CHAPTER IV

RESULTS AND DISCUSSION

Results

The biochemical profile, oxidative stress parameters and N-acetyl-β-D-glucosaminidase activity (NAG) concentration of 203 renal disease patients and 89 healthy control were nondiabetes subjects were parametric statistic and represented in mean ± SD. From the table 2 showed statistic data of renal disease patients and healthy control subjects. We compared the difference of those clinical characteristics in four groups using Post Hoc test. Almost of renal disease patient showed mildly and moderately renal insufficiency while BUN and creatinine levels were expressed as normal levels. The creatinine levels and dialysis of renal disease patient were stratified into three stages. 89 of healthy control were nondiabetes subjects (G1), 79 of renal disease had stage II: creatinine < 6 mg/dl and nondialysis (G2), 51 of renal disease had stage III: creatinine >6 mg/dl and nondialysis (G3), 73 of renal disease had stage IV: creatinine > 6 mg/dl and dialysis (G4). Patient with renal disease were significantly higher in age (53.16 ± 6.44 ; G2, 51.73 ± 7.40 ; G3, 40.49 ± 8.26 ; G4) and healthy control subject (G1) 42.88 ± 8.67, $P<0.001$, waist circumstance (84.43 ± 10.87 ; G2, 86.96 ± 13.83 ; G3, 92.31 ± 17.59 ; G4) and healthy control subject (G1) 75.49 ± 7.37, $P<0.001$, BMI (23.22 ± 4.47 ; G2, 21.09 ± 4.72 ; G3, 23.76 ± 4.13 ; G4) and healthy control subject (G1) 21.67 ± 3.21, $P<0.001$, systolic pressure (133.93 ± 19.96 ; G2, 137.25 ± 22.78 ; G3, 138.21 ± 22.03 ; G4) and healthy control subject (G1) 114.94 ± 12.07, $P<0.001$.

Results of biochemical parameters show table 2 that renal disease patient had significantly increased in WBC (8,379.75 ± 2,806.74 ; G2, 9,964.71 ± 15,062.18 ; G3, 6,963.01 ± 2,337.65 ; G4) and healthy control subject (G1) 6,329.33 ± 1,796.42, $P<0.001$, Glucose (124.43 ± 88.89 ; G2, 105.82 ± 35.45 ; G3, 130.47 ± 53.00 ; G4) and healthy control subject (G1) 83.03 ± 9.00, $P<0.001$, BUN (44.86 ± 19.88 ; G2, 85.10 ± 33.45 ; G3, 71.77 ± 29.51 ; G4) and healthy control subject (G1) 11.24 ± 3.50, $P<0.001$, creatinine (3.14 ± 0.91 ; G2, 8.20 ± 2.96 ; G3, 10.33 ± 3.80 ; G4) and healthy

control subject (G1) 0.74 ± 0.18, P<0.001, uric acid (8.54 ± 2.11 ; G2, 9.00 ± 2.38 ; G3, 7.48 ± 2.08 ; G4) and healthy control subject (G1) 5.18 ± 1.09, P<0.001, triglyceride (202.51 ± 165.17 ; G2, 164.12 ± 90.98 ; G3, 162.40 ± 131.95 ; G4) and healthy control subject (G1) 94.52 ± 34.38, P<0.001, LDL-C (88.64 ± 48.82 ; G2, 92.02 ± 37.64 ; G3, 75.51 ± 36.02 ; G4) and healthy control subject (G1) 104.64 ±21.65, P<0.001, HbA1C (6.26 ± 4.27 ; G2, 6.01 ± 1.22 ; G3, 6.15 ± 1.58 ; G4) and healthy control subject (G1) 5.42 ± 0.75, P<0.001, microalbumin (MB) (30.21 ± 52.23 ; G2, 105.79 ± 91.97 ; G3, 117.74 ± 87.00 ; G4) and healthy control subject (G1) 2.51 ± 8.38, P<0.001, and the renal disease patient had significantly lower in Hb (10.73 ± 1.81 ; G2, 10.95 ± 2.49 ; G3, 10.56 ± 2.19 ; G4) and healthy control subject (G1) 13.39 ± 1.43, P<0.001, Hct (32.81 ± 5.44 ; G2, 33.18 ± 7.30 ; G3, 32.20 ± 6.64 ; G4) and healthy control subject (G1) 40.67 ± 4.01, P<0.001, platelet (228,405.06 ± 71,365.71 ; G2, 229,196.08 ± 87,197.71 ; G3, 190,726.03 ± 65,646.58 ; G4) and healthy control subject (G1) 236,730.34 ± 65,758, P<0.001, HDL-C (42.95 ± 12.41 ; G2, 38.02 ± 13.06 ; G3, 35.49 ± 11.91 ; G4) and healthy control subject (G1) 53.29 ± 11.79, P<0.001, and eCrCl (22.83 ± 9.53 ; G2, 8.05 ± 3.36 ; G3, 7.39 ± 3.17 ; G4) and healthy control subject (G1) 101.59 ± 26.59, P<0.001.

Result of oxidative stress biomarker (lipid hydroperoxide and malondialdehyde), total antioxidant capacity shown table 2 that renal disease patient had significantly increased in oxidative stress biomarkers (lipid hydroperoxide and malondialdehyde) TBARS (10.01 ± 5.01 ; G2, 8.69 ± 3.77 ; G3, 13.33 ± 6.35 ; G4) and healthy control subject (G1) 7.05 ± 4.98, P<0.001, Lipid hydroperoxide; LOOH (9.13 ± 4.43 ; G2, 7.70 ± 3.57 ; G3, 9.39 ± 3.42 ; G4) and healthy control subject (G1) 8.47 ± 2.79, P<0.001. Total antioxidant capacity of renal disease patient had significantly decrease TAC (0.36 ± 0.18 ; G2, 0.34 ± 0.14 ; G3, 0.18 ± 0.71 ; G4) and healthy control subject (G1) 0.43 ± 0.17, P<0.001. Result of tubular damage, urine N-acetyl-β-D-glucosaminidase activity ; NAG shown table 2 that renal disease patient had significantly increased NAG (34.92 ± 18.80 ; G2, 40.74 ± 17.77 ; G3, 43.88 ± 21.92 ; G4) and healthy control subject (G1) 12.52 ± 9.29, P<0.001.

Table 2 Clinical parameters in healthy and renal disease patients

parameter	G1	G2	G3	G4	P Value
	n=89	n=79	n=51	n=73	
Age (year)	42.88 ± 8.67	53.16 ± 6.44*	51.73 ± 7.40**	49.49 ± 8.26***	< 0.001
WC (cm)	75.49 ± 7.37	84.43 ± 10.87*	86.96 ± 13.83**	92.31 ± 17.59***	< 0.001
BMI (kg/m^2)	21.67 ± 3.21	23.22 ± 4.47*	21.09 ± 4.72**	23.76 ± 4.13***	< 0.001
Systolic (mmHg)	114.94 ± 12.07	133.93 ± 19.96*	137.25 ± 22.78**	138.21 ± 22.03***	< 0.001
Diastolic (mmHg)	73.60 ± 9.68	74.39 ± 11.75	79.18 ± 12.85	77.10 ± 13.65	0.029
WBC (10^5 µl)	6,329.33 ± 1,706.12	8,379.75 ± 2,806.74*	9,964.71 ± 15,062.18**	6,963.01 ± 2,337.65***	< 0.001
Hb (g/l)	13.39 ± 1.43	10.73 ± 1.81*	10.95 ± 2.49**	10.56 ± 2.19***	< 0.001
Hct (%)	40.67 ± 4.01	32.81 ± 5.44*	33.18 ± 7.30**	32.20 ± 6.64***	< 0.001
Plt (10^5 µl)	236,730.34± 65,758	228,405.06± 71,365.71*	229,196.08± 87,197.71**	190,726.03± 65,616.58***	< 0.001
Glucose (mg/dl)	83.03 ± 9.00	124.43 ± 88.89*	105.82 ± 35.45**	130.47 ± 53.00***	< 0.001
BUN (mg/dl)	11.24 ± 3.50	44.86 ± 19.88*	85.10 ± 33.45**	71.77 ± 29.51***	< 0.001
Creatinine (mg/dl)	0.74 ± 0.18	3.14 ± 0.91*	8.20 ± 2.96**	10.33 ± 3.80***	< 0.001
Uric acid (mg/dl)	5.18 ± 1.09	8.54 ± 2.11*	9.00 ± 2.38**	7.48 ± 2.08***	< 0.001
Cholesterol (mg/dl)	173.27 ± 18.52	173.14 ± 91.44	167.43 ± 55.98	148.64 ± 51.02	0.036
Triglyceride (mg/dl)	94.52 ± 34.38	202.51 ± 165.17*	164.12 ± 90.98**	162.40 ± 131.95***	< 0.001
HDL-C(mg/dl)	53.29 ± 11.79	42.95 ± 12.41*	38.02 ± 13.06**	35.49 ± 11.91***	< 0.001
LDL-C(mg/dl)	104.64 ± 21.65	88.64 ± 48.82*	92.02 ± 37.64**	75.51 ± 36.02***	< 0.001
HbA1C (%)	5.42 ± 0.75	6.26 ± 1.27*	6.01 ± 1.22**	6.15 ± 1.58***	< 0.001
MB (mg/dl)	2.51 ± 8.28	30.21 ± 52.23*	105.79 ± 91.97**	117.74 ± 87.00***	< 0.001
MBCT	26.40 ± 105.06	404.88 ± 716.47	1,339.38 ± 1,323.03	5,722.66 ± 27,842.94	0.043
eCrCl	101.59 ± 26.59	22.83 ± 9.53*	8.05 ± 3.36**	7.39 ± 3.17***	< 0.001
TBARS (µmol/l)	7.05 ± 4.98	10.01 ± 5.01*	8.69 ± 3.77	13.33 ± 6.35***	< 0.001
LOOH(µmol/l)	8.47 ± 2.79	9.13 ± 4.13	7.70 ± 3.57	9.39 ± 3.42	0.037
TAC (mmol/l)	0.43 ± 0.17	0.36 ± 0.18*	0.34 ± 0.14**	0.18 ± 0.71***	< 0.001
NAG (mol/l)	12.52 ± 9.29	34.92 ± 18.80*	40.74 ± 17.77**	43.88 ± 21.92***	< 0.001

Note: G1 = healthy control, G2 = CT < 6 mg/dl Non hemodialysis , G3 = CT > 6 mg/dl Non hemodialysis, G4 = CT > 6 mg/dl hemodialysis

* = $p < 0.001$ G2 compair G1, ** = $p < 0.001$ G3 compare G1, *** = $p<0.001$ G4 compare G1

WC, waist circumference; BMI, Body mass index; WBC, white blood cell; Hb, hemoglobin; Hct, hematocrit: Plt, platelet; BUN, blood urea nitrogen; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; MB, microalbumin; MBCT, microalbumin cratinine ratio; eCrCl, estimated creatinine clearance; TBARS, thiobarbituric acid reactive substance; LOOH, lipid hydroperoxide; TAC, total antioxidant capacity; NAG, N-acetyl-β-D-glucosaminidase activity.

The correlation among of NAG activity, eCrCl with other parameter of dialysis patient

The NAG activity was positive correlation between creatinine, r = 0.178 (p = 0.018), microalbumin, r = 0.333 (p<0.001), and eCrCl was negative correlaton between BUN, r = -0.554 (p<0.001), creatininie, r = -0.736 (p<0.001), and microalbumin, r = -0.315 (p<0.001, respectively) as shown in Table 3.

Table 3 Correlation among of NAG activity, eCrCl with other parameter of dialysis patients

Correlation between parameters		Correlation coefficient	
		r	*P*- value
NAG activity	creatinine	0.178	0.018
	microalbumin	0.333	<0.001
eCrCl	BUN	-0.554	<0.001
	creatinine	-0.736	<0.001
	microalbumin	-0.315	<0.001

The correlation among of oxidative stress biomarker with other parameter of dialysis patients

The TBARS was positive correlation between creatinine, r = 0.163 (p = 0.020), cholesterol, r = 0.190 (p = 0.007), and triglyceride, r = 0.368 (p<0.001), lipid hydroperoxide (LOOH) was positive correlation between cholesterol, r = 0.144 (p=0.04), triglyceride, r = 0.497 (p<0.001), and HbA1C, r = 0.242 (p<0.001, respectively), as shown in Table 4.

Table 4 Correlation among of oxidative stress biomarker with other parameter of dialysis patient

Correlation between parameters		Correlation coefficient	
		r	*P*- value
TBARS	Creatinine	0.163	0.02
	Cholesterol	0.190	0.007
	Triglyceride	0.368	<0.001
LOOH	Cholesterol	0.144	0.04
	Triglyceride	0.497	<0.001
	HbA1C	0.242	<0.001

The correlation among of oxidative stress biomarker, total antioxidant capacity with NAG activity of dialysis patient

The TBARs was positive correlation between LOOH, r = 0.250 ($p<0.001$), and NAG activity, r = 0.189 (p = 0.012), and negative correlation between eCrCl, r = -0.142 (p = 0.043), total antioxidant capacity, r = -0.065 (p = 0.359, respectively) as shown in Table 5.

Table 5 correlation among of oxidative stress biomarker, total antioxidant capacity with NAG activity of dialysis patient

Correlation between parameters		Correlation coefficient	
		r	*P*- value
TBARS	LOOH	0.250	<0.001
	NAG activity	0.189	0.012
	eCrCl	-0.142	0.043
	Total antioxidant capacity	-0.065	0.359

Discussion

The results show that lipid profiles, BUN, creatinine, glucose, HbA1C, uric acid, and microalbumin of renal disease patients had elevated compare with healthy control. This result showed that patients with renal disease developed dyslipidemia which typical findings are high triglyceride, low HDL-C, and increased LDL-C [36, 57, 58]. The prevalence of abnormalities in the composition of lipoprotein in CRF is higher than in the general population and dyslipidemia in CRF is characterized by reduced concentration of HDL-C and increased concentration of triglyceride and LDL-C [59]. The start of therapy decreases uremic symptoms, but its influence on the uremic dyslipidemia is not clear. Diepeveen et al found no differences in plasma total cholesterol, triglyceride, and LDL-C in CRF compared to healthy control [59], and had elevated glucose [60], HbA1C [21, 23, 61, 62, 63]. Chronic in take of diets with a high glucose or high of fat promotes the development of insulin resistance, the glycated hemoglobin (HbA1C) level, a classical index of glycemic stress [64]. As the result of lipid profiles, glucose levels, HbA1C, body mass index and blood pressure of renal disease were greater than healthy control subject. These result showed that renal disease patients had complication from metabolic syndrome [65, 66] and increase production of free radical. The metabolic syndrome (insulin resistance syndrome) [67] consists of a constellation of metabolic abnormalities that confer increased risk of cardiovascular disease (CVD) and diabetes mellitus (DM). The major features of the metabolic syndrome include central obesity, hypertriglyceridemia, low HDL-C, hyperglycemia, and hypertension [68]. Patient with metabolic syndrome had excessive accumulation of fat in the body or in the abdominal cavity that is the major determinant of insulin resistance [67]. The formation of glycated hemoglobin is nonenzymatic and slow, and has been considered to reflect the degree of glycation of other proteins that are exposed to circulation glucose [64]. Hyperglycemia has been shown to generate superoxide radicals from autooxidantion of glucose, which contrinutes to increase in lipid peroxidation [64]. In general, it is the result of increased oxidative stress in renal disease patients [24, 44]. Therefore, measurement of lipid peroxidation products or the end products of lipid peroxidation, for example lipid hydroperoxide (LOOH) [54], and malondialdehyde [40], is used to estimate damage to lipid [38]. Lipid peroxidation is marker of oxidative stress, which disrupts the

structural integrity of cell membranes. One of the most often used biomarker to investigate the oxidative damage in lipid is the measurement of thiobarbituric acid reactive substances (TBARS), which includes MDA as their major compound [5]. For oxidative stress biomarker, the results showed that renal disease patients had elevated both lipid hydroperoxide and malondialdehyde [69]. The oxidative stress biomarker (LOOH, TBARS) come from lipid peroxidation process [49], especially from damaged of arterial cell wall. Excessive free radical production has been implicated in the hypertension and lipid peroxidation process leaded to pathogenesis of atherosclerosis. There is increasing evidence that elevation of oxidative stress and associated oxidative damages are mediator of vascular injury in various cardiovascular pathologies including atherosclerosis, in our result TBARS levels were higher in HD patients when compared to the healthy control. The extracorporeal dialytic therapies have been suggested to impose an addition oxidative stress to patients on dialysis by complement and leukocytes activation on dialyzer membrane leading to increased generation of reactive oxygen species (ROS) [70]. ABTS assay is presented as method of assessing total antioxidant capacity and is considered to be a useful indicator of the system is ability to regulate damage due to ROS [71]. Several studies suggest that there is enhancement of lipid peroxidation and decrease of antioxidant defense in the course of CRF progression, which could promote oxidative damage in the kidneys [72]. The antioxidation in human serum provides greater protection against free radical attacks than any antioxidant alone [73]. Then determining the total antioxidant capacity of biological fluids can determine the capacity of the system to withstand oxidative stress [74]. This study determined level of total antioxidant capacity in renal disease patients compare with healthy control and results of studies showed level of total antioxidant capacity of renal disease patients was lower than healthy control similar to other studies [56, 73, 74]. Moreover, in dialysis patients, reduced concentration of endogenous antioxidants, e.g., vitamin C, E [75] may be inadequate to counter oxidant activity. Mayer et.al., found that supplemention with antioxidant should be of some benefit for dialysis patients and the necessity for such a supplementation is highest at the beginning of the dialysis session [76]. Chronic kidney disease (CKD) is defined by estimated GFR (eGFR) [77]. Historically, serum urea and creatinine will be elevated and particular help in confirming that this is due to

CKD. Reversible causes of renal impairment should always be considered, and it is critical to identify patients with progressive decline in renal function over only a few months, and whose underlying disease is most likely to be treatable. Formulae predicting glomerular filtration rate and creatinine clearance are also useful but have limited precision. These equations are now being reported routinely by biochemistry laboratories, resulting is increase were recognition of previously undiagnosed renal disease. The N-acetyl-β-D-glucosaminidase (NAG activity), the inflammatory marker is also significantly elevated in renal disease patients in the present study [33]. One possible cause may be from the glomerulosclerosis that results from the influx and accumulation of inflammatory cells (monocytes and macrophages), with mesangial cells responding in the similar manner to vascular smooth muscle cells [12]. Then, renal disease and cardiovascular disease should share similar risk factors. NAG activity is marker widely distributed lysosomal enzyme located in the renal proximal tubules [11]. Our data showed that increase of NAG activity levels [12] were significantly associated with the decline in eCrCl and GFR, and increase with oxidative stress marker [78]

In conclusion, our results demonstrated that oxidative stress markers are significantly increased in dialysis patients. Increased ROS levels are associated with decreased antioxidant levels and consequently with profound impairment of oxidative stress in diabetic renal function. We propose that the oxidative stress alters glomeruli function, in the progression of diabetes with hypertension and induces renal dysfunction, it has reported that antioxidant treatment attenuated the renal dysfunction, suggesting the beneficial effect of antioxidant treatment in renal disease patients [75]. And we propose that a useful early marker of the N-acetyl-β-D-glucosaminidase (NAG) of renal injury associated with the decline in eCrCl and GFR.