THE ROLE OF GLOMERULOSCLEROSIS AND TUBULAR ATROPHY AS DETERMINING FACTOR FOR REDUCED KIDNEY FUNCTION IN KIDNEY STONE DISEASE

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Background: Kidney fibrosis including glomerulosclerosis (GS) and tubulointerstitial fibrosis (TIF) or tubular atrophy (TA) may be predictor of kidney dysfunction. Estimated glomerular filtration rate (eGFR) is a common laboratory examination to estimate the kidney function. The aim of this research is to determine relationship between histologic features namely glomerulosclerosis (GS) and tubular atrophy (TA), and estimated glomerular filtration rate (eGFR) in kidney stone disease. **Methods:** A cross-sectional study in a total of the 63 patients with kidney stone consisted of 25 (39.7 %) males and 38 (60.3 %) females, aged (51 ± 11 years), Blood urea nitrogen (BUN) 16 (8-62) mg/dl, serum creatinine 1.26 (0.47- 6.76) mg/dl and eGFR 61.4 ± 32.1 ml/min. Histologic features showed GS index 6 (0-30) and TA index 892 ± 333. There was significant correlation between eGFR and GS index dan TA index(r -0,577; p=0.001 and 0,514;p = 0,001, consecutively). Multivariate regresion equations were eLFG = 67,21 -1.63 (GS index) and eLFG = 67,21 + 0,01 (TA index). Estimated GFR may be used as a marker of glomerulosclerosis and tubular atrophy in obstructive nephropathy of kidney stone patients.

Keywords: kidney; fibrosis; filtration; glomerulosclerosis, tubular atrophy.

INTRODUCTION

Renal fibrosis is a main feature of the microscopic anatomy of renal pathology, characterized by glomerulosclerosis (GS) and tubulointerstitial fibrosis or tubular atrophy (TA), with clinical manifestations of chronic kidney disease (CKD). This condition may progress to end-stage renal disease (ESRD) which required renal replacement therapy such as dialysis and kidney transplantation. The most common causes of CKD are glomerulonephritis, metabolic disease, obstructive nephropathy, interstitial nephritis and nephropathy diseases including cystic kidney disease.[1] The incidence and prevalence of CKD in the world is on the rise, threatening to ESRD for the next decade, not all countries are able to overcome this problem. In the UK, the incidence and prevalence of ESRD approximately 10% and 60 %, in Europe an average 13 % and 70 %, and in the United States approximately 33 % and 140 %.[2] Prevalence ESRD in Indonesia, from 2002 to 2006, is increasing from 10 % to 20 %.[3], and prevalence of CKD in Bali with the formula modification of diet in renal disease (MDRD) approximately 6 %.[4]

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End stage renal disease in kidney stone are a common clinical problem has given a significant impact on the socio-economic. Patients with kidney stones may progress to ESRD. The proportion of kidney stone disease associated with ESRD approximately 3.2%.[5] Very limited evidence suggests that there is a relationship between kidney stones and the development into CKD,[6] and the kidney stone is a risk factor of ESRD not be clear.[7] Relationship between kidney stone and kidney function loss show that there is absolute increase in renal adverse outcomes associated with kidney stones. ESRD unadjusted rate was 2.48 per million people per day in people with one or more episodes of kidney stones compared to 0.52 per million people per day in the people without kidney stones.[8]

Kidney stone is a common clinical problem and potentially preventable, therefore, accurate assessment and prompt intervention is required. Histopathologic findings found by post surgical biopsy and microscopic examination may be important to predict residual renal function and long-term outcome. This study is aimed to elucidate microscopic finding in the specimens of kidney tissue obtained by biopsies during nephrolitectomy in kidney stone patients.

MATERIALS AND METHODS

Samples from kidney stones patient during nephrolitectomy has been reported in a cross-

sectional study. Samples were consecutively selected during surgery at Sanglah General Hospital in Denpasar Bali. An adequate amount of kidney tissue was taken which enough to prepare more than 30 glomeruli and tubuli of the kidney. The GS and TA were examined with under light microscope and using x 200 magnification, section of kidney biopsy were stained with Massontrichrome in Department of Pathology-Anatomy, Sanglah General Hospital Denpasar Bali-Indonesia by experienced pathologist. Five ml blood samples were drawn in each subject for an examination of estimated glomerular filtration rate (eGFR), which was calculated by CKD-EPI in an accredited Prodia Laboratory. Glomerulosclerosis index was defined the number of glomerulosclerosis counted in microscopic fields until total of 30 glomeruli had been counted. Tubular atrophy index was defined number of total tubuli in microscopic fields until 30 glomeruli had been counted. TA index equal number of tubular atrophy per total 30 normal glomeruli in microscopic field. Estimated CKD-EPI was calculated by Cockcroft-Gault formula. Plasma creatinine concentrations were examined by Jaffe methods.

Patient's characteristics were expressed using descriptive statistics. Correlation and regression analysis were used to analyze relationship between GS index and TA index to eGFR. Strength of relationship between independent variable and dependent variable was determined by correlation coefficient (r), relationship between independent variable and dependent variable was expressed using linear regression model. Significant level (alpha) was set by probability less than 5%.

RESULTS

Sixty-three kidney stone patients were operated and kidney biopsy was be done from January to December 2013 consisted of 25 (39.7%) males and 38 (60.3) females, blood urea nitrogen (BUN), serum creatinine (SC) concentrations, eGFR, GS and TA can be seen completely in Table1.

Table1
Characteristics of the study subjects (N =63)
Male 25 (39.70%), Female 38 (60.30%)

Parameters	Mean±SD	Median (interquartile)
Ages (Y)	51± 11	48 (25-84)
BUN (mg/L)	19.7±13.1	16 (8-62)
SC (mg/L)	1.7±1.3	1.26 (0.47-6.76)
eGFR (ml/min)	61.4±32.1	59 (9-126)
GS Index	9.1±8.9	6 (0-30)
TA Index	892 ± 333	895 (118-1581)

Remarks: eGFR (estimated glomerular filtration rate); GS = Glomerulosclerosis/30glomerulus; TA = The number of tubular /30 glomeruli.

Regression equation of reduction of one unit glomerulosclerosis (GS) index and estimated glomerular filtration rate can be seen in Figure 1. Figure 1 reveals equation of this correlation, i.e. eGFR = 67.21+-1.95 (GS), it means that every unit of reduction of one unit glomerulosclerosis (GS) index will increase the estimated glomerular filtration rate (eGFR) by 1.95ml/min. There was significant and negatively strong correlation between eGFR as dependent variable and independent variable GS index (r=-0.577; *p*=0.001)
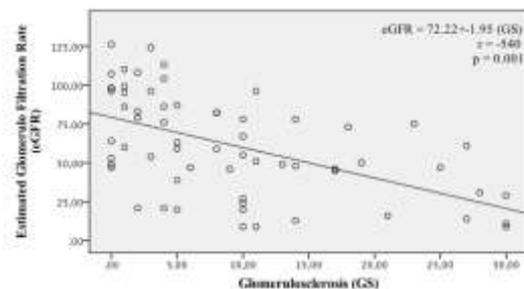

Figure1
Correlation between Estimated Glomerulofiltration Rate (eGFR) with Glomerulosclerosis(GS)

Correlation between estimated glomerulo filtration rate (eGFR) and Tubuloatrophy (TA) was presented in Figure 2.
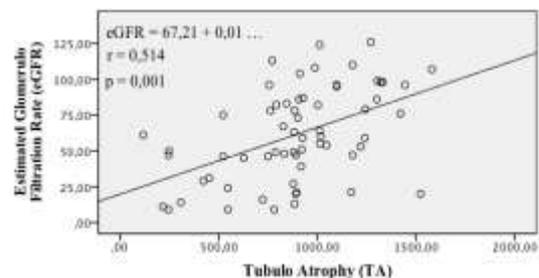

Figure 2
Correlation between estimated glomerulofiltration rate (eGFR) and Tubuloatrophy (TA)

Figure 2 showed of regression equation: eGFR=67.21+0.01(TA), it mean that every unit of increase of one unit glomerulosclerosis (GS) index will increase the estimated glomerular filtration rate (eGFR) by 0.01ml/minutes. There was significant and positive strong correlation between eGFR as dependent variable and independent variable GS index (r=0.514; *p*=0.001)

Multivariate linear analysis of eGFR as dependent variable and GS index and TA index as independent variable was listed in Table 2.

Table 2 showed that although both GS index and TA index were independent factors for eGFR, it revealed that only GS index as an independent factor. It means that either GS index or TA index was a confounder. This confounder can be

identified using covariance matrix which can be seen in Table 3.

Table 2
Multivariate Linear Analysis of eGFR as dependent variable and GS index and TA index as independent variables

Independent variables	B*	p
GS index	-1.629	0.028
TA index	0.010	0.599

Table3 Matrix Covariance between Glomerulosclerosis(GS) and tubular atrophy (TA) to estimate glomerular filtration rate (eGFR)

Tubular atrophy (TA)			
Glomeruloscl erosis (GS)			r = -0.816 p < 0.001
Glomerulo filtration rate (eGFR)		r = -0.577 p < 0.001	r = 0.514 p < 0.001
	Glomerulo filtration rate (eGFR)	Glomerulos clerosis (GS)	Tubular atrophy (TA)

Table 3 reveals that the interdependency between covariate (eGFR, GS index and TA index). GS index was a true independent factor for eGFR, and it can be explained that TA index and GS index were inter-related each other, while, both TA index and GS index were significantly related to eGFR in bivariate analysis, and only GS index was significantly related to GFR in multivariate analysis.

DISCUSSION

This study shows that glomerulosclerosis, tubular atrophy and reduced kidney function are milestones of kidney stone. There was significant and negatively strong correlation between eGFR as dependent variable and GS index as independent variable and strong correlation between estimated glomerulo filtration rate (eGFR) and tubuloatrophy (TA) index as independent variable, however, only GS index was significantly related to GFR in multivariate analysis. This phenomenon can be explained by the finding that TA index and GS index were inter-related each other.

Long term obstruction of upper urinary tract produces an increase in intratubular pressure and back leaked urine flow. Extravasation of the urine into intertitial tissue may trigger inflammation cascade and eventually interstitial fibrosis and tubular atrophy. It is thought than tubular atrophy produced glomerulosclerosis. This study found that both tubular atrophy and glomerulosclerosis are co-existed and interrelated each other, however, glomerulosclerosis is an independent factor for reduced kidney function. It is plausible, since most

of kidney function is run by filtration function by the glomeruli of the kidney. Obstructive nephropathy by kidney stone in our study may explain the mechanism of kidney damage started from the tubulus of the kidney.

Incidence of kidney stones in the world in men more often, ranging from1.5-3.0 times compared to women, about 2.5 times in Japan and about 1.2 times in Iran.[10] It is also reported that incidence of renal fibrosis in women, caused by obstructive uropathy less than men, most likely due to the presence of the hormone estradiol in women who can act as antifibrotic through regulation of angiotensin receptor type-2 (AT-2) and inducible nitric oxide synthase (iNOS).[11]

Chronic kidney disease (CKD) is a gradual progressive loss of kidney function leading to end-stage renal failure which requires kidney replacement for survival. The prevalence of this disease is increasing worldwide. Glomerular filtration rate (GFR) has a paramount diagnostic and staging role in the Kidney Disease. Glomerular filtration rate (GFR) cannot be measured directly. Variety methods of estimated GFR were reported and the best estimate of GFR can be obtained by measuring the rate of clearance of a given substance from the plasma. That substance must be able to achieve a stable plasma concentration, be freely filtered across the glomerulus, not be secreted, reabsorbed, synthesized, or otherwise metabolized by the renal tubules, and not be impacted by any other means of removal from the plasma. The most widely used serum creatinine based formulas in adults for estimated GFR (eGFR) are the Cockcroft-Gault (CG) and Modification of Diet in Renal Disease Study (MDRD).[12] Chronic kidney disease (CKD) is a characterized by progressive and irreversible loss of renal function. The increasing inability of the kidneys to properly clear the blood of waste products eventually results in the end-stage renal disease (ESRD) and requiring renal replacement therapy such as dialysis and transplantation to prevent azotemia, systemic organ damage and death. Regardless of etiology, the number of nephrons decreases during the progression of CKD and the space formerly occupied by glomeruli and tubuli becomes replaced with an extracellular matrix through a fibrotic process largely resembling scarring. The remaining nephrons increase their filtration rate in order to maintain the excretory need of the organism. Renal dysfunction appears when the remaining nephrons cannot cope with the sustained extra load. However, over time the adaptive mechanisms contribute to the deterioration of the remnant nephrons and irreversible loss of renal function.[13] Over the threshold of kidney cells injured that can not be tolerated, will cause a impaired in kidney function. It seems that tubulointerstitium were the key role in the development of these events. Direct

injury, increased cell metabolism, or various stimulation that caused of renal dysfunction will be activated tubular cells, further interact with network elements and interstitial inflammatory cells, that caused pathological changes in the renal parenchymal. Tissue response to these changes caused progressive loss of kidney function.[14]

The mechanism of kidney damage in is also seen in Wistar rat model of kidney stone with CKD,[15] which has shown that renal insufficiency associated with glomerulosclerosis and tubulointerstitial fibrosis. The most significant difference found in apoptosis of renal tubular cells with CKD. Apoptotosis glomerular cells 10 fold ($p \le 0.006$), tubular cells 26 fold ($p < 0.0001$) and interstitial cells of 5 fold ($p = 0.001$) than controls on day 120. There is a strong correlation between the severity of tubular atrophy and tubulointerstitial fibrosis with tubular apoptosis levels (r = 0.970, R2 = 0.941, $p \le 0.001$). The author found, that every reduction of glomerulosclerosis in 30 glomeruli will improve the estimation of glomerular filtration rate (eGFR) by1.95ml/minutes ml/minutes as explained in Figure 1 and each additional of one tubule in 30 glomerular will be able to improve the estimation of glomerular filtration rate (eGFR) by 0.01 ml/min (Figure 2) .

Comparison of four morphometric technique with two visual methods fibrosis score on renal biopsy with trichrome dye was concluded that the two best techniques for clinical trials, namely morphometric assessment of collagen III and visual slides with trichrome dye.[16] Microscopic pathological changes reported in models of pig kidney occurs 5 to 6 weeks after the obstruction and chronic obstruction of the human kidney. Microscopic changes include collapse (atrophy) glomerular and tubular atrophy, interstitial fibrosis, and proliferation of connective tissue.[17] The author reported sixty three patients with kidney stones (chronic obtruction) post surgical biopsied kidney speciments. Kidney function measured by eGFR (CKD - EPI) and microscopic changes glomerulosclerosis (GS) and tubular atrophy (TA) of the kidneys wereexamined with Masson trichrome staining.

Stevens and Levey (2009), measured GFR using iothalamate in urine and iohexol clearance in plasma compared with inulin clearance. Those techniques are recommended and widely applied in the clinical setting.[18] Evaluation of renal function with estimated GFR are very important for the diagnosis and treatment of patients with CKD. Fomula Cockcroft - Gault and modification of diet renal disease formula (MDRD) are most commonly used with some limitations.[19]Repair renal tubular cells was reported by Cochrane et al. (2005), showed that after 10 days of unilateral ureteral obstruction (UUO), kidney volume decrease by 32.6 % ($p< 0.001$), after 1 week of obstruction free

UUO (R-UUO), there was a significant recovery of renal volume and occupied by the repaired tissue by 54.7%(P<0.05), epithelialization of tubular cells and the presence of brush border membrane. There was a further increase at 2 weeks of R-UUO (by 78.6%, P <0.01). At 6 weeks of R-UUO, there was no significant difference in kidney volume compared with sham-operation controls (83.7 vs. 100 %).[20] In a cohort study, Murea Mariana et al. (2010), has observed histopathologic changes in renal biopsy including glomerulosclerosis and tubulointerstitial fibrosis. In the CKD group glomerulosclerosis occurred in 69 % and tubulointerstitial fibrosis in 73%. Control group had lower glomerulosclerosis and tubulointerstitial fibrosis (3% and 6%, respectively).[21]

CONCLUSION

In this study shows a significant negative correlation significant between eGFR and GS index, and correlation between eGFR and TA index. GS index is a true independent factor for eGFR. It is also showed that TA index and GS index were inter-related. Further research is needed the molecular basis of specific changes happen in obstructive kidney stone and long term impact on clinical outcomes including kidney failure.

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