

# Assessment of urinary epidermal growth factor level in patients with chronic kidney disease

## Abstract

**Background:** It has been suggested that urinary epidermal growth factor (EGF) originates from kidney and the urinary excretion rates of EGF decreases in patients with acute renal failure in the acute phase, and subsequently increases during the recovery.

**Objective:** To evaluate predictive potential of urinary EGF level in patients with chronic kidney disease of different stages according to the National Kidney Foundation (KDIGO) guidelines.

**Material and Methods:** A 24-hour urine was obtained from patients with chronic kidney disease (n=59) and age/sex matched healthy controls (n=21). EGF levels in urine were measured with competitive ELISA kit.

**Results:** Urinary EGF level was found to be lower in the patient group than those in the control group ( $p<0.01$ ). It was negatively correlated with serum creatinine. When patients with chronic kidney disease were classified according to eGFR (estimated glomerular filtration rate) categorization (KDIGO), urinary EGF in the patient with stage 3a was found to be higher than those with stage 4 ( $p<0.01$ ). Predictive performance of urinary EGF was evaluated by ROC curve. Using the cut off value of 2.94ng/mg creatinine of urinary EGF, 59% sensitivity, 71% specificity were obtained.

**Conclusion:** Urinary EGF seems a promising marker to reflect the kidney damage in the early stage in patients with chronic kidney disease.

**Keywords:** chronic kidney disease, urinary epidermal growth factor, glomerular filtration rate, ROC curve

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**Abbreviations:** CKD, chronic kidney disease; GFR, glomerular filtration rate; HPT, hyperparathyroidism; HIVAN, HIV-associated nephropathy; MMP-2, matrix metalloproteinase-2; FGF-2, fibroblast growth factor-2; TALDC, thick ascending limb and distal convoluted cells

## Introduction

Chronic kidney disease (CKD) is characterized by progressive loss of functioning nephrons and their replacement with fibrotic tissue over a period of months or years. The causes of CKD are mainly diabetes, hypertension and inflammatory kidney diseases. It is a life threatening disease; if not treated by dialysis or kidney transplant, CKD results in death. CKD has a long latent period in which the disease is clinically silent. Diagnosis and treatment is based mainly on clinical examination and biochemical markers assessing kidney function. Currently, glomerular filtration rate (GFR) and albuminuria are the ideal markers to assess kidney function. Unfortunately patients with CKD may have no symptom until the glomerular filtration rate (GFR) falls below 15ml/min.<sup>1</sup> New biomarkers which are able to reflect kidney damage at an earlier stage is required to improve management and decrease the morbidity and mortality of the patients with CKD.

Growth factors are the peptides which stimulate cell division and differentiation. They have mitogenic effects on epithelial and mesothelial cells. Epidermal growth factor (EGF) is expressed in many organs and is found in the urine.<sup>2</sup> The kidney is one of the major sites of EGF production. The thick ascending limb of Henle's loop and the distal convoluted tubule are the main sites of EGF production.<sup>3</sup> In kidney, EGF has several biological functions such as modulation

of cell growth, regulation of cellular metabolism and glomerular haemodynamics and renal repair after injury.<sup>4</sup> Recovery from tubular damage requires activation of regenerative mechanisms to lead re-epithelialization of the amaged tubules. EGF is shown to stimulate recovery in experimental acute renal failure.<sup>5</sup>

A large part of the urinary EGF is originated from kidney whereas a small amount is derived from plasma.<sup>6</sup> It was reported that urinary excretion of EGF is decreased in acute renal failure and returns to previous levels after the healing.<sup>7-9</sup> This decrease is attributed to tubular damage. On the light of previous data, we hypothesized that if urinary EGF has the renal origin, EGF concentration in the urine may reflect the number of functional nephrons and it may be a good marker for assessment of renal function. Therefore, determination of urinary EGF concentration and evaluation of the relation between urinary EGF and currently used renal function markers were purposed in patients with CKD.

## Material and methods

This study includes 59 non-dialyzed patients with different stages of CKD admitted to Cerrahpasa Medical Faculty Hospital, Department of Nephrology. The patients who are followed-up more than 6 months were included by the study. The control group was constituted by age-matched 21 healthy volunteers. All study cases were evaluated by standard physical examination, blood pressure measurement, and routine clinical laboratory tests including serum urea, creatinine, proteins, lipids, liver function tests, eGFR and proteinuria (estimated glomerular filtration rate and proteinuria with KDIGO classification). A total of 29 patients had diabetes mellitus. Cases who had thyroid

disorder, acute or chronic inflammatory disease, liver disease and malignancy were excluded from the study. Patient and control characteristics were given in the Table 1. Cerrahpasa Medical Faculty Ethical Committee approval was given in accordance with the principles of the Declaration of Helsinki, and informed consent was obtained from patient and control cases.

A 24-hour urine was obtained from all study cases and stored at  $-70^{\circ}\text{C}$  as aliquots. Urinary EGF levels were measured with competitive ELISA kit from Assaypro, USA (catalog no: EI1001-1). Measurements were performed according to manufacturer's instructions.

Statistical analysis was performed with SPSS 22 software package. EGF levels of the study groups were expressed as median (min-max). Since EGF levels show abnormal distribution, comparison between patient and control groups was performed by nonparametric Mann-Whitney U test. Comparisons between the groups of CKD patients with different stages were performed by Kruskal Wallis test. Correlations between urinary EGF level and renal markers were examined by Spearman correlation coefficient. The receiver-operating characteristic (ROC) analysis was performed to determine the optimal cutoff value in terms of sensitivity and specificity for detection of urinary EGF by ELISA. A value of  $P < 0.05$  was considered as significant.

**Table 1** Demographic and clinical data of the control and patient groups

	Patient group	Control group
Age	54(42-64)	52(41-64)
Sex		
Female	28	11
Male	31	10
Serum urea (mg/dl)	87(30-270)	30(10-50)
Serum creatinine(mg/dl)	2,57(0,94-6,13)	0,84(0,63-1,19)
Glucose(mg/dl)	126(28-336)	89(74-134)
Total cholesterol(mg/dl)	209(125-457)	205(146-295)
TG(mg/dl)	164(35-604)	125(30-458)
LDL(mg/dl)	134(65-355)	130(72-229)
eGFR (ml/min/1.73 m square)	48(13-89)	109(92-122)
Proteinuria (mg/day)	2048,94(74,50-12223,53)	147,28(75,38-306,38)

## Results

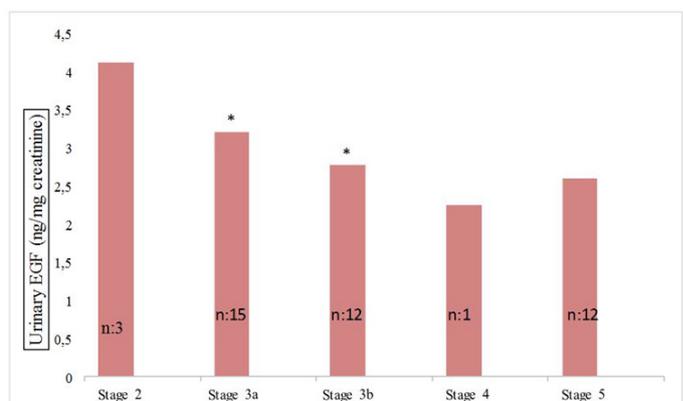
Urinary EGF excretion was expressed as ratio of urinary EGF/urinary creatinine concentration in 24-h urine (EGF/Cr). Urinary EGF

excretion was found to be lower in the CKD group than those in the control group ( $p < 0.01$ ) (Table 2). In the CKD group, EGF excretion was negatively correlated with serum creatinine ( $r = -0.32$ ,  $p < 0.01$ ) but no further correlation was found between urinary EGF and other renal markers. Estimated glomerular filtration rates (eGFR) were calculated according to the National Kidney Foundation (KDIGO) guidelines to detect the degree of kidney disease and patients were classified according to eGFR categorization (KDIGO table).<sup>10</sup> Stage 1: Normal GFR but some evidence of kidney damage e.g. abnormal urinalysis or histological changes,  $\text{GFR} > 90 \text{ ml/min/1.73 m square}$ ; Stage 2: Mild chronic renal failure,  $\text{GFR} = 60-89 \text{ ml/min/1.73 m square}$ ; Stage 3: Moderate chronic renal failure,  $\text{GFR} = 30-59 \text{ ml/min/1.73 m square}$ ; 3a: hypertension and secondary hyperparathyroidism (HPT)  $\text{GFR} = 45-59 \text{ ml/min/1.73 m square}$ , 3b: hypertension, secondary HPT plus anemia 30-44,  $\text{GFR} = \text{ml/min/1.73 m square}$ ; Stage 4: Severe chronic renal failure,  $15-29 \text{ ml/min/1.73 m square}$  Stage 5: End stage renal disease and renal replacement therapy has to be considered,  $< 15 \text{ ml/min/1.73 m square}$ . A total of 3 patients were in the stage 2, 15 were in the stage 3a, 12 were in the stage 3b, 16 were in the stage 4 and 12 were in the stage 5. When EGF/Cr was evaluated as respect with the eGFR categories, urinary EGF level in the patients with stage 2 could not be compared with the urinary EGF levels in those with advanced stages due to the insufficient number of cases in the stage 2, ( $n=3$ ). However, urinary EGF level in the patients with the stage 3a and 3b were found to be higher than those in the stage 4 ( $p < 0.01$  for both) (Figure 1). Upon determination low urinary EGF level in the CKD group, predictive performance of urinary EGF test was evaluated by ROC curve. The ROC curve was generated using urinary EGF values obtained from 59 patients with CKD and 21 controls (Figure 2). We found 0.697 (0.569-0.825) within 95% confidence interval [CI] for urinary EGF from the area under the curve ( $p = 0.008$ ). Using the cut off value of 2.94ng/mg creatinine of urinary EGF, 59% sensitivity, 71% specificity, 85% positive predictive value, 38% negative predictive value and 60 % accuracy were obtained.

**Table 2** Urinary EGF Levels in the study groups

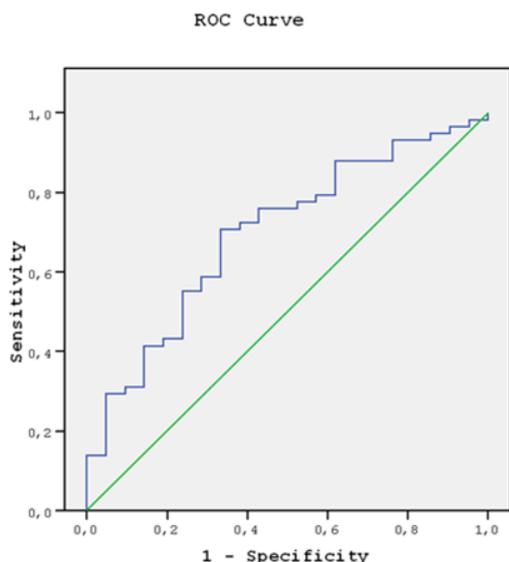
	Patient group (n:59)	Control group (n:21)
Urinary EGF (ng/mg creatinine)	2,74 (1,12-6,21)*	3,66 (1,84-5,60)

\* $P < 0,01$  versus control group



**Figure 1** Urinary EGF in Patients With Chronic Kidney Disease in Different Stages.

\* $P < 0,01$  versus stage 4



**Figure 2** ROC Curve for the Predictive Potential of Urinary EGF Levels in Patients with Chronic Kidney Disease. Using the cut off value of 2.94 ng/mg creatinine of urinary EGF, 59% sensitivity, 71% specificity, 85% positive predictive value, 38% negative predictive value and 60% accuracy were obtained.

## Discussion

Urinary EGF excretion is thought as a marker of tubular function. Some investigators suggest that EGF excretion can be reduced even in the early stages of the development of renal disease. Decreased EGF excretion in patients with CKD was detected at the second half of the 1980s for the first time.<sup>11</sup> The theory that urinary EGF originates from nephrons per se, and plasma derived EGF constitutes only a small part of urinary EGF was supported by the findings obtained from studies carried on patients with diabetic nephropathy.<sup>12-14</sup> The results of these preliminary studies showed a progressive decline in EGF excretion in diabetic patients with varying degrees of nephropathy. GFR was found to be well correlated with EGF excretion per minute in patients with diabetic renal failure ( $r = 0.63$ ,  $p < 0.001$ ).<sup>13</sup>

Urinary EGF is suggested as a reliable biomarker to follow the outcome of infants with acute kidney injury (AKI).<sup>6,15,16</sup> Infants with AKI have lower levels of EGF when compared with the infants without AKI.<sup>15</sup> Hoffman et al. reported that urinary EGF measured during the recovery stage, may identify critically ill newborns with ongoing renal injury.<sup>6</sup> Human immunodeficiency virus (HIV)-infected children have a risk of developing HIV-associated nephropathy (HIVAN), which is usually seen during late stages of infection in children with a high viral load.<sup>16</sup> Growth factors released into the urine of children with HIVAN may reflect the extent and activity of the renal lesions that are the characteristic of this disease. Soler Garcia et al.,<sup>17</sup> examined urine levels of EGF, fibroblast growth factor-2 (FGF-2) and matrix metalloproteinase-2 (MMP-2) in HIV-infected children. They found a decreased EGF level in the urine of children with HIVAN in association with increased urinary levels of FGF-2 and MMP-2, when compared with HIV-positive or -negative children without renal disease. Kwon et al.,<sup>7</sup> reported that increased urinary EGF level predicted renal functional recovery, adult patients with AKI with the lowest EGF levels more likely to have either slow or no recovery of kidney function after AKI.

Recent developments in the field of molecular biology clarified

the regenerative role of EGF during renal repair in response to injury. Baer et al.,<sup>3</sup> examined the growth and differentiation promoting effects of EGF, hepatocyte growth factor, insulin-like growth factor-1, and basic fibroblast growth factor on human renal thick ascending limb and distal convoluted cells (TALDC) *in vitro*. Only EGF was found to have a stimulative effect on signal transduction and proliferation of cultured human TALDC. EGF was shown to exert this effect via phosphorylation of MAPK (ERK1/2) (mitogen-activated protein kinase) which are the protein kinases mediating cell proliferation.<sup>3</sup> Lindenmeyer et al.,<sup>18</sup> reported that EGF mRNA level, and immunohistochemical staining of EGF were reduced in tubulointerstitial tissue obtained from patients with diabetic nephropathy, and EGF mRNA level was negatively correlated with proteinuria. In another study, the degree of tubulointerstitial damage in patients with various forms of chronic renal disease was shown to be correlated with decreased EGF mRNA level. In addition, for a 10 U decrease in the glomerular filtration rate, an estimated decrease of 15 % in EGF mRNA level was noted.<sup>19</sup>

Stangou et al.,<sup>20</sup> determined the reduced EGF excretion in immunoglobulin A nephropathy patients, compared to controls. According to their findings, reduction in EGF excretion was in association with progression of stage and a cut off 0.05pgEGF/mg urine creatinine level can distinguish between progressors and non-progressors immunoglobulin A nephropathy. They concluded that EGF excretion can be a predictive marker and its serial measurements may give information about disease outcome and the effect of treatment. Obstructive nephropathy is an evolving disease in which renal damage continues even after relief of the obstruction. Recently, urinary EGF/MCP-1 (monocyte chemotactic protein-1) ratio was suggested as an useful early biomarker of progressive renal damage for obstructive nephropathy, and can have a potential role in predicting the long-term renal function outcome.<sup>21</sup> In a study examining urinary EGF level in patients with ischemic acute renal injury, urinary EGF level was found to be decreased in the patients in comparison to controls; increased urinary EGF was shown to predict renal functional recovery on day 7 with a 90% sensitivity and 64% specificity using the cut off value of 2.17ng/mg urine creatinine of urinary EGF, and death within 3 months with a 74% sensitivity and 88% specificity using the cut off value of 1.48ng/mg urine creatinine.<sup>7</sup>

Recently, the most satisfying results about predictive potential of urinary EGF were obtained by Ju et al.<sup>22</sup> They found that urinary EGF amount showed significant correlation with intrarenal EGF mRNA level, interstitial fibrosis/tubular atrophy, eGFR, and rate of eGFR loss in patients with CKD. They suggested the urinary EGF as an independent prognostic marker of CKD progression which can add predictive power to traditional clinical prognostic markers of the disease.

In the present study, in agreement with previous studies, urinary EGF level was found to be lower in the patients with CKD than those in the control group. The urinary EGF was found to be weakly correlated with serum creatinine ( $r = -0.32$ ,  $p < 0.01$ ), likely derived from limited sample size of the study. The finding that, urinary EGF level in patients with stage 3a was higher than those with stage 4 is promising. However as a limitation, number of the cases was small so that urinary EGF level in patients with stage 2 could not be compared with the urinary EGF levels in patients with advanced stages. Due to similar reason results of the ROC curve analysis were not promising; sensitivity, specificity, negative predictive value and accuracy were low. In a large-scale study including a large number of patients at every stage will be helpful to assess predictive potential of EGF for CKD.

In conclusion, biomarkers which reflect the kidney damage at the early stage are currently being investigated. Urinary EGF level seems a promising marker to detect early renal damage. It may also have a prognostic potential. Regenerative potential in patients with diabetic nephropathy is low. In the case of inadequate treatment renal function loss is more rapid in patients with diabetic nephropathy when compared to CKD patients with other causes.<sup>23</sup> Subsequent measurements of urinary EGF may be an important marker for progression in patients with diabetic nephropathy.

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## Conflict of interest

The author declares that there is no conflict of interests regarding the publication of this article.

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