

# Differential associations of circulating peroxiredoxins levels with indicators of glycemic control in type 2 diabetes mellitus

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**Abstract. – OBJECTIVES:** Oxidative stress is an important patho-physiological mechanism in the development and complications of type 2 diabetes mellitus. To counteract oxidative stress the peroxiredoxin enzyme system exists in body cells. Whether diabetic state and/or glycemic control affects circulating levels of peroxiredoxins (PRDXs) needs to be elucidated. This study planned to assess PRDXs plasma levels of isoforms 1, 2, 4 and 6 in type 2 diabetes and their potential associations with glycemic control and insulin resistance.

**PATIENTS AND METHODS:** Plasma/serum samples were obtained from type 2 diabetic patients (n=53, 28F/25M) and control non-diabetic subjects (n=25, 7F/18M). According to HbA1c diabetics were divided into well-controlled (HbA1c < 7, n=19) and poorly-controlled (HbA1c > 7, n=34). PRDXs isoforms and insulin were measured using ELISA.

**RESULTS:** Compared to those of the control subjects, plasma levels of PRDXs1, 2, 4 and 6 were higher in diabetics. Poorly-controlled had lower levels of PRDXs2, 4 and 6 compared to well-controlled patients. PRDXs2 and 6 plasma levels negatively correlated with fasting blood sugar and HbA1c. No associations were detected with other isoforms and glycemic status or other parameters.

**CONCLUSIONS:** The high levels of PRDXs in diabetes may suggest their chaperone function and their differential association with indicators of glycemic control may suggest their biomarker role and different mechanisms of action that warrants further investigations.

*Key Words:*

Peroxiredoxins, Type 2 diabetes mellitus, Oxidative stress, Glycemic control.

duced antioxidant capacity results in oxidative stress which is known to play a role in various diseases, such as diabetes mellitus. Growing body of evidence provides a link between free radicals and oxidative stress in the pathogenesis of type 2 diabetes mellitus (T2DM) and development of its complications<sup>1-5</sup>. In addition, hyperglycemia-induced oxidative stress was reported to promote inflammation through increased endothelial cell damage, micro-vascular permeability, and increased release of pro-inflammatory cytokines, ultimately leading to evolution of diabetic complications<sup>6,7</sup>. A role of the glycemic equilibrium on the pro-oxidant/antioxidant balance has been previously investigated. Indeed, metabolic disturbances and oxidative stress seem to be tightly related; improved glycemic control is associated with lowering of the pro-oxidant status<sup>8</sup>. However, total normalization of the parameters of oxidative stress appears not to be reached by glycemic control alone, indicating continued oxidant injury despite optimal control of the diabetes<sup>9</sup>. Nevertheless, whether good glycemic control improves the antioxidant status needs to be further elucidated.

To reduce the concentration of ROS inside and outside of the cells, protective antioxidant systems have been developed<sup>10</sup>. One of such naturally occurring antioxidants is the peroxiredoxin family (PRDXs)<sup>11</sup>.

PRDXs are a group of antioxidant proteins containing essential catalytic cysteine that use thioredoxin to scavenge hydrogen peroxide, lipid hydroperoxides and peroxynitrite<sup>11</sup>. They function to play a potent defense mechanism maintaining redox balance under both normal and oxidative stress conditions. They also possess a chaperone activity<sup>12</sup>. Six isoforms (PRDXs1 to 6) have been identified and are distributed at sites of

## Introduction

An imbalance of redox homeostasis with elevated reactive oxygen species (ROS) and/or re-

ROS production, including the cytosol, mitochondria, and peroxisomes<sup>11</sup>.

A circumstantial evidence demonstrated attenuation of the antioxidant capacity in diabetic patients due to reduced activity of antioxidant enzymes including superoxide dismutase, catalase and glutathione peroxidase<sup>13,14</sup>. However, little is known about the activity of the novel antioxidative enzymes PRDXs in diabetes<sup>15,16</sup>. Therefore, the present study was designed to provide baseline data in relation to the circulating levels of PRDXs isoforms (PRDXs1, 2, 4 and 6) and their relevance to markers of glycemic control and insulin resistance in T2DM patients.

## Patients and Methods

### Study Population

In the present study 53 patients suffering from T2DM were recruited at the Primary Care Clinic at King Khalid University Hospital. Diabetic patients were subdivided according to HbA1c level into well-controlled (HbA1c ≤ 7) and poorly-controlled (HbA1c > 7) group. Patients who had acute onset of clinical or laboratory signs of acute infection, myocardial infarction, stroke, trauma, or surgical procedure in the last 6 months were excluded. Patients with coexisting malignant tumor, hepatic disease, end stage renal disease (dialysis) or immune suppression were also excluded. The full medical record of the patients was registered with detailed physical status and routine clinical laboratory tests. The duration of diabetes was between 7-10 years. Presence of complications as neuropathy, nephropathy and

retinopathy was exclusion. Diabetic patients were receiving anti-diabetic and antihypertensive agents. Control group involved twenty-five non-diabetic subjects. The study was carried out in accordance with the institutional Review Board regulations of Medical College at King Saud University.

### Blood Samples and Determination of PRDXs

Blood samples were taken after 10-12 hours of overnight fasting via antecubital vein into native, ethylenediaminetetraacetic acid (EDTA) tubes. The samples were centrifuged and aliquots of serum and plasma were stored at -70°C until assayed. PRDXs1, 2, 4 and 6 were measured in the plasma of patients and control subjects using commercially available ELISA kits (Wuhan EIAab Science Co., Ltd., China) and PRDX1 (Abfrontier, China) following manufacturer's instructions. Fasting insulin levels were determined in serum of patients and controls using ELISA kits according to the manufacturer's instructions (R&D Systems, Inc., Minneapolis, MM, USA).

Fasting blood sugar (FBS) was analyzed at KKUH Biochemistry Central Lab (Konelab Intelligent Diagnostics Systems, Konelab Corporation, Finland). And HbA1c was measured by using Helena Glyco-Tek Affinity Column method (Helena Biosciences, Colima Avenue, Sunderland Enterprise Park, UK). Index of basal insulin resistance was assessed using the homeostasis model (HOMA/IR), originally described by Matthews et al, 1985<sup>17</sup>, in which HOMA/IR (mmol/L x μIU/ml) = fasting glucose (mmol/L) x fasting insulin (μIU/ml)/22.5.

**Table 1.** Clinical data of control subjects (non-diabetic) and all diabetic patients.

	Control (non-diabetic) n = 25	Diabetic n = 53	p-value
Age	54.15 ± 15.39	61.33 ± 11.14	0.01
Gender (F/M)	8/17	28/25	
Serum Insulin (IU/ml)	13.17 ± 5.86	17.75 ± 14.94	0.206
FBS (mg/dL)	4.71 ± 0.544	9.67 ± 4.09	0.001
HOMA/IR	2.82 ± 1.437	7.14 ± 6.2	0.005
PRDX1 (ng/ml)	16.77 ± 3.899	21.92 ± 5.77	0.001
PRDX2 (ng/ml)	20.37 ± 8.61	36.61 ± 14.966	0.001
PRDX4 (pg/ml)	2696 ± 1972	3835 ± 1454	0.005
PRDX6 (pg/ml)	238 ± 111	311 ± 111	0.008

Results are expressed as mean±SD. p-value < 0.05 is considered statistically significant. Significance between two groups was determined by unpaired Student's t-test. FBS: fasting blood pressure, PRDX; peroxiredoxin1, 2, 4, and 6.

**Table II.** Effect of glycemic control on clinical and laboratory parameters in controlled (HbA1c < 7) and poorly-controlled diabetics (HbA1c > 7).

	Controlled diabetics HbA1c < 7 n = 19	Poorly-controlled HbA1c > 7 n = 34	p-value
Age	59 ± 12	62 ± 10.5	0.347
Gender (F/M)	(9/10)	(19/15)	
Serum Insulin (IU/ml)	15.97 ± 14.9	18.34 ± 15.05	0.74
FBS (mmol/L)	5.85 ± 1.2	10.96 ± 3.91	0.01
HOMA/IR	4.24 ± 3.78	8.11 ± 6.68	0.023
PRDX1 (ng/ml)	19.84 ± 6.768	23.09 ± 4.858	0.049
PRDX2 (ng/ml)	40.9 ± 13.75	34.2 ± 15.27	0.117
PRDX4 (pg/ml)	4442 ± 1428	3496 ± 1374	0.022
PRDX6 (pg/ml)	351 ± 127	289 ± 95	0.047

Results are expressed as mean±SD. *p*-value < 0.05 is considered statistically significant. Significance between two groups was determined by unpaired Student's *t*-test. BMI: body mass index; FBS: fasting blood pressure; PRDX: Peroxiredoxin1, 2, 4, and 6.

### Statistical Analysis

Data entry and statistical analysis was performed using SPSS statistical software (version 18) (SPSS Inc., Chicago, IL USA). Error bar was used to compare mean and 95% confidence interval (CI) of plasma level of PRDXs among groups. Pearson correlation was used to determine correlations of PRDXs with other variables and correlation line graphs were performed. Normally distributed values are presented as mean±SD. Students-*t* test was used for comparison between mean values of 2 groups, Chi-square ( $X^2$ ) test was used to compare qualitative variables. *p* value of < 0.05 was set as the level of significance.

## Results

### Clinical Characteristics of Study Subjects and PRDXs Levels

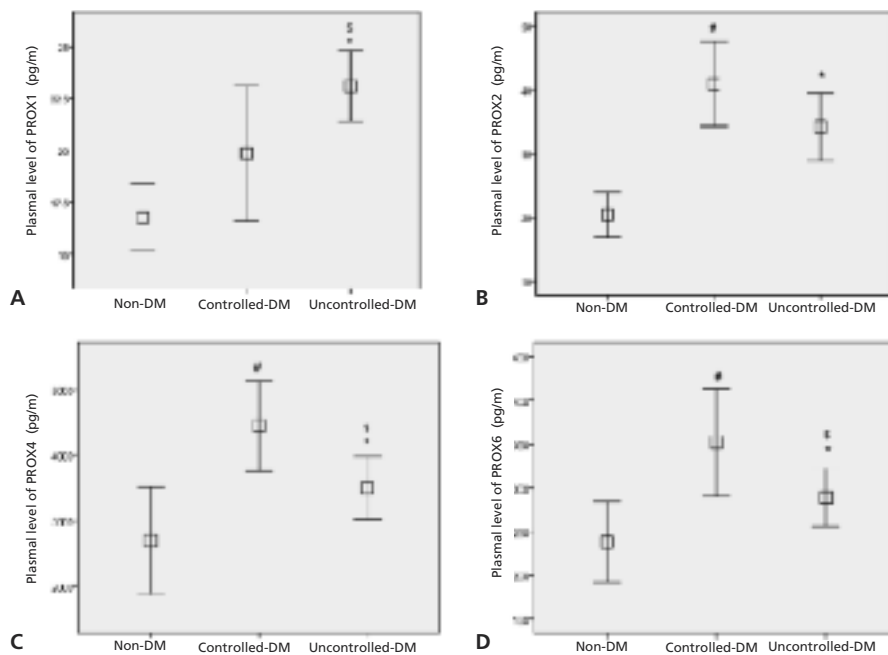
Table I shows the biochemical and clinical characteristics of the study groups comparing the control non-diabetic with all diabetic subjects. The diabetic patients demonstrated metabolic alterations including HOMA/IR, and FBS level compared with control non-diabetic subjects. Circulating plasma levels of PRDXs1, 2, 4 and 6 were significantly higher in diabetic patients compared to non-diabetics. There was no difference between diabetic patients and control subjects in serum insulin.

### Effect of Glycemic Control on PRDXs Levels in Diabetic Patients

Many prospective studies have confirmed that serum hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) concentration is an important predictor of glycemic control<sup>18</sup>. Therefore, in this study diabetic patients were divided into two groups according to HbA<sub>1c</sub> so that controlled diabetic had HbA<sub>1c</sub> < 7 and the poorly-controlled group had HbA<sub>1c</sub> > 7. Table II shows that the poorly-controlled diabetic group had significantly higher FBS and HOMA/IR than well-controlled group.

When comparing the three subgroups: poorly-controlled, well-controlled and the control healthy non-diabetic subjects, interesting results were detected in the levels of plasma PRDXs (Figure 1 a-d). Plasma levels of PRDX1 were markedly higher in poorly controlled diabetic patients (23.09 ± 4.858 ng/ml) than both non-diabetic subjects (16.8 ± 3.9 ng/ml), as well as controlled diabetic patients (19.84 ± 6.79 ng/ml, *p* < 0.01). Therefore, glycemic control brought PRDX1 plasma level to near normal control values with no difference detected between non-diabetics and controlled diabetics (Figure 1 a).

On the other hand, the plasma level of PRDX2 was significantly higher in both controlled (40.92 ± 13.75 ng/ml) and poorly-controlled diabetic patients (34.2 ± 15.26 ng/ml) compared with non-diabetic subjects (20.4 ± 8.6 ng/ml) (*p* < 0.001). PRDX2 level was not affected by the glycemic control in the diabetic patients (Figure 1b).



**Figure 1.** Plasma levels of Peroxioredoxins in non-diabetic control subjects and in controlled or poorly-controlled diabetic patients. **(A)** PRDX1, **(B)** PRDX2, **(C)** PRDX4, and **(D)** PRDX6 plasma circulating levels. Results are expressed as mean±SD. \* $p < 0.05$  vs non-diabetic (Non-DM); # $p < 0.05$  vs non-diabetic (Non-DM); \$ $p < 0.05$  vs controlled diabetic (controlled-DM).

Plasma PRDX4 reported higher levels in controlled diabetics ( $4442.36 \pm 1428.55$  pg/ml) and poorly-controlled diabetics ( $3496.78 \pm 1374.12$  pg/ml) compared to non-diabetic subjects ( $2696.9 \pm 1972$  pg/ml),  $p = 0.003$ ). However, poorly-controlled diabetic patients had lower levels of PRDX4 with respect to well-controlled diabetics ( $p < 0.05$ ) (Figure 1c).

PRDX6 also reported higher circulating levels in controlled ( $351.83 \pm 127$  pg/ml) and poorly-controlled diabetic patients ( $288.89 \pm 95.34$  pg/ml) compared to non-diabetic subjects ( $238.16 \pm 111.49$  pg/ml),  $p = 0.004$ . Similar to PRDX4, poorly-controlled diabetic patients had lower plasma level than controlled diabetics ( $p < 0.05$ ) (Figure 1d). These results suggest that PRDX isoforms are differentially affected by the glycemic control.

#### **Correlation of Circulating PRDXs Plasma Levels and Indicators of Glycemic Control**

We evaluated whether associations exist between plasma PRDXs and markers of glycemic control; HbA1c, HOMA/IR, FBS and insulin in diabetic patients. PRDX2 & 6 negatively correlated with FBS ( $p = 0.03$ ,  $r = -0.308$  and  $p =$

$0.049$ ,  $r = -0.281$ , respectively) (Figure 2) and HbA1c levels ( $p = 0.033$ ,  $r = -0.303$ , and  $p = 0.029$ ,  $r = -0.309$ , respectively) (Figure 3). PRDX6 positively correlated with fasting serum insulin (Table III). No significant correlations were detected with any of the other isoforms of PRDXs with insulin or HOMA/IR.

## **Discussion**

Oxidative stress, a situation with increased ROS production and/or decreased antioxidant defense mechanisms, is evident in the pathogenesis of diabetes<sup>14</sup> and its complications<sup>19</sup>. The present study investigated four members of the PRDX family and demonstrated that PRDX1,2,4 and 6 are markedly raised in T2DM patients. Because there are no available data regarding the average normal range of circulating PRDXs, we assume that the levels reported here for the healthy control subjects are not pathological as we stick to selection criteria for control and diabetic subjects. Previous studies in patients with T2DM revealed a decrease in antioxidant defenses and an increase in oxidative damage markers, especially in complicated state<sup>19</sup>. However, similar results to

**Table III.** Clinical data of control subjects (non-diabetic) and all diabetic patients.

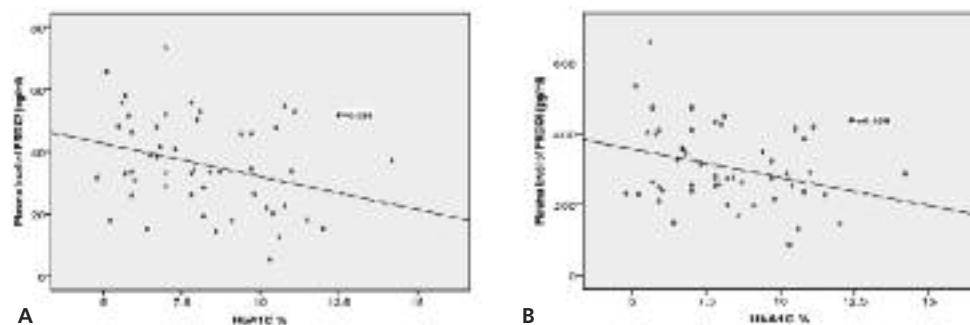
		PRDX1	PRDX2	PRDX4	PRDX6
Homa_IR	Pearson Correlation	-.093	.030	.139	.056
	Sig. (2-tailed)	.519	.835	.337	.697
Insulin (IU/ml)	Pearson Correlation	-.274	.289	.252	.450**
	Sig. (2-tailed)	.072	.057	.099	.002
	Sig. (2-tailed)	.000	.498	.812	.789
FBS (mmol/L)	Pearson Correlation	.031	-.308*	-.138	-.281*
	Sig. (2-tailed)	.831	.030	.341	.048
HbA1c	Pearson Correlation	.120	-.303*	-.102	-.309*
	Sig. (2-tailed)	.405	.033	.481	.029

FBS: fasting blood pressure; PRDX: Peroxiredoxin1, 2, 4, and 6.  $p$ -value < 0.05 is considered statistically significant.

ours were reported by Brinkmann, et al<sup>15</sup>, who observed that PRDX1, in erythrocytes, attained higher activity in T2DM than non-diabetics. Savu et al<sup>20</sup>, also found high total antioxidant capacity and higher concentration of lipid peroxidation markers in patients with T2DM. To the best of our knowledge, this is the first study measuring the circulating levels of the antioxidant PRDXs in diabetes mellitus. The increase in the anti-oxidant levels, observed in the present work, despite the well known increase in the oxidative stress in diabetes, suggests a possible adaptive response, probably due to the increased production of the  $O_2^-$ , which would lead to an augmentation in the production of  $H_2O_2$ . This mechanism, by its turn, would probably require a higher activity of antioxidant enzymes.

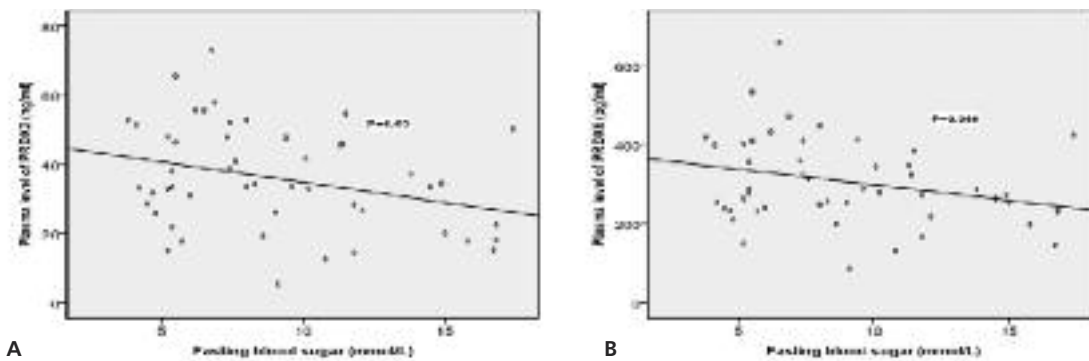
However, poorer diabetic control resulted in higher levels of PRDX1 but depression of PRDX4 and 6, as reported in the current investigation. These results can be explained by the dif-

ferent mechanistic responses of PRDXs isoforms to uncontrolled diabetic condition and signaling pathways inside the cells. Of interest, plasma levels of PRDX2 and 6 showed negative association with FBS and HbA1c, suggesting that poor glycemic control has significant impact on exhausting the cellular antioxidant capacity, possibly due to  $H_2O_2$  production. This  $H_2O_2$  when in high concentrations is associated with lesions in the pancreatic beta cells, causing disturbance both in cell signaling and gene expression<sup>21</sup>. However, we did not make any quantification of  $H_2O_2$ . In diabetic patients, the observed inverse correlations between fasting glucose and HbA1c with PRDX2 and 6, respectively, may suggest them as indicators of the critical role of the glycemic control and the occurrence of oxidative stress and depletion of antioxidants. Thus, it is plausible to suggest that the evident lack of glycemic control, despite the use of pharmaceuticals for this purpose, may promote oxidative



**Figure 2.** The association of plasma level of PRDX2 (**A**) and PRDX6 (**B**) with HbA1c ( $r = -0.303$ ,  $p = 0.033$  and  $r = -0.309$ ,  $p = 0.029$ , respectively).





**Figure 3.** Effect of fasting blood sugar on plasma levels of PRX2 and PRDX6. PRDX 2 (a) & PRDX6 (b) negatively correlated with fasting blood sugar ( $r = -0.308$ ,  $p = 0.03$  and  $r = -0.281$ ,  $p = 0.049$ , respectively).

stress through consumption/inactivation of antioxidative enzymes as PRDXs as demonstrated here and in other studies<sup>22</sup> or by activating enzymes such as NADPH oxidase<sup>23</sup>. Those could account, at least partially, for the redox imbalance and oxidative stress observed with poorer glycemic control.

The absence or inefficiency of defenses against ROS, oxidative stress would occur, leading to the activation of cellular mechanisms involved in the stress response, such as NFkB, p38MAPK, and JNK/SAPK, which would stimulate the production of inflammatory cytokines involved in diabetic complications and in pancreatic beta cells dysfunction, thus, intensifying defective insulin production<sup>24</sup>. It is well established that impaired cell glucose metabolism affects mitochondrial function and enhances reactive species production<sup>25</sup>, which seems to be implicated in the cases of insulin resistance and endothelial dysfunction which results in the persistence of the metabolic imbalance observed in diabetes<sup>26</sup>. In the current work the lack of association of insulin resistance with any of peroxiredoxins could be due to lack of heterogeneity of the diabetic population and relatively small population size.

### Conclusions

Overall, the present findings introduce the concept that altered plasma levels of PRDX1, 2, 4 and 6 occur more with lack of glycemic control in diabetic patients, which is clear for PRDX2 and PRDX6 as they were significantly related to FBS and glycosylated hemoglobin. The poorer the glycemic control the lower the level of these two members, which might suggest their bio-

marker role. The high levels of PRDXs in diabetes may suggest their chaperone function and the differential association with indicators of glycemic control warrants further investigations of their mechanisms of action. Whether these biomarkers could be used as indicators of disease progress and their role in the therapeutic management of T2DM and understanding the pathogenesis of the disease, they need future studies.

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### Conflict of Interest

The Authors declare that they have no conflict of interests.

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