1α, SOD, and Gal-3 levels in relation to patients with T2D. Therefore, the aim of this research was to explore serum HIF-1α, SOD, and Gal-3 levels in relation to T2D, as well as to examine the potential association of these biomarkers with demographic data in T2D patients. We examined serum HIF-1α, SOD, and Gal-3 levels and their correlation with anthropometric measurements, blood pressure, pulse, metabolic parameters, micro- and macro-complications, diabetes education, diet, time elapsed since diabetes diagnosis, exercise, and kidney and liver function tests.

Materials and Methods

The clinical protocols were approved by the Institutional Review Board of Bolu Izzet Baysal University (date/number: January 4, 2022, and 2021/318) and conducted accordance with the ethical principles of the Declaration of Helsinki, after the patients signed an informed consent form. A total of 50 T2D patients (male/female: 24/26; age:18–75) and 30 controls (male/female: 10/20) matched for age, gender, and body mass index were enrolled in this study. The mean age and BMI were (patients/controls: 55 ± 10.8 / 49.7 ± 7.5 years) and (patients/controls: 31.3 ± 5.2 / 25.5 ± 3.8 kg/m^2), respectively. Control individuals who responds to the questionnaire were eligible for inclusion in the study if they voluntarily agreed to participate. The exclusion criteria in the control group were a diagnosis of T2D or the use of antidiabetic medications. Patients with endocrine disorders other than T2D, and those with CVD, hypertension, heart failure, cancer, stroke, amyloidosis, pregnancy, gout, acute infection, chronic liver, kidney, rheumatological, hematological, pulmonary hypoxia, thyroid, or autoimmune conditions, as well as those who had experienced acute coronary disease in the past six months, nursing mother, and

patients under 18 years of age were excluded from the study. The diagnosis of T2D was made according to the American Diabetes Association criteria [5]. The questionnaire (which included questions regarding demographic data, acute/chronic diseases, medication use, lifestyle habits, diabetic diet, exercise, whether fasting blood glucose (FBG) and HbA1c targets were known, diabetes education, time elapsed since diabetes diagnosis, comorbidities, history of acute and chronic diabetic complications, and adherence to diabetic treatment) was administered to both patient group and the control group. Anthropometric measurements, including height, weight, and venipuncture, were performed by the same researcher on the same morning. Body mass index [BMI] was calculated as weight in kilograms divided by height squared [kg/m^2]. Blood pressure was measured for each participant. After an 8-hour overnight fast, 10 mL of venous blood was drawn via venipuncture into serum separator/clot activator tubes. Blood samples were centrifuged at 4000 rpm for 10 minutes, and the resulting serum samples were stored at -80°C until analyzed. HbA1c levels were measured in a blood sample collected in K_2EDTA tubes. All routine analysis (complete blood count, blood urea and serum creatinine, fasting plasma glucose, plasma insulin, routine and microscopic urine analysis, glomerular filtration rate, urine albumin-to-creatinine ratio, and lipid profile) were performed on the serum samples using commercial kits (Architect c8000) according to standardized procedures. Serum Gal-3 (Cat. No.: ELK1312 Lot:20357315440), SOD (Cat. No.: ELK 5615 Lot:20357325757), and HIF-1α (Cat. No.: ELK1603 Lot:20357316103) levels were measured using ELISA kits.

Statistical analysis

All statistical analyses and graphical visualizations were performed using R software (R Foundation for Statistical Computing, Vienna, Austria). Data manipulation and visualization procedures were conducted using the tidyverse, ggplot2, and ggpubr packages. The normality of the data distribution was assessed using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Continuous variables with a normal distribution were expressed as mean ± standard deviation (SD), while non-normally distributed variables were presented as medians and interquartile ranges (IQR; 25th–75th percentiles). Categorical variables were reported as frequencies and percentages (n, %). To compare continuous variables between the T2D and healthy control groups, the independent samples t-test was used for normally distributed data, and the Mann-Whitney U test was employed for non-normally distributed variables. Given the non-normal distribution of the primary biochemical markers (HIF-1α, SOD, and Galectin-3), non-parametric methods were prioritized for these specific analyses. For categorical data, including gender, lifestyle factors, and the prevalence of complications, the chi-square test or Fisher's exact test was used as appropriate. The diagnostic performance of serum HIF-1α, SOD, and Galectin-3 in predicting T2D was evaluated using Receiver Operating Characteristic (ROC) curve analysis with the pROC package. The Area Under the Curve (AUC) with 95% confidence intervals (CI) was calculated for each biomarker. Optimal cutoff values were determined using the Youden's Index, and the corresponding sensitivity and specificity values were reported. To investigate the relationships between serum biomarkers and metabolic parameters such as HbA1c, BMI, GFR, Spearman's rank correlation analysis was conducted separately for the control and T2D groups, and correlation-matrices were generated using the ggcorrplot package. For the evaluation of

diabetic complications within the patient cohort, subgroup analyses were restricted to categories with a sample size ≥ 10 (hypertension, retinopathy, and nephropathy) to ensure sufficient statistical power and reliability. Inferential testing was omitted for subgroups with n < 10 (neuropathy and diabetic foot) due to sample size limitations. A two-tailed p-value of < 0.05 was considered statistically significant for all analyses.

Results

The clinical and demographic characteristics of the study participants are presented in Table 1. While gender and lifestyle factors, including smoking, alcohol consumption, and exercise, were comparable between groups, T2D patients were older ($p = 0.003$) and had a higher BMI ($p = 0.001$) than controls. Metabolic assessments showed that FBG, HbA1c, triglycerides, and total cholesterol were significantly elevated in the T2D group ($p = 0.001$, all of them). While the HDL-cholesterol levels were similar in the two groups, LDL-cholesterol levels in patients were significantly higher than in the control group ($p = 0.002$). Regarding renal function, GFR was significantly lower in patients ($p = 0.015$), although no significant differences were observed in creatinine or urea levels. Additionally, liver function tests, including AST and ALT, remained similar between the two groups ($p = 0.727$; $p = 0.162$, respectively).

Table 1
Basic demographic, lifestyle, and clinical characteristics of patients and controls

Variables	Patients (n = 50)	Controls (n = 30)	P
Age (Year)	56.0 (49.2–62.8)*	49.0 (46.0–51.8)*	0.003 [a]
Gender (Male/female; %)	50 (24/26; 48/52)	30 (10/20; 33/67)	0.293 [b]
BMI (kg/m^2)	31.3 (5.2)	25.5 (3.8)	0.001 [c]
Life Style (n, %)			
Cigarette (Yes)	2 (4.0)	3 (10.0)	0.358 [d]
Alcohol (Yes)	—	1 (3.3)	0.375 [d]
Exercise (Yes)	3 (6.0)	3 (10.0)	0.667 [d]
Metabolic Parameters			
HbA1c (%)	7.0 (6.7–9.2)*	5.4 (5.1–5.7)*	0.001 [a]
FBG (mg/dL)	118 (105–164)*	92 (87–100)*	0.001 [a]
Kidney and Lipid Profile			
GFR (ml/dk/1.73m^2)	90.1 (19.2)	101.5 (20.4)	0.015 [c]
Creatinine (mg/dL)	0.80 (0.70–0.90)*	0.75 (0.70–0.90)*	0.617 [a]
Urea (mg/dL)	27.5 (25.0–38.8)*	24.5 (20.2–34.8)*	0.066 [a]
Triglycerides (mg/dL)	134 (102–189)*	86 (52–116)*	0.001 [a]
LDL-Cholesterol (mg/dL)	116 (95–141)*	90 (80–111)*	0.002 [a]
HDL-Cholesterol (mg/dL)	48.0 (41.2–59.8)*	55.0 (48.2–62.8)*	0.092 [a]
Total Cholesterol (mg/dL)	200 (177–220)*	160 (104–185)*	0.001 [a]
Liver Function Tests			
AST (U/L)	18.0 (15.0–23.3)*	17.5 (15.0–25.3)*	0.727 [a]
ALT (U/L)	20.0 (14.0–30.3)*	17.0 (10.0–25.5)*	0.162 [a]
Complications (n, %)			
Hypertension	18 (36.0)	—	0.001 [d]
Retinopathy	14 (28.0)	—	0.001 [d]

Variables	Patients (n = 50)	Controls (n = 30)	P
Nephropathy	11 (22.0)	—	0.006 [d]
Neuropathy	5 (10.0)	—	—
Diabetic Foot	2 (4.0)	—	—

Note: Continuous variables are presented as Mean (SD) for normally distributed data, and as Median (Q1 - Q3) for non-normally distributed data (indicated with an asterisk *). Categorical variables are expressed as number and percentage [n (%)].

Abbreviations: BMI, body mass index; HbA1c, glycated hemoglobin; FBG, fasting blood glucose; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein, AST, aspartate aminotransferase; ALT, alanine aminotransferase, Q1–Q3, interquartile range (25th–75th percentiles).

[a, c] Continuous variables were compared using Mann-Whitney U test (for non-normal distribution) or Student's t-test (for normal distribution).

[b] Chi-square test was used for gender distribution.

[d] Fisher's Exact test was applied for lifestyle factors (smoking, alcohol, exercise, hypertension, retinopathy, nephropathy) due to low cell frequencies.

Significance level was set at $p < 0.05$.

Serum levels of HIF-1α, SOD, and Galectin-3 for both groups are summarized in Table 2 **and** Fig. 1. All three biomarkers were significantly elevated in T2D patients compared to the control group ($p = 0.001$). Specifically, the median concentration of HIF-1α was 1057 ng/mL in the patient group versus 650 ng/mL in controls. Similarly, SOD activity (159.6 vs. 78.6 U/mL) and Galectin-3 levels (760 vs. 394 ng/mL) were significantly higher in patients with T2D ($p = 0.001$ for both).

Table 2
Comparison of serum biomarker levels between patient and control groups

Biomarker	Patients (n = 50)	Controls (n = 30)	P
HIF–1α (ng/mL)	1057 (862–1308)*	650 (208–769)*	0.001
SOD (U/mL)	159.6 (106.1–271.2)*	78.6 (69.5–100.3)*	0.001
Galectin–3 (ng/mL)	760 (431–1937)*	394 (287–696)*	0.001

Note: Data are presented as Median (Q1 – Q3) as they showed non–normal distribution (indicated with an asterisk *).

Abbreviations: HIF–1α, hypoxia–inducible factor–1 alpha; SOD, superoxide dismutase, Q1 – Q3, interquartile range (25th – 75th percentiles).

Statistical Analysis: p–values were determined using the Mann–Whitney U test.

Significance level was set at $p < 0.05$.

The diagnostic potential of serum HIF-1α, Galectin-3, and SOD for predicting T2D was evaluated using ROC curve analysis (Table 3, Fig. 2). All biomarkers demonstrated statistically significant diagnostic accuracy ($p = 0.001$). HIF-1α exhibited an AUC of 0.877 (95% CI: 0.792–0.963) with a sensitivity of 72.0% and a specificity of 96.7% at a cut-off of > 905 ng/mL. SOD followed with an AUC of 0.812 (sensitivity 80.0%, specificity 76.7%, cut-off > 103 U/mL). Galectin-3 showed an AUC of 0.744 and achieved a specificity of 100% at a cut-off of > 875 ng/mL, with a sensitivity of 44.0%.

Table 3
Diagnostic performance and ROC curve analysis of serum biomarkers for predicting patient group

Biomarker	AUC (95% CI)	Cut–off	Sensitivity (%)	Specificity (%)
HIF–1α (ng/mL)	0.877 (0.792–0.963)	> 905	72.0	96.7
SOD (U/mL)	0.812 (0.718–0.907)	> 103	80.0	76.7
Galectin–3 (ng/mL)	0.744 (0.635–0.854)	> 875	44.0	100

Note: AUC, Area under the curve; CI, Confidence interval. Cut–off values were determined using the Youden index.

Statistical Analysis: ROC curve analysis was performed to evaluate the diagnostic accuracy of markers.

All of the AUC values are statistically significant ($p < 0.001$).

Spearman correlation analyses were conducted separately for the healthy control and T2D patient groups (Fig. 3). In the healthy control group, no statistically significant correlations were observed between serum HIF-1α, SOD, or Galectin-3 levels and variables including HbA1c and BMI. Similarly, within the T2D patient group, no significant correlations were identified between these biomarkers and HbA1c levels. The distribution of complications across the study groups is detailed in Table 1. While no microvascular or macrovascular complications were present in the healthy control group, various complications were observed within the T2D cohort. Finally, the relationship between serum biomarkers and specific diabetic complications was analyzed as presented in Table 4. Statistical comparisons of serum biomarkers were performed for complication subgroups with a sample size of $n \geq 10$. In patients with retinopathy ($n = 14$), median levels of HIF-1α, SOD, and Galectin-3 did not differ significantly from those without the complication. Similarly, no statistically significant variations were observed in relation to the presence of nephropathy ($n = 11$) or hypertension ($n = 18$). A composite analysis comparing patients with at least one microvascular complication ($n = 16$) to those without any documented complications ($n = 34$) also showed no significant differences in the investigated biomarker levels.

Table 4
Subgroup analysis of serum biomarkers based on diabetic complication status within the patient cohort

Complication	Biomarker	Present (n)	Absent (n)	P
Retinopathy (n = 14)	HIF–1α (ng/mL)	1099.2 (1016.7–1513.4)	1026.5 (759.2–1281.4)	0.136
	SOD (U/mL)	159.6 (108.5–415.2)	156.0 (108.1–252.0)	0.705
	Galectin–3 (ng/mL)	564.8 (421.2–1754.4)	767.1 (581.2–2245.2)	0.347
Nephropathy (n = 11)	HIF–1α (ng/mL)	1123.5 (984.0–1460.2)	1041.0 (785.4–1312.0)	0.214
	SOD (U/mL)	162.4 (110.2–388.5)	155.2 (106.4–264.0)	0.682
	Galectin–3 (ng/mL)	612.0 (450.5–1820.0)	745.5 (560.2–2180.5)	0.412
Hypertension (n = 18)	HIF–1α (ng/mL)	1085.0 (1010.5–1490.2)	1035.4 (765.8–1295.5)	0.187
	SOD (U/mL)	158.2 (107.4–405.6)	157.1 (109.2–248.5)	0.841
	Galectin–3 (ng/mL)	598.4 (435.6–1780.2)	755.0 (572.4–2210.8)	0.395

Note: Data are presented as Median (Q1 – Q3). Statistical significance was determined using the Mann–Whitney U test for comparisons between groups with and without each complication.

A p–value < 0.05 was considered statistically significant.

Due to the limited sample size and to ensure statistical power, inferential analysis was restricted to complication subgroups with ≥ 10 (hypertension, retinopathy, and nephropathy).

Abbreviations: HIF–1α, hypoxia–inducible factor 1–alpha; SOD, superoxide dismutase; T2D, type 2 diabetes; Q1 – Q3, interquartile range (25th – 75th percentiles).

Discussion

T2D is the end point of a progressive insulin secretory defect against a background of insulin resistance. Its prevalence has increased dramatically worldwide. Our study is the first to report an association between serum Gal-3, HIF-1α, and SOD levels in patients with T2D. Subjects with T2D demonstrated elevated serum Gal-3, HIF-1α, and SOD levels compared to the control group. Elevated levels of these parameters may be selectively associated with insulin resistance, hypoxia, endothelial dysfunction, coronary artery diseases, cardiomyopathy, arrhythmias, and cerebrovascular and peripheral artery diseases in patients. However, the underlying mechanisms linking Gal-3, HIF-1α, and SOD to T2D remain to be determined.

HIF-1α, a transcription factor that plays a key role in neovascularization, the development of beta-cell mass in the embryonic pancreas, and beta cell protection, is associated with insulin signaling pathway and is regulated via the way the protein kinase B signaling pathway [6]. Inflammatory reactions in the body are closely linked to diabetes. The relationship between T2D and the T allele in exon 12 of the HIF-1α gene was demonstrated by Nagy and his colleagues. Some studies have shown that all diabetic mechanisms are affected by hypoxia, and that the stability and activity of the HIF-1α protein are altered by hyperglycemia, diabetic cells and tissues are more vulnerable to hypoxia, and HIF-1α has become a risk factor for cardiovascular diseases in diabetes [7, 8]. Oxygen homeostasis is critical for the survival of all living cells. Complex adaptive mechanism under hypoxia conditions have evolved to ensure cellular survival. The transcript factor HIF-1α plays a major role in maintaining cellular oxygen homeostasis by directly activating hundreds of target genes. We hypothesize that serum HIF-1α levels may be elevated in our patients in response to hypoxia that progresses with diabetes. Consistent with our findings, some studies have shown that patients with T2D who have elevated HIF-1α levels have a worse prognosis than those with lower concentration, as high HIF-1α levels increase the risk of diabetic complications [9]. Additionally, previous study showed that higher blood HIF-1α concentrations in T2D patients may play a role in vascular calcification, including both intimal and medial calcification, and that intimal calcification of the coronary arteries is most commonly associated with atherosclerosis lesions [10]. Liu X et al. demonstrated that serum HIF-1α prevents endothelial injury by mediating ischemia and reperfusion in mice with T2D [11]. Liu N et al. reported that increased demand for insulin secretion due to insulin resistance can induce hypoxia in islets in T2DM cell culture models [12]. On the other hand, some studies have shown that there is evidence of dysregulated HIF-1α expression in islets from both rodents and human with diabetes, and that this leads to hypoxia and the activation of hypoxia-responsive pathways in pancreatic β-cell lines and isolated mouse islets [13,14]. Additionally, some studies have shown that the metabolism of VEGF and erythropoietin, as well as the supply of endothelial reperfusion, are activated by HIF-1α to meet the energy requirements of cells [3]. Hong-Tao Yang et al. revealed that blood HIF-1α acts in conjunction with VEGF for facilitate reperfusion in mice with diabetic retinopathy [15]. The two animal studies mentioned above can serve as a reference for our study. Hariharan T et al. showed that the development of diabetic foot is related to high glucose concentration and decreased HIF-1α levels [16]. In light of the information mentioned above, blood HIF-1α levels are closely associated with vascular endothelial dysfunction, hypoxia, particularly in response to ischemia/reperfusion, disease activity, and complication in type 2 diabetes mellitus. Galectin-3 is primarily synthesized by activated fibroblasts and vascular cells, functioning as a biomarker for inflammation, fibrosis, macrophage infiltration, adhesion, proliferation, apoptosis, tissue damage and the extracellular matrix [17]. Elevated serum Gal-3 levels were reported to be positively associated with age, potentially via the inflammatory signaling cascade that causes β-cell fibrosis and impairs insulin secretion, and were also shown to be higher in patients with related obesity in a study by Weigert et al., and are related to the progression of diabetes complications due to its ability to bind the advanced glycation end products (AGEs) and advanced lipoxidation end products (ALEs) that accumulate in target organs [18,19]. It has emerged as a key player in the pathogenesis of common disease conditions, including cancer and immune/inflammatory and

metabolic disorders. Recent data have suggested a close relationship between Gal-3 concentration and worsening albuminuria in patients with T2DM [20]. To date, the association between Gal-3 and T2DM has not been fully understood. The findings of this study suggest that increased Gal-3 concentration may be implicated in diabetes complication such as insulin resistance, β-cell damage, endothelial dysfunction, age, and glycemic status in patients with T2DM. In our view, Gal-3 may have a high binding affinity for AGEs and has been suggested to participate in the degradation of AGEs. We believe that a key aspect of Gal-3 is its ability to bind AGEs and ALEs that accumulate in target organs and exert their toxic effects by triggering pro-inflammatory and pro-oxidant pathways. Studies using mouse models have shown that targeted disruption of the Gal-3 gene results in accelerated diabetic complications, including AGEs and ALEs [21]. In agreement with our study, Pejnovic et al. demonsrated that Gal-3 knockout mice fed a high-fed diet exhibited increased inflammation in the pancreatic islets due to AGE accumulation, which may be responsible for impaired glucose regulation [22]. Our interpretation aligns with the aforementioned study showing that increased blood Gal-3 reduces the adaptive immune response to overnutrition, which leads to adipose tissue inflammation and oxidative stress, thereby protecting against obesity-associated T2D. Pang et al. found that increased Gal-3 levels were associated with elevated blood glucose concentration in mice fed a standard high-fat diet. Moreover, they found that, in these animals, systemic inflammation and increased fat mass occurred following the onset of impaired glucose homeostasis, which was not accompanied by decreased insulin sensitivity in the suggesting that Gal-3 modulates β-cell function, irrespective of obesity-related inflammation [23]. Vora et al. revealed that Gal-3 has been suggested to play a more prominent role in insulin secretion than in insulin resistance due to a profibrotic mechanisms [24]. Some studies indicate that oxidative stress is a significant risk factor in T2D as well as in the development of diabetic complications [25]. Diabetes is associated with increased of reactive oxygen species (ROS) and antioxidant enzymes such as SOD. SOD is a key enzyme for scavenging free radicals in the body. The increased SOD activity in our study may be explained by the enhanced synthesis of the antioxidant defense system in response to excessive free radical generation. The presence of oxidative damage in the body is associated with the increased production of reactive oxygen species such as superoxide anion radical [26]. Thus, the increase in SOD activity in our patients suggests a possible adaptive response, likely due to the increased production of the superoxide anion radical.

With regard to BMI, HbA1c %, fasting glucose, insulin, LDL-c, TG, ALT, AST and GFR, our results show a significant increase in BMI, HbA1c %, fasting glucose, insulin, LDL-c and TG, but no differences in ALT, AST and GFR between patients with diabetes and controls, indicating impaired metabolism in patients with type 2 diabetes. In line with our findings, some studies have shown that fasting glucose, TG, and LDL-c levels are elevated in patients with type 2 diabetes compared to controls [27]. Additionally, other studies have demonstrated that patients with type 2 diabetes have higher risk of dyslipidemia than non-diabetics, and dyslipidemia is a major risk factor for cardiovascular disease [28,29]. Some studies have suggested that hyperglycemia, obesity smoking, and a sedentary lifestyle are associated with various metabolic and cellular functions, including dyslipidemia, endothelial dysfunction, and disturbance in

cardiac metabolism [30,31]. The patients included in this study with elevated BMI, HbA1c %, fasting glucose, insulin, LDL-c and TG are more likely to have uncontrolled diabetes. An increased incidence of diabetic microvascular complications such as retinopathy (28%), nephropathy (22%), neuropathy (10%) and diabetic foot (4%) in our patients was accompanied by an increase in disease duration, fasting blood glucose, and HbA1c levels. The typical patient with type 2 diabetes mellitus is overweight and the age-adjusted relative risk for diabetes begins to increase at certain BMI values. Additionally, the risk of diabetes increases with rising BMI values in patients. The marked increase in the prevalence of obesity is a major contributor to the increased prevalence of T2D. In this study, ROC curve analysis of serum HIF-1α, Gal-3 levels, and SOD activity showed high AUC with high sensitivity and specificity in T2D patients compared to controls. According to ROC curves analysis, serum HIF-1α, SOD, and Gal-3 levels can be used as predictors for T2D patients.

Conclusions

In summary, our work leads to several important conclusions. First, we have reported, for the first time, that there are marked increases in Gal-3, HIF-1α, and SOD levels in T2D patients. Second, we have demonstrated that Gal-3, SOD, and HIF-1α have diagnostic value in T2D patients. Finally, increases in these parameters in T2D patients have profound implications for diagnosis, treatment, and follow-up, particularly regarding mechanisms that are important for next generation treatment protocol. The data suggested that elevated Gal-3, HIF-1α, and SOD levels are likely downstream genes associated with a combination of insulin resistance and impaired β-cell function. The study demonstrates a significant increase in Gal-3, HIF-1α, and SOD in patients with T2D. Additionally, the results of the ROC curve analysis revealed high sensitivity of serum Gal-3, HIF-1α, and SOD in the T2D patients. Furthermore, Gal-3, SOD, and HIF-1α can be used as predictors for diagnosis in patients with T2D.

Declarations

Authorship contribution statement

Conceptualization, validation, data curation, and methodology: ÇK, MG, ÖMY and SY; software and formal analysis: ÇK and SY; investigation, resources, writing, visualization and project administration: ÇK and MG.

Declaration of competing interest

All authors have read and agreed to the published version of the manuscript.

Data availability

Data will be made available within the paper.

AI statement

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Figures

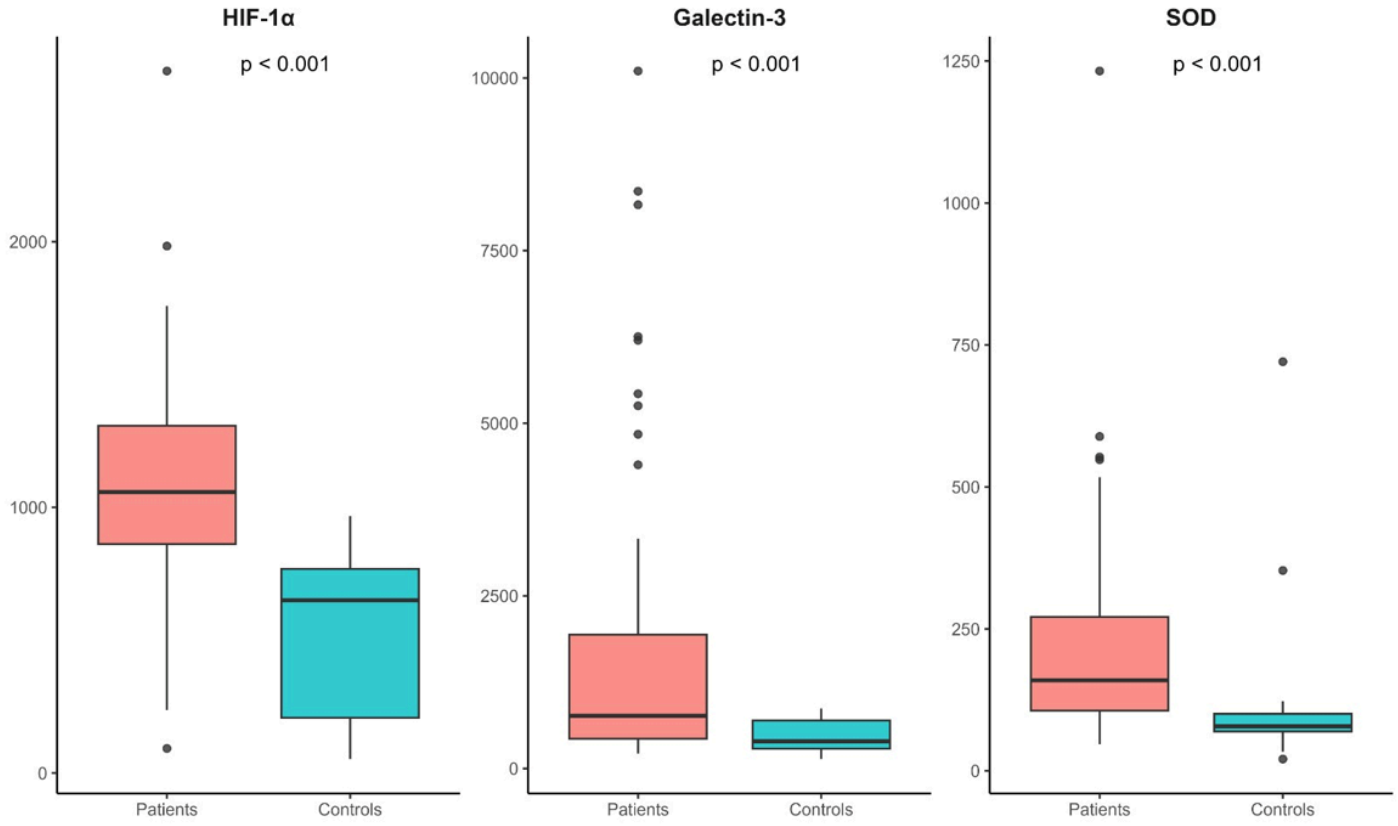


Figure 1

Levels of galectin-3, hypoxia-inducible factor-1 alpha,and superoxide dismutasein patients with type 2 diabetesmellitus and controls.

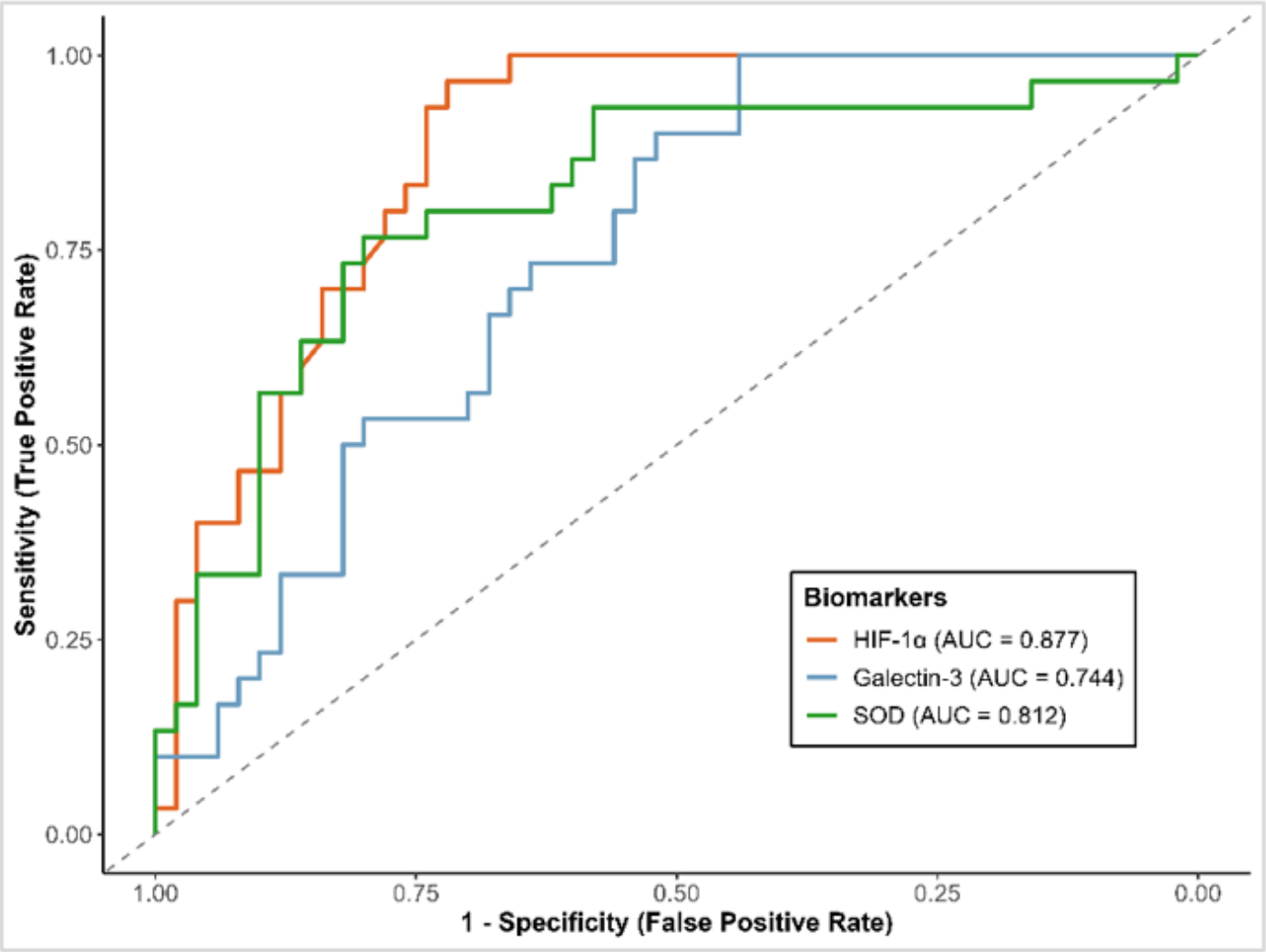


Figure 2

Receiver operating characteristic curve analysis of serum galectin-3, hypoxia-inducible factor-1 alpha, and superoxide dismutase for the prediction of type 2 diabetes.

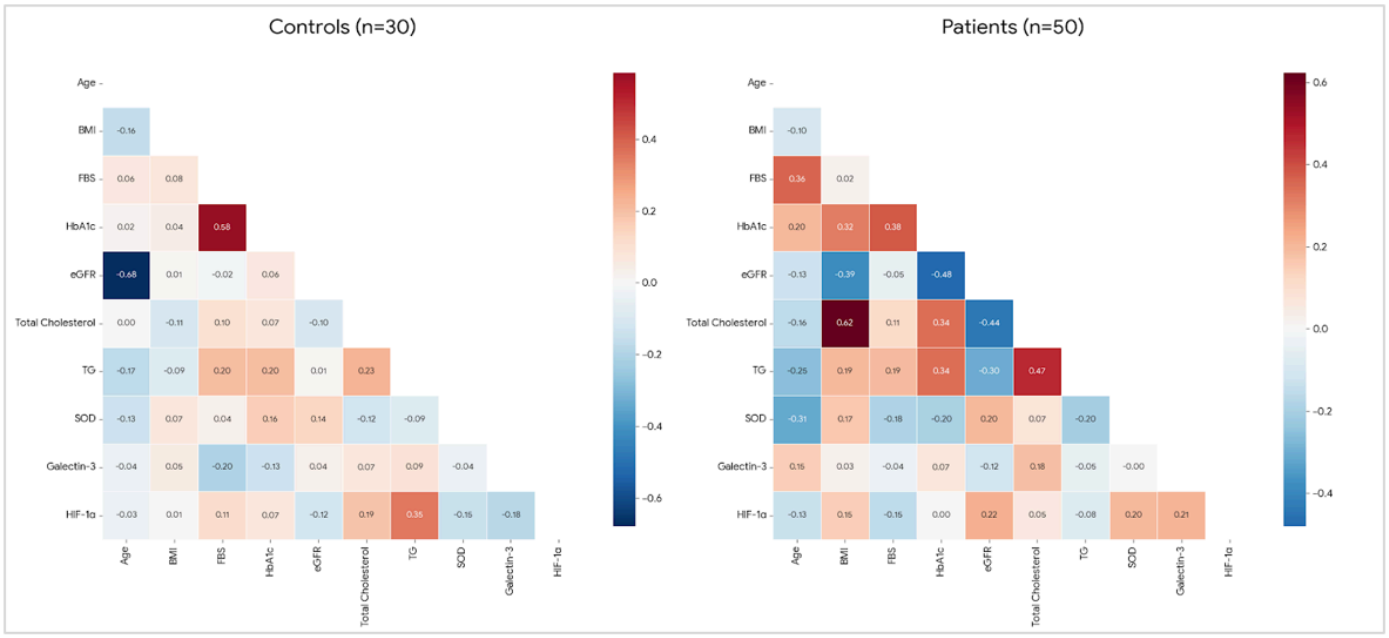


Figure 3

Spearman correlation heat maps of clinical and biochemical parameters in study groups.