

Original Paper

Urine Epidermal Growth Factor, Monocyte Chemoattractant Protein-1 or Their Ratio as Biomarkers for Interstitial Fibrosis and Tubular Atrophy in Primary Glomerulonephritis

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Key Words

Biomarker • Chronic kidney disease • EGF • Epidermal Growth Factor • Fibrosis • Glomerulonephritis • Kidney • NGAL • Neutrophil gelatinase-associated lipocalin • MCP-1 • Monocyte chemoattractant protein-1 • Tubulointerstitial

Abstract

Background/Aims: The degree of tubular atrophy and interstitial fibrosis (IFTA) is an important prognostic factor in glomerulonephritis. Imbalance between pro-inflammatory cytokines such as monocyte chemoattractant protein-1 (MCP-1) and protective cytokines such as epidermal growth factor (EGF) likely determine IFTA severity. In separate studies, elevated MCP-1 and decreased EGF have been shown to be associated with IFTA severity. In this study, we aim to evaluate the predictive value of urinary EGF/MCP-1 ratio compared to each biomarker individually for moderate to severe IFTA in primary glomerulonephritis (GN). **Methods:** Urine samples were collected at biopsy from primary GN (IgA nephropathy, focal and segmental glomerulosclerosis, minimal change disease, membranous nephropathy). MCP-1 and EGF were analyzed by enzyme-linked immunosorbent assay. **Results:** EGF, MCP-1 and EGF/MCP-1 ratio from primary GN, all correlated with IFTA (n=58). By univariate analysis, glomerular filtration rate, EGF, and EGF/MCP-1 ratio were associated with IFTA. By multivariate analysis, only EGF/MCP-1 ratio was independently associated with IFTA. EGF/MCP-1 ratio had a sensitivity of 88%

and specificity of 74 % for IFTA. EGF/MCP-1 had good discrimination for IFTA (AUC=0.85), but the improvement over EGF alone was not significant. **Conclusion:** EGF/MCP-1 ratio is independently associated IFTA severity in primary glomerulonephritis, but the ability of EGF/MCP-1 ratio to discriminate moderate to severe IFTA may not be much better than EGF alone.

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Introduction

Chronic kidney disease (CKD) is an important cause of morbidity and mortality worldwide resulting in elevated risks for end-stage renal disease (ESRD), cardiovascular disease, and death [1]. Primary glomerulonephritis (GN) consists of a group of disorders that together constitutes one of the leading causes of ESRD [2]. An imbalance between pro-inflammatory and protective mediators is critical in the pathogenesis of GN and loss of renal function [3]. Independent of the primary disease, glomerular injury causes activation of common profibrotic pathways characterized by tubular atrophy and interstitial fibrosis leading to progressive nephron loss [4, 5]. The rates of decline in renal function in GN differ widely and are more closely associated with the severity of the tubulointerstitial disease than with the glomerular lesions [3-5]. Traditional clinical parameters such as the degree of proteinuria, hypertension and reduced glomerular filtration rate (GFR) cannot fully identify patients with significant tubulointerstitial involvement at risk of more rapid progression. At present, the extent of interstitial fibrosis and tubular atrophy (IFTA) can only be assessed by a renal biopsy. Because a biopsy is invasive, it may be delayed and cannot be performed serially to monitor disease progression.

Noninvasive biomarkers with close correlations to the severity of pathologic lesions would be useful to guide and monitor therapy. Multiple potential biomarkers have been associated with renal impairment, but currently, there are no universally accepted biomarkers to predict tubulointerstitial involvement [3]. For example, neutrophil gelatinase-associated lipocalin (NGAL), a 25 kDa protein produced by the injured tubular epithelia and widely used as a biomarker for acute kidney injury [6], has been found to be elevated in patients with glomerular diseases and tubulointerstitial damage [7, 8]. However, other factors such as co-existing acute tubular injury or proteinuria might alter the relationship of urinary NGAL with tubulointerstitial lesions [6].

Monocyte chemoattractant protein-1 (MCP-1) is a potent chemokine that promotes recruitment of inflammatory cells such as monocyte/macrophage into the kidney and contribute to tubulointerstitial involvement by promoting the release of several mediators including transforming growth factor-beta and [9, 10]. Increased urinary excretion of MCP-1 have been observed in various glomerular diseases with correlations observed between urinary levels and degree of tubulointerstitial involvement [11, 12]. On the other hand, epidermal growth factor (EGF), a peptide growth factor produced by the ascending loop of Henle and the distal convoluted tubule, seem to have a protective role in tubulointerstitial damage. Decreased renal EGF expression has been found in chronic kidney disease [13], and decreased urine EGF levels have been found in IgA nephropathy (IgAN) patients with advanced histopathological lesions [14, 15].

Combined use of biomarkers with opposing actions such as EGF and MCP-1 may offer additional information compared to either cytokine alone. Previously, the ratio of urinary EGF and MCP-1 was found to correlate better with renal prognosis than either biomarker alone in patients with IgAN [15]. However, the clinico-pathological relationship of the ratio of these cytokines have been not tested in other primary GN.

In this study, we aim to test the hypothesis that the ratio of EGF and MCP-1 in the urine is more closely associated with the severity of tubulointerstitial lesions than each biomarker individually in common primary GN. In addition, we will compare the relationship of these biomarkers and tubulointerstitial lesions with urine NGAL.

Materials and Methods

Patient population

Healthy controls were recruited from volunteers with no chronic illnesses including hypertension or kidney diseases after detailed history, physical examination and routine laboratory tests including urinalysis, serum creatinine and fasting glucose.

We enrolled patients with primary GN who underwent a renal biopsy for clinical indications at Ramathibodi Hospital. Patients with kidney transplants, acute kidney injury, secondary causes of GN such as diabetic nephropathy, lupus nephritis were excluded. Written informed consent was obtained. The study was conducted according to the Declaration of Helsinki, and approved by the Ethics Committee of the Faculty of Medicine, Ramathibodi Hospital.

Hypertension was defined as systolic blood pressure (SBP) \geq 140 mmHg or diastolic blood pressure (DBP) \geq 90 mmHg or use of oral antihypertensive medication [16]. *Nephrotic range proteinuria* was defined as UPCr more than 2000 mg/mg according to KDIGO criteria [17]. Estimated glomerular filtration rate (eGFR in ml/min/1.73m²) was calculated by using the CKD-EPI equation [18]. *Low GFR* was defined as eGFR < 60 [19].

Sample preparation and laboratory measurements

Blood and second morning urine samples were collected under sterile conditions from patients on the day of the biopsy. None of the subjects had urinary tract infections at the time of sample collection. Urine samples were centrifuged at 3000 rpm for 10 minutes at 4°C and the supernatant stored in aliquots at -80°C. Common biochemical parameters were measured in a laboratory in compliance with ISO 15189. Creatinine in blood and urine was measured by enzymatic method (Dimension ExL analyzer, Siemens Healthcare Diagnostics, Newark, DE USA). Urine protein was measured by modified pyrogallol red-molybdate method.

Urine MCP-1 and EGF measurements

Urinary MCP-1 and EGF measurements were performed using sandwich ELISA kit (Quantikine ELISA Immunoassay, R&D systems) according to the manufacturer's instructions. Samples were measured in duplicate by a researcher unaware to the clinical history using TECAN Infinite M200 Pro microplate reader and results calculated using Magellan Tracker software. The intra-assay coefficients of variation were 2.6 % for MCP-1 and 3.2 % for EGF. The stability of the two cytokines was tested by measuring the concentrations of the cytokines at the time of collection and again after 6 months storage. We did not observe any significant differences in the concentrations between the two measurements. EGF and MCP-1 values were normalized for creatinine (ng per mg Cr) and EGF/MCP-1 ratio was reported as ng/ng.

Neutrophil gelatinase-associated lipocalin (NGAL)

Urine NGAL was measured in the same urine aliquots using a chemiluminescent microparticle immunoassay (CMIA) kit (The ARCHITECT Urine NGAL assay). Coefficient of variation at the low (20.2 ng/mL), medium (196.7 ng/mL) and high (1174.4 ng/mL) urine NGAL levels were 4.4%, 3.0% and 2.2% for intra-assay variation, respectively while the inter-assay were 2.1%, 1.7% and 1.4%, respectively. NGAL was expressed as NGAL ng/mg Cr.

Pathologic studies

Kidney biopsies were fixed in histological fixative (Glyo-Fixx, Thermo scientific, USA), paraffin embedded, and sections (2 μ m) were processed for light microscopy (hematoxylin and eosin, periodic acid-Schiff, Masson's trichrome, and silver staining), immunofluorescence, and electron microscopy. Glomerular diseases were classified according standard criteria [17].

The severity of interstitial fibrosis and tubular atrophy (IFTA) was assessed semi-quantitatively by a nephropathologist blinded to the laboratory and biomarker data as a proportion relative to the total section area as follows: none (0), <5%; mild (1+), 5-25%; moderate (2+), 26-50%; and severe (3+), >50%.

Statistical analysis

The STATA version 13 software was used for analyses. Data are shown as mean \pm SD for normally

Table 1. Patient characteristics by severity of interstitial fibrosis and tubular atrophy. None to Mild group (Grades 0+1) and Moderate to Severe group (Grades 2+3). Data as mean ± SD or median (25, 75 percentile)

Characteristics	Severity of Interstitial Fibrosis and tubular atrophy			P value
	All	None-Mild	Moderate-Severe	
Number (%)	58	42 (72%)	16 (28%)	
Age (years)	42 ± 15	43 ± 15	38 ± 14	0.3
Female (%)	36 (62%)	27 (64%)	9 (56%)	0.8
SBP (mmHg)	136 ± 20	135 ± 21	137 ± 18	0.7
DBP (mmHg)	80 ± 11	80 ± 11	79 ± 11	0.8
eGFR (ml/min per 1.73 m ²)	73 ± 30	82 ± 28	49 ± 22	<0.001
Proteinuria (g/g Cr)	2.19 (0.77,4.78)	1.93 (0.59,4.76)	2.93 (1.77,5.59)	0.17
MCP-1/ (ng/mg Cr)	0.34 (0.20,0.62)	0.31 (0.18,0.53)	0.57 (0.32,0.93)	0.031
EGF (ng/mg Cr)	8.7 (5.0,16.7)	11.7 (7.5,18.8)	4.4 (2.4,7.6)	<0.001
EGF/MCP-1 ratio (ng/ng)	24.3 (8.9,61.6)	32.6 (17.0,81.7)	6.9 (2.9,15.8)	<0.001
NGAL (ng/mg Cr)	20.3 (11.2,47.7)	19.2 (9.28,44.6)	36.8 (15.2,98.3)	0.095

SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Cr, creatinine; MCP-1, monocyte chemoattractant protein-1; EGF, epidermal growth factor; NGAL; Neutrophil gelatinase-associated lipocalin

distributed values, median (interquartile range) for non-normally distributed values or percentages as appropriate. Spearman coefficients were used to evaluate correlations between two variables. Continuous variables between 2 groups were compared by unpaired t test or Mann-Whitney U test, and the chi-square test was used for categorical variables. The adjusted risk estimates of the biomarkers individually or as a ratio to identify moderate to severe IFTA were calculated by multivariate logistic regression after adjustments for traditional risk factors for kidney disease progression. The parameters in the multivariate model were selected if $p < 0.1$ in the univariate analysis. The area under the curve (AUC) of the Receiver-operating characteristic (ROC) graph was used to evaluate the discrimination of moderate to severe IFTA from none to mild IFTA for the biomarkers individually or as a ratio. Differences between AUC of the biomarkers were compared by DeLong test [20]. Optimal cut-offs for each biomarker was determined by Youden index [21]. P values < 0.05 were considered significant.

Results

Clinical characteristics and MCP-1 and EGF levels in healthy controls and GN patients

The GN group (n=58) comprised of IgA nephropathy (IgAN, n= 21), focal segmental glomerulosclerosis (FSGS, n= 13), Minimal change disease (MCD, n= 13) and Membranous nephropathy (MN, n= 11). Thirty-six patients (62%) were female and mean age was 42 ± 15 years (table 1). Forty patients were hypertensive, 30 had nephrotic range proteinuria and 23 had low GFR.

Eighteen healthy controls (10 females) with mean age 38 ± 6 years were recruited. As expected eGFR was higher in controls compared to GN patients [eGFR: CON, 104 ± 14 vs. GN, 73 ± 30, $p < 0.001$].

Urine monocyte chemoattractant protein-1 was considerably higher in GN compared to control subjects [MCP-1 (ng/mg Cr): GN, 0.34 (0.20, 0.62) vs. CON, 0.07 (0.05, 0.13), $p < 0.001$]. Despite differences in eGFR, urine epidermal growth factor levels were similar between GN and controls [EGF (ng/mg Cr): GN, 8.7 (5.1, 16.7) vs. CON, 8.1 (4.1, 18.7), $p = 0.6$]. The EGF/MCP-1 (ng/ng) ratio was about 3 folds lower in GN [EGF/MCP-1: GN, 24.3 (8.9, 61.6) vs. 97.1 (71.1, 205.1) ng/ng, $p < 0.001$] (figure 1).

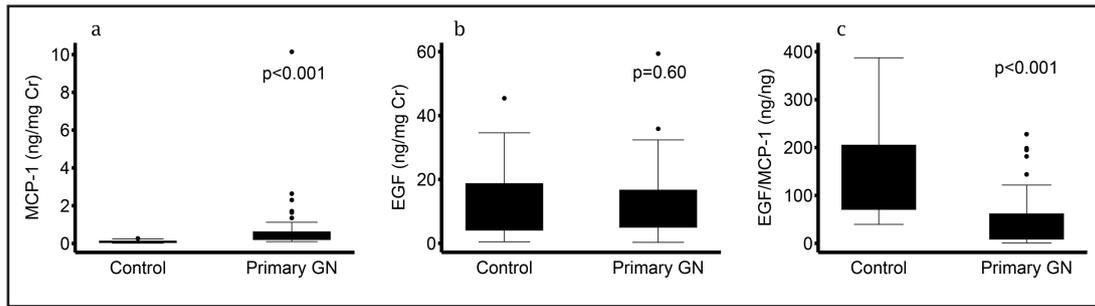


Fig. 1. Levels of Biomarkers in patients with primary glomerulonephritis vs. controls: a) MCP-1, b) EGF, c) EGF/MCP-1. a) MCP-1 (ng/mg creatinine) b) EGF (ng/mg creatinine), c) EGF/MCP-1 (ng/ng).

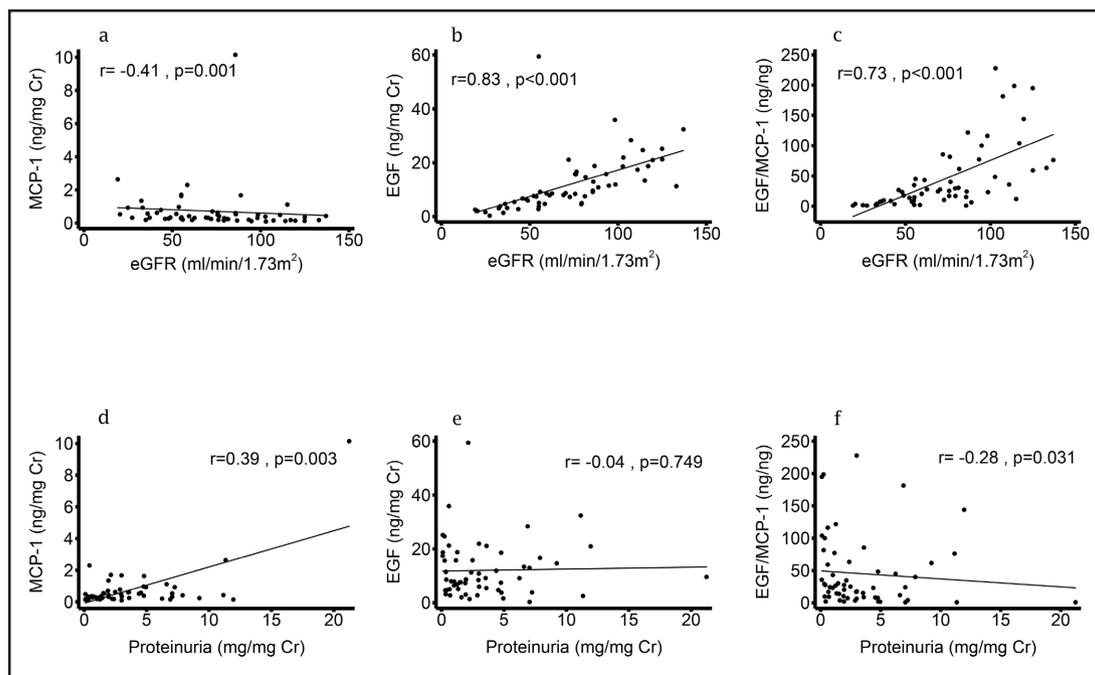


Fig. 2. Relationship between biomarkers and classical index of kidney function. Estimated glomerular filtration rate (a-c) and proteinuria (d-f).

Urinary MCP-1 and EGF levels and clinical correlations in GN

In GN patients (figure 2), MCP-1 showed a moderate inverse correlation with eGFR ($R = -0.41$, $p = 0.001$) and a moderate positive correlation with proteinuria ($R = 0.39$, $p = 0.003$). By contrast, EGF did not correlate with proteinuria, but showed a strong positive correlation with eGFR ($R = 0.83$, $p < 0.001$). EGF/MCP-1 ratio showed a strong correlation with eGFR ($R = 0.73$, $p < 0.001$) and a significant inverse correlation with proteinuria ($R = -0.28$, $p = 0.031$).

When patients were categorized according to proteinuria levels, MCP-1 was found to be twice as high in subjects with nephrotic range proteinuria compared to lower degrees of proteinuria [MCP-1: *Nephrotic*, 0.52 (0.25, 0.93) vs. *Sub-nephrotic*, 0.30 (0.19, 0.39) ng/mg Cr, $p = 0.01$]. By contrast, EGF and EGF/MCP-1 ratio were not different between nephrotic and sub-nephrotic subjects [EGF: *Nephrotic*, 10.2 (4.7, 16.6) vs. *Sub-nephrotic*, 7.9 (5.1, 16.6) ng/mg Cr, $p = 0.7$, and EGF/MCP-1 ratio: *Nephrotic*, 17.4 (6.5, 48.3) vs. *Sub-nephrotic*, 27.8 (14.3, 79.5) ng/ng, $p = 0.13$].

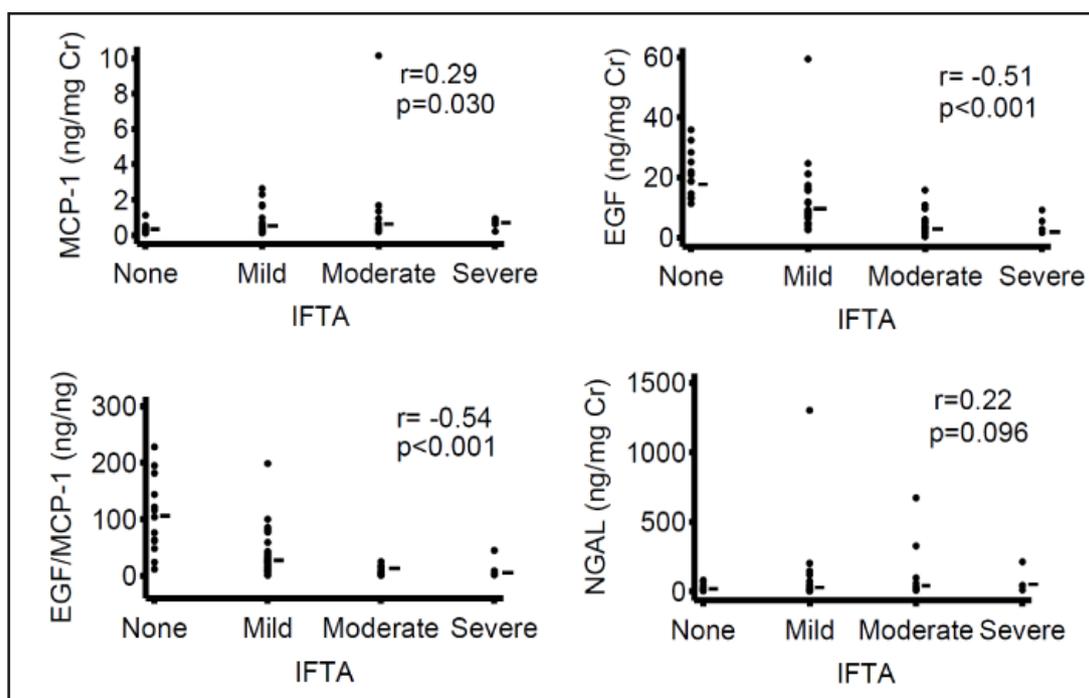


Fig. 3. Relationship between biomarkers and severity of interstitial fibrosis and tubular atrophy (IFTA): a) MCP-1 (ng/mg creatinine) b) EGF (ng/mg creatinine), c) EGF/MCP-1 (ng/ng), d) NGAL (ng/ mg creatinine). Horizontal bar denotes median value.

When patients were categorized according to kidney function, patients with low GFR (eGFR<60) had slightly higher MCP-1 compared to those with preserved GFR (eGFR≥60) [MCP-1: *Low GFR*, 0.55 (0.32, 0.97) vs. *Preserved GFR*, 0.31 (0.18, 0.50) ng/mg Cr, p=0.003]. By contrast, EGF and EGF/MCP-1 were 3 to 5 folds lower in patients with low GFR compared to those with preserved GFR [EGF: *Low GFR*, 4.7 (2.8,6.8) vs. *Preserved GFR*, 14.6 (8.7,21.0) ng/mg Cr, p<0.001, and EGF/MCP-1 ratio: *Low GFR*, 8.3 (2.3,17.7) vs. *Preserved GFR*, 43.1 (24.2,100.1) ng/ng, p<0.001].

Relationship of MCP-1 and EGF with interstitial fibrosis and tubular atrophy

IFTA was graded into 4 groups grade 0, n=13 (22 %); grade1, n= 29 (50%); grade2, n=12 (21%); and grade3 n=4 (7%). There was a considerable overlap in the values between the groups for each biomarker (figure 3.) MCP-1 showed a modest positive correlation with IFTA severity (R=0.29, p=0.03), EGF showed a strong inverse correlation with IFTA (R= -0.51, p<0.001), and EGF/MCP-1 showed a strong inverse correlation with IFTA (R= -0.54, p<0.001).

Relationship of NGAL with clinical pathological parameters and other biomarkers

NGAL was higher in GN vs controls (NGAL (ng/mg Cr): GN, 20.3 (11.2, 47.4) vs. CON, 4.3 (3.7, 4.6) vs p<0.001).

In patients with GN, NGAL correlated strongly with proteinuria (R= 0.48, p<0.001), but not with GFR (r=-0.05, p=NS). The levels of NGAL in each IFTA group are shown in figure 3. NGAL tended to correlate with IFTA, but this was not significant (R= 0.22, p=0.096). NGAL correlated positively with MCP-1 (R=0.37, p=0.004), but not with EGF (R=0.02, p=NS) or EGF/MCP-1 (R=-0.16, P=NS).

Table 2. Univariate and multivariate analysis of traditional and biomarkers for moderate to severe interstitial fibrosis and tubular atrophy

Parameters	Univariate			Multivariate		
	OR	95%CI	P	OR	95%CI	P value
SBP (mmHg)	1.01	0.98-1.03	0.6			
eGFR (ml/min per 1.73m ²)	0.95	0.92-0.98	0.001			
Proteinuria (g/g Cr)	1.08	0.94-1.23	0.3			
MCP-1 (ng/mg Cr)	1.59	0.74-3.42	0.2			
EGF (ng/mg Cr)	0.77	0.64-0.92	0.003			
EGF/MCP-1 ratio (ng/ng)	0.92	0.87-0.98	0.008	0.92	0.87-0.98	0.008
NGAL (ng/mg Cr)	1.00	0.99-1.00	0.5			

SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate; Cr, creatinine; MCP-1, monocyte chemoattractant protein-1; EGF, epidermal growth factor; NGAL; Neutrophil gelatinase-associated lipocalin

EGF/MCP-1 ratio is independently associated with Moderate to Severe IFTA

Because of low number of subjects, we combined patients into 2 groups according to the severity of IFTA: None to Mild (grades 0 and 1) and Moderate to Severe (grades 2 and 3). There were no significant differences in baseline proteinuria, systolic blood pressure or other clinical characteristics between 2 groups except that eGFR was lower in the Moderate to Severe group (table 1). MCP-1 was significantly higher, whereas EGF and EGF/MCP-1 were significantly lower in None to Mild compared to Moderate to Severe IFTA group. NGAL tended to be higher in Moderate to Severe IFTA group (P= 0.095).

Univariate analysis of clinical and biomarkers showed that eGFR, EGF and EGF/MCP-1 ratio were associated with Moderate to Severe IFTA (table 2). Of note, MCP-1 and NGAL were not significantly associated with Moderate to Severe IFTA. In multivariate logistic regression analysis, only EGF/MCP-1 ratio was an independently associated with Moderate to Severe IFTA.

Discrimination of Moderate to Severe IFTA

ROC curve was constructed to evaluate the discriminatory values of urinary biomarkers for Moderate to Severe IFTA (figure 4). MCP-1, EGF and EGF/MCP-1 ratio had significant discriminatory values. An optimum cut off value of 0.32 ng/mg Cr for MCP-1 yielded sensitivity of 81 % and specificity of 52 % for moderate to severe IFTA. An optimum cut off value for EGF of 10.8 ng/mg Cr had a sensitivity of 94 % and specificity of 55 %. An optimum cut-off of 17.7 ng/ng for EGF/MCP-1 ratio had sensitivity of 88 % and specificity of 74%. Overall, the AUC (95% confidence interval) were: MCP-1, 0.69 (0.54-0.83); EGF, 0.83 (0.71-0.95) and EGF/MCP-1, 0.85 (0.75-0.96). The AUC for MCP-1 tended to be lower compared to EGF/MCP-1 (p=0.07), whereas EGF and EGF/MCP-1 were comparable (p=NS).

By comparisons, NGAL tended to have discriminatory value for Moderate to Severe IFTA, but this was not significant (NGAL AUC: 0.65 (0.49-0.81), p=0.08).

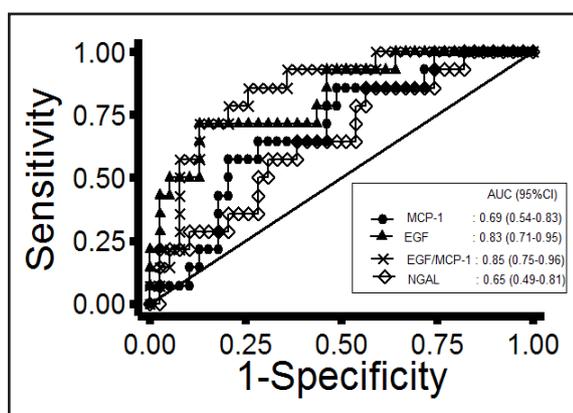


Fig. 4. Receiver operating characteristic curve for moderate to severe interstitial fibrosis and tubular atrophy or different biomarkers in primary glomerulonephritis.

Discussion

The major findings of this study are that the pro-inflammatory cytokine, MCP-1 and the potentially protective cytokine, EGF, and the EGF/MCP-1 ratio correlated with the severity of tubulointerstitial lesions and eGFR in primary GN. EGF/MCP-1 ratio, MCP-1, but not EGF, correlated with the severity of proteinuria. Although by multivariate analysis, EGF/MCP-1 ratio was an independently associated with IFTA, there was negligible improvement in ROC for discriminating moderate to severe IFTA when EGF/MCP-1 was compared to EGF alone. This data suggests that urinary EGF might be a useful biomarker for moderate to severe IFTA in primary GN, but measurement of MCP-1 may not provide useful, additional information.

MCP-1 plays a major role in renal diseases progression through multiple processes beyond monocyte/macrophage recruitment [9, 10]. Targeted inhibition of MCP-1 led to reductions in interstitial fibrosis and renal disease progression in experimental models [22, 23]. Infiltrating macrophage represent an important source for urine MCP-1 in GN, but resident glomerular or tubular cells also contribute significantly [24]. Urine MCP-1 levels have been shown to correlate with proteinuria, renal histology and prognosis in patients with proliferative glomerulonephritis [24, 25], lupus nephritis [26], diabetic nephropathy [27], and IgAN [11, 14]. We have extended previous findings to show that MCP-1 correlated positively with proteinuria, IFTA, and inversely with GFR in non-inflammatory primary GN including FSGS and MGN. The correlation of MCP-1 with IFTA severity was weaker than EGF. There was considerable overlap in MCP-1 values across histological grades. Indeed, MCP-1 was not associated with moderate to severe IFTA by univariate analysis, and a weaker discriminator of moderate to severe IFTA by ROC analysis. The lack of a strong predictive role for IFTA may reflect a more direct role of MCP-1 on macrophage infiltration and glomerular proliferation than on tubulointerstitial fibrosis as observed in some previous studies [9, 24].

Exogenous EGF enhanced tubular cell repair process in an animal model of acute renal injury suggesting that EGF may regulate tubular recovery to damage [28]. Reduced urine EGF levels have been observed in diabetic nephropathy, children with CKD, and IgAN [14, 29, 30]. Urine EGF was shown to correlate inversely with the severity of tubulointerstitial lesions in IgAN [14]. Recently, renal EGF mRNA expression was identified as being independently associated with tubulointerstitial grade using a transcriptome-driven approach, and urine EGF levels was shown to be predictive of ESRD in patients with various kidney diseases [31]. Our study showed a similarly strong association of urinary EGF with GFR and IFTA in primary GN. The lack of association of EGF with proteinuria is similar to other studies consistent with a predominantly role of EGF as a marker of tubulointerstitial function than of glomerular lesions [15, 31].

Augmented NGAL production by the damaged distal tubules may be a defensive compensatory response to prevent tubular cell apoptosis. Although widely used as a biomarker for acute kidney injury, NGAL has also been studied as a potential biomarker for chronic tubulointerstitial damage [6]. Previous studies have shown that urine NGAL is elevated in proportion to the degree of tubulointerstitial lesions in patients with chronic glomerular diseases [7, 8]. In our study, NGAL was elevated by several folds in GN patients compared to normal controls, although the levels observed in these patients were at least an order of magnitude lower than values reported in acute kidney injury consistent with previous studies [6]. NGAL tended to correlate with the degree of IFTA, and this correlation would probably have reached statistical significance with larger numbers of patients. In contrast to the weaker relationship of NGAL with tubulointerstitial lesions, NGAL was strongly associated with proteinuria, which is consistent with previous studies [7, 8]. Both passive loss of circulating NGAL through the damaged glomeruli, and interference of megalin-cubilin dependent reabsorption of NGAL at the proximal tubule by increased filtered albumin could increase urinary NGAL excretion in proteinuric patients independent of the severity of tubular damage, and account for the less robust relationship of NGAL with tubulointerstitial lesions compared to EGF in this study [6].

The current inability to easily identify patients at high risk for loss of renal function still represent a major obstacle to improving outcomes in CKD. There is still a lack of consensus on the best biomarker(s) to track tubulointerstitial injury. Optimal biomarkers that reflect the histopathological severity could provide a means for selecting patients at the highest risk of progression for more aggressive therapy and for monitoring the success of therapy on renal fibrosis. By promoting chemotaxis and inflammation, MCP-1 production results in the release of a number of autocrine factors and cytokines, including angiotensin II and transforming growth factor- β accelerating the development of interstitial fibrosis by increasing the extracellular matrix, epithelial to mesenchymal transition, cell infiltration and tubular apoptosis [3-5, 9, 10]. On the other hand, EGF has been shown to promote tubular regeneration and protect against tubular apoptosis or tubulointerstitial fibrosis in renal injury or obstruction models [32-34]. Because of their opposing effects on fibrosis, the balance of EGF and MCP-1 may be a better predictor of IFTA severity and renal disease progression than either cytokine alone. Previously, EGF/MCP-1 ratio was found to correlate with renal function, and was an independent predictor of renal disease progression in obstructive uropathy supporting the value of these biomarkers in determining the severity and prognosis in patients with chronic tubulointerstitial disease [35]. EGF/MCP-1 ratio was also shown to correlate with renal function in IgAN [15], with greater sensitivity and specificity for predicting long term outcome compared to either cytokine on its own [15]. Our study identified similar relationships of EGF/MCP-1 ratio with GFR and proteinuria in patients with common primary GN. There was a strong inverse relationship of EGF/MCP-1 ratio with IFTA. In the multivariate analysis, EGF/MCP-1 ratio was independently associated with IFTA even with the inclusion of traditional clinical factors and each cytokine individually in the model. The results of this study support a role for EGF as a good candidate biomarker for moderate to severe IFTA. It is worth noting however, that there was considerable overlap in EGF levels between patients with mild IFTA and normal controls suggesting that EGF may perform less well in discriminating patients with early tubulointerstitial damage. Our study suggests that while EGF/MCP-1 was an independently associated with IFTA, the overall improvement in specificity and discrimination of EGF/MCP-1 over EGF for moderate to severe IFTA was not significant. Thus, for the purposes of identifying IFTA noninvasively, measurement of MCP-1 may not be worthwhile given the additional costs of the assay. However, additional studies are necessary to fully assess the role of EGF/MCP-1 as a biomarker in predicting long term outcome or response to therapy.

To our knowledge, this is the first study to evaluate the relationship of IFTA and EGF/MCP-1 in common primary GN. This study has several limitations. This was a cross-sectional study from a single center involving small numbers of subjects. A larger study population would be necessary to determine the clinical benefits of using EGF/MCP-1 compared to EGF as a predictor the long term outcome in different types of GN.

Conclusions

Urinary EGF/MCP-1 ratio is independently associated with tubulointerstitial severity in primary glomerulonephritis. However, the benefit of EGF/MCP-1 ratio over EGF alone at discriminating renal histological grade is not clearly demonstrated in this study especially when the additional costs of the MCP-1 assay is considered. By contrast, NGAL appears to be strongly associated with proteinuria, and less useful as a biomarker of tubulointerstitial disease severity compared to EGF. Further prospective studies are needed to support our evaluate role of EGF or EGF/MCP-1 as candidate biomarkers to guide to therapy in various types of GN.

Disclosure Statement

None of the authors have any conflict of interest. The results presented in this paper have not been published previously in whole or part, except in abstract format.

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