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Original Article

Correlation of oxidative stress and inflammatory markers with the severity of sickle cell nephropathy

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Abstract

Background: Reactive oxygen species have been shown to mediate inflammatory process and may be involved in lipid peroxidation.
Methods: This study evaluates superoxide dismutase, glutathione peroxidase, catalase, malondialdehyde, C- reactive protein and fibrinogen in the serum of patients with sickle cell disease and their correlation with renal insufficiency. Superoxide dismutase, glutathione peroxides and C - reactive protein were assayed using sandwich ELISA technique while malondialdehyde and fibrinogen were determined using thiobarbituric reactive substance and turbidometric technique, respectively.
Results: The study group consisted of 40 patients with sickle cell disease along with macroalbuminuria, 16 with chronic kidney disease and 144 sickle cell disease controls. Superoxide dismutase, glutathione peroxidase and catalase were decreased while malondialdehyde, C-reactive protein and fibrinogen were increased in patients with sickle cell disease along with renal insufficiency. These parameters correlated with the severity of renal disease.
Conclusion: Oxidative stress and inflammatory parameters correlate with sickle cell disease nephropathy.

Keywords: Inflammation, oxidative stress, sickle cell disease nephropathy

Résumé

Arrière-plan: Oxygène réactive espèces ont été montrés pour arbitrer le processus inflammatoire et peuvent être impliquées dans les lipides peroxidation.
Méthodes: Cette étude évalue Superoxyde dismutase, Glutathion peroxydase, catalase, malondialdehyde, C-réactive protéine et fibrinogène dans le sérum de patients atteints de maladie de cellule faucille et leur corrélation avec l'insuffisance rénale.
Superoxyde dismutase, peroxydes glutathion et C - réactive protéine ont été testé à l'aide de sandwich technique ELISA, tandis que malondialdehyde et fibrinogène ont été déterminés à l'aide de thiobarbituric substance réactive et technique turbidometric, respectivement.
Résultats: Groupe l'étude se composait de 40 patients atteints de maladie de cellule faucille avec macroalbuminuria, 16 avec la maladie rénale chronique et contrôles de maladie de cellule faucille 144. Superoxyde dismutase, Glutathion peroxydase et catalase ont diminué tandis que malondialdehyde, C - réactive protéine et fibrinogène ont augmenté chez les patients atteints de maladie de cellule faucille avec insuffisance rénale. Ces paramètres en corrélation avec la gravité de la maladie rénale.
Conclusion: Stress oxydant et paramètres inflammatoire corrélation avec la faucille cellule maladie NEPHROPATHIE.

Mots-clés: L'inflammation, stress oxydant, maladie de cellule faucille NEPHROPATHIE

Introduction

The kidney of patients with sickle cell disease is affected by both hemodynamic changes of chronic anemia and by the consequences of vaso-occlusion

which are especially marked within the renal medulla.[1] As a result, there are many abnormalities in renal structure and function. Functional changes occur in patients with sickle cell disease (SCD) as the patients increase in age.[2] Proteinuria, severe anemia and hematuria were reported to be reliable predictors of chronic renal failure in SCD.[3] Patients with sickle cell disease in steady state have been observed to generate large amount of free radicals,[4,5] hence the end organ damage of SCD may have more complex pathogenetic mechanism than those due to the mere sludging action of sickle cells.[6] Study on free radical participation in glomerular leakage of high molecular weight proteins emphasized the general relationship between free radicals and the induction of microvasculopathy in a variety of tissues.[6]

Reactive oxygen species (ROS) has been reported to mediate inflammatory process and may be involved in oxidative reactions such as lipid peroxidation and protein oxidation.[4,7] They may greatly increase the inflammatory response and may also contribute to tissue damage. To counter the destructive effects of these oxidants, there are endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), which help to detoxify ROS.[8]

There is little or no information on the oxidative stress and inflammatory markers in adult Nigerians with sickle cell nephropathy. The aim of this study, therefore, was to evaluate serum Cu/ZnSOD, CAT, GPX, malondialdehyde (MDA), C- reactive protein (CRP) and fibrinogen in adult Nigerians with sickle cell nephropathy and correlate these oxidative stress and inflammatory parameters with the severity of the disease.

Materials and Methods

Subjects: The protocol used for the study was approved by the ethical committee of Aminu Kano Teaching Hospital. Patients and control subjects gave informed consent before enrollment into the study. Two hundred SCD patients made up of 100 males aged 23.9 ± 5years and 100 females aged 21.8 ± 4.9 years were consecutively recruited. The study group consisted of 44 SCD patients with macroalbuminuria, 16 SCD patients with chronic kidney disease and 144 SCD controls. Twenty HbAA control subjects mean aged 22 ± 2.6 years without renal insufficiency and 20 HbAA subjects mean aged 48.6 ± 15.2 years with chronic kidney disease (CKD) were also evaluated. Sociodemographic data as well as complete physical examination findings were obtained with the help of a structured questionnaire.

Collection and preparation of samples: Random urine sample was obtained for analysis using combi-9 commercial dipstick, which was used to test for biochemical urinalysis.

Ten milliliters (ml) of blood was collected and 3 ml was dispensed into a bottle containing potassium ethylene diamine tetra acetic acid (EDTA) for full blood count estimation, while 2 ml was dispensed into a tube containing 0.2 ml of 3.8% sodium citrate for fibrinogen estimation. The remaining 5 ml of blood was put into a plain tube. The blood was allowed to clot and serum was obtained after centrifugation at 3000 rpm for 5 minutes.

Assays: Total and differential leukocyte count was done using an automated blood cell counter (coulter counter CELL DYE 3700).

Urea and creatinine were evaluated using colorimetric techniques by Randox laboratories, UK. Glomerular filtration rate (GFR) was calculated using Cockcroft-Gault formula.[9] Superoxide dismutase and glutathione peroxidase were assayed in serum using ELISA technique supplied by Northwest life science specialties, Vancouver, Canada while CAT was estimated using a kit by SIGMA, Missouri, USA. C-reactive protein and fibrinogen were evaluated using a kit by Anogen, Ontario, Canada and spectrophotometric rapid techniques[10] respectively.

Statistical analysis: Results are expressed as mean ± SD and were analyzed by Students t-test. Pearson correlation coefficient was calculated to test the strength of association between the parameters and severity of disease. Values of $P<0.05$ were considered as statistically significant.

Results

Oxidative stress markers are shown in Table 1. When the patients with macroalbuminuria was compared with controls, statistically significant increase ($P<0.01$) was observed for MDA, while significant decreases ($P<0.001$) were observed for GPX, Cu/ZnSOD and CAT. On the other hand in SCD patients with CKD, statistically significant increase($P<0.001$) was observed for MDA, while antioxidant enzymes were further decreased ($P<0.001$) when compared with controls.

Table 2 indicates that CRP and fibrinogen were increased ($P<0.001$) in the serum of HbSS macroalbuminuria patients and CKD patients compared with controls. Also, urea and creatinine were increased in the macroalbuminuria patients (NS) and CKD patients ($P<0.001$) when compared

with controls, while the eGFR was decreased in macroalbuminuria patients (NS) and CKD ($P<0.001$) when compared with controls.

Table 3 shows the oxidative stress markers in control HbSS, HbAA and CKD HbAA and CKD HbSS. Oxidative stress markers were significantly different ($P<0.001$) in CKD HbAA compared with control HbAA and in CKD HbSS compared with control HbSS. The mean values of Cu/ZnSOD and CAT in control HbAA were significantly increased compared with control HbSS. The mean ages of CKD HbSS and CKD HbAA subjects were significantly increased ($P<0.001$) compared with their respective controls.

Table 4 shows inflammatory markers in controls HbSS, HbAA and CKD HbSS and CKD HbAA subjects. The mean values of all evaluated inflammatory markers except eGFR were significantly increased in CKD HbSS and CKD HbAA subjects compared with their respective controls, while the mean values of eGFR were significantly decreased in CKD HbSS and CKD HbAA subjects compared with the controls. The mean values of serum urea, creatinine and eGFR were significantly increased ($P<0.001$) in control HbAA subjects compared with control HbSS.

The hematological indices as shown in Table 5 indicate that there was a significant decrease ($P<0.001$) in hemoglobin and absolute lymphocyte levels in macroalbuminuria and CKD patients compared with controls. There was a significant increase ($P<0.001$) in absolute neutrophil in macroalbuminuria and CKD patients compared with controls, and also there was an increase ($P<0.001$) in the level of platelets in CKD patients compared with

Table 1: Oxidative stress markers in serum of SCD patients with macroalbuminuria, CKD and controls (mean ± SD)

Oxidative stress Markers	Controls HbSS	Macroalbuminuria HbSS	*P*-value	CKD HbSS	*P*-value
No of subjects	144	40		16	
Age (years)	21.6±3.2	20.8±4.2	NS	32.6±3.0	*P*<0.001
Malondialdehyde (mmol/l)	2.5±0.4	3.82±1.0	*P*<0.01	5.8±0.4	*P*<0.001
Glutathione Peroxidase (mU/ml)	9.6±0.9	8.3±3.0	*P*<0.001	2.81±0.24	*P*<0.001
Superoxide Dismutase (ng/ml)	32.5±4.2	25.4±4.6	*P*<0.001	18.3±2.8	*P*<0.001
Catalase (μmol/min/ml)	156±5.9	152±1.9	*P*<0.001	148±1.06	*P*<0.001

Table 2: Serum levels of inflammatory markers, urea, creatinine and eGFR in SCD patients with macroalbuminuria, CKD and controls (mean ± SD)

Inflammatory Markers	Controls HbSS	Macroalbuminuria HbSS	*P*-value	CKD HbSS	*P*-value
No of subjects	144	40		16	
C-reactive protein (μg/ml)	1.120±0.02	1.23±0.1	*P*<0.001	1.81±0.05	*P*<0.001
Fibrinogen (mg/dl)	299±9.1	307±6.0	*P*<0.001	317±4.1	*P*<0.001
Urea (mmol/l)	2.6±0.25	3.4±0.2	NS	14.0±2.8	*P*<0.001
Creatinine (μmol/l)	59.2±10.2	63±27	NS	496±78	*P*<0.001
eGFR (ml/min)	103±22	101±2.5	NS	14.5±2.0	*P*<0.001

Table 3: Oxidative stress markers in controls HbSS, HbAA, CKD HbSS and CKD HbAA

Oxidative stress Markers	Controls HbSS	Control Hb AA	CKD HbSS	CKD HbAA
No. of subjects	144	20	16	20
Age (years)	21.6±3.2	22.0±2.6	32.6±3.0*	48.6±15.2*
Malondialdehyde (mmol/l)	2.5±0.4	2.4±0.2	5.8±0.4*	5.03±0.8*
Glutathione Peroxidase (mU/ml)	9.6±0.9	10.3±2.7	2.81±0.24*	4.35±1.8*
Superoxide Dismutase (ng/ml)	32.5±4.2	34.5±1.6*	18.3±2.8*	17.2±12.0*
Catalase (μmol/min/ml)	156±5.9	163±5.8*	148±1.06*	153±3.0*

*$P<0.001$

Table 4: Inflammatory markers in controls HbSS, HbAA, CKD HbSS and CKD HbAA

Inflammatory Markers	Control HbSS	Control HbAA	CKD HbSS	CKD HbAA
No. of subjects	144	20	16	20
C-reactive protein (μg/ml)	1.12±0.02	0.87±0.9	1.81±0.05*	1.48±0.08*
Fibrinogen (mg/dl)	299±9.1	291±29.8	317±4.1*	318±2.0*
Urea (mmol/l)	2.6±0.25	5.10±0.1*	14.0±2.8*	22.5±5.2*
Creatinine (μmol/l)	59.2±10.2	69±2.12*	496±78*	872±58.2*
eGFR(ml/min)	103±22	112±2.5*	14.5±2.0*	16.2±9.8*

*$P<0.001$

Table 5: Hematological indices in SCD patients with macroalbuminuria, CKD and controls

	Control HbSS	Macroalbuminuria HbSS	*P*-value	CKD	*P*-value
No. of subjects	144	40	-	16	-
Age (years)	21.6±3.2	20.8±4.2	-	32.6	$P<0.001$
Hematocrit (%)	20.1±5.9	19.1±3.9	NS	18.7±1.19	NS
Hemoglobin (g/dl)	7.0±2.1	6.25±0.9	$P<0.001$	6.1±0.2	$P<0.001$
Total leukocyte Count ($x10^{9/l}$)	11.7±4.05	11.8±3.2	NS	11.9±1.04	NS
Red blood cells Count ($x10^{12/l}$)	2.43±0.6	2.19±1.0	NS	2.07±0.2	$P<0.001$
Platelet count ($x10^{9/l}$)	373±135	348±92	NS	428±221	$P<0.001$
Mean cell Hemoglobin (pg)	29.6±2.6	30.2±2.3	NS	31.9±6	$P<0.005$
Mean cell volume(fl)	82.2±6.9	84.9±4.2	NS	87±0.9	$P<0.001$
Mean cell hemoglobin Conc. (g/dl)	36.4±2.1	35.7±3.3	NS	36.6±1.5	NS
Absolute lymphocyte Count ($x10^{9/L}$)	4.0±1.3	3.2±0.6	$P<0.001$	2.8±0.4	$P<0.001$
Absolute neutrophil Count ($x10^{9/L}$)	5.2±1.7	6.0±0.8	$P<0.001$	6.4±0.6	$P<0.001$
Absolute Monocyte Count ($x10^{9/L}$)	0.5±0.2	0.4±0.03	$P<0.001$	0.4±0.04	$P<0.001$
Absolute eosinophil Count ($x10^{9/L}$)	0.2±0.1	0.2±0.02	NS	0.2±0.1	NS

controls. Urea and creatinine correlated positively with MDA, CRP and fibrinogen and negatively with Cu/ZnSOD, GPX and CAT ($P<0.001$).

Discussion

The data showed that there were increases in stress and inflammatory markers in SCD patients with renal insufficiency. Studies have shown increased serum levels of acute phase protein and oxidative stress parameters in SCD patients in a steady state[10,11] and sickle cell crisis.[12] Renal insufficiency is also associated with increased levels of acute phase proteins[13] and oxidative stress markers[14] in patients with normal hemoglobin. No information to the best of our knowledge is available on the levels of inflammatory and oxidative stress markers in SCD patients with renal insufficiency in Nigeria. In this study, it was observed that CRP and fibrinogen were increased in subject with renal insufficiency and were associated with increased urea and creatinine levels. The mechanism through which inflammation could lead to decline in renal function is not well understood. Cytokines could act directly on the endothelium and mesangium of the glomerulus.[13] The development of animal model of SCD has provided useful information in understanding the pathophysiology of renal insufficiency in SCD. Studies have shown that kidney in SCD is susceptible to hypoxia because of occlusion of blood flow in the vasa recta which may lead to medullary and papillary necrosis and fibrosis.[15]

The renal medulla favors red cell sickling due to the presence of hypertonic environment and acidosis leading to hypoxic damage.[16] There are evidences which suggest that prolonged glomerular hyperfiltration due to any cause especially SCD could lead to glomerular damage resulting in glomerular sclerosis, proteinuria and progressive renal disease.[17] Proteinuria, as observed in these patients with renal insufficiency, may act in synergy

with oxidative stress and inflammation to initiate, and accelerate the progression of renal disease. Chronic exposure of renal tubular epithelium to high levels of filtered plasma proteins may cause tissue injury. Remuzzi and Bertani[17] suggested that filtered plasma protein taken up by tubular epithelium stimulate inflammatory genes, release inflammatory and vaso-active substances into the renal interstitium that induce scarring and sclerosis. The observed elevated CRP and fibrinogen in this study is consistent with similar investigations.[18,19] Increased levels of CRP and fibrinogen were observed in control SCD patients compared with control HbAA subjects [Table 4]. Studies have shown elevation of CRP in sickle cell disease compared with subjects with normal hemoglobin.[20-22] Many have documented elevation of inflammatory markers in SCD patients,[12] but this study showed solid association of chronic inflammation with a specific organ complication in SCD. Altered pro-inflammatory cytokine levels in the plasma of SCD patients during both steady state and acute vaso-occlusive crisis have been reported,[12,13] but no consistent pattern of cytokines involvement in SCD has emerged that correlates with specific clinical outcomes. However, these cytokines in turn could be responsible for driving the low grade or chronic inflammatory response, evidenced by the presence of mild-moderate baseline elevations of acute phase reactants such as CRP.[21]

We would like to acknowledge that these results could be confounded by large age differences between CKD and other SCD patients studied. Cockcroft-Gault formula was used to estimate glomerular filtration rate in this study. Some workers have recently suggested that Cockcroft-Gault formula is not accurate for lean individuals from Sub-Saharan Africa.[23] This opinion probably requires further investigation.

We observed a decrease in antioxidant enzymes in SCD patients with renal insufficiency. This result is in agreement with that of some studies,[20] but different from others,[24,25] who observed no change in oxidative stress parameters in renal disease patients on hemodialysis. The increase in oxidative stress and markers of inflammation may be due to anemia found more commonly in SCD patients than renal disease patients on hemodialysis. We observed low hemoglobin level and increased platelet level in SCD patients with renal insufficiency compared with controls. This observation is consistent with those of other authors[1] who considered low hemoglobin level as a marker of inflammation. The finding that anemia may directly affect renal function is supported by Silverberg *et al*,[25] who observed that treatment of anemia in heart failure led to improvement in both cardiac and renal functions.[13] Increase in progression of renal insufficiency often results in a decline in erythropoietin secretion, because of the loss of peritubular fibroblasts within the renal cortex that synthesize erythropoietin. This glycoprotein hormone stimulates erythroid progenitor cells within the bone marrow to produce red blood cells. Inappropriate secretions of this hormone in CKD often lead to anemia. Red cell survival may also be reduced under this condition.[26]

The results obtained from this study support the hypothesis that inflammatory and oxidative stress markers contribute to the pathophysiology of glomerulopathy in SCD. Other contributing factor to the pathophysiology of glomerulopathy in SCD is possible iatrogenic acceleration by analgesic medications. Studies have noted that morphine induces mesangial cell proliferation and glomerulopathy *via* kappa-opioid receptors,[27] while others have speculated on nonsteroidal anti-inflammatory drugs (NSAIDs)-induced damage.[28,29]

Conclusion

This study shows increased generation of ROS in SCD patients with renal insufficiency, which is demonstrated by decreased serum activity of antioxidant enzymes. There were also increased markers of inflammation, which correlated significantly with the severity of renal disease. These may increase the mortality and morbidity associated with SCD.

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