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OBSTRUCTIVE NEPHROPATHY OF KIDNEY STONE: The Role of Caspase-3, Transforming Growth Factor-β and Tumor Necrosis Factor-α in Kidney Fibrosis

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Objective: Kidney stones are one of the causes of obstructive nephropathy and chronic kidney disease (CKD), characterized by the presence of hydronephrosis and kidney dysfunction. The histhopathologycharacteristics of CKD is kidney fibrosis, which consists of glomerulosclerosis (GS), tubulointerstitial fibrosis (TIF) and tubular atrophia (TA).This research aims to find out that the role of caspase-3, TGF-β and TNF-α as risk factors for kidney fibrosis in obstructive nephropathy of kidney stone. **Methode:** A total of 82 samples of kidney biopsy patientsduring kidney stone surgery in Sanglah General Hospital Denpasar-Bali from February antil December 2013 wereapplied match paired based on age and sex divided in to 41 samples as case and 41 samples as control. Determination of caspase-3, TNF-α and TGF-β1 were carried out using Kit Methode and kidney fibrosis stained with Masson's Trichrome. **Results:** Bivariate analysis test indicates that OR caspase-3 activity and kidney fibrosis were 3.50; 95%CI (2.95-4.19); p=0.001, OR levels of TGF-β and kidney fibrosis were 2.00; 95% CI (1.72-2.34); p=0.04 andOR levels of TNF-α and kidney fibrosis were 0.60; 95% CI (0.53-0.68); p=0.14. On the other hand, multivariate analysis test OR for caspase-3 activity , levels of TGF-β, levels of TNF-α and kidney fibrosis after adjustment of hydronephrosis, eLFG, and Hb were 2.43; 95% CI (0.86 – 6.90); p=0.09, 2.14; 95% CI (0.74-6.18); *p*=0.16 and 1.12; 95% CI (0.40-3.16); *p*=0.82. **Conclusion:** In obstructive nephropathy of kidney stone patients with high activity of caspase-3 have 3.5 fold risk to gain kidney fibrosis and with high levels of TGF-β have a 2 fold risk to gain kidney fibrosis. However, high levels of TNF-α was not as a risk factor to gain kidney fibrosis. These results can be used as a based data to run further research for obtaining new strategy for managing kidney fibrosis.

Keywords: obstructive nephropathy, obstructive kidney stones, kidney fibrosis, chronic kidney disease.

INTRODUCTION

Kidney fibrosis is a histhopathologic characteristic of progressive chronic kidney disease (CKD) regardless of the cause and was believed as prognosis predictor of kidney dysfunction. Histhopathologic of kidney fibrosis consists of glomerulosclerosis (GS), tubulointerstitial fibrosis (TIF) and tubular atrophia (TA). Kidney stone is one cause of kidney obstructive, obstructive nephropathy and chronic kidney disease.

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Obstructive nephropathy of kidney stone was also identified by the present of hyronephrosis and kidney dysfunction. Hydronephrosis were characterized by tubular dilatation, tubular cells atrophic, interstitial fibrosis and loss of kidney parenchymal with clinical manifestation of reducing kidney function. Hydronephrosis can be as a marker of kidney stone obstruction.[1] Kidney stone is a risk factor of chronic kidney disease.[2,3]

Currently, incidence and prevalence of CKD increase worldwide and threatening to be end stage kidney disease for the next decade and not too much country has an ability to protect problems posed.[4] Prodjosudjadi and Suhardjono,[5] reported that CKD incidence who underwent hemodialisis in

Indonesia from the year of 2002 to 2006 were around 1.5% to 3.1%. On the other hand, their prevalence reported around 1.0% to 2.4%. Raka-Widiana,[6] reported that CKD prevalence in Bali were 6%. Bowolaksono, et al.,[7] reported that kidney function reversibility for mild kidney hydronephrosis was 2.8 fold compare to severe kidney hydronephrosis and for unilateral kidney obstruction was 1.8 fold compare to bilateral kidney obstruction.

Fibrogenesis divided into three phase,[8] i.e. phase-1 (induce phase) is a macrophage activated phase and elaborates some cytokines (TGF-β, IL-I, PDGF and TNF-α); recruitment of immune cells (B-cells, T-cells, eosinophil) and elaborates cytoplasm factors (IL-2, IFN-γ); stimulates motility and fibroblast proliferation; disollution, matrix phagocytosis and stimulates epithelium tubular cells. Phase-2 (matrix disposition phase) is chronic inflammation phase marked by the occurrence of synthesis, secretion and fibronectin, collagen type-I and III deposition. Phase-3 (resolution phase) is resolution phase or depressing inflammation process, where decline of matrix synthesis occurs and stimulates matrix proteolysis. Kidney fibrosis process was very complex involving simultaneous interaction of some cellular mediator and molecular. Destructive fibrosis occurs on solid organ such as kidney, cardiac and liver. Pathophysiology of tubular interstitial fibrosis were grouped into four phase,[9] i.e. 1) injury phase and cellular activation, 2) fibrogenic signal phase, 3) fibrogenic phase and 4) kidney destruction phase. Tissue destruction and loss of tissue capillary leads to decline blood perfusion and oxygen transport to tissue and ended with fibrosis.[10] Hypoxia inducible factor-1 (HIF-1) and HIF-2 are basic-helix-loop-helix transcription factors that enables cells to survive in less oxygen environment by regulates energy metabolism, vascular remodeling, erythropoiesis, proliferation and cells apoptosis. HIF activation stimulates epithelial transition to mesenchymal and kidney fibrogenesis. Kidney fibrosis was determined by tubular interstitial fibrosis on kidney parenchymal leads to kidney dysfunction.[11] General mechanism of kidney fibrosis is epithelial to mesenchymal transition (EMT), in which tubular epithelial cells were transformed to mesenchyme fibroblast, migrates to interstisial parenchymal together with local and circulating cells. Proteinuria and hipoxia regulate the EMT mechanism. TGFβ-1 through smooth muscle actin dorsophila (SMAD) pathway possibly together with hypoxia inducible factors (HIF) regulate EMT molecule mechanism. Hepatocyte Growth Factor (HGF) and Bone Morphogenetic Factor-7 (BMF-7) are inhibitor of EMT molecule protects interstisial fibrosis in an experimental and clinic. Obstruction and initial kidney injury induce cellular and molecular activation.[12] This activation leads to accumulation excess of extra cellular matrix (ECM). Cellular activation includes epithelial to mesenchimal transicion (EMT), fibroblast activation, monocyte/macrophage infiltration, and apoptosis. In addition, molecular activation includes activation of transforming growth factor betha (TGF-β), Angiotensin-II (Ang-II), connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF), endothelin-1 (ET-1), tumor necrosis factor alpha (TNF-α), and interleukin-1 (IL-1). Cytokine anti-fibrotics such as hepatocyte growth factor (HGF), bone morphogenetic protein-7 (BMP-7), interferon gamma (IFN-γ), and insulin-like growth factor-1 (IGF-1) or some metalloproteinases matrix protease (MMP), plasminogen activator, and cathepsins lisosomal are possibly to reduce kidney fibrosis. Cellular and molecular pathophysiologic activation of kidney fibrosis is very complexes process. Caspase-3 and 8 correlate to kidney cells apoptosis, caspase increased in kidney obstruction in line to kidney cells apoptosis.[13] TNF-α is a cytotoxic cytokine that induce apoptosis besides its role in kidney inflammation. When this cytokine bind to its receptor (TNFR1) and associated death domain bind to TNFR1 (TRADD) will activate some signal path ways and stimulate activation of caspase-8 that play a role in apoptosis occurrence. Other alternative, complex TNFR1-TRADD activates nuclearfactor kappa-B (NF-kB) that has ability as pro and antiapoptosis depends on their cellular environment.

MATERIALS AND METHODS

The research design was a matched paired case-control study. The research was approved by reginal ethical review board in Denpasar Bali by the Research Ethics Committee of the Faculty of Medicine Udayana University / Sanglah Hospital Denpasar. Kidney biopsi was undertaken in 84 kidney unit as a one-step procedure through the kidney stone surgery approach. Kidney biopsy with fibrosis (N = 42) were used in cases and kidney biopsy without fibrosis (N = 42) were used as controls.

Estimation of kidney fibrosis, the kidney fibrosis was determined by experienced pathologist. Using a 200 magnification, sections stained with Masson's Trichrome and the data were collected from a minimum series of 12 randomly selected fields in the cortex, or such number of fields until 30 glomeruli had been counted were scored as follows. For glomerulosclerosis, a normal glomerulus scored 0; mild glomerulosclerosis (GS) affecting up to 25% of the glomerular tuft scored 1;

moderate GS affecting between 25% and 50% of the tuft scored 2; and severe GS affecting in excess of 50% of the tuft scored 3. Tubulointerstitial fibrosis was defined and scored as: normal tubules with approximately 1000 tubule cells scored 0; mild tubular atrophy (TA), reduced tubular cells affecting up to 25% of the section scored 1; moderate TA, reduced tubular cells affecting 25% to 50% of the section scored 2; and severe TA, reduced tubular cells exceeding 50% of the section scored 3.[15]

Examination of the caspase-3 activity is uses by caspase-3 assay kit colorimetric. The renal biopsy tissue was weighed, cut into small pieces, were given 2 ml of lysis buffer, and stirred with micropestle, centrifuged at 5000 x g, 4^0 C for 10 minutes. Supernatant (sample) was separated from the pellet and the samples stored in deep freezer (minus 70-80^0 C)until to reach of 41 samples of each case and control. The examination of TNF-α and TGF-β1 with ELISA. Renal biopsy tissue was given 1 ml of assay buffer (1x assay buffer: 20 mM HEPES, pH 7.4, 2 mM EDTA, 0.1% CHAPS, 5 mM DTT). The homogenate was centrifuged at 3.000 x g, 4^0 C for 15 minutes. The supernatant was stored at minus 70-80^0 C until it was time for the assay (41 samples of each case and control).

RESULTS

Characteristic of Match Paired Case-Control Study

A number of 82 patients were match paired case-control study within 41 samples for case and 41 samples for control. These two groups comprise of 14 (34.1%) kidney female and 27 (65.9%) kidney male. Mean age of case group was 50.66± 10.55 years (25-74 years) and 49.85±11.59 years (25-78 years) for control. The complete data were listed in Table1.

Characteristic of Histopathologic Fibrosis Variable

Masson's Trichrome staining of kidney tissue biopsy and microscope light, 200 fold magnification. Cheratin and muscle fiber (red color), collagen and bone (blue color or green), cytoplasmic Cell (red bright or pink) and nuclei cell (dark brown or black) as can be seen in Figure 1.

Characteristic Variables of Case and Control Study

All data were analyzed for their normality using Kolmogorov-Smirnov Test. It was observed that, the only caspase-3 activity data of case and levels of TNF-α for control were normally distributed (p = 0.094) and (p = 0.134). Mean of caspase-3 activity, TNF-α and TGF-β for case were 0.60 ± 0.50pmol/minute, 948.87 ± 1108.06 pg/ml and4879.37 ± 6101.61 pg/ml, respectively.

Table1
Characteristic of Match Paired Case-Control Study

Characteristic	Case (n=41)	control (n = 41
Age, Year (Mean ± SD)	51 ± 11	50 ± 12
Sexes		
Female (n)	14	14
Male (n)	27	27
Grade of Obstruction		
Hydronephrosisgrade-I (n)	10	11
Hydronephrosisgrade-II (n)	19	24
Hydronephrosisgrade-III (n)	12	6
BMI, kg/m^2 (Mean ± SD)	23.26 ± 2.76	23.07 ±3.82
Haemoglobin, mg/dL (Mean ± SD)	11.00 ± 3.83	13.23 ± 2.77
White blood leucocytes (WBC) sel/mL x10^3	9.67 ± 5.73	9.76 ± 5.05
BUN, mg/dL (Mean ± SD)	23.34 ± 13.88	13,59 ± 5.06
Creatinin serum, mg/dL (Mean ± SD)	2.15 ± 1.60	1.11 ± 0.22
eLFG, mL/minute (Mean ± SD)	48.98 ± 26.09	81.15 ± 25.71
Uric acid serum, mg/dL (Mean ± SD)	5.61 ± 2.81	6.23 ± 2.36

SD = standard deviation; n = number of sample; p= Tests of Normality Kolmogorov-Smirnov; BMI= Body Mass Index; eLFG= estimation of Glomerulus Filtration Rate.

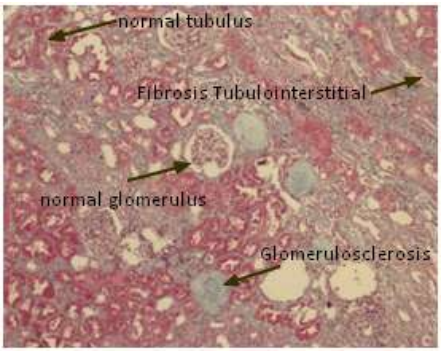

Figure 1
Kidney Fibrosis with Masson's Trichrome Staining

Their 50 persentil (median) for these parameters were 0.49 pmol/minute; 500.10 pg/ml and 1760.21pg/ml, respectively. On the other hand, for control group these values were 0.31± 0.24 pmol/minute; 2226.33±1893.17 pg/ml and 3046.75 ± 4479.62 pg/ml, respectively, with median of each parameter were 0.18 pmol/minute; 1396.30 pg/ml and 323.36 pg/ml. The complete data of caspase-3 activity, levels of TNF-α and TGF-β kidney tissue were listed on Table 2

Table2
Descriptive of Case and Control Study

Variable	K-S	Mean ± SD	Persentil		
	*p**		25	50	75
Case (n=41)					
Caspase-3 pmol/minute	0.094	0.60 ± 0.50	0.17	0.49	0.88
TNF-α pg/ml	0.029	948.87 ± 1108.06	253.41	500.10	1185.54
TGF-β pg/ml	0.036	4879.37 ± 6101.61	308.99	1760.21	7545.20
Control (n=41)					
Caspase-3 pmol/minute	0.015	0.31± 0.24	0.12	0.18	0.54
TNF-α pg/ml	0.134	2226.33 ± 1893.17	743.63	1396.30	3906.41
TGF-β pg/ml	0.001	3046.75 ± 4479.62	199.80	323.36	5275.57

K-S = Kolmogorov-Smirnov Test
*Data were normally distributed $p > 0.05$

Correlation between Caspasae-3, TNF-α and TGF-β to Kidney Fibrosis before and after adjustment with eLFG, Hb and Hydronephrosis

Correlation between caspase-3 activity, levels of TNF-α and TGF-β to Kidney Fibrosisshows that risk of patients with high caspase-3 activity probably have 3.5 fold to gain kidney fibrosis compare to patients with low caspase-3 activity and statistically significant (p=0.001). Probability of patients with high caspase-3 activity to gain kidney fibrosis was 78%.[16] Risk of patients with levels of high levels of TGF-β probably have 2 fold to obtain kidney fibrosis compare to patients with low levels of TGF-β and statistically significant (p=0.042). It can be said that the probability of patients with high level of TGF-β to gain kidney fibrosis was 66.6%.[16] In addition, risk of patients with high levels of TNF-α to gain kidney fibrosis were about 0.5 fold compare to patients with low levels of TNF-α and statistically insignificant (p=0.137).

Resume of correlation between Caspase-3 activity, TNF-α and TGF-β to kidney fibrosis before and after adjustment of eLFG, Hb and hydronephrosis was presented in Table 3. The table reveals that for unadjusted RO of Caspase-3 activity was 3.5 and statistically significant (95% CI = 2.95-4.19; p = 0.001). However, after adjustment RO adjusted obtained was 2.4 and insignificant statistically (95% CI = 0.86-6.90; p = 0.09). Unadjusted RO for TNF-α was 0.60 and insignificant statistically. After adjustment, RO adjusted was 1.1 and insignificant statistically. Furthermore, RO unadjusted for TGF-β was 2.0 and significant statistically (95% CI = 1.72-2.34; p = 0.04). After adjustment, RO adjusted obtained was slightly increase to be 2.1 and insignificant statistically.

DISCUSSION

Bivariate Correlation between caspase-3 and Kidney Fibrosis

Table 3
Resume of correlation between Caspasae-3, TNF-α and TGF-β and Kidney Fibrosis before and after adjustment of eLFG, Hb and Hydronephrosis

Variables	RO Unadjusted	95% CI	*p*	RO Adjusted	95% CI	*p*
High Caspase-3 activity (pg/minute)	3.5	2.95-4.19	0.001	2.4	0.86-6.90	0.09
High levels of TNF-α (pg/ml)	0.6	0.53-0.68	0.14	1.1	0.40-3.16	0.82
High levels of TGF-β (pg/ml)	2.0	1.72-2.34	0.04	2.1	0.74-6.18	0.16

This study indicates that risk factor of high caspase-3 activity towards kidney fibrosis incidence in obstructive kidney stone patients was indicated by its ratio odds (RO) 3.5within 95% CI (2.954 – 4.189) and statistically significant (p = 0.001). Clearly, risk of patients group with high caspase-3 activity has a probability of 3.5 fold to gain kidney fibrosis, patients with high caspase-3 activity probably 78% has a risk to gain kidney fibrosis, and it can be determined its population atributable risk (PAR) was 0.47.[17]This means that around 47 % proporsi of case in total population could be protective to gain kidney fibrosis if the risk factor was eliminated. Therefore, the impact in community was elimination of high caspase-3

activity as a risk factor of kidney fibrosis will protect a number of 47% kidney fibrosis.

Since kidney fibrosis is a prognosis factor of kidney dysfunction. Yang et al.,[15] reported that in their case-control study of kidney rat with sham surgery (control) and subtotal nefrektomi (case), that caspase-3 activity increase in case starting on the day-30 and reach optimum on the day-120 around 2.5 fold of control.Specific characteristic of apoptosis is the present of caspase-8 and 9 activities as initiator, meanwhile, the present of caspase-3 and caspase-6 as executor. All cells were equipped with intrinsic death and life signals, imbalance of these signal leads to apoptosis.[18] Apoptosis initiation in mammal is generally through intrinsic pathway involving mitochondria and extrinsic pathway involving death receptor. The present of activated caspase was as a marker of apoptosis cells.[18] Research by Wu, et.al.[19] Tissue protectif effect of helix B surface peptide (HBSP) on ischemic reperfusi and cyclosporine A (CsA) in kidney injury. HBSP is a derivate of erythropoietin.Research on rat model (control groups, ischemic groups, ischemic reperfusion (IR) groups and 3 treatment groups). 1) Ischemic reperfusion (IR) groups + CsA groups, 2) IR + HBSP groupsand 3) IR + CsA + HBSP groups). Their research obtained was active caspase-3 cells detected by Immunostaining of Myeloperoxidase (MPO) significantly increase on ischemic and IR groups compare to control group. This value increase much more significantly on IR+CsA groups in contrast to group IR+HBSP decrease significantly. Caspase-3 mRNA expression measured using real-time quantitative reverse-transcriptase polymerase chain reaction (qPCR) indicates significantly increase of caspase-3 mRNA on IR+CSA groups compare to ischemic groups, however, decrease significantly on IR+CSA+HBSP groups.

Bivariate Correlation between levels of TNF-α and Kidney Fibrosis

Risk factor of high levels of TNF-α kidney tissue towards kidney fibrosis incident was determined on the basis of ratio odds which was found 0.6 with 95% CI (0.527-0.684). This indicates that risk of patients group with high levels of TNF-α kidney tissue to obtain fibrosis was 0.5 fold even though is not significant statistically (p = 0.137).

TNF-α as an initial trigger of inflammation, induces other cytokines production and other molecule adhesion (ICAM and VCAM). Main source of TNF-α after chronic kidney injury was macrophage and kidney tubular cells.[20] TNF-α is a cytotoxic cytokine, induce apoptosis and inflammation depends on cellular surrounding.[13] The author assumes that chronic obstruction cause tubular cells apoptosis, meanwhile one of the main source of TNF-α on chronic injury or chronic obstruction was tubular kidney cells, therefore, this will leads to lower levels of TNF-α on case than on control. This finding was in line to Morimoto et al., (2008)[21] research. They compared kidney fibrosis on unilateral ureter (UUO) between wild-type rat (control) and rat with TNF-α deficient (case) measured of imunohistochemistry, enzyme-linked immunoassay (ELISA), and real-time polymerase chain reaction (PCR). They obtained that there was no significant difference of kidney fibrosis wide (UUO) in 2 weeks between case and control. However, there was wider increase of kidney fibrosis on case than in control after 4 weeks. Imunohistochemistry, ELISA and real-time PCR analysis show increase of extracellular matrix on case. This was due to increase of expression regulation of TGF-β1 and Snail as a result of increase of macrophage infiltration. Real-timePCR indicates increase of TNF-α tipe-2 receptor expression after 4weeks of UUO that explain the present of different of kidney fibrosis wide between kidney fibrosis of TNF-α deficientrat (case) and wild-type rat (control). In chronic kidney fibrosis, TNF-α depress macrophage infiltration that induced TNF-α tipe-2 receptor expression, therefore, fibrosis improvement on wild type (control) occurred. TNF-α is a pleiotropic cytokine that play an important role in kidney inflammation disease, such as lupus nefritis, anti-neutrophil cytoplasmic antibody (ANCA) and rejection of kidney allograft. TNF-α is also play an important role in immunoregulation needed for maintaining body homeostasis immunity. Complexes biologic function of TNF-α was arranged by two receptors, i.e. TNFR1 and TNFR2. Function of TNFR2 is stimulating leukosit or macrophage infiltration to injury site. On the other hand, TNFR1 plays a role in immunoregulation of lupus nephritis of animal model in which deficient of TNFR1receptor worsen this disease. In human, role of proinflammation and immunoregulalation of TNF-α can be determined through anti-TNF-α therapy in inflammation patients caused by rheumatoid arthritis, apparently was very helpful to reduce the disease symptom. On the other hand, this kind of therapy for curing lupus and multiple sclerosis resulted in the symptom of the diseases increase. This condition leads to anti TNF-α therapy for inflammation kidney disease still debatable. In general, complexes biology therapeutic of TNF-α was not completely understood yet. Further understanding of TNF-α receptors function will help to frame out the dualistic role of proinflammation and immunoregulation of TNF-α. [22,23]

Bivariate Correlation between Levels of TGF-β and Kidney Fibrosis

Risk factor of high levels of TGF-β kidney tissue towards kidney fibrosis in this study was determined by obtaining ratio odds. The ratio odds obtained was 2.0 with 95% CI (1.719-2.340). This indicates that risk of patients with high levels of TGF-β kidney tissue has a risk of 2 fold to gain kidney fibrosis and was found significant statistically (p = 0.042). Probabillity of patients who have high levels of TGF-β kidney tissue to gain kidney fibrosis were 66.6%.[16] It can be said that kidney stone obstructive patients with high levels of TGF-β kidney tissue have a risk of 2 fold to gain kidney fibrosis.

TGF-β is a growth factor that plays an important role in regulate of cells growth, differentiation, inflammation, and improvement of tissue. Quantification of levels of TGF-β in active biology on tissue is very important to picture mechanism involves in varies of physiology and pathology processes. However, direct measurement of bioactive TGF-β on tissue needs an appropriate method. Based on Khan (2012)[24] research levels of total TGF-β in serum between bioassay and enzim-linked immunosorbent assay (ELISA) was not significantly different. However, levels of bioactive TGF-β measured using ELISA was significantly lower than measured by bioassay. Total levels of TGF-β on an organ including cardiac, hearth, and kidney relatively equivalent, however, the levels were slightly higher on cardiac and kidney compare to in liver. For example, levels of bioactive TGF-β and total levels of TGF-β of homogenate kidney rat were 0.84 and 1.36 ng/ml protein, respectively. Levels of total TGF-β measures using ELISA method was 60% higher than its bioactive form. It was believed that increase levels of latent and active TGF-β1 can be used as a marker of the present of fibrosis.[25] During fibrogenesis almost every cell expressed TGF-β1 receptor in which TGF- β1 is very important to activate fibrogenic cells, therefore, TGF-β1 affecting alls fibrosis process. There are three sources of fibrogenic cells, i.e. resident fibroblast activated, fibroblast/fibrosit derivate from bone marrow, and fibroblast produced from epithel to mesenkimal transision (EMT). TGF- β1 was also as one of the most potent chemoattractants for macrophage and mieloid cells. The main sources of TGF-β1ininjured tissue was tissue macrophage and recruited macrophage (infiltrating macrophage). The others cells secreteTGF-β1 are parenchyma cells apoptosis and miofibroblast. Multi function of TGF-β cytokine during fibrogenesis was formation of fibroblast tissue, healing and improvement of parenchyma tissue organ injured.[26] Chronic kidney stone obstructive caused damage of tubular epithelial cells as a trigger of fibrogenic cytokine secretion and recruitment of inflammation cells and activation of renin-angiotensin (RAS). RAS also stimulates inflammation, including cytokine expression, growth factors and reactive oxygen species (ROS). RAS, especially angiotensin-II (Ang-II) induce vascular inflammation, endothelial dysfunction, up-regulation of adhesion molecule and infiltrating cells recruitment, normally macrophage.[27] In normal condition, kidney fibroblast was present inactive form, however, in kidney injury, fibroblast will change to miofibroblast form and proliferated, recruitment of myelo-monocytic cells from bone marrow that produce TGF-β1. Furthermore, TGF-β1 induces collagen-producing cells activation. Collagen expression in kidney fibrosis is around 50% contributes from resident fibroblast, around 35% from epitheel-to mesenchimal transition (EMT) and 14% to 15% from bone marrow cells.[26] Kidney fibrosis reversibility was probably occurredin chronic nefrotoksisity caused by cyclosporin. Termination of chronic nefrotoksisity could be caused by cyclosporin that indicates the improvement of kidney fibrosis. However, whether kidney fibrosis could become a normal kidney architecture was still unsolved and point of no return in kidney fibrosis has not been determined yet. Kidney fibrosis is a main characteristic of chronic kidney disease regardless of initial cause and kidney fibrosis can be determined as a prognosis factor of kidney function. TGF-β plays an important role in kidney fibrosis patogenesis and therapy TGF-β intervention has succeeded and tolerable in animal model. However, this intervention could probably have an adverse affect to induce systemic inflammation due to its dual role pro-fibrosis and strong anti-inflammationof TGF-β.[28]

Multivariate CorrelationCaspase-3, TNF-α and TGF-β to Kidney Fibrosis afterAdjustment of Hydronephrosis, Hemoglobin and eLFG

Odd Ratio (OR) Caspasae-3, TNF-α and TGF-β to kidney fibrosis before adjustment of eLFG, Hb and hydronephrosis respectively, Unadjusted OR Caspase-3 was 3.5 and significant statistically (95% CI = 2.95-4.19; p = 0.001) and adjusted OR was 2.43 and statistically significant (95% CI = 0.86-6.90; p = 0.09). Unadjusted OR TNF-α was 0.6 statistically is insignificant and adjusted OR obtained was 1.1 and was also insignificant statistically. Unadjusted OR for levels of TGF-β was 2.0 and statistically significant (95% CI = 1.72-2.34; p = 0.04). Adjusted ORTGF-β was slightly increased to 2.1, however, is insignificant statistically. OR of Variabel Caspase-3 and TGF-β which were

significant in bivariate analysis becomes insignificant in multivariate analysis. This is probably due to the present of compounding variable. In addition, OR for eGFR obtained higher compare to Caspase-3, TNF-α and TGF-β and significant statistically and also consistent.

Glomerulo filtration rate (GFR) examination has an important diagnostic role in kidney disease. GFR could not be measured directly. Varies methods to estimate GFR has been reported and the best result was obtained by measuring plasma certain compound clearance. The compound should be able to reach stable concentration in plasma and freely filtrate in glomerulus and not secreted, absorbed, synthesized or metabolism by tubular kidney cells.

The most formulae based on creatinine serum applied to estimate GFR on adults was Cockcroft - Gault (CG) and modified diet formulae to study renal disease (MDRD).[13] Regardless of its etiology, number of nefrondecreases during the present of chronic kidney disease and space that initially was placed by glomerulus and tubulus changes with extracellular fibrosis tissue. The remaining nefron increases rate of filtration to maintain its function. Kidney dysfunction appears when remaining nephron is not capable to control extra and continues load. Over time and adaptive mechanism, the damage of remaining nephron will be occurred and lose of permanently kidney function.[29]

Damaging of kidney cells over tolerable value will lead to irreversible kidney function. It seems that tubulointerstitium plays an important role in the development of this incidence. Direct damage and increase cells metabolism or injury stimulation of varies forms caused kidney dysfunction, activated tubular cells and finally interacted to interstitial tissue elements and inflammation cells resulted in pathology changes of kidney parenchyma. Tissue response due to kidney injury will lead to lose of progressive kidney function.[30]

CONCLUSION

Based on result and discussion of the reseach can be concluded:

Obstructive nephropathy kidney stone with high activity of caspase-3 patients have a risk of 3.5 fold to gain kidney fibrosis. Obstructive nephropathy kidney stone with high levels of TGF-β patients have a risk of 2 fold to gain kidney fibrosis. Obstructive nephropathy kidney stone with high levels of TNF-α patient were not as a risk factor to gain kidney fibrosis.

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