ORIGINAL ARTICLE

Association of microRNA-192, pentraxin-3, and transforming growth factor-beta1 with estimated glomerular filtration rate in adults with diabetic nephropathy

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Abstract

Objective Nephropathy is among the most pervasive complications of diabetes; it frequently results in end-stage renal disease and even death. However, current biomarkers for diabetic nephropathy (DN) have limited diagnostic utility. Thus, this present study aims to examine the associations of estimated glomerular filtration rate (eGFR) with plasma concentrations of microRNA-192 (miR-192), pentraxin-3 (PTX-3), and transforming growth factor-beta1 (TGF-β1) to identify biomarkers able to distinguish late-stage from early-stage DN.

Methods In total, 50 healthy volunteers and 271 diabetes patients were enrolled in this study. Participants were stratified into seven groups according to eGFR and glycated hemoglobin (HbA1c), healthy controls, diabetes without DN (G1), diabetes with mild renal impairment (G2), and 4 DN grades (G3a, G3b, G4, and G5).

Results DN groups exhibited increases in serum miR-192 ($p < 0.05$), PTX-3($p < 0.05$), TGF-β1($p < 0.05$), malondialdehyde ($p < 0.05$), and xanthine oxidase ($p < 0.05$) levels and reductions in glutathione-s-transferase ($p < 0.05$) and superoxide dismutase ($p < 0.05$) compared to healthy controls. Among patients, eGFR was negatively correlated with miR-192, PTX-3, and TGF-β1, and positively correlated with HbA1c. In receiver operating characteristic curve analysis, miR-192 and PTX-3 demonstrated good diagnostic performance in distinguishing early from advanced DN.

Conclusion Elevated serum miR-192 and PTX-3 are associated with lower eGFR in DN, suggesting their utility as diagnostic and prognostic biomarkers.

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Introduction

The most prevalent complication of type 1 and type 2 diabetes mellitus is diabetic nephropathy (DN), which will develop in approximately 40% of all diabetic patients. Moreover, DN progressed to end-stage renal disease (ESRD), a leading cause of diabetes-related mortality [1]. The chronic hyperglycemia characteristic of diabetes often results in excessive glucose metabolism and the overproduction of reactive oxygen species (ROS), which can destroy crucial cellular macromolecules, including structural proteins, enzymes, membrane lipids, and DNA. Ultimately, this oxidative damage results in organelle dysfunction, especially mitochondrial dysfunction and energy failure [2]. In addition, enhanced polyol pathway activity and activation of protein kinase C (PKC) and nicotinamide-adenine-dinucleotide-phosphate-oxidase (NADPO) may result in the formation of

advanced glycosylation end products, which increase ROS generation and kidney damage [3].

Diabetic nephropathy (DN) characterized by excessive urinary albumin secretion, reduced glomerular filtration rate, and gradual kidney function decline, ultimately leading to kidney failure [4]. However, microalbuminuria is not a reliable diagnostic indicator of DKD. Hence, there is a need for more effective biomarkers to predict, diagnose, and monitor the disease.

Noncoding RNAs (ncRNAs), in particular, long noncoding RNAs (lncRNAs) and microRNAs (miRNAs), provide significant contributions to the pathophysiology of renal disorders [5, 6]. MicroRNAs are highly conserved noncoding RNAs of 18–24 nucleotides that silence genes post-transcriptionally to control expression [7]. MicroRNA-192 (miR-192) is among the most highly expressed miRNAs in the renal cortex [8], suggesting potential contributions to DN pathogenesis. Transforming growth factor-beta-1 (TGF-b1) is an immune mediator that reduces inflammation by interfering with Toll-like receptor-dependent signaling, thereby preventing or reversing macrophage activation [9]. There are 33 cytokines in the TGF family, and they function by forming dimeric type I and type II serine/threonine kinase receptors [10]. These receptors control the expression of genes involved in the epithelial–mesenchymal transition, angiogenesis, immune system regulation, and growth arrest [11]. Given the contributions of oxidative stress and chronic inflammation to DN, we speculated that serum TGF-b1 concentration may also be associated with the DN stage. Finally, we have also explored the long noncoding RNA pentraxin-3 (PTX-3) as a potential biomarker. Long-noncoding RNAs are ncRNAs greater than 200 nucleotides that act as the primary regulators of gene expression by increasing or decreasing mRNA stability [12]. By blocking the angiogenic fibroblast growth factor reaction, PTX-3 prevents angiogenesis, induces restenosis, and advances atherosclerotic lesions [13]. In cardiovascular and renal disorders, PTX-3 has been identified as a sensitive biomarker of innate immunity and localized inflammatory responses [14, 15].

To evaluate the potential utility of serum miR-192, PTX-3, and TGF-β1 as biomarkers for diabetic nephropathy, we have examined the associations between serum concentrations and kidney function as measured by eGFR in a cohort of diabetic adults with DN of variable severity.

Methods and Materials

Study design

In total, 271 outpatients with type 2 diabetes mellitus (T2DM) were enrolled from the specialized diabetes and nephrology clinic of the Internal Medicine Department,

Beni-Suef University Hospital, Beni-Suef, Egypt. Eligible patients were divided into six severity groups according to eGFR [16]. Fifty healthy adult males and females matched for age and sex were included as normal controls. This study was conducted following the Declaration of Helsinki and clinical practice recommendations, and study protocols were approved by the hospital Ethics Committee (BSU: 7–2021). Blood samples were collected between November 2020 and June 2021 after study approval. During laboratory visits, patient body parameters were also measured.

Inclusion criteria

Healthy controls ($n = 50$) were selected based on the absence of significant health-related issues and no signs of diabetes/prediabetes [glycated hemoglobin (HbA1c) below 5.7%, fasting glucose level below 110 mg/dl, and postprandial glucose level below 140 mg/dl] or kidney dysfunction [eGFR ≥ 90 mL/min/ 1.73 m^2)]. Diabetic patients were divided into a non-nephropathy group (G1: HbA1c > 6.5%, eGFR ≥ 90 mL/min/ 1.73 m^2, $n = 46$), a mild DN group (G2: HbA1c > 6.5%, eGFR 60–89 mL/min/1.73 m^2, $n = 50$), and a DN group (HbA1c > 6.5%, eGFR < 60 mL/min/ 1.73 m^2). In turn, the DN group was stratified into severity groups as follows: G3a: HbA1c > 6.5%, eGFR 45–59 mL/min/1.73 m^2 ($n = 43$); G3b: HbA1c > 6.5%, eGFR 30–44 mL/min/1.73 m^2 ($n = 40$); G4: HbA1c > 6.5%, eGFR 15–29 mL/min/1.73 m^2 ($n = 45$); G5 [ESRD]: HbA1c > 6.5%, eGFR < 15 mL/min/1.73 m^2 ($n = 47$).

Exclusion criteria

Participants with a history of acute and chronic infections, malignancy, hepatic disease, diabetic retinopathy, and other endocrine dysfunctions were excluded from this study.

Biochemical assays

After an overnight fast, two 4-mL blood samples were obtained from all participants, one in ethylenediamine tetraacetic acid (EDTA)-treated tubes and the other in plain ones. Samples in plain ones were incubated at room temperature for 30 min and centrifuged at 4,000 g for serum isolation; meanwhile blood samples containing EDTA were frozen at −80 °C until DNA extraction and HbA1c% determination. Glycated hemoglobin was measured using kits from Stanbio (Boerne, Texas, USA), blood glucose, creatinine, urea, uric acid, sodium, potassium, and calcium were measured in serum samples using dedicated kits from Spinreact (Girona, Spain). Fasting insulin was measured using radioimmunoassay kits from Diagnostic Products Corporation (Los Angeles, CA, USA). Insulin resistance was measured according to the homeostatic model (HOMA-IR),

where HOMA-IR = [(Fasting Insulin, μU/ml) × (Fasting Glucose, mmol/L)]/22.5 according to Matthews et al. [17]. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to estimate eGFR in adults [18]. Malondialdehyde (MDA) levels were measured using the thiobarbituric acid method [19], xanthine oxidase (XO) activity using the methodology of Ozer et al. [20], and superoxide dismutase (SOD) and glutathione-s-transferase (GST) activities according to a previously described method [21] using kits from Biodiagnostic (Giza, Egypt).

MicroR-192, PTX-3, and TGF-β1 assays

The total RNA was isolated from the serum samples using the Direct-zol RNA Miniprep Plus kit (Cat # R2072, Zymo Research, Irvine, CA, USA), and RNA quality was assessed using a Beckman dual spectrophotometer (Brea, CA, USA). Isolated RNA samples were reverse-transcribed; then, expression levels were measured by quantitative real-time PCR using a One-Step RT-PCR kit (Cat # 12594100, Thermo Fisher, USA) and the following primer sequences: F: 5′TGACCTATG AATTGACAGCCGT-3′ and R: 5′ ATCCAGTGCAGGGTC CGA-3′ for miR-192; F: 5′ AATGCTGTGTCTCTGTCA-3′ and R: 5′ ACATACCAATAACAATGAACAATG-3′ for PTX-3; F: 5′AACACATCAGAGCTCCGAGAA-3′ and R: 5′GTC AATGTACAGCTGCCGCAC-3′ (NM-000660.2) for TGF-β1; F: 5′GGCGGCACCACCATGTACCCT-3′ and R: 5′ AGG GGCCGGACTCGTCATACT-3′ (NM-001101.3) for β-actin (the internal control for TGF1-β); F: and R: for GAPDH (the internal control for PTX-3). Expression of miRNA-192 was normalized to U6 expression. The RQ of each target gene was quantified and normalized to the specified internal control according to the calculation $2 - \Delta\Delta Ct$ [22].

Statistical analysis

All statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS) 22.0 (SPSS Inc., Chicago, IL, USA). Demographic and clinical parameters are presented as mean ± standard error of the mean (SEM). Group means were compared via one-way analysis of variance with post hoc Duncan's multiple range tests for pairwise comparisons. A $p < 0.05$ was considered statistically significant for all tests. Associations between eGFR and target gene expression levels were evaluated via Pearson's correlation coefficient. Receiver operating characteristic (ROC) curves were constructed by plotting sensitivity on the Y-axis versus 1-specificity on the X-axis at various cut-off values. The diagnostic accuracy for each cut-off was evaluated by measuring the area under the ROC curve (AUC). At least 50% of performance was considered adequate.

Results

The average age of the study population was 49.40 ± 13.50 years, and the majority were males (51.71%). There were marked variations in body mass index (BMI), systolic blood pressure, and diastolic blood pressure among the healthy control, diabetic without DN (G1), diabetic with mild impairment (G2), and diabetic nephropathy (G3a, G3b, G4, and G5) groups ($p < 0.05$) (Table 1). Compared to the healthy controls, all diabetic groups exhibited significantly higher fasting blood sugar (FBS), HbA1c%, Homa-IR, and serum urea, creatinine, uric acid, and potassium levels. Additionally, there were significantly lower levels of serum insulin, serum calcium, and eGFR ($p < 0.05$). Sodium levels were found to be significantly higher in G2, G3a, G3b, and G4 groups as compared to healthy controls (Table. 1). On the other hand, a significant decline ($p < 0.05$) was noted in sodium concentrations at G5 (ESRD) compared to healthy controls and patients in earlier stages of DN (G2, G3a) (Table 1).

The findings demonstrate that individuals with diabetes have significantly higher levels of MDA and XO, indicating the presence of chronic oxidative stress. Moreover, all diabetic patient groups showed considerably lower levels of SOD and GST compared to healthy controls, as depicted in Fig. 1. Notably, as DN progressed to its later stages (G3b, G4, G5), MDA and XO levels continued to rise significantly ($p < 0.05$). SOD and GST activities showed significant declined, as shown in Fig. 1A, B, C, D for the early stages (G2 and G3a).

Serum expression levels of miR-192, PTX-3, and TGF-β1 were significantly elevated in DN stages (G3a, G3b, G4, G5) as compared to healthy controls (Fig. 2) and in later stages (G3b, G4, G5) compared to early stages (G2, and G3a) (Fig. 2A, B, C). Moreover, miR-192, PTX-3, and TGF-β1 levels were negatively correlated with eGFR in stages G2, G3a, G3b, G4, and G5 (for G2; miR-192: $r = -0.643$, $p < 0.001$; for PTX-3: $r = -0.523$, $p < 0.001$; for TGF-β1: $r = -0.570$, $p < 0.001$) (Table 2), whereas these correlations did not reach significance in G1. The glycemic biomarker (HbA1c) was also determined to be positively correlated with serum expression levels of miR-192, PTX-3, and TGF-β1 (Table 2) in all diabetic stages (G1, G2, G3a, G3b, G4, and G5), and these correlations were higher through disease progression from diabetic stage (G1) (miR-192: $r = 0.794$, $p < 0.001$; PTX-3: $r = 0.722$, $p < 0.001$; TGF-β1: $r = 0.706$, $p < 0.001$) to ESRD (G5) (miR-192: $r = 0.913$, $P < 0.001$; PTX-3: $r = 0.878$, $p < 0.001$; TGF-β1: $r = 0.889$, $p < 0.001$).

Serum PTX-3 discriminated participants with DN from healthy controls with 96.68% sensitivity and 100% specificity (AUC = 0.993; 95% confidence interval [CI] = 0.985–1.001;

Table 1 Demographic, diabetic and kidney profiles of healthy controls and diabetic-nephropathy groups

Group Parameter	Healthy controls	G1	G2	G3a	G3b	G4	G5
Age (Year)	43.71 ± 1.15^a	51.08 ± 0.66^b	56.12 ± 0.89^c	60.02 ± 0.91^d	63.02 ± 0.98^e	63.88 ± 0.81^e	59.06 ± 0.85^d
Gender, no. (%)							
Male	27 (54)	20 (43)	26 (52)	20 (46)	23 (57)	24 (53)	26 (56)
Female	23 (46)	26 (47)	24 (48)	23 (54)	17 (43)	21 (47)	21 (44)
BMI (Kg/m^2)	28.06 ± 0.33^a	33.08 ± 0.52^{cd}	32.16 ± 0.63^{bcd}	31.41 ± 0.41^{bc}	33.62 ± 0.65^d	30.36 ± 0.52^b	31.28 ± 0.59^{bc}
SBP (mmHg)	124.22 ± 1.08^a	125.69 ± 0.87^{ab}	130.94 ± 1.31^b	140.76 ± 1.65^c	142.13 ± 1.88^c	143.12 ± 2.37^c	146.26 ± 3.04^c
DBP (mmHg)	82.98 ± 0.55^a	82.91 ± 0.58^a	85.88 ± 0.75^{ab}	88.18 ± 0.98^b	88.85 ± 1.08^b	88.24 ± 1.57^b	87.16 ± 1.71^b
FBS (mg/dl)	82.06 ± 0.92^a	165.61 ± 3.73^{bc}	194.12 ± 7.62^d	181.21 ± 7.28^{cd}	190.30 ± 6.42^d	163.84 ± 3.24^b	173.23 ± 5.88^{bc}
HbA1c (%)	4.73 ± 0.05^a	8.81 ± 0.09^b	9.67 ± 0.16^d	9.12 ± 0.16^{bc}	9.82 ± 0.13^d	9.44 ± 0.10^{cd}	9.21 ± 0.17^c
Fasting insulin (mLU/L)	11.25 ± 0.09^e	10.52 ± 0.08^d	8.43 ± 0.05^c	8.07 ± 0.04^b	7.96 ± 0.04^{ab}	7.82 ± 0.04^a	7.88 ± 0.05^a
HOMA-IR	2.27 ± 0.03^a	4.29 ± 0.09^f	4.04 ± 0.16^{ef}	3.61 ± 0.15^{cd}	3.74 ± 0.12^{de}	3.16 ± 0.06^b	3.35 ± 0.10^{bc}
Creatinine (mg/dl)	0.92 ± 0.02^a	0.94 ± 0.01^a	1.01 ± 0.02^{ab}	1.26 ± 0.02^b	1.58 ± 0.04^c	3.09 ± 0.07^d	7.22 ± 0.26^e
Urea (mg/dl)	22.26 ± 0.46^a	23.89 ± 0.52^a	27.84 ± 0.65^a	36.37 ± 1.04^b	51.22 ± 1.98^c	88.55 ± 2.74^d	114.70 ± 3.73^e
Uric acid (mg/dl)	4.33 ± 0.06^a	4.98 ± 0.10^b	5.36 ± 0.11^b	5.79 ± 0.12^c	6.11 ± 0.26^{cd}	6.42 ± 0.15^d	6.51 ± 0.19^d
Sodium (mEq/l)	140.66 ± 0.66^b	144.36 ± 0.72^c	144.90 ± 0.75^c	147.09 ± 0.97^{cd}	149.35 ± 1.42^d	146.80 ± 1.78^{cd}	134.43 ± 0.80^a
Potassium (mEq/l)	4.39 ± 0.07^a	4.42 ± 0.06^a	4.51 ± 0.07^a	4.54 ± 0.07^a	4.76 ± 0.08^b	6.13 ± 0.06^d	5.72 ± 0.09^c
Calcium (mg/dl)	9.47 ± 0.07^d	9.09 ± 0.07^c	9.04 ± 0.09^c	8.59 ± 0.10^b	8.98 ± 0.11^c	9.03 ± 0.11^c	7.90 ± 0.10^a
eGFR (mL/min/1.73 m^2)	92.93 ± 0.31^f	90.92 ± 1.85^f	70.11 ± 0.81^e	52.31 ± 0.64^d	39.66 ± 0.72^c	20.07 ± 0.49^b	7.87 ± 0.36^a

Data were expressed as mean $\pm$ SE. Values that share the same superscript symbol are not significantly different

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FBS* fasting blood sugar, *HbA1c%* glycated hemoglobin, *HOMA-IR* homeostatic model assessment for insulin resistance, *eGFR* estimated glomuler filtration rate, *G1* stage 1 (kidney damage with normal or increased GFR $\geq$ 90), *G2* stage 2 (kidney damage with mildly decreased GFR 60–89), *G3a* stage 3a (moderately decreased G FR 30–59), *G3b* stage 3b (moderately to severely decreased GFR 30–44), *G4* stage 4 (severely decreased GFR 15–29), *G5* stage 5 (kidney failure, GFR < 15), all GFR inmL/min/1.73m^2

$p < 0.001$; Fig. 3A), thus suggesting its utility as a diagnostic marker. Indeed, serum PTX-3 level discriminated late stages of DN (G3b, G4, G5) from early stages (G2, G3a) with 97.83% sensitivity and 94.41% specificity (AUC = 0.994, $p < 0.001$; Fig. 3B). Similarly, serum miR-192 differentiated participants with DN from healthy controls and late stages of DN (G3b, G4, G5) from early stages (G2, G3a), as shown in Fig. 3C, D.

Discussion

Chronic hyperglycemia can induce DN, which, in turn, can progress to chronic kidney failure and even death [23]. Microalbuminuria is the standard biomarker for early DN detection and diagnosis, but its efficiency in estimating disease stage remains limited [24]. To effectively diagnose and treat DN in its early stages, it is imperative to identify more sensitive indicators of DN progression. Here, we show that serum levels of miR-192 and PTX-3 can distinguish early- from late-stage DN with high sensitivity and specificity among Egyptian diabetes patients with widely varying eGFR and other clinicodemographic parameters such as BMI, SBP, and DBP. In addition, there were linear increases in FBS,

HbA1c%, creatinine, urea, uric acid, and potassium with DN severity, consistent with other Egyptian studies [25, 26], while fasting insulin, calcium, and eGFR declined with DN severity. Serum sodium was also significantly higher in G2, G3a, G3b, and G4 compared to healthy controls but lower in stage 5 (ESRD) compared to earlier stages. Therefore, these factors may yield even more accurate staging and prognosis.

Elevated blood sugar levels have the potential to trigger a surge in ROS production, leading to harmful effects on the renal kidney tubes and podocytes. With time, this damage can transform into renal fibrosis [27]. Malondialdehyde and XO levels were significantly higher, while SOD and GST levels were noticeably lower in all diabetic patient groups as compared to healthy controls, in accordance with Lodhi and colleagues [28], who found that diabetic rats had considerably higher MDA and lower SOD levels than the controls. Additionally, there were substantial differences in MDA, XO, SOD, and GST levels between the early and late stages of DN. Patients with ESRD were found to have higher serum XO activity than healthy individuals and those with chronic renal failure [29]. Our findings are also consistent with Bessa and coworkers, who reported that GST, SOD, glutathione peroxidase (GPx), catalase (CAT), and glutathione (GSH) were negatively correlated with albumin creatinine
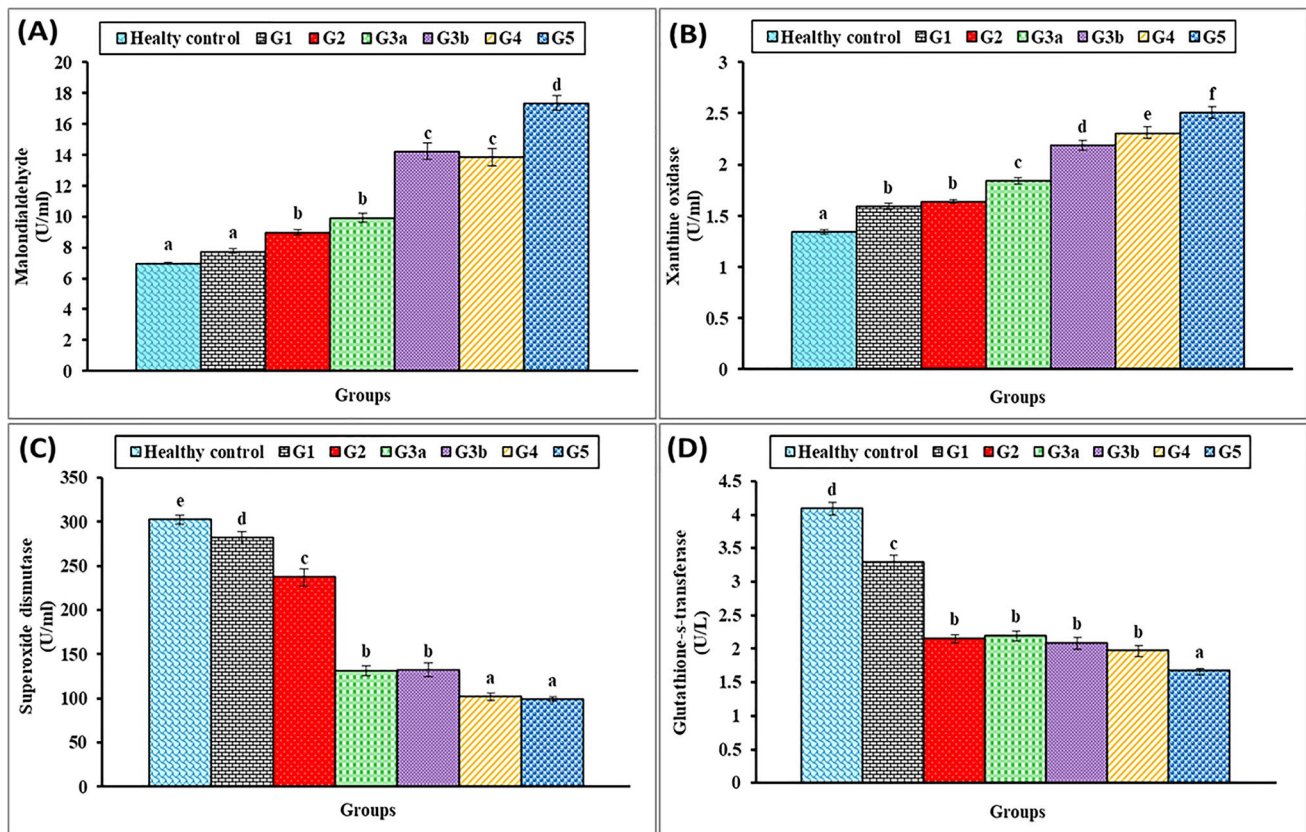
Fig. 1 Oxidative stress profiles of healthy controls, diabetic and diabetic nephropathy groups. Data are expressed as mean ± SEM. Insignificant differences between two groups according to Duncan's post hoc multiple comparison tests are indicated by the same superscript symbol

ratio and positively with eGFR, two crucial indices of renal function [30]. These findings indicate that oxidative stress is a chronic process that contributes substantially to the progression of DN.

Noncoding RNAs are essential for many cellular functions, as more than 30% of the human genome is regulated by lncRNAs and miRNAs [31]. MicroRNA-192 has been widely expressed in the human kidney and is critical for maintaining normal kidney function [32]. Expression of miR-192 was elevated significantly in all DN stages relative to healthy controls, and there was a significant difference between late and early stages, consistent with Saadi and colleagues [33], who found higher blood levels of miR-192 in participants with more advanced DN than T2DM patients with normal albuminuria. They also found a strong inverse relationship between eGFR and miR-192 and a positive correlation between miR-192 levels and the degree of glycemia as indicated by HbA1c. In the current study, miR-192 levels were positively correlated with HbA1c in all investigated groups and negatively correlated with eGFR except for stage 1. Chien and colleagues reported no observable change in serum miR-192 levels between type 2 diabetes patients with and without kidney disease. However, compared to patients

with moderately elevated urinary albumin excretion (UAE), those with considerably elevated UAE had significantly greater serum miR-192 [34]. One of the potential mechanisms by which miR-192 could impact DKD and kidney fibrosis is through E-box repressor Smad-1 interacting protein (Zeb2), which attaches to E-box enhancer elements in the Col1a2 gene and accelerates collagen production in response to TGF-β1 [35]. As DN progresses, elevated TGF-β1 expression in renal cells would induce both fibrosis and hypertrophy [36]. Other miRNAs (miR-216a/217 and miR-200 family) may also altered by this process (Zeb1/2 targeted by miR-192). Moreover, Akt kinase may stimulate fibrosis via miR-192 as Akt stimulation in mouse mesangial cells (MCs), which could result in the elevation of DKD markers like extracellular matrix (ECM) genes as well as hypertrophy and apoptosis inhibition [37]. Consequently, miR-216a can target RNA binding protein (Ybx1) and a P-bodies component, leading to TGF-β1-induced collagen expression in mouse MCs [38].

The long pentraxin PTX-3 is a member of the same family as serum amyloid P and hs-CRP [39]. In response to vascular inflammation, PTX-3 expressed in vascular endothelial cells, primary proximal renal tubular epithelial cells,
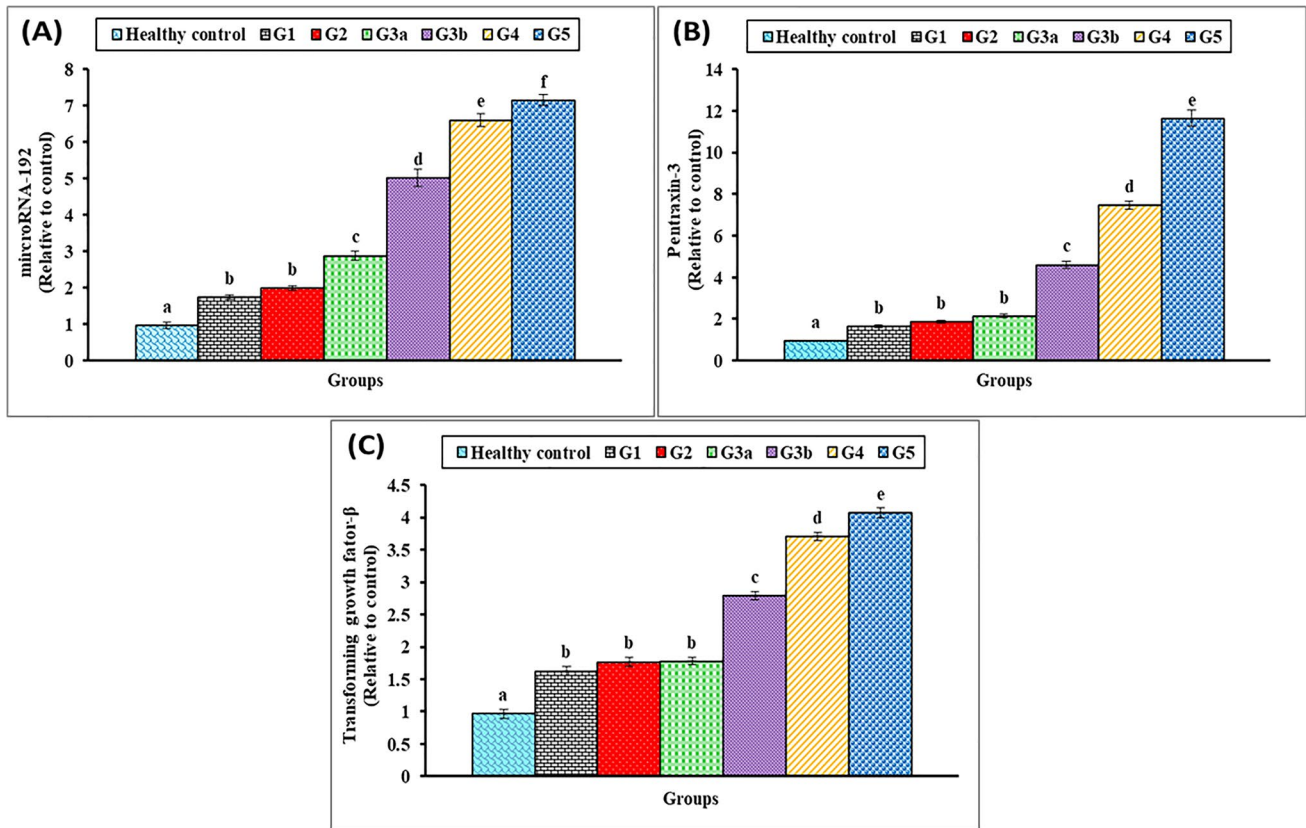
Fig. 2 Serum levels of (**A**) microRNA-192, (**B**) pentraixn-3, (**C**) transforming growth factor-beta in healthy controls, diabetic and diabetic nephropathy groups. Data are expressed as mean ± SEM. Insig-nificant differences between two groups according to Duncan's post hoc multiple comparison tests are indicated by the same superscript symbol

Table 2 Correlations between miR-192, PTX-3 and TGF-β with eGFR and HbA1c% among diabetic and diabetic-nephropathy groups

Parameters	eGFR						HbA1c%					
Groups	miR-192		PTX-3		TGF-β		miR-192		PTX-3		TGF-β	
	r	p	r	p	r	p	r	p	r	p	r	p
G1	0.090	> 0.05	0.112	> 0.05	0.061	> 0.05	0.794	< 0.001***	0.722	< 0.001***	0.706	< 0.001***
G2	−0.643	< 0.001***	−0.523	< 0.001***	−0.570	< 0.001***	0.837	< 0.001***	0.732	< 0.001***	0.732	< 0.001***
G3a	−0.776	< 0.001***	−0.724	< 0.001***	−0.710	< 0.001***	0.838	< 0.001***	0.833	< 0.001***	0.757	< 0.001***
G3b	−0.842	< 0.001***	−0.860	< 0.001***	−0.876	< 0.001***	0.892	< 0.001***	0.926	< 0.001***	0.933	< 0.001***
G4	−0.929	< 0.001***	−0.932	< 0.001***	−0.943	< 0.001***	0.934	< 0.001***	0.952	< 0.001***	0.949	< 0.001***
G5	−0.949	< 0.001***	−0.921	< 0.001***	−0.944	< 0.001***	0.913	< 0.001***	0.878	< 0.001***	0.889	< 0.001***

Correlation was significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively

G1 stage 1 (kidney damage with normal or increased GFR ≥ 90), *G2* stage 2 (kidney damage with mildly decreased GFR 60–89), *G3a* stage 3a (moderately decreased GFR 30–59), *G3b* stage 3b (moderately to severely decreased GFR 30–44), *G4* stage 4 (severely decreased GFR 15–29), *G5* stage 5 (kidney failure, GFR < 15), all GFR in mL/min/1.73 m^2

primary MCs, and renal fibroblasts [40]. The expression of PTX-3 was markedly elevated in all DN stages but to a greater extent in the late stages than in the early stages. These findings are in line with earlier studies by Dawood and colleagues and Wang and colleagues, who both reported a substantial elevation in PTX-3 levels with DN progression

and suggested that PTX-3 can act as a biomarker for both prognostic and diagnostic purposes before the onset of overt chronic kidney disease (CKD) [15, 41]. By preventing fibroblast growth factor signaling, PTX3 prevents angiogenesis, increases restenosis, and increases advanced atherosclerotic lesions [13]. In this present study, PTX-3 and HbA1c were
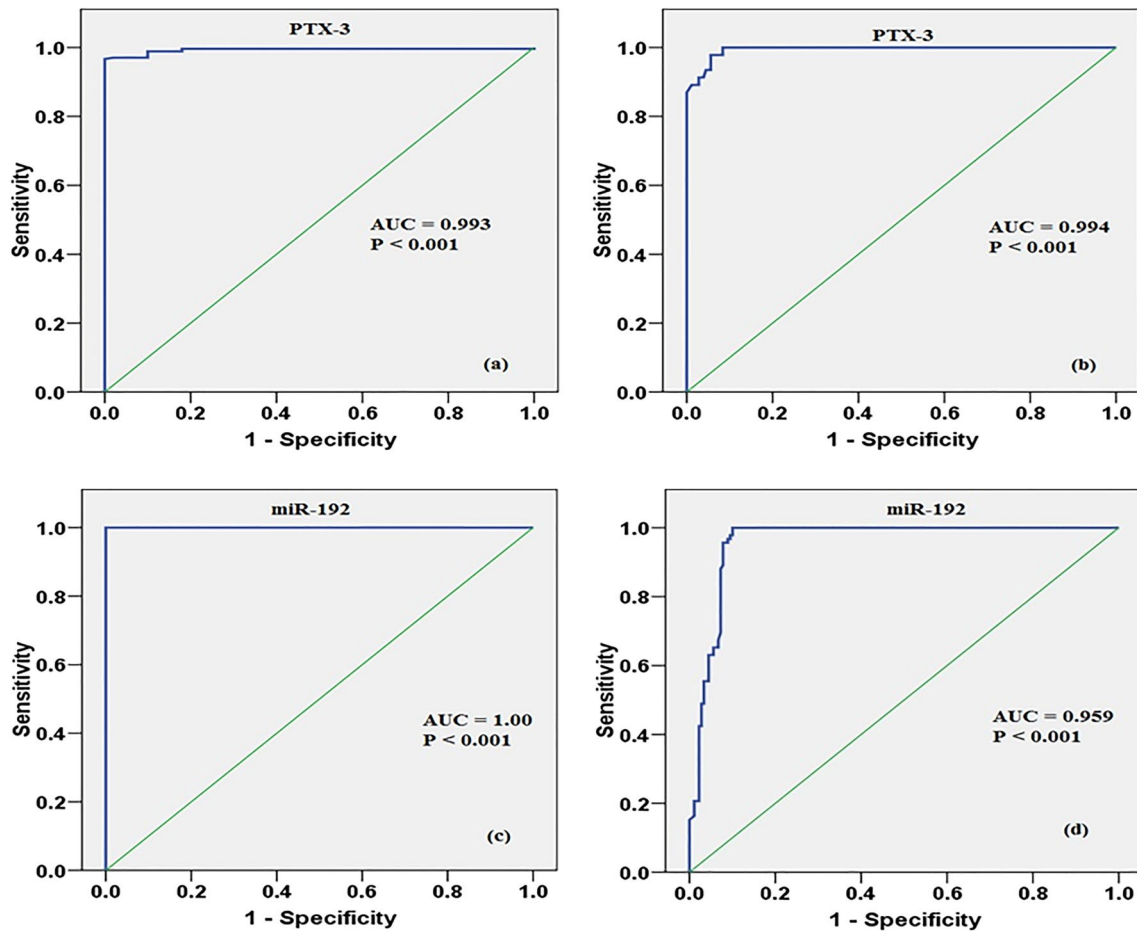
Fig. 3 Receiver operating characteristic (ROC) curves for pentraixn-3 and microRNA-192 in (**a, c**) early diabetic groups and (**b, d**) late-stage diabetic nephropathy groups

determined to be strongly and positively correlated during all DN stages, consistent with Takashi et al. [42], who found that PTX-3 was significantly higher in patients with diabetes and has a notable correlation with HbA1c and UAE. There was a negative correlation between eGFR and PTX-3. Tong et al. [43] also observed higher PTX-3 levels in CKD patients than in healthy controls. Furthermore, PTX-3 was positively associated with protein-energy wasting, cardiovascular disease, and mortality and negatively correlated with eGFR in these patients.

Transforming growth factor-beta may also contribute to the pathogenesis of DN. In the proximal tubules, TGF-β1 stimulates renal cell hypertrophy, controls the formation of ECM molecules such as type I and type IV collagen, and induces the production of chemokines [44]. Further, TGF-β1 also slows down matrix disintegration by blocking proteases, thereby accelerating the development of glomerulosclerosis and tubulointerstitial fibrosis [45]. In addition to TGF-β1, ROS generation activates NF-κB, angiotensin II/ TGF-β1/ smad, and PKC signaling pathways, which can induce ECM

protein accumulations and fibrosis. Further, signaling elements, including PKC, TGF-β1, and angiotensin II, also promote ROS generation, causing oxidative stress damage and DN [46]. ROS accumulation induces the expression of numerous pro-fibrotic growth factors, including TGF-β1, VEGF, and connective tissue growth factors, which accelerate ECM protein formation and kidney dysfunction [47].

Expression of TGF-β1 was elevated in all stages of DN and higher in late-stage than early-stage patients. These results align with Qiao et al. [48] Shukla et al. [49], who reported that serum TGF-β1 expression was elevated in type 2 diabetes patients and considerably higher in T2DM patients with nephropathy. There were a strong positive correlation between TGF-β1 and HbA1c in all DN stages and a negative correlation with eGFR. Shaker et al. found positive correlations between TGF-β1 and glucose and HbA1c levels [50]. Additionally, John and Yadla reported that TGF-β elevation in T2DM patients was associated with the severity of kidney damage [51].

Finally, the results of the ROC analysis demonstrate that serum miR-192 and PTX-3 can accurately distinguish DN patients from healthy controls, with serum miR-192 exhibiting diagnostic potential as it can also discriminate late from early stages. These results align with Saadi et al. [33], who reported that miR-192 effectively distinguished T2DM patients with and without DKD and that plasma expression correlated positively with albuminuria. Hence, miR-192 and serum PTX-3 may be promising circulating biomarkers that can effectively detect and monitor disease progression at an early stage.

This current study has several limitations, including the sample size of each DN grade. In addition, other variables known to be associated with DN, such as smoking, various medications, degree of physical activity, and inflammatory mediators, were not examined or controlled.

Conclusion

Serum miR-192, PTX-3, TGF-β1, MDA, and XO were elevated, while SOD and GST levels lowered in DN patients relative to healthy controls. Moreover, It has been noted that the alterations in the later stages of the disease are notably more substantial compared to the initial stages. Additionally, plasma expression levels of miR-192, PTX-3, and TGF-β1 were negatively correlated with eGFR and positively correlated with HbA1c. Our findings suggest that miR-192 and PTX-3 can act as diagnostic biomarkers for discriminating patients in the late stages of DN from those in the early stages.

Declarations

Ethics approval and consent to participate This study was conducted in compliance with the Declaration of Helsinki, and approved by the (BSU: 7-2021). Written informed consent was obtained from each participant in the study.

Patient consent for publication Patient consent for publication was covered by the informed consent document.

Competing interests The authors declare that they have no competing interests.

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