Assesment of C- Reactive Proteins and Markers of Oxidative Stress in Patients with Chronic Kidney Failure

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ABSTRACT

Chronic kidney failure (CKF) results from progressive loss of kidney function over a period of time. It is characterized by low glomerular filtration rate and the associated metabolic problems consequently haemodialysis is the mainstay of management of these patients prior to kidney transplant. CKF is associated with enhanced oxidative stress but scarce reports exist on the status of oxidative stress in these patients before haemodialysis. This therefore, formed the basis of this research. Fifty newly diagnosed non-dialyzed chronic kidney failure patients and thirty age and sex-marched healthy individuals participated in this study. Plasma sample was used to determine total antioxidant potential (TAP), total plasma peroxides (TPP) and urinary albumin, creatinine by standard spectrophotometric methods while oxidative stress index (OSI) was a ratio of TAP and TPP. CRP was quantified by single radial immunodiffusion method. Mid stream urine samples were assayed the same day for urinary albumin and creatinine. Statistical analysis was performed using two-tail student's t-test. Correlations between analytes were investigated using Pearson correlation co-efficient. All data were considered significant at p< 0.05. Significant increases were observed in the levels of total antioxidant potential (TAP) urinary albumin, urinary albumin / creatinine ratio in CKF patients compared with the controls. The urinary creatinine among CKF patients was significantly lower. Positive correlations was found between OSI and CRP. Significantly high level of TAP in undialyzed CKF patients ameliorates the oxidative effects of TPP.

Key words: *Chronic kidney disease, oxidative stress index, total plasma peroxides, CRP*

Kronik böbrek yetmezliği olan hastalarda C-reaktif protein ve oksidatif stres belirteçlerinin değerlendirilmesi.

ÖZET

Kronik böbrek yetmezliği (KBY) zaman içinde böbrek fonksiyonlarında ilerleyici kayıp sonucu gelişir. Düşük glomerüler filtrasyon hızı ile karakterizedir ve ek metabolik problemler ve sonuçta hemodiyaliz böbrek transplantasyonu öncesi bu hastaların yönetiminde ana yoldur. KBY artmış oksidatif stresle birliktedir, ancak bu hastalarda hemodiyaliz öncesi oksidatif stres durumuyla ilgili çok az bildiri mevcuttur. Bu nedenle bu durum bu çalışmanın temelini oluşturmuştur. Elli yeni tanılı diyalize girmeyen KBY hastası ve otuz yaş ve cinsiyet uyumlu sağlıklı birey bu çalışmaya katıldı. total antioksidan potansiyeli (TAP), total plazma peroksidaz (TPP) ve idrar albümin, kreatinin standart spektrofotometrik method ile plazma örnekleri kullanılarak değerlendirildi ve oksidatif stres indeksi TAP ve TPP'nin oranı olarak ölçüldü. CRP single radial immündifüzyon metoduyla ölçüldü. Orta akım idrar örnekleri aynı gün idrar albümin ve kreatinin ölçümü için çalışıldı. İstatistiksel analiz two tail studet's test kullanılarak yapıldı. Korelasyonlar Pearson correlation co-efficient kullanılarak araştırıldı. Bütün veriler p<0.05 olduğunda istatistiksel olarak anlamlı kabul edildi. Kontrollerle karşılaştırıldığında KBY hastalarının TAP, idrar albümin, idrar albümin/kreatinin oranlarında belirgin artışlar izlendi. KBY hastaları arasında idrar kreatinini belirgin olarak düşüktü. OSI ve CRP arasında pozitif korelasyon bulundu. Diyalize girmeyen KBY hastalarında belirgin yüksek düzeyde TAP TPP'nin oksidatif etkilerini artırır.

Anahtar kelimeler: *Kronik böbrek hastalığı, oksidatif stres indeksi, total plazma peroksidleri, CRP*

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INTRODUCTION

Chronic kidney failure (CKF) is the 9th leading cause of death in the USA (1). It is commonly associated with the complications of auto-immunity, toxins, toxic chemicals, chronic glomerulonephritis, hypertensive nephrosclerosis and diabetes mellitus (2-4). CKF is formed as a result of progressive loss of kidney function over a period of months or years, and characterized by low glomerular filtration rate (GFR <15ml/min/1.73m^2) and the associated metabolic problems (5). The exact prevalence rate of CKF in Nigeria is not known (3), but hospital based data in Nigeria expressed as ratios of hospital admission showed figures between 1.6 and 8% (6). The possible causes of oxidative stress in chronic kidney diseases include the activation of neutrophils, macrophages, vascular cells, various glomerular cells and complement activation, iron overload, increase of advanced glycation end-points and increased homocysteine (7-9). The removal during dialysis of antioxidant hydro-soluble factors (i.e. urea, vitamin C etc) has been implicated as the cause of oxidative stress in dialyzed CKF patients. High free radical load contributes to the pathogenesis of ischemia reperfusion injury in the kidney (10) and plays a role in a variety of kidney diseases such as glomerulonephritis and tubulointerstitial nephritis that progress to end stage kidney failure (11). Accumulating evidences suggest that chronic kidney failure is associated with enhanced oxidative stress (12). The target of oxidative stress in CKF patients are muscle lipids and proteins, thus contributing to the skeletal muscle disease in the patients. Endothelial dysfunction and atherogenesis are possible consequences of oxidative stress in CKF patients (13). Following kidney injury, glomerular filtration of albumin is increased and the re-absorption and degradation of albumin by tubules are decreased, resulting in increased levels of intact albumin in the urine (14). C-reactive protein (CRP) production is part of the non-specific acute-phase response to most forms of inflammation, infection, and tissue damage (15). It is a marker of inflammation (16) and has emerged as a strong independent risk marker for CKF patients (17). The potential causes of chronic inflammation in CKF patients include reduced renal clearance or increased production of pro-inflammatory cytokines (18), reduced renal clearance of advanced glycation end products (19), the atherelerosclerosis process, chronic heart failure, inflammatory diseases and infections (20). Several evidences available link oxidative stress, cardiovascular diseases and renal damage. Others elucidate the causes of oxidative stress after haemodialysis, but scarce reports exist on the status of oxidative stress in chronic kidney failure patients before haemodialysis. To bridge this gap in knowledge, the present study was designed to determine the plasma levels of total antioxidant potential, total plasma peroxide, oxidative stress index, C-reactive proteins, urea, urinary albumin, urinary creatinine and urinary albumin / creatinine ratio in CKF patients.

MATERIAL AND METHODS

Patients

Fifty patients with chronic kidney failure and thirty age and sex-marched healthy individuals (as controls) participated in this study. The CKF patients were newly diagnosed non-dialyzed patients recruited from the outpatient clinic of the Renal Unit of Medicine Department, University College Hospital, Ibadan while the controls were healthy subjects (unrelated to the patients) from the General Out Patient Department of the hospital for other reasons apart from kidney related problems. Informed consent was obtained from each participant before recruitment in to the study. Participants with cancer, active liver disease or immune complex diseases were excluded. 5ml of venous samples were taken from the anticubital vein of each participant. Plasma was obtained by centrifugation and stored at -70 0C until assay. Mid stream clean catch urine samples were collected in sterile universal bottle and assayed the same day for urinary albumin and creatinine.

Methods

Total antioxidant potential (TAP) was determined using the ferric reducing / antioxidant power (FRAP) assay (21). 1.5 ml of working pre-warmed 37^{o}C FRAP reagent (300mM acetate buffer - pH 3.6, 10mM 2,4,6- tripyridyl-s-triazine in 40mM HCl and 20mM $FeCl_3$ at ratio 10:1:1) was vortex mixed with 50µl of test sample and standards. Absorbance was read at 593 nm against a reagent blank. The result was reported as µmol Trolox equiv. / L. Total plasma peroxide levels was determined from the reaction of ferrous-butylated hydroxytoluene-xylenol orange complex (F0X-2 reagent) with plasma hydrogen peroxide, which yields a colour complex that was measured spectrophotometrically at 560mm. H_2O_2 was used as standard. 1.8ml of F0X-2 reagent was mixed with 200µ1 of plasma. This was incubated at room temperature for 30 minutes. 100µM H_2O_2 was used as stan-

dard. The mixture was centrifuged and the supernatant separated for reading at 560nm (21). OSI, an indicator of the degree of oxidative stress, is the percent ratio of the TPP to the TAP values (2). The urinary albumin concentration was determined by bromocresol green method as previously descibed (22). CRP was quantified by single radial immunodiffusion method (15). A volume of an optimally diluted anti-CRP antiserum was mixed with noble agar and poured on glass plate. Wells of equal diameters were cut in the antibody-agar mixture. The wells were filled with test or standard sera. After incubation, the diameters of precipitin rings were measured using a Hyland viewer with a micrometer eyepiece. Urinary creatinine: was determined by modified Jaffe reaction (23).

Statistical analysis

Statistical analysis of the data was performed by two-tail student's t-test. Correlations between analytes were investigated using Pearson correlation co-efficient. All data were considered significant at $p < 0.05$.

RESULTS

There were 29 males and 21 females in the CKF group, age range 17-75 years, BMI= 37.9±3.9 kgm/m^2, mean systolic BP was 144.3±18.0 mmHg while diastolic BP was 90.8±8.0 mmHg) The controls had 13 males and 17 females, age range, 20-75 and mean age, 40.6±5.4 years. Forty percent (40%) of the patients had diabetic nephropathy while the other either had hypertensive related kidney disease. Table 1 shows significantly higher plasma levels of total antioxidant potential (1292.84±330.75 vs.993.00±242.52), CRP (8.44±4.68 vs. 4.40±0.82, urea 115.70±59.46 vs. 24.55±6.93, in CKF patients compared with the controls ($p<0.05$, 0.000, 0.040, and 0.000) respectively. The increases in the plasma levels of total plasma peroxide and oxidative stress index were not statistically significant in the CKF patients when compared with the controls. The urinary creatinine among CKF patients was significantly lower $p< 0.001$, while the urinary albumin, urinary albumin/creatinine ratio were significantly higher when compared with the controls. ($p= 0.022$, 0.002 respectively) In Table 2, positive correlations was found between OSI and CRP ($r=0.318$, $p=0.025$); TPP and OSI ($r=0.988$, $p=0.000$); TPP and CRP ($r=0.313$, $p=0.027$).However there were no correlations between TPP, OSI and TAP with serum creatinine, urea and urinary albumin.

DISCUSSION

The present study shows significantly higher level of CRP in patients with CKF patients recruited for this study. The significantly higher level of CRP observed in these patients corroborates the reports of Abraham et al, Zebrack et al, Ortegar et al, (16,17,23) who reported significantly higher levels of CRP in CRF patients. Since higher level of CRP is an index of inflammation (24), the observed increase CRP level in our CKF patients demonstrates inflammatory responses in these patients. The inflammatory responses in these patients could be due to increased production of pro-inflammatory cytokines (18), reduced renal clearance of advanced glycation end-products (19), the atherelosclerosis process, chronic heart failure, inflammatory diseases and infections associated with CKF (20). There was a relative increase in the mean level of TPP in our CKF patients, however this did not reach significance when compared with the controls. This high TPP level may be due to significantly higher levels of TAP and plasma urea in CKF patients. Since TAP is an index of all antioxidant molecules in the

***Table 1**. Plasma levels (mean ± SD) of CRP, total antioxidant potential and markers of oxidative stress in patients with chronic renal failure.*

Variable	*CKF Patients (n:50)*	*Control (n:30)*	*t-value*	*p-value*
Total Plasma Peroxide	*12.09±12.31*	*6.23±1.41*	*1.225*	*0.225*
Total antioxidant potential	*1292.84±330.75*	*993.0±242.52*	*3.672*	*<0.01**
Oxidative stress index	*0.87±1.24*	*0.665±0.20*	*0.730*	*0.468*
C-reactive protein	*8.44±4.68*	*4.40±0.82*	*2.280*	*0.040**
Urea	*115.70±59.46*	*24.55±6.93*	*6.808*	*<0.01**

p is significant at < 0.05

***Table 2**. Correlations of total C- reactive protein and markers of oxidative stress in patients with chronic renal failure.*

	r-values	p-values
TPP and OSI	0.988	<0.01*
TPP and CRP	0.313	0.027*
OSI and CRP	0.318	0.025*

**Correlation is significant at < 0.05 level*

plasma, accumulation of urea, uric acid, other antioxidant hydro-solubles and stable vitamins A and E in CKF patients could contribute to the high plasma antioxidant potential (25,26). These antioxidant hydro-solubles and stable vitamins A and E commonly found in chronic renal failure are free radical scavengers that keep the level of TPP at a relative physiological level. The fact that TPP in CKF patients in this study was not significantly higher than in controls could be due to the detoxifying effect of the TAP on the free radicals (TPP). Although the TAP in this study was not significantly elevated, yet it supports the reports of previous authors showing elevated levels of free radicals in CKD patients (27,28). It can therefore be hypothesized that plasma retention of antioxidant hydro-soluble in end stage chronic kidney failure counteracts the radicals generated in the patients. It can also be assumed that the relative increase in the levels of OSI and TPP observed in this study may lead to a significant oxidative stress as the kidney failure progresses. Therefore, further work is needed to determine the levels of oxidative stress biomarkers in various stages/severities of CKD if they are to become useful clinical tools. Significantly higher level of urinary albumin was observed in CKF patients when compared with controls. This observation agrees with the reports of Glassock and Jong et.al (29,30) who associated the increased urinary albumin excretion in CKF with increased permeability of glomeruli to circulating albumin by basement abnormalities or podocyte disorders. The observed low urinary creatinine level in our CKF patients could be due to progressive destruction of nephrons (4) leading to reduced urinary excretion. Also, the significantly higher urinary protein/creatinine ratio could be due to increased urinary loss of albumin in the presence of reduced urinary creatinine excretion resulting from progressive destruction of the nephrons in our CKF patients. Significant positive correlations were observed between TPP and OSI, TPP and CRP, OSI and CRP in CKF patients. Previous reports show that a positive correlation exists between CRP and lipid peroxidation (a marker of oxidative stress) with a negative correlation between alpha-tocopherol and CRP in CKF patients (18). The correlations observed between CRP and markers of oxidative stress in the present study could be due to inflammatory episodes and reduced glomerular function in CKF patients. Sofic et al. (31), reported that serum creatinine and uric acid levels enhance the total antioxidant capacity of the serum.

In conclusion, the results of this study show that the significantly high level of TAP in CKD patients counteract the oxidative effects of TPP. It could therefore be hypothesized from this study that oxidative stress is under control in un-dialyzed CRF patients until the hydro-soluble antioxidants are removed from the system by dialysis.

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