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Urinary Epidermal Growth Factor Excretion: A Useful Prognostic Marker for Progression of Renal Damage in Children

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In this study, we determined urinary Epidermal Growth Factor (EGF) concentrations in children with Glomerulonephritis with normal kidney functions, Acute Renal Failure (ARF) and Chronic Renal Failure (CRF). We investigated 32 children, 18 males and 14 females, of 9.3±2.3 years (mean±standard deviation), who were followed in the Nephrologic Unit of the Pediatric Department of the Al-Azhar University. Results were compared to 16 healthy controls age and sex matched. Children with ARF (group I_b) and CRF (group I_c) had significantly lower urinary EGF concentrations as well as urinary EGF/creatinine ratio (EGF/Cr). In contrast, children with nephritis (group I_a) with normal renal function had normal urinary EGF levels and urinary EGF/Cr. There were significant positive correlation between urinary EGF, EGF/Cr and both Glomerular Filtration Rate (GFR) and creatinine clearance, also There was a significant negative correlation between EGF/Cr, serum creatinine in patients with acute and chronic renal failures.

Key words: Epidermal growth factor, acute renal failure, chronic renal failure, glomerular filtration rate, creatinine clearance

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INTRODUCTION

Human Epidermal Growth Factor (EGF) is a 6 KDa peptide consisting of 53 amino acids including 6 cysteines forming 3 disulphide bridges (Chang and Li, 2002). EGF is synthesized as large glycosylated precursor (prepro-EGF) with a total length of 1217 residues. EGF operates by binding to the EGF receptor, which is 170 KDa transmembrane glycosylated protein with tyrosine kinase activity (Leahy, 2004).

Human EGF is present in most extracellular fluid and secretions including plasma, saliva, amniotic fluid, milk and urine (Plebani *et al*., 1997; Xiao *et al*., 2002). The highest concentration-approximately 2-10 ng mL^{-1} is found in urine, while plasma concentrations are near the undetectable limit which is less than 20 pg mL^{-1}. This difference suggests the hypothesis that urinary EGF (uEGF) derives from kidney synthesis and secretion (Callegari *et al*., 1988). In fact, the localization of prepro-EGF to the thick ascending loop of Henle and the distal nephron suggests EGF synthesis in this region (Gesualdo *et al*., 1996). On the other hand immunohistochemical studies on human or on mouse kidney have demonstrated that EGF is mainly located into the tubular epithelial cells of the thick ascending loop of Henle, the distal convoluted tubule, the collecting ducts and the proximal tubules, as well as in the human glomerulus (Poulsen *et al*., 1986; Lakshmanan *et al*., 1992).

Growth factors contribute to renal injury/repair in several ways including proliferative responses, leading to regeneration after acute tubular injury and to reparative, often sclerotic, outcomes after other forms of glomerular and tubular renal injury. Growth factors, such as EGF, may also have glomerular hemodynamic consequences, as infusion of EGF is reported to acutely reduce glomerular filtration rate. Renal EGF expression, however, is rapidly diminished in several forms of renal injury including acute glomerular injury (Chesney, 2005), cystic kidney diseases (Veizis and Cotton, 2005) and renal andurothelial malignancy (Paule and Brion, 2003).

This study was conducted to estimate urinary excretion of epidermal growth factor and EGF/Cr in children with different renal diseases and to evaluate the relation between EGF excretion and kidney functions.

MATERIALS AND METHODS

Patients: The present study included two groups of children:

Group I: Comprised 32 children with different renal diseases, they were 18 males and 14 females. Their ages ranged between 4 and 16 years with a mean 9.3±2.3 years. This group was subdivided into 3 groups.

Group I_a (glomerulonephritis group with normal kidney functions): It include 11 children (7 males and 4 females) and their ages ranged from 7-13 years. Five patients had acute glomerulonephritis (verified by clinical and laboratory findings), 3 patients had systemic lupus nephritis (verified by clinical, laboratory and biopsy findings), 2 patients had diabetic nephritis (verified by history, clinical and laboratory findings) and 1 patient had IgA nephropathy as verified by renal biopsy.

Group 1_b (acute renal failure): It included 10 patients on early stage of acute renal failure (6 males and 4 females) and their ages ranged from 7.5-16 years.

Group 1_c (chronic renal failure): It included 11 patients (5 males and 6 females), their ages ranged from 4-15 years. One patient was on heamodyalsis.

Group II (normal control group): It included 16 children (9 males and 7 females), their ages ranged from 4-14 years.

Methods: All cases were subjected to:

- Full clinical history.
- Thorough clinical examination including anthropometrics measurements were performed. Body surface area was calculated (Rigalleau *et al*., 2004)

Investigations included:

- Complete blood picture: (using Automated Coulter Counter T-660).
- Complete urine analysis.
- Kidney function test: blood urea, serum creatinine (Schuck *et al*., 2004).
- Urinary creatinine (Vernaglione *et al*., 2003).
- Creatinine clearance (Pong *et al*., 2005).
- Glomerular filtration rate (Stevens and Levey, 2005).
- Urinary EGF was measured by ELISA technique for all patients and controls. The kit supplied from Biosource Europ SA (Tsau and Chen, 1999). Aseptically collected urine of 24 h voided directly into a sterile container centrifuged to remove particulate mater and stored at −20°C until assayed. Analysis was performed by quantitative sandwich enzyme linked immune sorbent assay technique, using commercially available kit. A monoclonal antibody specific for EGF has been pre-coated onto

a microplate. Standards and samples were pipetted into the wells and any EGF present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for EGF was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and colour developed in proportion to the amount of EGF bound by the immobilized antibody. The colour development was stopped and the intensity of the colour was measured. The mean intra and inter assay coefficient of variation (CV) were 5.0 and 5.3%, respectively.

Statistical analysis: A standard computer program (SPSS for Window, Release 10.0, SPSS, Chicago, IL) was used for data entry and analysis. Student t-test was applied to the data conforming to normal distribution. Correlation coefficient (r) was used to determine the relationships between different quantitative values. For all tests a probability <0.05 was considered significant.

RESULTS

In children with different renal diseases, there were significant increase in serum creatinine and blood urea in groups with acute and chronic renal failure as compared to normal control group and there were significant decrease in urinary creatinine, creatinine clearance (CC), GFR, mean RBCs count and hemoglobin concentration in renal failure groups (group $I_{b,c}$) compared to normal control group (Table 1).

Children with acute renal failure (group I_b) and chronic renal failure (group I_c) had significantly lower urinary EGF concentrations as well as urinary EGF/creatinine ratio (EGF/Cr) as compared to normal control (group II). In contrast, children with nephritis with normal kidney function had normal urinary EGF levels and urinary EGF/Cr (Table 2).

In patients with acute and chronic renal failures, there were significant positive correlation between urinary EGF, EGF/Cr and both GFR and creatinine clearance, also there were a significant negative correlation between EGF, EGF/Cr and serum creatinine in the same groups of patients (Table 3, 4).

DISCUSSION

The functional integrity of the mammalian kidney is vital total body homeostasis, as the kidney plays a principal role in the excretion of metabolic wastes and

Table 1: Descriptive data of the children with renal diseases (group $I_{a,b,c}$ and control group) (mean±SD)

Characteristics	Group I_a (n = 11)	Group I_b (n = 10)	Group I_c (n = 11)	Group II (n = 16)
Mean ages (years)	10.20±2.30	9.80±2.10	10.30±3.10	8.09±2.7
Systolic blood pressure (mmHg)	100.00±20.6	128.00±22.2*	150.00±29.2**	85.50±10
Diastolic blood pressure (mmHg)	72.00±10.7	98.25±12.5*	101.11±10.6*	55.80±10
Serum creatinine (mg dL^{-1})	0.90±0.40	6.77±2.17*	10.13±3.57*	0.60±0.13
Urinary creatinine (mg dL^{-1})	87.67±12.84	42.93±9.09*	29.81±15.28**	127.73±26.03
CC (mL min^{-1}/1.73 m^2)	44.30±31.35	4.21±3.52***	2.11±1.71***	56.32±12.1
GFR	89.15±22.98	10.98±3.29***	8.83±4.65***	116.70±20
Blood urea (mg)	32.00±12.52	68.53±13.54**	131.00±37.24***	19.00±2.7
Hb (mg dL^{-1})	10.10±2.62	7.55±2.31*	6.77±0.89**	11.90±1.9
RBCs (10^{12} cc^{-1})	4.00±1.56	2.90±0.57*	2.50±0.46**	4.70±0.6

*: Significant p<0.05. **: High significant p<0.01. ***: Very high significant p<0.001. Hb: Hemoglobin. RBCs: Red Blood Cells. cc: Creatinine clearance

Table 2: Urinary EGF and EGF/Cr in all studied groups (mean±SD)

Parameters	Group I_a (n = 11)	Group I_b (n = 10)	Group I_c (n = 11)	Group II (n = 16)
EGF ng mL^{-1}	30.35±1.4	5.50±0.85**	4.82±1.30**	38.7±1.70
EGF/Cr (ng mg^{-1} creatinine)	0.34±0.1	0.14±0.09**	0.16±0.08**	0.3±0.06

**: High significant p<0.01

Table 3: Correlation coefficient (r) between EGF and different variables among patients groups

Parameters	Group I_a EGF	Group I_b EGF	Group I_c EGF
GFR	r = 0.137	r = 0.652*	r = 0.841**
Serum creatinine	r = -0.145	r = -0.667*	r = -0.646*
Creatinine clearance	r = 0.319	r = 0.671*	r = 0.653*

*: Significant p<0.05, **: High significant p<0.01

Table 4: Correlation coefficient (r) between EGF/Cr ratio and different variables among patients groups

Parameters	Group I_a EGF/Cr	Group I_b EGF/Cr	Group I_c EGF/Cr
GFR	r = 0.012	r = 0.640*	r = 0.853**
Serum creatinine	r = 0.005	r = -0.668*	r = -0.647*
Creatinine clearance	r = 0.022	r = 0.656*	r = 0.59*

*Significant p<0.05, **: High significant p<0.01

in the regulation of extracellular fluid volume, electrolyte composition and acid base balance. In addition, the kidney synthesizes and releases hormones, such as rennin and erythropoietin and metabolizes vitamin D_3 to the active 1.25 dihydroxy vitamin D_3 form. An insult to the kidney therefore, could disturb any or all of these functions and could have profound effects on total body metabolism (Chesney, 2005). Growth retardation is a common problem in chronic renal failure. A great majority of children are already growth retarded when CRF is initially noted especially if renal impairments began during rapid growth in the first year of life, several factors have been identified; including acidosis, anemia, hypertension and reduced caloric intake. If the patient is on dialysis, decreased intake can be further exacerbated by increased losses of nutrients into the dialysis, especially amino acids and water soluble vitamins (particularly vitamin C, pyridoxine and folic acid) (Druml, 2001; Fouque, 2003).

In this study there was significant decrease in mean weight and mean height in children with chronic renal failure (group I_c) as compared to normal control group (group II) $p<0.001$. These findings of growth retardation agree with many previous published reports (Tsau and Chen, 1999; Nouwen *et al*., 1994).

As regard, the hematological results, significant decrease in the mean RBC counts and hemoglobin concentration were found in ARF group (I_b) and CRF group (I_c) as compared to children who had nephritis with normal kidney functions.

This is in agreement with Montini *et al*. (1990), who reported that anemia is a common manifestation of renal failure and usually correlates with severity of the diseases. It contributes to many of the non-specific symptoms of renal failures. Several mechanisms are implicated in its pathogenesis which include, relative deficiency of erythropoietin, diminished erythropoietin due to toxic effects of ureamia on marrow precursor cells, reduced red cell survival, increased blood loss due to capillary fragility and poor platelet function and reduced dietary intake and absorption of iron and other haematinics (Chavers *et al*., 2004).

In the present study urinary levels of EGF were significantly lower in renal failure groups (group-I_b and group-I_c) as compared with control group. This agree with the results reported by Lev-Ran *et al*. (1990) and Tsau and Chen (1999). They found that children with CRF had a significantly lower daily urine EGF concentration.

Growth factors contribute to renal injury/repair in several ways including proliferative responses, leading to regeneration after acute tubular injury and to reparative, often sclerotic, outcomes after other forms of glomerular and tubular renal injury. Growth factors, such as EGF, may also have glomerular hemodynamic consequences, as infusion of EGF is reported to acutely reduce glomerular filtration rate. Renal EGF expression, however, is rapidly diminished in several forms of renal injury including acute glomerular injury (Chesney, 2005).

It has been reported that nephrotoxic and ischemic renal injury reduces prepro-EGF mRNA and urinary EGF excretion in rats with ARF (Safirstein *et al*., 1989; Safirstein, 2004).

In the present study, urinary levels of EGF were markedly lower in patients with ARF than in normal control. These results were similar to that reported by Taira *et al*. (1992) and Nouwen *et al*. (1994).

The urinary EGF-creatinine ratio (EGF/Cr) has been traditionally used to express urinary excretion. In the present study this ratio was also significantly decreased in renal failure groups (group-I_b and group-I_c). This is in agreement with many previous studies (Tsau and Chen, 1999; Tasu *et al*., 1996). There were many trails to explain the mechanism of reduced urinary levels of EGF among patients with renal failure. Tiara *et al*. (1992), concluded that, decreased urinary EGF can not be described to decreased clearance resulting from impaired renal functions.

In rats, removal of submandibular glands and the duodenal Brunner's glands, organs known to produce EGF, had no influences on the level of EGF in urine (Dubiel *et al*., 1992). By immunohistochemistry EGF can be visualized in the mouse kidney. Furthermore, serum EGF levels are very low and only 10% of intravenously injected mouse EGF is excreted in urine (Jung *et al*., 2005). Chen and Liu (1997) mentioned that: the mechanism of reduced EGF excretion could be attributed to renal damage. This mechanism was reasoned that renal ischemia affects both transcriptional and post-transcriptional effects of EGF and decreased renal prepro-EGF mRNA production.

In the present study, there were no significant difference in the urinary EGF levels in patients with ARF and CRF (Table 2). So we postulated that urinary EGF does not have any utility in distinguishing ARF from CRF. This observation was also reported by Taira *et al*. (1992).

Acute renal failure is a reversible form of organ failure (Price *et al*., 2003). Safirstein *et al*. (2004) postulated that the decreased urinary EGF excretion is believed to result from reduced renal EGF production secondary to acute tubular injury and may return to normal levels after complete clinical recovery.

Glomerulonephritis is one of the leading causes of both acute and chronic renal failure (Sugaru *et al*., 2005). In this study there was a non significant decrease in urinary level of EGF among nephritis group compared to normal control, this can be contributed to that our patients were in the early stage of the disease. This agree with the results reported by Taira *et al*. (1993).

Torffvit *et al*. (1998) in their study on patients with glomerulonephritis and patients with diabetic nephropathy and Ranieri *et al*. (1996) on patients with IgA nephropathy, reported that EGF was decreased and might be a valuable prognostic marker for the progression of the renal damage in nephropathy.

Regression analysis of the present results revealed a negative significant correlation between urinary EGF levels, EGF/Cr ratio (separately) and serum creatinine concentration among patients with acute as well as chronic renal failure. Also these groups of patients showed a positive significant correlation between urinary levels of EGF and EGF/Cr ratio with each of creatinine clearance and GFR. These results were similar to that reported by Mattila *et al*. (1986) and Tsau and Chen (1999).

A probable explanation of our findings is that urinary EGF originates only in the kidney and its rate of formation and excretion depends on the number of functioning nephrons.

CONCLUSIONS

From the present results we concluded that, urinary level of EGF was significantly decreased among patients with acute and chronic renal failure, the presence of negative correlation between urinary EGF levels and serum creatinine concentration on one side and positive correlation between its level and each of creatinine clearance and GFR on opposite side, denoted that its urinary level depends on the number of functioning nephrons and reflecting the degree of kidney damage.

Further study will needed to estimate the role of urinary EGF in the evolution of renal lesions after injury and the in recovery stage of acute renal failure.

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