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ORIGINAL ARTICLE

E-cadherin and periostin in early detection and progression of diabetic nephropathy: epithelial-to-mesenchymal transition

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Abstract

Background Diabetic nephropathy (DN) is a severe complication of diabetes mellitus (DM). Many mechanisms are involved in its development; one of these mechanisms is epithelial-to-mesenchymal transition (EMT). During EMT, losing of the epithelial biomarkers like E-cadherin and increasing of mesenchymal biomarkers like periostin are very characteristic.

Methods The study included 19 healthy controls and 71 DN patients categorized according to their urinary albumin-to-creatinine ratio (UACR) into 19 normoalbuminuric (UACR < 30 mg/g), 37 microalbuminuric (UACR 30–300 mg/g), and 15 macroalbuminuric (UACR > 300 mg/g) patients. Fasting plasma glucose (FPG), glycated hemoglobin (HbA_{1C}%), serum creatinine (Cr), and urea were measured. E-cadherin and periostin were measured by ELISA and compared among groups.

Results Concerning E-cadherin levels, in comparison to control group, there were significantly decreased in all groups (0.94, 0.52, and 0.14 ng/mL in normoalbuminuria, microalbuminuria, and macroalbuminuria groups; respectively). For periostin levels, nonsignificant increase in normoalbuminuria (0.32 ng/mL) than control group (0.3 ng/mL) was observed. There was a significant increase in other groups with the highest values in macroalbuminuria group (1.66 ng/mL). E-cadherin and periostin were correlated with each other ($r = -0.353$, $P < 0.001$). UACR was negatively correlated with E-cadherin and positively correlated with periostin. ROC curve analyses showed that the AUC to diagnose established microalbuminuria using E-cadherin was 0.998 (95% CI 0. 932–1), and using periostin was 0.833 (95% CI 0.709–0.919).

Conclusion Serum E-cadherin and periostin could be considered as reliable biomarkers involved in DN pathogenesis and linked to its stages.

Keywords Diabetic nephropathy · E-cadherin · EMT · Periostin

Introduction

Diabetes mellitus (DM) is considered one of the most important public health challenges, as at the last three decades the number of people with DM has almost doubled [1].

One of the major devastating complications of DM is diabetic nephropathy (DN) which became the leading reason of end-stage renal failure [2]. DN is a syndrome characterized by high quantities of urinary albumin excretion, loss of glomerular filtration rate, presence of many glomerular lesions, accumulation of the extracellular matrix (ECM), and thickening of basement membranes that lead to renal fibrosis [3]. Notably, DN spreads rapidly as the number of patients was 382 million in 2013 and is estimated to achieve 592 million by 2035 [4].

Epithelial-to-mesenchymal transition (EMT) is one of the underlying mechanisms of DN in which loss of epithelial cell markers like cadherins and increase of mesenchymal markers like vimentin and fibronectin occur. This process is induced by fibrogenic mediators such as transforming growth factor beta (TGF-β) [5, 6].

The epithelial cells lose their morphology by becoming elongated with spindle shape according to four consequent steps: first, loss of epithelial cell adhesion; second, expression of fibrotic features; third, disruption of tubular basement membrane by matrix metalloproteinases; fourth, enhancement of cell migration [7, 8].

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The cadherin family is one of the most critical epithelial cell-to-cell adhesion molecules [9]. Among 50 cadherins, the best described are type one cadherins such as E-cadherin. E-cadherin is a transmembrane integral protein which is found at the adherent junction of the epithelial cells. E-cadherin is abundant in the distal tubules, but does not exist or is found only at very low amounts in the proximal tubules [10]. E-cadherin is essential for the assessment and regulatory of kidney transplant [7]. In the early stages of EMT, losing of E-cadherin expression occurs and the separation of cells within the epithelial sheet happens [8].

Many biomarkers induce expression of mesenchymal phenotype in renal cells such as periostin. The original name of periostin is osteoblast-specific factor-2 (Genbank D13664) which was categorized as a soluble ECM protein that was expressed in bone [11]. Periostin has been lately discovered as a tissue biomarker of kidney impairment [12]. Periostin is unobserved in the normal renal tubules, and is discharged when distal tubules are damaged. It was found that, during the renal injury, periostin was highly expressed in tubulointerstitial areas [13].

The previous studies demonstrated that the renal expression and urinary excretion of periostin increased in rats after chronic kidney disease (CKD) and in the kidneys of mice with diabetes [14]. The objective of the present study is to inspect the circulatory level of E-cadherin and periostin in type 2 diabetic patient with nephropathy and examine their possible interrelation to address the role of EMT in the pathophysiology of DN, and, furthermore, to study the association of E-cadherin and periostin with the degree of renal impairment in DN patient.

Methods

This study was conducted on 90 subjects including both men and postmenopausal women. Their age ranges from 45 to 55 years. Before the start of the study, a written informed consent was taken from all study participants.

The subjects were distributed into two groups: Group I was 19 control apparently healthy volunteers and group II was diabetic patients that were suffering from type 2 DM for at least 10 years.

Enrolled diabetic patients were from the National Institute of Diabetes and Endocrinology (NIDE), Cairo, Egypt. A detailed clinical history was taken with complete general and systemic examinations were done.

Group II was sub-divided in agreement with the patient urinary albumin/creatinine ratio (UACR) into Group II a: 19 diabetic patients with normoalbuminuria (UACR < 30 mg/g), Group II b: 37 diabetic patients with microalbuminuria (UACR 30–300 mg/g), and Group II c: 15 diabetic patients with macroalbuminuria (UACR > 300 mg/g).

Exclusion criteria

Subjects with type 1 diabetes mellitus, pregnancy, an active urinary tract infection, neoplastic disorders, severe liver disease, infection (acute or chronic), autoimmune conditions, congestive heart failure, ischemic heart disease, and patients with any evidence of renal impairment due to cause other than diabetes were excluded.

Study design and methods

Morning random volume spot urine specimens were obtained from all subjects to assess UACR by total proteins and creatinine colorimetric kits (Chemelex, SA, Barcelona, Spain). Total protein to creatinine was converted to UACR using an equation according to Teo et al. [15].

Blood sampling and biochemical evaluation

10 mL fasting blood samples were collected from all subjects. Plasma collected on sodium fluoride vacutainer tubes were designed for plasma preparation to measure fasting plasma glucose (FPG); however, EDTA vacutainers were used for glycated hemoglobin (HbA_{1C}%) determination. Plain vacutainers were used for serum preparation for urea and creatinine measurement. Serum aliquots were then stored at − 80 °C for further investigation of E-cadherin and periostin.

Commercially available spectrophotometric kits (Bioscope Diagnostic, Cairo, Egypt) were used for routine biochemical measurements at the Biochemistry Laboratory, Faculty of Pharmacy, Ain Shams University. HbA_{1C} % assay was done using crystal chem's hemoglobin A_{1C} kit.

Periostin was measured using human POSTN/OSF-2(Periostin) ELISA Kit (Elabscience, China) as stated by sandwich-ELISA technique. The micro-ELISA plate was pre-coated with antibodies specific to human POSTN/OSF-2. Standards and samples were pipetted into corresponding wells to bind to the antibodies. Biotinylated detection antibodies targeting human POSTN/OSF-2 and avidin–horseradish peroxidase (HRP) conjugate were then pipetted successively into each well. After incubation, washing for unbound components was done. When the substrate solution is pipetted, only wells having human POSTN/OSF-2, biotinylated antibody, and avidin–HRP complex were colored blue. Then, sulfuric acid stop solution was added. Optical density (OD) was measured with spectrophotometry at a wavelength of 450 nm ± 2 nm. The OD value was proportional to human POSTN/OSF-2 concentration.

E-cadherin was measured using human E-Cadherin ELISA Kit (Elabscience, China) according to sandwich-ELISA method. The principle of this kit was similar to periostin kit.

Statistical analysis

After data collection and revision, the Statistical Package for Social Science (IBM SPSS) version 23 was used for data analysis. Parametric data were presented as mean and standard error of mean (SEM) and non-parametric data as median with interquartile range. Comparisons between groups were done by ANOVA test for parametric data, and by Kruskal–Wallis test for non-parametric data. Correlation between parameters was done using Spearman correlation coefficients. Multivariate linear backward regression analysis was used for assessment variables that most affect the level of periostin and E-cadherin. Receiver-operating characteristic (ROC) curve depicting the ability to discriminate between healthy control and DN patients was plotted for periostin and E-cadherin with determination of area under the curve (AUC). The confidence interval was set to 95%. All *P* values reported were two-tailed and *P* at 0.05 was considered significant; while that at 0.01 and 0.001 is highly significant.

Results

Among the 90 cases studied, 19 subjects were the control group (UACR 17 (12.5–21 mg/g), 19 subjects were normoalbuminuria group (UACR 19.9 (17–22 mg/g), 37 subjects were microalbuminuria group [UACR 51 (39–104 mg/g)], and 15 subjects were macroalbuminuria group [UACR 400 (320–473 mg/g)]. The anthropometric, clinical, and biochemical parameters of the study participants are existing in Table 1. No significant differences in age were observed in the studied groups; control was (52.58 ± 0.67) years, normoalbuminuria was (55 ± 1.79) years, microalbuminuria was (54.97 ± 1.16) years, and macroalbuminuria was (56.67 ± 1.67) years. Furthermore, no significant differences were observed in sex, systolic (SBP), or diastolic blood pressure (DBP) among diabetic groups. Excluding diabetes duration that was highly significant in macroalbuminuria group. All diabetic groups were having highly significant values of both FPG and HbA_{1C}% than the control group. In comparison to control, FPG increased in normoalbuminuria to 197%, in microalbuminuria to 192.59%, and in macroalbuminuria to 224.28%. HbA_{1C}% also increased to 134.32, 139.83, and 143.85%, respectively. Regarding serum urea, all groups were significantly having higher values than the control group and the macroalbuminuria group showed

Table 1 Main anthropometric and metabolic parameters of the studied groups

Groups	Control (Group I)	Normoalbuminuria (Group IIa)	Microalbuminuria (Group IIb)	Macroalbuminuria (Group IIc)
Anthropometric parameters				
N	19	19	37	15
Age (years)	52.58 ± 0.67	55.00 ± 1.79	54.97 ± 1.16	56.67 ± 1.67
Sex (M/F) (%)	M = 9 (47.4%)	M = 12 (63.2%)	M = 23 (62.2%)	M = 7 (46.7%)
	F = 10 (52.6%)	F = 7 (36.8%)	F = 14 (37.8%)	F = 8 (53.3%)
Diabetes duration (years)	–	6.47 ± 0.61†	8.65 ± 0.76†,‡	15.87 ± 0.85†,‡, §
SBP (mmHg)	130.47 ± 3.76	131.1 ± 4.4	136.35 ± 3.86	146.67 ± 4.57
DBP (mmHg)	82.32 ± 2.18	84.95 ± 2.06	83.86 ± 1.89	89.13 ± 2.21
Metabolic parameters				
FPG (mg/dL)	89.05 ± 2.14	175.47 ± 13.20†	171.51 ± 14.03†	199.73 ± 10.59†
HbA_{1C}%	4.72 ± 0.2	6.34 ± 0.33†	6.6 ± 0.26†	6.79 ± 0.22†
Kidney parameters				
UACR	17 (12.5–21)	19.9 (17–22)	51 (39–104)†, ‡	400 (320–473)†,‡, §
Serum urea (mg/dL)	29.63 ± 1.75	76.21 ± 4.95†	85.53 ± 5.58†	115.3 ± 9.66†,‡, §
Serum creatinine (mg/dL)	0.9 (0.8–1)	0.76 (0.63–1.03)	0.7 (0.62–0.97)	2.9 (2.6–3.1)†, ‡, §

Parametric biomarkers are represented as mean ± SEM and non-parametric biomarkers are represented as median with interquartile range. Group I: control subjects; Group IIa: normoalbuminuria; Group IIb: microalbuminuria; Group IIc: macroalbuminuria

SBP systolic blood pressure, *DBP* diastolic blood pressure, *FPG* fasting plasma glucose, *HbA_{1C}%* glycated hemoglobin, *UACR* urinary albumin/creatinine ratio

†Significantly different from control at $P < 0.001$

‡Significantly different from normoalbuminuria at $P < 0.001$

§Significantly different from microalbuminuria at $P < 0.001$

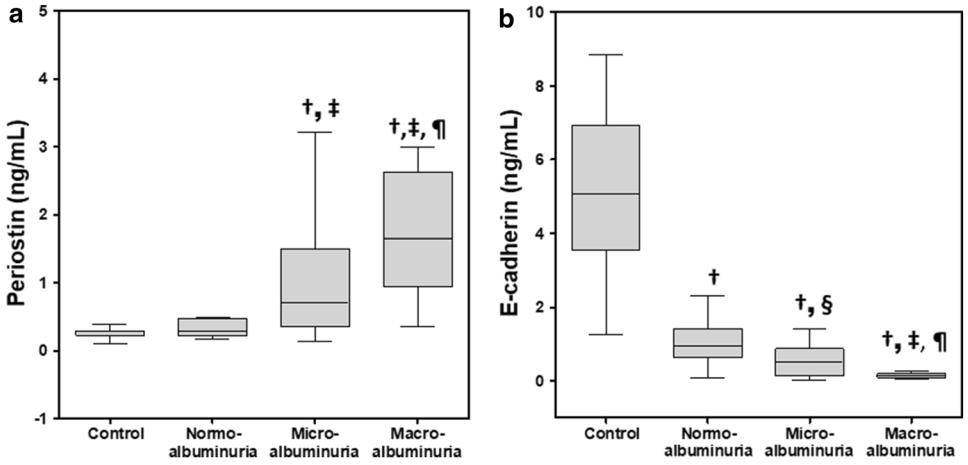

Fig. 1 Serum levels of (**a**) periostin and (**b**) E-cadherin in diabetic nephropathy patients. †Significantly different from control at $P < 0.001$. ‡Significantly different from normoalbuminuria at $P < 0.001$. §Significantly different from normoalbuminuria at $P < 0.05$. ¶Significantly different from microalbuminuria at $P < 0.05$

Table 2 Correlation analysis of periostin and E-cadherin with creatinine, urea, UACR, and $HbA_{1C}\%$

	Creatinine		Urea		UACR		$HbA_{1C}\%$	
	r	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value
Periostin	0.185	0.081	0.299	0.004	0.563	0.000	0.289	0.006
E-cadherin	−0.077	0.470	−0.485	0.000	−0.719	0.000	−0.43	0.000

UACR urinary albumin-to-creatinine ratio, *$HbA_{1C}\%$* glycated hemoglobin%

significant higher values than all other groups, normoalbuminuria group increased to reach 257.17%, microalbuminuria increased to reach 288.66%, and macroalbuminuria increased to reach 389.13% with respect to the control group. Concerning serum creatinine, no significant difference among groups was found except in macroalbuminuria group which had significantly higher values compared to all other three groups. With regard to serum periostin, as shown in Fig. 1, although there was no significant difference between normoalbuminuria [0.32 (0.27–0.49) ng/mL] and control group [0.3 (0.23–0.31) ng/mL] in periostin level, a tendency towards higher values was observed in normoalbuminuria patients. There were significant differences between remaining groups, microalbuminuria increased to 503.3% [0.74 (0.36–1.5) ng/mL], and macroalbuminuria increased to 563.3% [1.66 (0.94–2.63) ng/mL] in comparison to the control group. Diversely, serum E-cadherin dramatically decreased from the control [5.07 (3.54–6.93) ng/mL] to 18.54, 10.45, and 3.15% in normoalbuminuria [0.94 (0.64–1.43) ng/mL], microalbuminuria [0.52 (0.14–0.87) ng/mL], and macroalbuminuria [0.14 (0.09–0.2) ng/mL], respectively.

Correlation analysis of periostin, E-cadherin, and other biochemical parameters

Correlation analysis showed highly significant positive correlations of levels of periostin with UACR (P value = 0.000), urea (P value = 0.004), and $HbA_{1C}\%$ (P value = 0.006).

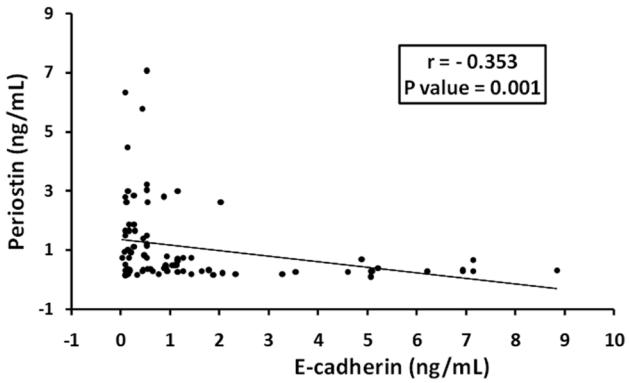

Fig. 2 Correlation between serum levels of periostin and E-cadherin

There were highly negative correlations between the level of E-cadherin with UACR (P value = 0.000), urea (P value = 0.000), and $HbA_{1C}\%$ (P value = 0.000). Furthermore, no significant correlation was observed for creatinine with periostin or with E-cadherin, as shown in Table 2. Interestingly, periostin and E-cadherin were significantly negatively correlated ($P = 0.001$) (Fig. 2).

Multivariate regression of E-cadherin and periostin

After carrying out multiple linear backward regression analysis using E-cadherin as the dependent variable and periostin and urea as independent variables, all indicated significantly negative associations with E-cadherin, as shown in Table 3.

Table 3 Multivariate linear backward regression of E-cadherin and periostin with others biomarkers

Dependent variables	Predictors	*B* (*r*)	*P* value
E-cadherin	Urea	−0.53	0.000
	Periostin	−0.21	0.017
Periostin	UACR	0.321	0.009
	E-cadherin	−0.277	0.026

UACR urinary albumin-to-creatinine ratio

Regarding periostin, considering E-cadherin and UACR as independent variables, there was a significant positive correlation between periostin with UACR and a significant negative correlation with E-cadherin, as shown in Table 3.

ROC curve analysis of serum E-cadherin and periostin

The ROC curve analyses of serum E-cadherin and periostin in diagnosing DN are depicted in Fig. 3. AUC to diagnose established microalbuminuria in type 2 DM using E-cadherin was 0.998 (95% CI 0. 932–1.000). The cut-off levels were ≤ 1.43 ng/mL. For periostin, the AUC to diagnose established microalbuminuria was 0.833 (95% CI 0.709–0.919). The cut-off levels were > 0.31 ng/mL.

Discussion

Diabetic kidney disease (DKD) is one of the most severe complications of diabetes, so late recognition or insufficient treatment may lead to end-stage renal disease and the need for renal transplantation. Although DN is progressive and irreversible, the previous studies proved that early recognition of the disease and initiation of nephro-protective treatment may delay its progression. Kidney function must be evaluated in a comprehensive manner in T2DM patients. The comprehensive evaluation should take into account not only GFR and albuminuria which indicates the possible damage to the filtration membrane, but also the function of renal tubules [16].

The glomerular injury is assumed to be the cause of the presence of albumin in the urine. Nevertheless, the existence of DKD in conditions of normoalbuminuria has made an opportunity for the development of early biomarkers for DKD, which makes us look for prevention or early treatment [17]. In the current study, we supposed that we have two renal biomarkers E-cadherin and periostin that would help in the early prevention of the disease.

Multiple mechanisms are involved in the processing of renal interstitial fibrosis. EMT is one of these mechanisms [18], which is a complex process in which epithelial cells lose their functional and phenotypic features to convert to myofibroblasts [19]. EMT is induced by increasing the expression of TGF-β1. This transformation is characterized by the activation of fibrotic features including α-smooth muscle actin and vimentin, accumulation of extracellular matrix and loss of the epithelial cell polarity, and intercellular adhesion molecules like E-cadherin [19, 20].

E-cadherin is considered one of the most vital molecules in cell-to-cell adhesion in epithelial tissues. It is localized on the surfaces of epithelial cells in areas of cell-to-cell connection known as adherent's junctions [21]. This loss of E-cadherin was confirmed in this study. The present study found changes on the level of E-cadherin in the control group, normoalbuminuria, microalbuminuria, and macroalbuminuria, respectively. We observed that there was a significant gradual decrease in serum level of E-cadherin reaching the lowest values in macroalbuminuria group.

The current study was in harmony with a recent study which used rats' paraffin-embedded renal sections which

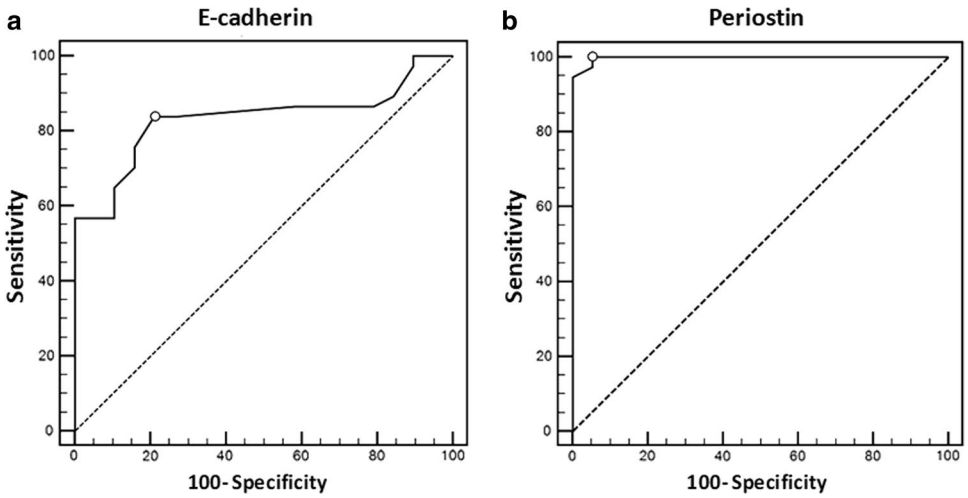

Fig. 3 ROC curve analysis for microalbuminuria for (**a**) E-cadherin and (**b**) periostin

were taken for immunohistochemical analysis, and they detected that both the gene and protein expression of E-cadherin in the group having renal fibrosis distinctly decreased [22].

This study demonstrated that E-cadherin levels decreased before normoalbuminuria and that proved that E-cadherin is an early kidney biomarker. These results come in agreement with another study which measured urinary soluble E-cadherin and its expression using immunohistochemical analysis on 162 Chinese patients with type 2 DM and they indicated that urinary soluble E-cadherin/creatinine significantly raised in the early stage of DN and steadily elevated with the progression of DN [23].

By performing Spearman's correlation, we found that there were significant negative correlations of E-cadherin with creatinine, albumin/creatinine ratio, and urea confirming the possible role of E-cadherin in renal function. In concert with these findings, positive correlations of urinary soluble E-cadherin/creatinine ratio with albuminuria and serum creatinine were previously demonstrated. It also showed high negative correlation with GFR [21]. In addition, our study is in harmony with Jiang et al. (2009), which indicated that urinary soluble E-cadherin/creatinine had high positive correlation with UACR and creatinine according to Pearson correlation analysis [23].

The mechanism that explains that negative correlation of serum E-cadherin with albuminuria and serum creatinine is renal ischemia and apoptosis. During the progress of renal impairment in DN, both ischemia and apoptosis of renal tubular epithelial cells increase [24]. Other studies have proved that degradation of E-cadherin by proteolytic enzyme activation is caused by ischemia and apoptosis [25]. Thus, the degradation prompted by renal injury could be one of the main causes for the decrease in serum E-cadherin. In addition, the immunohistochemical staining revealed that the expression of E-cadherin was evidently decreased in renal tubular epithelial cells of DN patients. However, the previous studies have exposed that EMT induced by TGF-β might suppress the expression of E-cadherin [26, 27], and the degradation and cleavage of E-cadherin could be another vital cause for decreased E-cadherin levels, but the exact mechanism still needs further clarification.

Upon performing regression analysis, we found that there was a strong negative correlation between E-cadherin and urea. Comparable results were reported in a previous study on Saudi Arabia's population which measured urinary E-cadherin on normoalbuminuria, microalbuminuria, and macroalbuminuria, and showed that there were positive correlations between E-cadherin and both creatinine and HbA_{1C}% [21].

Periostin is considered as one of the mesenchymal biomarkers. Some studies have proved a local linkage between periostin and diabetic vascular complications. A study demonstrated that serum periostin is an independent risk factor and significantly associated with diabetic retinopathy in patients with T2DM [28]. During renal injury, periostin significantly increased in tubulointerstitial areas, so urinary periostin was a degree of the loss of renal tubular cells in response to diverse renal damages [29]. The previous studies suggested that periostin increases TGF-β1 expression and can induce extracellular matrix deposition [30]. In addition, administering TGF-β1 to renal epithelial cells increases periostin expression and stimulated EMT [31].

This study showed that serum periostin was highly correlated with the severity of DN. Although no significant difference between normoalbuminuria and control groups in periostin level was observed, there was a slight increase in periostin level than control group. Moreover, we found a highly significant increase in other remaining groups than control group reaching the highest value in macroalbuminuria group. Our study was in harmony with Um et al. (2017) who proved that increasing in the expression of periostin in both kidneys of mice and in unilateral ureter obstruction mouse models was correlated with renal fibrosis severity [32].

Also in accord with our results, urinary periostin was increased in normoalbuminuria compared with healthy controls in Satirapoj et al. (2015). This study indicated that significant differences were detected between the urinary periostin values in the each phase of DN ($P < 0.001$) compared with the control group reaching the highest value in macroalbuminuria group [14].

To discuss the value of periostin as a biomarker of DN development, we made Spearman's correlation and regression analysis and we found strong association of periostin with UACR and urea. The data from the previous studies on DN proved that mRNA expression of periostin levels was strongly correlated with proteinuria [33]. Moreover, the increase in urinary periostin levels was correlated unswervingly with proteinuria among proteinuric and non-proteinuric patients in CKD [34]. Therefore, the increase of serum periostin level underlines its value as a critical biomarker for DN.

In the current study, the results of the ROC curve done for microalbuminuria group detected high values for AUC that confirmed high sensitivity and specificity for diagnosing DN. Our study is in harmony with Satirapoj et al. (2012) which made the ROC curve analysis of urinary periostin for normoalbuminuric, microalbuminuric, and macroalbuminuric type 2 diabetic patients, and confirmed that there was moderate-to-high sensitivity and specificity for diagnosing DN as concluded from the AUC [13]. Therefore, serum periostin showed moderate-to-high sensitivity and specificity for diagnosing DN.

The current investigation may be the first to demonstrate that there was a strong negative correlation between the

serum level of both periostin and E-cadherin. However, further studies on larger population size are recommended to clearly define their clinical usefulness.

Conclusion

Serum levels of the epithelial marker; E-cadherin and the mesenchymal marker; periostin could be considered as reliable biomarkers of renal injury and they are associated with the degree of renal damage in type 2 diabetic patients. They were negatively correlated. These findings highlight the involvement of EMT in the pathogenesis and progression of DN.

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Compliance with ethical standards

Conflict of interest All the authors have declared no competing interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Human Ethical Review Committee, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt, and the NIDE, Cairo, Egypt at which the studies were conducted (Approval number 122/2015) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

1. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. Nat Rev Endocrinol. 2011;8:228–36.
2. Gaballa MR, Farag YMK. Predictors of diabetic nephropathy. Cent Eur J Med. 2013;8:287–96.
3. Lim AKH. Diabetic nephropathy? Complications and treatment. Int J Nephrol Renovasc Dis. 2014;7:361–81.
4. Leon BM, Maddox TM. Diabetes and cardiovascular disease: epidemiology, biological mechanisms, treatment recommendations and future research. World J Diabetes. 2015;6:1246–58.
5. Lan HY, Chung ACK. TGF-β/Smad signaling in kidney disease. Semin Nephrol. 2012;32:236–43.
6. Gewin L, Zent R. How does TGF? Mediate tubulointerstitial fibrosis? Semin Nephrol. 2012;32:228–35.
7. Lee S, Han SM, Kim J, Chung K, Han K. Science expression of E-cadherin in pig kidney. J Vet Sci. 2013;14:381–6.
8. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial–mesenchymal transition. Nat Rev Mol Cell Biol. 2014;15:178–96.
9. Maître J-L, Heisenberg C. Three functions of cadherins in cell adhesion. Curr Biol. 2013;23:626–33.
10. Stephenson RO, Yamanaka Y, Rossant J. Disorganized epithelial polarity and excess trophectoderm cell fate in preimplantation embryos lacking E-cadherin. Dev Stem Cells. 2010;137:3383–91.
11. Chijimatsu R, Kunugiza Y, Taniyama Y, Nakamura N, Tomita T. Expression and pathological effects of periostin in human osteoarthritis cartilage. BMC Musculoskelet Disord. 2015;16:1–12.
12. Takada M, Ban Y, Yamamoto G, Ueda T, Saito Y, Nishimura E. Periostin, discovered by nano-flow liquid chromatography and mass spectrometry, is a novel marker of diabetic retinopathy. Biochem Biophys Res Commun. 2010;399:221–6.
13. Satirapoj B, Wang Y, Chamberlin MP, et al. Periostin: novel tissue and urinary biomarker of progressive renal injury induces a coordinated mesenchymal phenotype in tubular cells. Nephrol Dial Transplant. 2012;27:2702–11.
14. Satirapoj B, Tassanasorn S, Charoenpitakchai M, Supasyndh O. Periostin as a tissue and urinary biomarker of renal injury in type 2 diabetes mellitus. PLoS One. 2015;10:e0124055.
15. Teo BW, Loh PT, Wong WK, Ho PJ, Choi KP, Toh QC, et al. Spot urine estimations are equivalent to 24 hour urine assessments of urine protein excretion for predicting clinical outcomes. Int J Nephrol. 2015;2015:156484. https://doi.org/10.1155/2015/156484.
16. Zyłka A, Gala-Błądzińska A, Dumnicka P, et al. Is urinary NGAL determination useful for monitoring kidney function and assessment of cardiovascular disease? A 12-month observation of patients with type 2 diabetes. Dis Mark. 2016;2016:8.
17. de Carvalho JAM, Tatsch E, Hausen BS, et al. Urinary kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin as indicators of tubular damage in normoalbuminuric patients with type 2 diabetes. Clin Biochem. 2016;49:232–6.
18. Liu Y. New insights into epithelial-mesenchymal transition in kidney fibrosis. J Am Soc Nephrol. 2010;21:212–22.
19. He J, Xu Y, Koya D, Kanasaki K. Role of the endothelial-to-mesenchymal transition in renal fibrosis of chronic kidney disease. Clin Exp Nephrol. 2013;17:488–97.
20. Flier SN, Tanjore H, Kokkotou EG, Sugimoto H, Zeisberg M, Kalluri R. Identification of epithelial to mesenchymal transition as a novel source of fibroblasts in intestinal fibrosis. J Biol Chem. 2010;285:20202–12.
21. Rizvi MR, Rukban M. E-cadherin-A surrogate marker and its relation to other biomarkers in early stages of diabetic nephropathy in eastern proving of Saudi Arabian population. J Glob Pharma Technol. 2016;8:18–26.
22. Chen H, Xu Y, Yang Y, Zhou X, Dai S, Li C. Shenqiwan ameliorates renal fibrosis in rats by inhibiting TGF-β 1/smads signaling pathway. Evidence Based Complement Altern Med. 2017;2017:9.
23. Jiang H, Guan G, Zhang R, Liu G, Cheng J, Hou X, Cui Y. Identification of urinary soluble E-cadherin as a novel biomarker for diabetic nephropathy. Diabetes Metab Res Rev. 2009;25:232–41.
24. Lorz C, Benito-Martín A, Boucherot A, et al. The death ligand TRAIL in diabetic nephropathy. J Am Soc Nephrol. 2008;19:904–14.
25. Bush KT, Tsukamoto T, Nigam SK. Selective degradation of E-cadherin and dissolution of E-cadherin-catenin complexes in epithelial ischemia. Am J Physiol Renal Physiol. 2000;278:847–52.
26. Burns WC, Twigg SM, Forbes JM, et al. Connective tissue growth factor plays an important role in advanced glycation end product—induced tubular epithelial-to-mesenchymal transition: implications for diabetic renal disease. J Am Soc Nephrol. 2006;17:2484–94.
27. Li JH, Wang W, Huang XR, Oldfield M, Schmidt AM, Cooper ME, Lan HY. Advanced glycation end products induce tubular epithelial-myofibroblast transition through the RAGE-ERK1/2 MAP kinase signaling pathway. Am J Pathol. 2004;164:1389–97.
28. Ding Y, Ge Q, Qu H, et al. Increased serum periostin concentrations are associated with the presence of diabetic retinopathy in patients with type 2 diabetes mellitus. J Endocrinol Invest. 2018;41:937–45.

29. Sen K, Lindenmeyer MT, Gaspert A, et al. Periostin is induced in glomerular injury and expressed de novo in interstitial renal fibrosis. Am J Pathol. 2011;179:1756–67.
30. Gordon ED, Sidhu SS, Wang ZE, et al. A protective role for periostin and TGF-β in IgE-mediated allergy and airway hyperresponsiveness. Clin Exp Allergy. 2012;42:144–55.
31. Mael-Ainin M, Abed A, Conway SJ, Dussaule J-C, Chatziantoniou C. Inhibition of periostin expression protects against the development of renal inflammation and fibrosis. J Am Soc Nephrol. 2014;25:1724–36.
32. Um JE, Park JT, Nam BY, et al. Periostin-binding DNA aptamer treatment attenuates renal fibrosis under diabetic conditions. Sci Rep. 2017;7:1–12.
33. Guerrot D, Dussaule JC, Mael-Ainin M, Xu-Dubois YC, Rondeau E, Chatziantoniou C, et al. Identification of periostin as a critical marker of progression/reversal of hypertensive nephropathy. PLoS One. 2012;7:e31974.
34. Satirapoj B, Witoon R, Ruangkanchanasetr P, Wantanasiri P, Charoenpitakchai M, Choovichian P. Urine periostin as a biomarker of renal injury in chronic allograft nephropathy. Transplant Proc. 2014;46:135–40.