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ORIGINAL ARTICLE

The correlation between blood oxidative stress and sialic acid content in diabetic patients with nephropathy, hypertension, and hyperlipidemia

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Abstract

This clinical study was designed to find out the correlation between oxidative stress and sialic acid (SA) content of plasma and RBCs in patients with type 2 diabetes. We evaluated SA concentration and oxidative stress biomarkers in healthy subjects and diabetic patients with and without complications in a cross-sectional survey. Significant changes in oxidative stress biomarkers and RBC-SA were revealed in the diabetic patients compared to those in the healthy group. Plasma SA significantly increased with an increase in lipid peroxidation of RBCs (LPO-RBC) ($P < 0.001$) in the diabetic patients without complication. RBC-SA significantly decreased with an elevation in LPO-RBC ($P < 0.001$) in all the diabetic patients and those with nephropathy. There was no significant correlation between plasma and RBC-SA and other oxidative stress biomarkers in the diabetic subjects. In multiple logistic regression analysis, RBC-SA was independently related to LPO-RBC in all the diabetic patients and those with nephropathy. We conclude that the induction of LPO-RBC in diabetic patients and those with nephropathy may influence the SA decomposition of RBC membrane, thereby altering its functions and transporter activities. Therefore, LPO-RBC and SA levels in RBCs can be used for prediction of diabetic nephropathy, and further studies to evaluate other factors contributing to desialylation of RBC membrane are justified.

Keywords Diabetic complications · Diabetes mellitus · Oxidative stress · Sialic acid · Type 2 diabetes

Introduction

Type 2 diabetes (T2D) is a metabolic disorder in endocrine system which is the consequence of insufficiency in insulin secretion, action, or both [1]. The prevalence rate of T2D as the most common type of diabetes is increasing over time throughout the world, especially in developing countries [2–4]. The number of diabetic patients in Iran is estimated to be over 9 million cases by 2030 [5]. Common biomarkers for diagnosis of diabetic people are to measure fasting blood glucose levels and oral glucose tolerance test, which are not good predictors for diabetic complication. Other proposed biomarkers such as glycosylated hemoglobin, fructosamine, and glycated albumin have limitations such as moderate sensitivity and specificity [6, 7]. Therefore, it is important to find out novel biomarkers accurately identifying those at risk for diabetic complications.

Extensive evidence in both experimental and clinical studies shows that oxidative stress plays an important role in the pathogenesis of diabetes through an increase in the production of reactive oxygen species (ROS) and decline in the antioxidant defense [8–10]. Induction of oxidative stress can contribute to the development of diabetic complications including neuropathy, nephropathy, retinopathy, etc. Free radicals in diabetes are formed by glucose oxidation, resulting in subsequent non-enzymatic glycation of proteins and oxidative degradation of glycosylated proteins [11, 12]. Free radicals in diabetes are formed by glucose oxidation, resulting in subsequent non-enzymatic glycation of proteins and oxidative degradation of

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glycosylated proteins [13]. ROS constantly created as a result of induction of oxidative stress through physiological metabolic processes and pathological conditions are very reactive molecules with unconjugated electrons in their outer orbital that react with various macromolecules such as lipids, carbohydrates, and proteins [14, 15].

One of the compounds targeted for oxidative stress is sialic acid (SA) [16, 17], a terminal component of non-reducing end of carbohydrate chains of glycoproteins and glycolipids [18]. SA is an essential compound in all cell membranes and plays an important role in maintaining the structure, permeability, and integrity of the cell membrane [19]. SA also affects cellular adhesiveness, antigenicity, transport process, action of some hormones, and catalytic properties of enzymes [20, 21]. It should be noted that high levels of SA in plasma have been reported in inflammatory processes, alcohol consumption, diabetes, renal diseases, and cancer [22, 23]. It has been suggested that changes in sialylation are associated with oxidative stress induced by several disorders including diabetes [24, 25]. Although the role of SA in diabetic conditions has been established, the mechanisms altering the blood SA levels in diabetic patients have remained unclear.

The present study was designed to evaluate the correlation between oxidative stress parameters and blood SA and their association with the development of diabetic complications, including nephropathy, retinopathy, neuropathy, and cardiovascular complications. To achieve these goals, the correlation among oxidative stress biomarkers including glutathione level, activity of superoxide dismutase (SOD) enzyme, lipid peroxidation (LPO), and SA content was evaluated in plasma and RBCs of diabetic patients with or without complications.

Materials and methods

Participants

This cross-sectional study was conducted on diabetic patients from 2016 to 2018 after obtaining the informed consent. Considering $\alpha = 0.5$ and $\beta = 0.20$, the mean and standard deviation ($X \pm \mathrm{SD}$) of SA were estimated 10.3 ± 3.2 and 24 ± 1.8 in diabetic and healthy subjects, respectively [26]. The sample size calculated on the basis of the following two mean comparison formula was at least 15 people in each group:

$$n = \frac{2\left(z_{1-\frac{\alpha}{2}} + z_{1-\beta}\right)^2 \delta^2}{d^2}.$$

The total number of patients enrolled to this study consisted of 161 subjects including 146 diabetic patients aged between 22 and 80 years which were recruited from Diabetes Centers in Afzalipour and Bahonar hospitals, Kerman, Iran and 15 healthy people in the same age range which were selected from the referents to Kerman Blood Transfusion Organization. The subjects were selected using convenience sampling. The inclusion criteria of the diabetic group were the presence of T2D alone or in combination with diabetic complications subjected to clinical examination to confirm the diagnosis of complications based on clinical features and blood biochemical tests. All diabetic patients with nephropathy had chronic renal failure with estimated Glomerular Filtration Rate (eGFR) lower than 15 ml/min/1.73 m^2 and were undergoing dialysis. The exclusion criteria for both groups were having a history of addiction, smoking, and alcohol consumption.

Blood sampling and preparation

Venous blood samples (5 ml) were taken from the participants in the morning after 8–10 h fasting using EDTA-containing tubes. Plasma were isolated after centrifugation at 3000*g* for 15 min and the precipitated RBCs were washed and a part of them were used for preparation of RBCs lysates. Another part of RBCs were mixed by 10 volume of 5 mM phosphate buffer pH 7.4 and kept on ice bath 30 min for isolation of RBCs membranes. The RBC membranes were then centrifuged at 20,000*g* for 40 min, and after removal of supernatant, the precipitants were washed and centrifuged again under the same condition. Finally, the RBCs' membranes were resuspended in 5 mM phosphate, pH 7.4. All samples were stored at − 80 °C for subsequent tests.

Measurement of glutathione content

According to Ellman colorimetric method [27], 750 ml of EDTA and 750 µL of TCA 10% were added to 1.5 ml of hemolysate and centrifuged at 3500*g* for 35 min. Then, 2.5 ml of Tris buffer was added to the supernatant. In addition, after addition of 0.5 ml 5,5-dithionitrobenzoic acid (DTNB, 10 mM in 0.1 M phosphate buffer, pH 8), it was incubated in the dark for 15 min at room temperature. The absorbance was read at 412 nm and total thiol content was expressed as µMol/mg protein according to the standard curve of glutathione.

Measurement of SOD enzyme activity

The rate of pyrogallol auto-oxidation was used to measure SOD activity [28]. To carry out this experiment, 610 µl of phosphate buffer was mixed with 90 µl of pyrogallol (2 mM pyrogallol in Tris–HCl buffer, pH 8.2), and the absorbance was determined kinetically at 420 nm alone and after the addition of 50 µl of sample. The amount of SOD needed for 50% inhibition of the pyrogallol autoxidation was considered as one unit of SOD activity and expressed as U/mg protein.

Measurement of LPO

To perform this experiment, malondialdehyde (MDA) level was measured by Draper and Hadley method with a slight modification [29]. The samples were deproteinized by trichloroacetic acid (TCA, 20%) and centrifuged at 4100*g* for 15 min. One ml of sulfuric acid 0.05% and 800 µl of thiobarbituric acid (TBA, 0.2%) solution were then added to the precipitant, and the tubes were placed in a boiling water bath for 30 min. Afterwards, 800 µl *n*-butanol was added to each well to extract the colored complex of MDA-TBA, and the absorbance was read by spectrophotometry at 532 nm. Using the MDA standard curve, the concentration of MDA was obtained in each sample and expressed as µMol MDA/mg protein.

Measurement of SA content

The SA content of RBC membrane (RBC-SA) and that of plasma (PSA) were measured according to Griess method [30]. Briefly, 100 µl of 0.04 M periodic acid was mixed with 500 µl diluted sample and cooled in an ice bath for 30 min at 0 °C. Then, 1.25 ml of resorcinol working solution (5 ml of 6.0% resorcinol solution, 0.12 ml of copper sulfate solution 0.1 M and 19.87 ml of distilled water, brought to a final volume of 50 ml with 10 M HCl) was added to the samples, mixed, and heated at 98 °C for 5 min. The tubes were cooled in the ice bath for approximately 2 min, 3.25 ml of *n*-butanol was added, and the tubes were placed in a water bath at 37 °C for at 3 min. The absorbance was measured at 625 nm, and according to the standard curve of *N*-(1-naphthyl) ethylenediamine, the SA value of each sample was expressed as mMol/mg protein in RBC membrane and µMol/l in plasma.

Statistical analyses

SPSS 16 was used to analyze the data. The descriptive results were shown as the mean ± SD and frequency (%). The normality of the data was examined by one-sample Kolmogorov–Smirnov test. The data were analyzed using one-way ANOVA, and the post hoc test was performed using Tukey multiple comparison. The correlation between different parameters was examined using Pearson's correlation coefficient. To measure factors associated with the level of PSA and RBC-SA, stepwise linear regression was used. Receiver operator characteristic (ROC) curve was plotted to assess whether PSA, RBC-SA, and oxidative stress biomarkers can be used as the biomarkers in the diagnosis of progression of diabetes complications. To determine the best cut point, Youden index was calculated for each biomarker with a significant area under the ROC curve above 0.5 [31]. *P* value less than 0.05 was considered significant.

Results

The demographic characteristics of the diabetic and healthy subjects are shown in Table 1. The average age of the participants was not significantly different among the groups. The duration of diabetes in the diabetic patients with nephropathy (18.0 ± 12.6) was significantly ($P < 0.001$) higher than that in the diabetic group without complications (9.0 ± 6.3).

As shown in Table 2, the RBC glutathione decreased significantly ($P < 0.05$) in the diabetic patients with nephropathy compared to that in the diabetic subjects without complication. There was no significant difference in the RBC glutathione among the other groups. The activity of SOD in all the diabetic patients with and without complications was significantly lower ($P < 0.001$) than that of the healthy subjects. The diabetic subjects with nephropathy showed a significant reduction in the SOD activity compared to the diabetic patients without complications ($P < 0.001$). All the diabetic subjects showed a significant increase ($P < 0.001$) in the lipid peroxidation of RBC in comparison with the healthy group but not with the diabetic patients without complications. Lipid peroxidation level was not significantly different among the diabetic patients with various complications. In addition, RBC-SA and PSA in the diabetic patients with all the complications were, respectively, lower and higher than those in the healthy group ($P < 0.001$).

Table 3 shows a significant positive correlation between PSA and LPO in the all diabetic subjects (r: − 0.297, P: 0.018). On the other hand, a significant negative correlation was shown between RBC-LPO and the level of

Table 1 Demographic characteristic of diabetic patients and healthy subjects

Variables	Healthy group (*N*:15)	Diabetic patients (*N*:146)			
		Without complication	Nephropathy	Hypertension	Hyperlipidemia
Sex, male/female (%)	9/6 (60/40)	11/9 (35.5/64.5)	10/20 (33.3/66.6)	12/43 (36.4/63.6)	10/31 (28.6/71.4)
Age, year	46.54 ± 24.23	53.9 ± 11	63 ± 10.7	58.9 ± 9.5	58.2 ± 9.7
Duration of diabetes, year	–	10.81 ± 9.11	18 ± 12.6	12.1 ± 7.7	10.4 ± 6.2
Fasting blood glucose, mg/dl	97.3 ± 12.5	168.7 ± 62.9	141.7 ± 40	142.5 ± 54.1	142.5 ± 45.3

Data are means ± SD for quantitative or frequency (%) for qualitative data

Table 2 Oxidative stress and plasma and RBC-sialic acid in the healthy and the diabetic subjects

Variables	Healthy group	Diabetic patients (*N*:146)			
		Without complication	Nephropathy	Hypertension	Hyperlipidemia
GSH, µmol/mg pro	741.64 ± 146.50	812.51 ± 405.1	573.53 ± 296.50†	805.40 ± 434.23	874.60 ± 482.93
SOD, U/mg pro	4.30 ± 0.36	1.92 ± 0.22***	0.84 ± 0.53*** †††	1.81 ± 0.61***	2.00 ± 0.40***
LPO, µmol/mg pro	1.20 ± 0.41	1.56 ± 0.31***	1.74 ± 0.34***	1.63 ± 0.32***	1.62 ± 0.20***
PSA, µmol/l	149.9 ± 47.30	175.00 ± 68.89*	174.13 ± 23.20*	176.00 ± 54.82 *	160.80 ± 48.61*
RBC-SA, mMol/mg pro	5.84 ± 1.43	4.20 ± 2.14*	4.00 ± 2.51*	4.14 ± 2.00*	3.93 ± 2.10*

Data are means ± SD

GSH glutathione, *LPO* lipid peroxidation, *PSA* plasma sialic acid, *RBC-SA* red blood cell-sialic acid, *SOD* superoxide dismutase

*$P < 0.05$ and ***$P < 0.001$ by ANOVA in comparison with control, $^{\dagger}P < 0.05$ and $^{\dagger\dagger\dagger}P < 0.001$ by ANOVA in comparison with DM

Table 3 Correlation between plasma and red blood cell sialic acid with oxidative stress biomarkers and duration of diabetes in the diabetic groups

Variables	Groups	PSA		RBC-SA	
		P	*r*	*P*	*r*
GSH	DM without complication	0.76	0.05	0.82	−0.04
	DM + nephropathy	0.22	0.23	0.75	0.06
	DM + hypertension	0.74	− 0.04	0.38	− 0.12
	DM + hyperlipidemia	0.81	− 0.03	0.38	− 0.12
	All diabetic subjects	0.62	− 0.04	0.61	− 0.04
SOD	DM without complication	0.29	0.19	0.68	0.08
	DM + nephropathy	0.15	− 0.27	0.86	0.03
	DM + hypertension	0.77	− 0.03	0.93	0.01
	DM + hyperlipidemia	0.45	0.1	0.55	− 0.08
	All diabetic subjects	0.89	− 0.01	0.85	0.02
LPO	DM without complication	0.02	0.3	0.3	− 0.19
	DM + nephropathy	0.66	0.08	0.03	− 0.34
	DM + hypertension	0.12	− 0.18	0.33	− 0.12
	DM + hyperlipidemia	0.74	0.05	0.8	− 0.03
	All diabetic subjects	0.14	− 0.12	0.04	− 0.17

Value (*r*) indicates correlation coefficient by Pearson's correlation analysis. *P* indicates level of significance. $P < 0.05$ is considered statistically significant

DM diabetes mellitus, *GSH* glutathione, *LPO* lipid peroxidation, *PSA* plasma sialic acid, *RBC-SA* red blood cell-sialic acid, *SOD* superoxide dismutase

RBC-SA in the diabetic patients (*r*: − 0.172, *P*: 0.038) and those with nephropathy (*r*: − 0.343, *P*: 0.036). The decrease in the activity of SOD and the glutathione level of RBCs did not show any correlation with PSA and RBC-SA.

Stepwise multiple regression coefficients are shown in Table 4 for PSA, RBC-SA, and the oxidative stress variables for all the diabetic subjects. In this model, RBC-LPO was significantly and negatively correlated with RBC-SA as the dependent variable in the diabetic patients and in the diabetic patients with nephropathy.

The diagnostic accuracy of PSA, RBC-SA, and the oxidative stress biomarkers was measured by ROC plot. As shown in Fig. 1 and Table 5, RBC-LPO at the best cut point of 1.45 µmol/mg protein, Youden index of 0.57, and area under the curve of 0.80 ($P < 0.00$) could differentiate the diabetic and non-diabetic subjects with 76% sensitivity and 80% specificity. The optimal RBC-LPO (AUC 0.68, $P < 0.02$), differentiating the group of diabetic patients from those with nephropathy, was 1.73 µmol/mg protein with the sensitivity of 67%, specificity of 75%, Youden index of 0.42, and AUC of 0.68.

Discussion

The findings of the current study showed oxidative stress induction along with PSA elevation and RBC-SA reduction in the diabetic patients. A novel finding of our manuscript was the significant correlation between SA content of RBCs membrane and peroxidation of RBCs membrane lipids in the diabetic patients with chronic renal failure. We did not find any correlation between PSA and the occurrence of the diabetic complications and alteration in the oxidative stress biomarkers. Furthermore, the result of ROC curve in the present study indicated that oxidative stress biomarkers and sialic acid content of plasma and RBCs were not good biomarkers in differentiation of diabetic patients from those with nephropathy, hypertension, and hyperlipidemia, but the LPO as an end product of RBCs' oxidative damage was more convincing that other biomarkers. Risk of chronic renal failure in diabetic patients is about 30% and develops decades after the onset of diabetes [32]. Krolewski et al. revealed that in majority of diabetic patients, the onset of progressive loss in renal function happens suddenly when patients have normal renal function and normal urinary albumin excretion [33]. Genetic and environmental factors, inflammation, and induction of oxidative damages are contributed in the development of renal failure [34]. Dysregulation of oxidative and mitochondrial enzymes leading to accumulation of

Table 4 Stepwise multiple linear regression coefficients and statistical significance for plasma sialic acid (PSA), red blood cell-bound sialic acid (RBC-SA) with other variables

Variables	Groups	PSA		RBC-SA	
		Regression coefficient	*P*	Regression coefficient	*P*
GSH	DM without complication	0.03	0.87	− 0.04	0.82
	DM + nephropathy	0.16	0.38	0.08	0.66
	DM + hypertension	− 0.05	0.65	0.02	0.85
	DM + hyperlipidemia	− 0.05	0.7	− 0.12	0.38
	All diabetic subjects	− 0.04	0.62	− 0.03	0.68
SOD	DM without complication	0.12	0.48	0.08	0.68
	DM + nephropathy	− 0.17	0.38	0.06	0.74
	DM + hypertension	− 0.15	0.19	0.08	0.51
	DM + hyperlipidemia	0.08	0.53	− 0.08	0.55
	All diabetic subjects	− 0.01	0.9	0.02	0.84
LPO	DM without complication	− 0.24	0.15	− 0.19	0.29
	DM + nephropathy	0.04	0.82	− 0.37	0.04
	DM + hypertension	− 0.12	0.3	− 0.17	0.14
	DM + hyperlipidemia	0.01	0.93	− 0.03	0.8
	All diabetic subjects	− 0.12	0.14	− 0.19	0.02

P indicates level of significance. $P < 0.05$ is considered statistically significant

GSH glutathione, *PSA* plasma sialic acid, *RBC-SA* red blood cell-sialic acid, *SOD* superoxide dismutase, *LPO* lipid peroxidation

free radicals and oxidative damage have a pivotal role in the acceleration of eGFR decline and the development of renal failure [35]. The inflammatory cytokines and oxidative stress biomarkers might be used for prediction of renal failure in diabetic patients [36].

Oxidative stress induction contributed to the development of diabetic complications through production of mitochondrial ROS [37–39]. In addition, it has been shown that inhibition of diabetes-induced oxidative stress can be an effective strategy in control and prevention of the onset and progression of diabetic complications [40, 41]. It has also been reported that total serum SA and PSA are in association with micro- and macrovascular complications including nephropathy, retinopathy, and hypertension in diabetic patients [42–46]. In consistence with the finding of the current study, the previous studies also demonstrated a negative correlation between SA content of RBC membrane and elevation of LPO products like MDA in diabetes [47, 48]. Elevation of oxidative stress during aging process in human has a positive and negative correlation, respectively, with PSA and RBC-SA [30]. Since RBC membrane undergoes various modifications through induction of oxidative stress in blood circulation, the negative correlation of RBC-SA with LPO may be due in part to detachment and degradation of RBC-SA from membrane as a result of the oxidative damage [30]. RBC-SA is thought to favor negative charge of RBC membrane and its biophysical properties which affect RBC interactions with the other blood cells and vascular wall [49]. It should be mentioned that desialylation of carbohydrate domain of RBC membrane is in association with development of macro and microvascular complications of diabetes by elevating RBC aggregation [50, 51].

The results of this study indicate that one of the mechanisms which can affect desialylation of RBC membrane is induction of oxidative damage of membrane lipids during development of diabetic nephropathy. Considering the potential of SA and oxidative stress biomarkers in diagnosis of diabetic conditions, further studies should be performed to evaluate other factors contributing to desialylation of RBCs' membrane including Na^+ and K^+ cation permeability, intracellular concentration of Ca^{2+}, enzymatic desialylation, and cytoskeletal changes in diabetic patients. In addition, also it is required to develop studies in diabetic patients with different stages of nephropathy for a better evaluation of the involvement of oxidative stress biomarkers in pathology of diabetic renal failure.

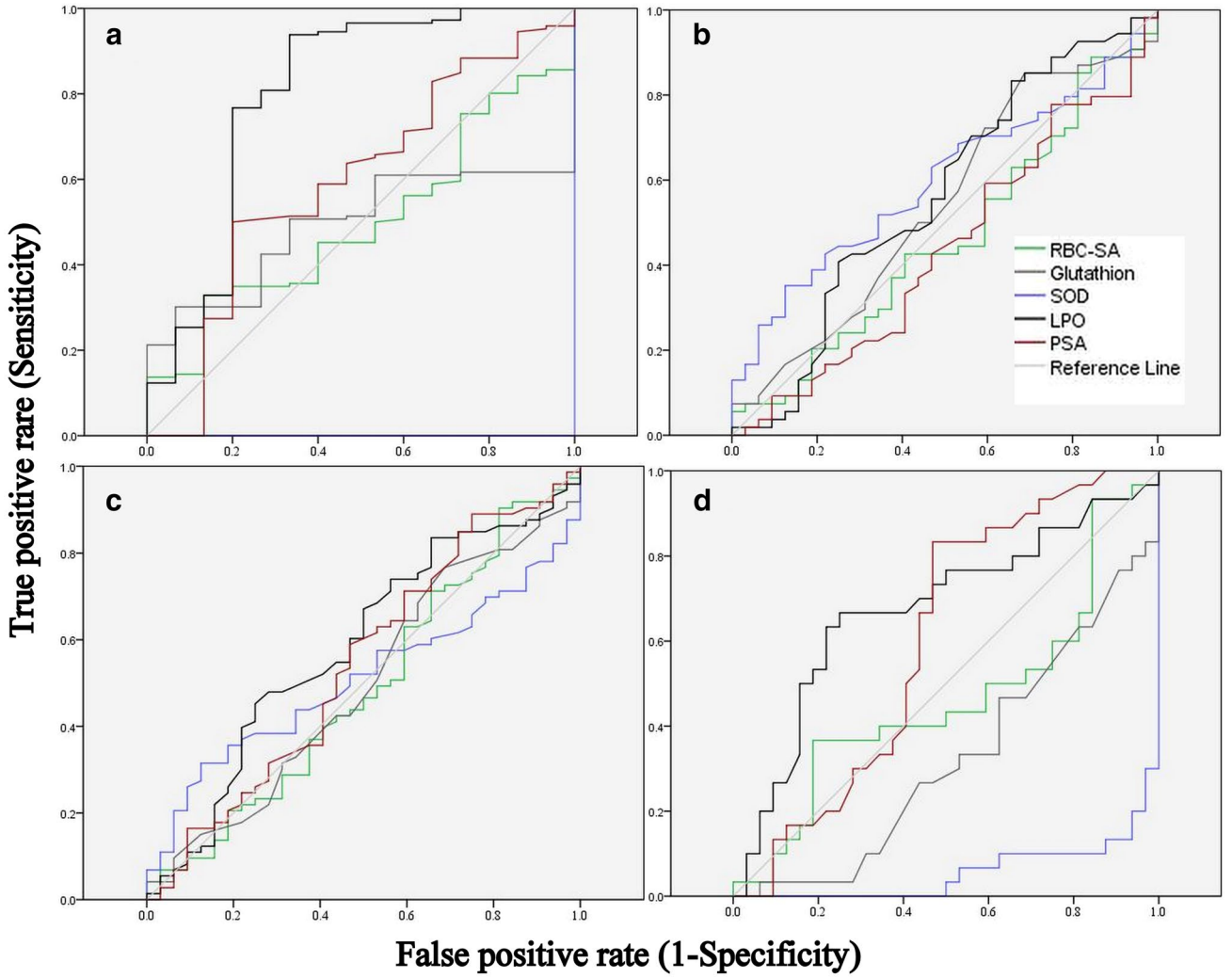

Fig. 1 Receiver-operating characteristic curve (ROC) of sensitivity plotted against 1-specificity of various biomarkers including RBC-sialic acid (RBC-SA) and plasma sialic acid (PSA), glutathione, superoxide dismutase (SOD), and lipid peroxidation (LPO) in **a** diabetic subjects, **b** diabetic patients with hyperlipidemia, **c** diabetic patients with hypertension, **d** diabetic patient with nephropathy

Table 5 Area under the curve (AUC) if the receiver-operating characteristic curve (ROC) for various biomarkers including RBC-sialic acid (RBC-SA), glutathione (GSH), superoxide dismutase (SOD), lipid peroxidation (LPO), plasma sialic acid (PSA) in the healthy, and diabetic groups

Groups	Variables	AUC ± SD	*P*
Diabetic patients against healthy group	RBC-SA	0.50 ± 0.07	0.99
	GSH	0.49 ± 0.05	0.91
	SOD	0.00 ± 0.00	0.00
	LPO	0.80 ± 0.08	0.00
	PSA	0.60 ± 0.08	0.23
Diabetic patients with hypertension against diabetic group	RBC-SA	0.49 ± 0.06	0.85
	GSH	0.50 ± 0.06	0.94
	SOD	0.51 ± 0.06	0.90
	LPO	0.58 ± 0.06	0.18
	PSA	0.54 ± 0.06	0.51
Diabetic patients with Hyperlipidemia against diabetic group	RBC-SA	0.46 ± 0.06	0.55
	GSH	0.54 ± 0.07	0.52
	SOD	0.60 ± 0.06	0.14
	LPO	0.56 ± 0.07	0.32
	PSA	0.44 ± 0.06	0.33
Diabetic patients with Nephropathy against diabetic group	RBC-SA	0.46 ± 0.08	0.61
	GSH	0.32 ± 0.07	0.02
	SOD	0.06 ± 0.03	0.00
	LPO	0.68 ± 0.07	0.02
	PSA	0.60 ± 0.07	0.10

P indicates that level of significance $P < 0.05$ is considered statistically significant

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest associated with this manuscript.

Human rights statement All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (the research ethics committee of Kerman University of Medical Sciences, approval date. 2016.10.17, approval no. IR.79. KMU.REC.1395.273) and with the Helsinki Declaration of 1964 and later versions.

Informed consent Informed consent was obtained from the participants before they were included in the study.

References

1. American Diabetes Association. Postprandial blood glucose. Diabetes Care. 2001;24(4):775–8.
2. Boyle JP, Honeycutt AA, Narayan KM, Hoerger TJ, Geiss LS, Chen H, Thompson TJ. Projection of diabetes burden through 2050: impact of changing demography and disease prevalence in the US. Diabetes Care. 2001;24(11):1936–40.
3. Animaw W, Seyoum Y. Increasing prevalence of diabetes mellitus in a developing country and its related factors. PLoS One. 2017;12(11):e0187670.
4. Zimmet P. Globalization, coca-colonization and the chronic disease epidemic: can the Doomsday scenario be averted? J Intern Med. 2000;247(3):301–10.
5. Javanbakht M, Mashayekhi A, Baradaran HR, Haghdoost A, Afshin A. Projection of diabetes population size and associated economic burden through 2030 in Iran: evidence from Micro-Simulation Markov model and Bayesian meta-analysis. PLoS One. 2015;10(7):e0132505.
6. Bonora E, Tuomilehto J. The pros and cons of diagnosing diabetes with A1C. Diabetes Care. 2011;34(Suppl 2):S184–S190190.
7. Dorcely B, Katz K, Jagannathan R, Chiang SS, Oluwadare B, Goldberg IJ, Bergman M. Novel biomarkers for prediabetes, diabetes, and associated complications. Diabetes Metab Syndr Obes. 2017;10:345–61.
8. Marra G, Cotroneo P, Pitocco D, Manto A, Di Leo MA, Ruotolo V, Caputo S, Giardina B, Ghirlanda G, Santini SA. Early increase of oxidative stress and reduced antioxidant defenses in patients with uncomplicated type 1 diabetes: a case for gender difference. Diabetes Care. 2002;25(2):370–5.
9. Karasu C. Glycoxidative stress and cardiovascular complications in experimentally-induced diabetes: effects of antioxidant treatment. Open Cardiovasc Med J. 2010;4:240–56.
10. Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol. 2003;17(1):24–38.
11. Trost S, LeWinter M. Diabetic cardiomyopathy. Curr Treat Options Cardiovasc Med. 2001;3(6):481–92.
12. Karunakaran U, Park KG. A systematic review of oxidative stress and safety of antioxidants in diabetes: focus on islets and their defense. Diabetes Metab J. 2013;37(2):106–12.
13. Wolff SP. Diabetes mellitus and free radicals. Free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. Br Med Bull. 1993;49(3):642–52.

14. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition. 2002;18(10):872–9.
15. Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol Rev. 2014;94(2):329–54.
16. Ogasawara Y, Namai T, Yoshino F, Lee MC, Ishii K. Sialic acid is an essential moiety of mucin as a hydroxyl radical scavenger. FEBS Lett. 2007;581(13):2473–7.
17. Cho A, Christine M, Malicdan V, Miyakawa M, Nonaka I, Nishino I, Noguchi S. Sialic acid deficiency is associated with oxidative stress leading to muscle atrophy and weakness in GNE myopathy. Hum Mol Genet. 2017;26(16):3081–93.
18. Varki A, Schauer R (2009) Sialic acids. In: Varki A et al. (eds) Essentials of glycobiology. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
19. Varki NM, Varki A. Diversity in cell surface sialic acid presentations: implications for biology and disease. Lab Investig. 2007;87:851.
20. Schauer R. Chemistry, metabolism, and biological functions of sialic acids. Adv Carbohydr Chem Biochem. 1982;40:131–234.
21. Traving C, Schauer R. Structure, function and metabolism of sialic acids. Cell Mol Life Sci. 1998;54(12):1330–499.
22. Sillanaukee P, Ponnio M, Jaaskelainen IP. Occurrence of sialic acids in healthy humans and different disorders. Eur J Clin Invest. 1999;29(5):413–25.
23. Nigam PK, Narain VS, Kumar A. Sialic acid in cardiovascular diseases. Indian J Clin Biochem. 2006;21(1):54–61.
24. Goswami K, Koner BC. Level of sialic acid residues in platelet proteins in diabetes, aging, and Hodgkin's lymphoma: a potential role of free radicals in desialylation. Biochem Biophys Res Commun. 2002;297(3):502–5.
25. Gorgun FM, Ozturk Z, Gumustas MK, Kokogu E. Melatonin administration affects plasma total sialic acid and lipid peroxidation levels in streptozotocin induced diabetic rats. J Toxicol Environ Health A. 2002;65(10):695–700.
26. Swithraa C, Sumathi S, Annapurn K, Asmathulla S. Evaluation of oxidative stress and protein bound sialic acid in diabetes with and without retinopathy. Int J Recent Trends Sci Technol. 2014;13(1):183–6.
27. Hu ML. Measurement of protein thiol groups and glutathione in plasma. Methods Enzymol. 1994;233:380–5.
28. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47(3):469–74.
29. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol. 1990;186:421–31.
30. Mehdi MM, Singh P, Rizvi SI. Erythrocyte sialic acid content during aging in humans: correlation with markers of oxidative stress. Dis Mark. 2012;32(3):179–86.
31. Marzban C. The ROC curve and the area under it as performance measures. Weather Forecast. 2004;19(6):1106–14.
32. Hahr AJ, Molitch ME (2015) Management of diabetes mellitus in patients with chronic kidney disease. Clin Diabetes Endocrinol 1:2
33. Krolewski AS, Skupien J, Rossing P, Warram JH. Fast renal decline to end-stage renal disease: an unrecognized feature of nephropathy in diabetes. Kidney Int. 2017;91(6):1300–11.
34. García-García PM, Getino-Melián MA, Domínguez-Pimentel V, Navarro-González JF. Inflammation in diabetic kidney disease. World J Diabetes. 2014;5(4):431–43.
35. Daenen K, Andries A, Mekahli D, Van Schepdael A, Jouret F, Bammens B. Oxidative stress in chronic kidney disease. Pediatr Nephrol. 2018;34(6):975–91.
36. Uwaezuoke SN. The role of novel biomarkers in predicting diabetic nephropathy: a review. Int J Nephrol Renovasc Dis. 2017;10:221–31.
37. Kumari S, Panda S, Mangaraj M, Mandal MK, Mahapatra PC. Plasma MDA and antioxidant vitamins in diabetic retinopathy. Indian J Clin Biochem. 2008;23(2):158–62.
38. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010;107(9):1058–70.
39. Matough FA, Budin SB, Hamid ZA, Alwahaibi N, Mohamed J. The role of oxidative stress and antioxidants in diabetic complications. Sultan Qaboos Univ Med J. 2012;12(1):5–18.
40. Kundu D, Mandal T, Nandi M, Osta M, Bandyopadhyay U, Ray D. Oxidative stress in diabetic patients with retinopathy. Ann Afr Med. 2014;13(1):41–6.
41. Rattan R, Nayak D. High levels of plasma malondialdehyde, protein carbonyl, and fibrinogen have prognostic potential to predict poor outcomes in patients with diabetic foot wounds: a preliminary communication. Int J Low Extrem Wounds. 2008;7(4):198–203.
42. Crook MA, Pickup JC, Lumb PJ, Giorgino F, Webb DJ, Fuller JH. Relationship between plasma sialic acid concentration and microvascular and macrovascular complications in type 1 diabetes: the EURODIAB Complications Study. Diabetes Care. 2001;24(2):316–22.
43. Nayak SB, Bhaktha G. Relationship between sialic acid and metabolic variables in Indian type 2 diabetic patients. Lipids Health Dis. 2005;4:15.
44. Crook MA, Tutt P, Pickup JC. Elevated serum sialic acid concentration in NIDDM and its relationship to blood pressure and retinopathy. Diabetes Care. 1993;16(1):57–60.
45. Lindberg G, Eklund GA, Gullberg B, Rastam L. Serum sialic acid concentration and cardiovascular mortality. BMJ. 1991;302(6769):143–6.
46. Abdella N, Akanji AO, Mojiminiyi OA, Al Assoussi A, Moussa M. Relation of serum total sialic acid concentrations with diabetic complications and cardiovascular risk factors in Kuwaiti Type 2 diabetic patients. Diabetes Res Clin Pract. 2000;50(1):65–72.
47. Yilmaz G, Yilmaz FM, Aral Y, Yucel D. Levels of serum sialic acid and thiobarbituric acid reactive substances in subjects with impaired glucose tolerance and type 2 diabetes mellitus. J Clin Lab Anal. 2007;21(5):260–4.
48. Vahalkar GS, Haldankar VA. RBC membrane composition in insulin dependent diabetes mellitus in context of oxidative stress. Indian J Clin Biochem. 2008;23(3):223–6.
49. Huang YX, Wu ZJ, Mehrishi J, Huang BT, Chen XY, Zheng XJ, Liu WJ, Luo M. Human red blood cell aging: correlative changes in surface charge and cell properties. J Cell Mol Med. 2011;15(12):2634–42.
50. Rogers ME, Williams DT, Niththyananthan R, Rampling MW, Heslop KE, Johnston DG. Decrease in erythrocyte glycophorin sialic acid content is associated with increased erythrocyte aggregation in human diabetes. Clin Sci (Lond). 1992;82(3):309–13.
51. Ferents IV, Brodyak IV, Lyuta MY, Sybirna NA. Structural and quantitative changes of carbohydrate chain of erythrocyte membrane glycoproteins in experimental diabetes mellitus after treatment with agmatine. Cytol Genet. 2013;47(4):252–60.