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Epidermal growth factor receptor and podocin predict nephropathy progression in type 2 diabetic patients through interaction with the autophagy influencer ULK-1

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ABSTRACT

Aims: Diabetic nephropathy (DN) that progress to end stage renal failure is a serious health problem. Autophagy is involved in DN pathogenesis. Finding renal prognostic biomarkers can help in the future renal status prevision. Therefore, the aim of current study was to evaluate and correlate circulating levels of autophagy regulator protein Unc-51-like kinase 1 (ULK-1) with the widely expressed receptor in mammalian kidney; epidermal growth factor receptor (EGFR); and the key functional podocyte protein podocin (PDCN).
Methods: Serum levels were assessed by ELISA in 72 type 2 diabetic patients classified according to their urinary albumin/creatinine ratio; 19 normoalbuminuric, 37 microalbuminuric and 16 macroalbuminuric patients; age and sex matched with 18 healthy controls.
Results: Microalbuminuria and macroalbuminuria patients exhibited decreased ULK-1, EGFR and PDCN levels. Only EGFR showed lower levels in normoalbuminuria compared with controls. ULK-1 and EGFR were significantly higher in normoalbuminuria compared with microalbuminuria and macroalbuminuria patients. ULK-1, EGFR and PDCN were correlated with each other and with some metabolic parameters.
Conclusions: ULK-1 with EGFR can predict early impairment in DN while PDCN can highlight progressive DN risk EGFR and PDCN may interact synergistically with ULK-1 in autophagy dysregulation as a pathogenic mechanism of DN induction and progression.

1. Introduction

One of the major health problems affecting 415 million people (215 million of males and 200 million of females) is type 2 diabetes mellitus (T2DM). It is believed that this number will increase to be 642 million in 2040.[1] Therefore with the frightful increase of T2DM, it is important to study pathogenesis of diabetic complications associated with it so that risk factors for diabetic complications can be diminished as early as possible.[1]

Many studies have raised more and more concerns about diabetic nephropathy (DN) which becomes a serious public health problem worldwide and affects around 35–40% of diabetic patients.[2] The progression of DN occurs over a period of 10–20 years, beginning from microalbuminuria and leading to end-stage kidney disease (ESKD).[2]

Recent pathways including autophagy are observed to be activated during the development of diabetes mellitus (DM); which is an evolutionary homeostatic process for cellular restoration in which protein aggregates and damaged organelles are recycled by the use of lysosomes[3] and individually or collectively regulate the induction and progression of DN.[2] Although the role of autophagy in DN remains to be elucidated, but raising evidence given that impairing the autophagy system is associated with the development of diabetic kidneys.[4] This afflicted autophagy affects the glomerular and tubular renal components, and supported by the accumulation of damaged mitochondria in the kidney.[4]

Negative regulation of autophagy is interpreted by alteration of some intracellular nutrient-sensing signaling pathways of the direct up-regulatory kinase Unc-51-like kinase 1 (ULK1).[5] This alteration is either done by activation of the mechanistic target of rapamycin (mTOR) signaling pathway or inhibiting the activated mitogen protein kinase (AMPK) signaling pathway that lead to inactivation of the ULK1.[6]

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It has been reported that the main cells affected in DN are the podocytes and proximal tubular cells.[7] One of the key functional proteins in podocyte slit diaphragm is podocin (PDCN) which podocytes require to express along with several specific proteins in a correct manner for their differentiation, as well as for the sustainment of their complex anatomy.[8]

Clinical studies have provided that a reduction in the number of glomerular podocytes is characteristic in patients with DN.[9] This reduction causes foot process widening and down regulation of PDCN protein leading to marked albuminuria.[9] A lot of data proved that podocytes have a higher level of constitutive autophagy than other intrinsic renal cells, therefore, dysregulation of basal autophagy is believed to play a crucial role in podocyte injury under high-glucose conditions.[10]

Epidermal growth factor receptor (EGFR), a member of the human ErbB family with an extracellular protein ligands, consists of four transmembrane receptors. Among the four ErbBs, EGFR is the prototypical receptor, and receptor activation leads to phosphorylation on specific tyrosine residues within the intracellular region.[11] It is ubiquitously expressed widely in mammalian kidney, including glomeruli, proximal tubules, cortical and medullary collecting ducts and its expression increases in both glomeruli and tubules specifically in diabetic patients.[11,12] Both in-vivo and in-vitro studies have shown that prolonged EGFR activation plays a role in mediating the differentiated epithelial cell function through enhancing renal tubular cell regeneration and accelerating the recovery of renal function after injury.[12] The phosphorylation sites on tyrosine residues act as an anchor for different signaling molecules, leading to the activation of intracellular pathways as AMPK and phosphoinositide 3-kinase (PI3K). This has a direct inhibitory effect on mTORC1 signaling which is one of the important pathways controlling cell autophagy in podocytes of diabetic patients and correlates with increased endoplasmic reticulum (ER) stress which finally led to the development and progression of DN.[13]

Therefore, the aim of present work was to investigate if EGFR and PDCN can address the pathophysiology of DN through determining their serum circulating levels, in addition to examining their possible correlation with impaired autophagy via evaluating serum ULK-1 levels. Finally, to explore whether EGFR and PDCN could be used to detect the degree of renal impairment in DN patients.

2. Subjects and methods

2.1. Study subjects

A total of 90 Egyptian patients (54 men and 36 post-menopausal women) aged between 45 and 65 were enrolled in the study. The study groups were classified as follows: 18 apparently healthy control subjects and 72 patients suffering from T2DM for maximum 15 years. They were recruited from the National Institute of Diabetes and Endocrinology (NIDE), Cairo, Egypt from November 2016 to March 2017. Laboratory analyses were performed at Biochemistry Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt. All participants gave their informed consent and the study was conducted in compliance with the approval of the Human Ethical Review Committee, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt and The NIDE, Cairo, Egypt in accordance to the declaration of Helsinki.

Urinary albumin-to-creatinine Ratio (UACR) was treated as a categorical variable by classifying type 2 diabetic patients into three groups as normoalbuminuria (UACR < 30 mg/g creatinine (gCr)), microalbuminuria (UACR 30–<300 mg/gCr) or macroalbuminuria (UACR > 300 mg/gCr) that obtained from their files during their monthly visit to the NIDE outpatient clinics and then confirmed by collecting a urine specimen. Control subjects (group I) were individuals who came with the patients for monitoring or working in the NIDE and had fasting plasma glucose (FPG) < 6.1 mmol/l, HbA_{1C} < 42 mmol/mol (6%) and UACR < 30 mg/gCr, BP < 120/80 mm Hg and had a negative history of any renal disease, group II ($n = 19$) composed of type 2 diabetic patients with normoalbuminuria, group III ($n = 36$) composed of type 2 diabetic patients with microalbuminuria while group IV ($n = 16$) composed of type 2 diabetic patients with macroalbuminuria.

The following subjects were not eligible to participate in the current study: subjects with acute and chronic inflammatory diseases, type 1 DM patients, cancer, acute and chronic infections, recent history of acute myocardial infarction, stroke, any renal disease other than DN or severe liver disease.

2.2. Blood sampling and biochemical assays

10 ml of blood sample was drawn from all participants after an overnight fasting, plasma collected on sodium fluoride containing tubes were used for FPG estimation while EDTA containing tubes were used for HbA_{1C} estimation. Urea and creatinine (cr) were estimated directly using vacutainer serum collecting tubes that were centrifuged for serum preparation. Serum aliquots were then stored at −80 °C for further investigation of (EGFR, PDCN and ULK-1).

Random spot urine samples were collected from all participants for estimation of UACR using total proteins and creatinine spectrophotometric kits (Chemelex, SA, Barcelona, Spain). FPG, serum urea and Cr. were measured at labs of biochemistry department, Faculty of Pharmacy, Ain Shams University using commercial spectrophotometric kits (Bioscope Diagnostic, Cairo, Egypt). HbA_{1C} was assayed with ion exchange chromatography using commercial spectrophotometric kits (Spectrum, Cairo, Egypt).

2.3. Measurement of ULK-1

Serum level was assayed using human ELISA kits (Cat. No.: SG-10727, SinoGeneclon Co., Ltd., China), according to sandwich ELISA method to quantify level of ULK1 in the sample in which purified human ULK1 antibody has been precoated onto the microtiter plate to make solid-phase antibody. Standards and test samples were added to the wells, biotinylated detection polyclonal antibody specific to ULK1 combined with labeled enzyme were added subsequently to form antibody-antigen-enzyme-antibody complex then followed by washing completely with buffer. Substrate solution was added to visualize the enzymatic reaction which became blue color. The reaction was terminated by the addition of a stop solution and the color change was measured at a wavelength of 450 nm. The concentration of ULK1 in the samples was then determined by comparing the O.D. of the samples to the standard curve.

2.4. Measurement of EGFR and PDCN

Serum levels of EGFR and PDCN were assayed using EGFR Human ELISA kits (Cat. No.: EK0327, Boster biological technology, CA, USA) and PDCN Human ELISA kits (Cat. No.: E-EL-H0912, Elabscience, China) respectively, based on standard sandwich ELISA technology. The principle of these kits is the same like ULK-1 Kit.

2.5. Statistical analysis

Data were collected, revised, computed and statistically analyzed using statistical package for social science (SPSS) version 20. (IBM Corp., Chicago, IL, USA). Normality was checked before any analysis using Kolmogorov-Smirnov test and then data were summarized as the mean ± SEM when their distribution found parametric or as the median with interquartile range [1st, 3rd quartile], when their distribution found non-parametric. Qualitative variables were presented as numbers and percentages and comparison was done between groups using Chi-square test.

Quantitative data with normally distributed parameters were compared using ANOVA followed by post hoc least significant difference (LSD) test.

Non-normally distributed data were analyzed by Kruskal-Wallis test followed by Man-Whitney U post hoc test. Spearman's rank correlation

coefficient and multivariate backward regression analysis were carried out between the measured parameters of the study participants. p values < 0.05 was considered statistically significant and <0.01 was considered highly significant.

3. Results

3.1. Demographic data and metabolic parameters of the studied groups

Among the 90 cases studied, group I was composed of 18 subjects with UACR 12.75 (10, 14) mg/gCr, group II was 19 type 2 diabetic patients with UACR 20 (15, 28) mg/gCr, group III was 37 patients with UACR 57 (42, 103) mg/gCr and group IV was 16 patients with UACR 388 (340, 466) mg/gCr. The baseline characteristics of all participants in the study were presented in Table 1. All groups were matched for their age and sex ($p = 0.057$ and 0.380) respectively. The three diabetic groups showed significantly higher SBP and DBP compared with control subjects while there was no significant difference between the three diabetic groups themselves, and also there was no significant difference in diabetes duration between group II and group III ($p = 0.582$) but group IV patients showed longer duration than group II and group III. All diabetic groups had significantly higher routine biomarkers (FPG, HbA_{1C}, urea and Cr.) compared with control subjects. Values of FPG increased in group II to 187%, in group III to 155% and in group IV to 219% in comparison to control group. HbA_{1C} also was increased to 133%, 137% and 152% correspondingly. There is no significant difference in FPG or HbA_{1C} among the three diabetic groups except for in group IV that was significantly increased to 11.11 (10.17, 12.83) mmol/l and 55 ± 3.6 mmol/mol (7.14 ± 0.33%) correspondingly compared with group III.

Concerning urea, values were increased in group II by 2.4 fold, in group III by 2.7 fold in comparison to control subjects. Only group IV showed profound elevation in their urea serum levels (43.32 ± 5.63 mmol/l) compared with group II (26.42 ± 1.61 mmol/l) and group III (29.71 ± 1.46 mmol/l). On the other hand, regarding Cr. levels, group IV only showed significant elevation by 3 fold (247 (209,274) μmol/l) in comparison to control group (79.6 (76.9, 88.4) μmol/l).

3.2. ULK-1, EGFR, PDCN levels in studied groups

As depicted in Fig. 1, serum levels of ULK-1, EGFR and PDCN decreased sharply in diabetic patients of group III and group IV compared with the control healthy subjects. For values of ULK-1 group III and group IV had been decreased to 76.5% and 60% respectively compared to control subjects. On the other hand levels of PDCN had been decreased to 90% and 76.6% and values of EGFR declined to 68.7% and 55.4% respectively, however there is no significant decline in serum values of ULK-1 and PDCN between group II and control subjects except for the EGFR that showed significant decline in group II to 75% compared with the control group. Serum values of ULK-1 and EGFR showed a significant decline in group III (307 ± 18.48 pg/ml) at $p < 0.01$ and (5540 ± 175 pg/ml) at $p < 0.05$ respectively and a sharp decrease in group IV (242 ± 18.05 pg/ml) and (4472 ± 204 pg/ml) at $p < 0.01$ respectively compared to group II for ULK-1 (385 ± 18.04 pg/ml) and for EGFR (6047 ± 126 pg/ml), however for serum levels of PDCN it showed only a significant decrease in group IV (14.88 ± 0.75 ng/ml) compared to group II (18.56 ± 0.63 ng/ml). Moreover, diabetic patients of group IV showed a remarkable decline in values of ULK-1 at $p < 0.05$ compared to group III, yet for EGFR and PDCN they showed a noteworthy decrease at $p < 0.01$ compared to group III.

3.3. Correlation analysis of ULK-1, EGFR and PDCN with other biochemical parameters of the study participants

As shown in Table 2, Spearman's correlation analysis revealed significant negative correlations between serum levels of ULK-1 and EGFR with FPG, HbA_{1C}, UACR and urea. Meanwhile, There were negative correlations between PDCN with FPG, HbA_{1C} and UACR. Interestingly, ULK-1 is highly positively correlated to both EGFR and PDCN. Additionally, EGFR is significantly positively correlated with PDCN as shown in Fig. 2.

Table 1
Main demographic data and metabolic parameters of the studied groups.

N		Control Subjects	T2DM Patient Groups		
		Group I	Group II	Group III	Group IV
		$n = 18$	$n = 19$	$n = 37$	$n = 16$
Demographic data					
Age (years)		51.28 ± 0.58	54.79 ± 1.93	56.11 ± 1.10	57.56 ± 2.43
Sex (M/F)		9/9	11/8	26/11	8/8
Duration (years)		–	7.42 ± 0.68	8.3 ± 0.65	14.4 ± 0.94[‡,§]
SBP (mm Hg)		116.89 ± 1.68	131.74 ± 5.11[*]	139.32 ± 3.67[**]	137.25 ± 6.50[**]
DBP (mm Hg)		70.22 ± 4.00	83.63 ± 2.41[**]	83.81 ± 1.80[**]	84.31 ± 2.80[**]
Metabolic parameters					
FPG	(mmol/L)	5.08 [4.61, 5.53]	9.5 [6.72, 12.79][**]	7.89 [5.83, 12.89][**]	11.11 [10.17, 12.83][**,π]
	(mg/dL)	91.5 [83, 99.5]	171 [121, 230][**]	142 [105, 232][**]	200 [183, 231][**,π]
HbA_{1C}	(mmol/mol)	28 ± 1.47	45 ± 3.18[**]	47 ± 2.6[**]	55 ± 3.6[**,†]
	(%)	4.69 ± 0.18	6.26 ± 0.29[**]	6.43 ± 0.24[**]	7.14 ± 0.33[**,†]
UACR (mg/g Cr)		12.75 [10, 14]	20 [15, 28][**]	57 [42, 103][**,‡]	388 [340, 466][**,‡,§]
Urea (mg/dL)		30.61 ± 1.72	74.00 ± 4.51[**]	83.22 ± 4.09[**]	121.34 ± 15.78[**,‡,§]
(mmol/L)		10.93 ± 0.61	26.42 ± 1.61[**]	29.71 ± 1.46[**]	43.32 ± 5.63[**,‡,§]
Cr (mg/dL)		0.9 [0.87, 1]	0.8 [0.68, 1][*]	0.8 [0.7, 0.98][*]	2.79 [2.36, 3.1][**,‡,§]
(μmol/L)		79.6 [76.9, 88.4]	70.7 [60.1, 88.4][*]	70.7 [61.9, 86.6][*]	247 [209, 274][**,‡,§]

Data are expressed as mean ± SEM or median [1st, 3rd quartile].
Group I, apparently healthy control subjects; Group II, normoalbuminuria diabetic patients; Gp III, microalbuminuria diabetic patients; Gp IV, macroalbuminuria diabetic patients; M, male; F, female; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA_{1C}, glycosylated hemoglobin A1c; UACR, urinary albumin creatinine ratio; Urea, serum urea; Cr, serum creatinine.

[*] Significant difference from Gp I at $p < 0.05$.
[**] Significant difference from Gp I at $p < 0.01$.
[†] Significant difference from Gp II at $p < 0.05$.
[‡] Significant difference from Gp II at $p < 0.01$.
[π] Significant difference from Gp III at $p < 0.05$.
[§] Significant difference from Gp III at $p < 0.01$.

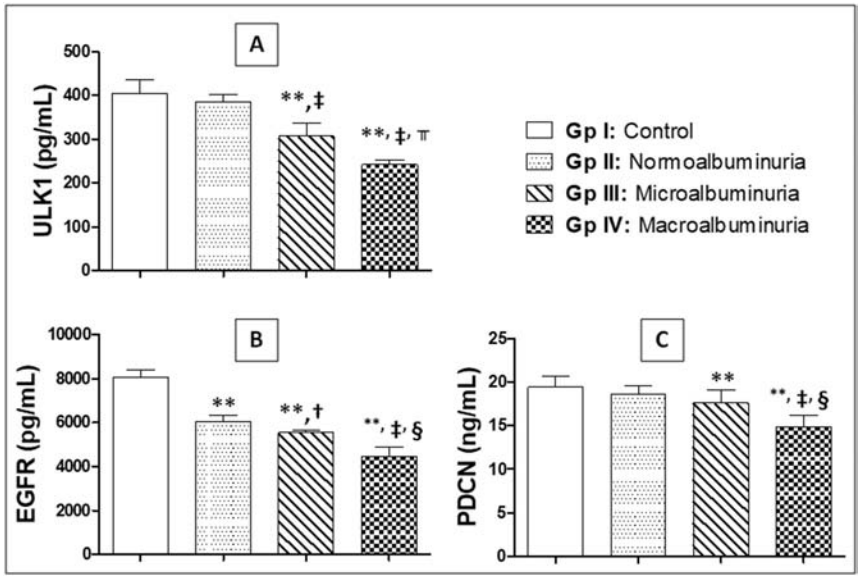

Fig. 1. Serum levels of (A): ULK-1; (B): EGFR and (C): PDCN in type 2 diabetic patients classified into four groups (Gp I: control, Gp II: normoalbuminuria, Gp III: microalbuminuria and Gp IV: macroalbuminuria group), according to their UACR. *Significant difference from Gp I at $p < 0.05$. **Significant difference from Gp I at $p < 0.01$. †Significant difference from Gp II at $p < 0.05$. ‡Significant difference from Gp II at $p < 0.01$. ᵀSignificant difference from Gp III at $p < 0.05$. §Significant difference from Gp III at $p < 0.01$.

3.4. Multivariate linear backward regression of ULK-1, EGFR and PDCN as dependent variables

On performing multiple linear backward regression analysis, as shown in Table 3, using ULK-1 as the dependent variable and FPG, HbA_{1C}, UACR, urea, EGFR and PDCN as independent variables only ULK-1 showed highly significant positive association with serum EGFR at ($\beta = 0.517$, $p = 0.000$). Regarding EGFR, considering FPG, HbA_{1C}, urea, ULK-1 and PDCN as independent variables, EGFR showed only significant negative correlation with HbA_{1C} ($\beta = -0.275$, $p = 0.001$) while showed positive association with serum ULK-1 and PDCN ($\beta = 0.228$, $p = 0.006$ and $\beta = 0.203$, $p = 0.016$ respectively). For the last dependent variable PDCN in comparison with FPG, HbA_{1C}, UACR, ULK-1 and EGFR as independent variables, it showed significant negative correlation with UACR and positive association with EGFR ($\beta = -0.373$, $p = 0.001$ and $\beta = 0.266$, $p = 0.014$ respectively).

4. Discussion

With the high propagation and increasing incidence of DN in patients with type 2 diabetes, different pathways had been forecasted to solve the mystery of disease progression and to establish new prognostic biomarkers involved in the pathogenesis of renal disease.

Autophagy is considered as one of the key master pathways of great interest that many studies are trying to elucidate its role in the pathogenesis of DN.[7] Hyperglycemia mediates alterations of nutrient sensing pathways involved in autophagy regulation through activation of mTOR signaling by inactivation of AMPK, which inhibits the ULK kinase complex and contribute to the pathogenesis of DN.[14,15] So we aimed in this study to appraise the attenuation in autophagy through measuring the serum levels of one of the autophagy regulator proteins ULK-1. In early stage of diabetes, hyperglycemia-induced intracellular stresses may activate autophagy as a compensatory response for cell survival. However, alteration of these pathways overwhelmingly suppresses autophagy in the kidney,[16,17] and this was observed in this study as assessed ULK-1 concentration that was not significantly altered in normoalbuminuria patients compared with control subjects however there was a detectable decrease in ULK-1 concentration in patients with albuminuria.

As It has been reported that podocytes and proximal tubular cells are the main cells affected in DN, an emerging evidence has suggested that impairing autophagy in these cells under high glucose condition is involved in DN through dysregulating the kidney homeostasis.[18,19] In our study we tried to hypothesize this relation through measuring PDCN and EGFR. Firstly, one of the podocyte specific proteins PDCN that was significantly decreased in diabetic patients with microalbuminuria and macroalbuminuria compared with control group. The marked UACR increase associated with the reduction in the slit diaphragm protein-PDCN that was supported by Qi and colleagues' study in which the glomerular expression of PDCN was barely detectable in the diabetic rats group obtained from the immunohistological tissue evaluation that was confirmed quantitatively using Western blotting analysis.[8] It was also accompanied by glomerular basement membrane (GBM) thickening in the kidney ultrastructure. As proved in many studies that in the late stages of human DN, decrease in glomerular podocytes is distinctive,[20] herein there was an apparent decline in serum levels of PDCN correlated with inhibiting the determining signal ULK-1 in autophagy pathway in macroalbuminuria patients that was not observed in other groups. So PDCN can be considered to be a marker of late stage DN as it was reported that tubulointerstitial damage rather than glomerular injury that is well correlated with the decline of the renal function.[21]

Table 2
Correlation analysis of the serum levels of ULK-1, EGFR and PDCN and the other metabolic parameters of the studied groups.

	FPG		HbA_{1C}		UACR		Urea	
	r	*p*-Value	r	*p*-Value	r	*p*-Value	r	*p*-Value
ULK-1	−0.270*	0.010	−0.331**	0.001	−0.514**	0.000	−0.285**	0.006
EGFR	−0.514**	0.000	−0.551**	0.000	−0.719**	0.000	−0.465**	0.000
PDCN	−0.264*	0.012	−0.357**	0.001	−0.551**	0.000	−0.161	0.131

* Correlation is significant at $p < 0.05$.
** Correlation is highly significant at $p < 0.01$.

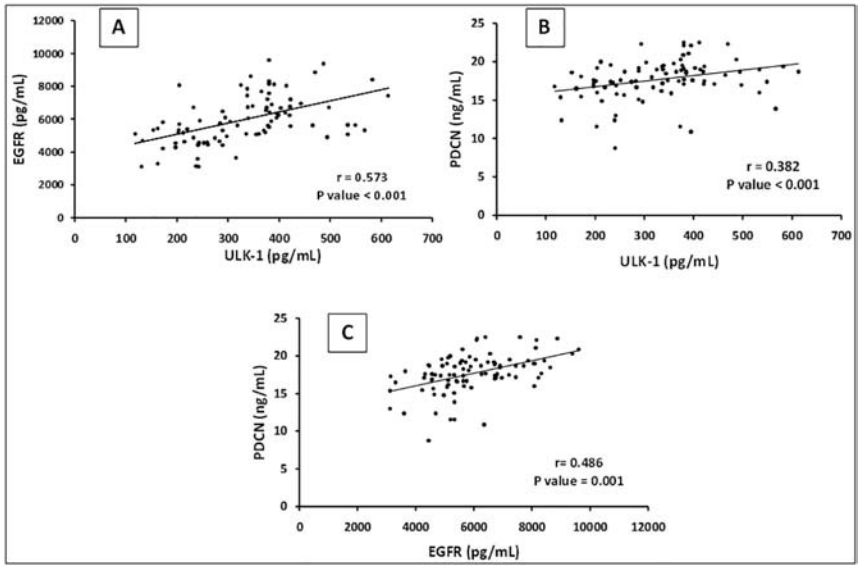

Fig. 2. Correlation analysis between (A): ULK-1 and EGFR levels; (B): ULK-1 and PDCN levels and (C): EGFR and serum PDCN levels.

Secondly, our work defined a significant role for EGFR in understanding the tubuloglomerular interactions underlying DN as it had shown a significant decline in diabetic patients starting from those with normoalbuminuria along with the different stages of UACR compared with healthy controls. This finding was in harmony with the Edinburgh Type 2 Diabetes Study (ET2DS), which included a representative cohort of type 2 diabetic patients aged 60 to 75 years with eGFR <60 ml/min per 1.73 m^2, 74% of them were normoalbuminuric.[22]

Inconsistent with the results of the current study, several studies showed strong evidence for the pathological role of persistent EGFR receptor activation in the development and progression of DN.[23,24] Also these controversy results have been found in other diseases as in breast cancer, head and neck squamous cell carcinoma where EGFR showed increased levels in cancer patients compared with healthy controls.[25,26] While other studies reported worse clinical outcomes in breast cancer patients with low circulating EGFR.[27,28]

Although the evidence on the prognostic relevance of EGFR is not conclusive, the majority of published studies showed that inhibition of EGFR activity by erlotinib-EGFR kinase inhibitor-led to such marked amelioration of the observed nephropathic changes.[24] So our observation might seem surprising at first, since decline of EGFR in the proximal tubules has been shown to predict poor progression of DN.[29] But although rodent models can be a useful source of discovering the pathogenesis of human disease however these rodent models of DN display only the earliest stages of human disease and consequently have limited translational potential for the detection of biomarkers.[29] The present results support a role for EGFR activation as an important mediator of renal repair as EGFR is decreased with the rapid loss of renal functions correlated positively with the other renal biomarker PDCN and with the autophagy regulator ULK-1. This was supported by previous studies that addressed EGFR activation as a well-described mediator of mTOR activity through activation of PI3K/AKT pathway.[30] The observed reduction in urinary low-molecular-weight EGF and in the EGF protein expression in the renal tubulointerstitium in patients with DN[31,32] which suggests that EGF may be a biomarker of healthy tubular mass, and indeed EGFR activation may be important in maintaining tubular cell health.

Correlation analysis confirmed the positive crosstalk between the assessed parameters PDCN and EGFR with the autophagy initiator ULK-1 as autophagy in DN patients is believed to be dysregulated by inhibition of AMPK pathway through two independent mechanisms: activation of mTORC1 and direct suppression of ULK-1.[6] EGFR may be depressed through inhibition of PI3K pathway that activates mTORC1[33] which is hypothesized to inhibit gene expression of the slit diaphragm proteins in podocytes as PDCN.[34]

In conclusion, EGFR can be considered as a useful biomarker to predict early stages of human renal disease even in the normoalbuminuria patients. Serum levels of ULK-1 with EGFR can predict the progression of renal impairment in type 2 diabetic patients. On the other hand, PDCN can be seen as a useful alarm for the risk of renal failure in these patients. Finally ULK-1, EGFR and PDCN were interrelated through impairment of autophagy in type 2 DN patients.

Table 3
Multivariate linear backward regression analysis using ULK-1, EGFR and PDCN as the dependent variable with others biomarkers.

Dependent variables	Predictors	β (r)	*p*
ULK-1 (pg/ml)	EGFR	0.517	0.000
EGFR (pg/ml)	HbA_{1C}	−0.275	0.001
	ULK1	0.228	0.006
	PDCN	0.203	0.016
PDCN (ng/ml)	UACR	−0.373	0.001
	EGFR	0.266	0.014

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