CHANGES IN ANTIOXIDANT STATUS ASSOCIATED WITH HAEMODIALYSIS IN CHRONIC KIDNEY DISEASE.

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TITLE: CHANGES IN ANTIOXIDANT STATUS ASSOCIATED WITH HAEMODIALYSIS IN CHRONIC KIDNEY DISEASE.

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ABSTRACT

Oxidative stress has been implicated in the pathogenesis, progression of chronic kidney disease (CKD) and development of cardiovascular complications. Hemodialysis (HD) has been found to contribute significantly to oxidative stress in CKD patients, though reports are conflicting.

OBJECTIVE

We evaluated the effects of one session of HD on the antioxidant capacity and lipid peroxidation in CKD patients.

METHOD

Thirty-six CKD patients requiring HD were recruited into this study. Participants were naïve to HD and each completed a session of three hours using polysulfone membrane dialyzers. Blood samples were collected before and after dialysis. Total antioxidant capacity (TAC) was measured by ferric reducing antioxidant power (FRAP) while malondialdehyde (MDA) was measured using thiobarbituric acid-reactive substance (TBARS). Comparison was made between pre HD and post HD values of TAC and MDA respectively, p value of <0.05 was taken as significant.

RESULT

Mean age and estimated glomerular filtration rate of subjects were 45 ±15 years and 6.3± 4.7mls/1.73m^2 respectively. There was significant decrease in the mean TAC from 1232.2 ± 495.6 µmol Trolox equiv/ to 832.4 ± 325.7 µmol Trolox equiv/L post-dialysis ($p< 0.001$) while MDA values were similar before and after HD (11.8 ± 1.8 vs 11.8 ± 2.331)µmol/L ($p> 0.05$). There was no significant association between changes in antioxidant status following HD with blood flow rate, ultrafiltration volume nor dialyzer per size.

CONCLUSION

A session of HD is associated with significant reduction of the total antioxidants capacity. There may be a need to conduct more studies on the benefit of antioxidant supplements in dialysis patients.

Keywords; oxidative stress, antioxidants capacity, hemodialysis, chronic kidney disease.

INTRODUCTION

Oxidative stress has been described to play an important role in disease progression and development of cardiovascular complications in chronic kidney disease (CKD) patients. CKD is characterized by an imbalance between pro-oxidant and anti-oxidant factors in favor of oxidants which predisposes them to increased risk of cardiovascular disease and increased mortality[1].

Though oxidative stress may be directly associated with renal insufficiency as observed in early stages of CKD, it is most pronounced in patients on dialysis[1]. A number of mechanisms have been postulated as being responsible for oxidative stress in these subjects; certain studies suggested an alteration of the oxidative stress phenomenon, by production of reactive oxygen species on the surface of dialysis membranes and activation of polymorphonuclear leukocytes in patients undergoing hemodialysis (HD)[2,3], another study suggested loss of antioxidant during the course of HD through the dialyzer membranes[4] ,while some studies claim uremia is generally associated with increased oxidative stress[5].

Haemodialysis, the commonest renal replacement therapy in developing countries, has been reported to induce repetitive bouts of oxidative stress primarily through membrane bio-incompatibility[6,7]. While CKD is a pro-oxidant state, HD may contribute significantly to oxidative stress in these patients.

Total antioxidant capacity is described as the sum total of all endogenous and exogenous antioxidant in a medium[8]. It consist of enzymes such as glutathione peroxidase, catalase, superoxide dismutase as well as macromolecules such as cerulosplasmin, uric acid, ferritin and a number of micromolcules such as ascorbic acid, alpha tocopherol and beta carotene. Combination of these components provides a better protection against reactive species than individual antioxidant.

Malondiadehyde (MDA) is an intermediate product of lipid peroxidation which serves as evidence of oxidation of lipids following HD. Elevated levels of MDA has been reported in CKD as evidence of free radical generation[9].

This study was designed to evaluate the effects of one session of HD using the standard polysulfone membrane on antioxidant status of newly-diagnosed hemodialysis- naive CKD patients who required HD.

Methodology

This study was conducted at the Kidney Care Centre, University of Medical Science, Ondo city between April 2015 and March 2016. Inclusion criteria were newly diagnosed adult stage 5 CKD patients who required HD and signed written consent to participate in the study. Exclusion criteria were those on antioxidant supplement and lipid lowering medications.

5ml of venous blood collected just before dialysis (as pre dialysis sample) and 30 minutes after completion of the first session of HD (as post dialysis). Samples were separated and kept at -2^{o}c till analysis. Glomerular filtration rate (GFR) was calculated using the MDRD formula which has been previously validated in Nigerian subjects.

Assay

Serum creatinine and urea were analyzed using standard spectrophotometric methods (using reagents commercially prepared by RANDOX).

Total antioxidant potential was analyzed using commercially prepared reagent using the ferric reducing antioxidant power (FRAP) assay method as described by Benzie and Strain[10]. 50uL of sample was reacted with 1.5 mL freshly constituted working reagent (2, 4, 6-tripyridyl-*s*- triazine and Ferric chloride hexahydrate) at 37^{0}C and result expressed in µmol Trolox equiv/L. MDA was measured using thiobarbituric acid-reactive substance (TBARS) as described by Varshnsey and Kale[11]. 100uL of deproteinized sample was added to 1mL of TBARS reagents and incubated at 100^{0}C for 20 min expressed in umol/L.

Statistical analysis.

Comparison between the mean values of TAC and MDA before and after HD was done using students' t-test. $p < 0.05$ was considered statistically significant in all comparisons.

Result

A total of 36 CKD patients requiring HD participated in this study, 29 were males and 7 were females. The mean age was 45 ±15 years, systolic blood pressure, diastolic blood pressure, packed cell volume, serum urea, creatinine, estimated GFR before HD were 148.9 ± 29.2mmHg, 86.4± 20.0mmHg, 21.8 ± 5.03%, 40.7 ± 19.1 mmol/L, 1420 ± 856.0 µmol/L, 6.3 ± 4.7mL/min/1.73m^2 respectively. Ultrafiltration volume and blood flow rate of the study participants were 1.69 ± 1.59 L and 302 ± 60.9ml/min respectively.

Etiology of CKD among the study subjects were hypertension in 12(33.3%), chronic glomerulonephritis in 11(30.6%), diabetes mellitus in 7(19.4) and others in 6 (16.7%) (Table 1).

There was significant decrease in the mean TAC from 1232.2 ± 495.6 to 832.4 ± 325.7 µmol Trolox equiv/L ($p<0.001$) after a single session of HD. There was no significant change in mean MDA values after a single HD session (11.8 ± 1.8 vs 11.8 ± 2.331 µmol/L($p>0.05$))

Blood flow rate, ultrafiltration volume and blood pressure did not significantly affect changes in MDA and TAC following HD ($p>0.05$) (Table 3 and 4).

Discussion

Antioxidants are important in patients with CKD to retard disease progression, and reduce the risk of premature development of cardiovascular disease (CVD) and death. Total antioxidant capacity is a measure of overall body defence against free radicals, it is made up of enzymatic and non-enzymatic components.

This study showed a significant reduction in the total antioxidant capacity in patients undergoing HD. Oxidative stress (OS) has been implicated in the pathogenesis of cardiovascular death and CKD patients are at increased risk of both OS and cardiovascular death. Incidence of cardiovascular events and death has been reported to increase soon after commencement of HD, however the mechanism responsible is not fully understood[12]. This study further suggests that loss of antioxidants across the semipermeable dialyzer bio-incompatible membrane during the course of HD may play a significant role in increased oxidative stress in patients undergoing HD and may partly contribute to increased mortality. This study is in consonance with observed significant difference in the levels of total antioxidants after HD in previous studies.[4,13] Significant imbalance in the amount of pro-oxidants and antioxidants have been shown to be prevalent among CKD patients, while this has been associated with uremia and production of inflammatory cells such as polymorphonuclear cells (PMNs), loss of antioxidants during dialysis procedure plays a significant role. Oxidative stress in HD patients is associated with extracorporeal treatments[14], repeated contact of blood cells with bio-incompatible membrane causing leukocyte activation and consequent increased formation of ROS, pro-inflammatory cytokine and pro-oxidants[15]. The inflammatory cells triggers increased secretion of myeloperoxidase and nicotinamide adenine dinucleotide phosphate oxidase that will also increase the production of ROS. Leakage of blood antioxidants through the dialysis membrane and higher consumption of antioxidant during HD has been described[16].

In this study, the levels of plasma MDA, which serves as evidence of lipid peroxidation were similar before and after HD. Plasma MDA is an intermediate product of lipid peroxidation and a marker of oxidative stress. The result of our study suggests that HD may not be associated with the removal of pro-oxidants molecules such as MDA in CKD patients. Though elevated levels of MDA has been demonstrated in patients with varying degrees of renal functions[9,17], this study showed that a session of HD did not yield a significant reduction in its plasma levels in CKD patients undergoing first session of HD. This is in

agreement with earlier study[18] who observed a non-significant difference following HD though it's not stated whether participants were incipient or have been on dialysis. In another study by Biasioli *et al*[19], post dialysis sample was collected 30 minutes after completing HD, MDA values were reported to be similar to those pre-dialysis. This study however recruited participants who were on regular HD.

In contrast to some studies that observed significant increase in lipid peroxidation following HD evidenced by elevated MDA level post-dialysis[4,20,21], our study did not show increased lipid peroxidation rather it did show inability of the HD to remove MDA (pro-oxidant molecule). The difference may be associated with duration of dialysis and method of selection of participants. Our study considered incipient dialysis patients and a duration of three hours while most other studies didn't state the duration of HD, some studies also considered patients on regular maintenance HD[11,21]. The difference may also be associated with the possibility of serum MDA value attaining equilibrium after HD as this study sampled post-dialysis 30 minutes after completing HD while others collected immediately after HD. This is similar to finding in a study that sampled post dialysis 30 minutes after completing HD[19] suggesting that time of collection may be associated with the difference.

This study showed that blood flow rate, ultrafiltration volume and blood pressure at the onset of dialysis did not significantly affect changes in TOC and MDA following HD. However, there are no previous reports to compare with our findings.

Our study confirmed significant decrease in total antioxidant capacity associated with HD that has been documented in previous studies and further suggests that the possible mechanism may be associated with loss of soluble components during HD. Possible cumulative effects of this coupled with non-clearance of MDA pose dialysis patients at increased risk of oxidative stress. This may enhance disease progression, cardiovascular complications and increase mortality among these patients. However, the use of vitamin E coated dialyzers has been suggested to reduce oxidative stress and endothelial dysfunction associated with HD[22].

CONCLUSION

This study has further shown that HD is associated with significant alteration in antioxidant status of CKD patients. Even a single session of HD may contribute to OS in CKD patients

through loss of antioxidants across dialyzer bio incompatible membrane. Clinical trials may be necessary to ascertain the probable beneficial effects of antioxidants supplements and antioxidant-coated dialyzers.

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Table1: Characteristics of Study Population.

Parameters	**Mean± Sd / n(%)**	
Age	45 ± 15 years	
Systolic blood pressure	148.9 ± 29.2 mmHg	
Diastolic blood pressure	86.4 ± 20.0 mmHg	
Packed cell volume	21.8 ± 5.03 %	
Serum urea	40.7 ± 19.1 mmol/L	
Serum creatinine	1420 ± 856.0 micromol/L	
Estimated GFR	6.3 ± 4.7 mL/min/1.73m^2	
Ultrafiltration volume	1.69 ± 1.59 L	
Blood flow rate	302 ± 60.9 ml/min	

Table 2: Aetiology of CKD among the participants.

Aetiology of CKD	n(%)
Hypertension	12 (32.4%),
Chronic glomerulonephritis	11 (29.7%)
Diabetes mellitus	7 (18.9)
Others	6 (16.2%).

CKD (Chronic Kidney Disease), GFR (Glomerular Filtration Rate)

Table 3: The effect of a single session of Haemodialysis on MDA and TOC

Parameter	Pre-dialysis	Post-dialysis	p value
TOC	1232.2 ± 495.6	832.4 ± 325.7	< 0.001
MDA	11.8 ± 1.8	11.8 ± 2.331	0.753

Table 4: Association between changes in MDA and ultrafiltration volume, blood flow rate and blood pressure

	p-value	R
Ultrafiltration volume	0.73	-0.07
Blood Flow Rate	0.75	-0.06
Systolic Blood Pressure	0.50	0.12
Diastolic Blood Pressure	0.43	-0.14

Table 5: Association between changes in TOC and ultrafiltration volume, blood flow rate and blood pressure

	p-value	R
Ultrafiltration volume	0.73	-0.04
Blood Flow Rate	0.53	-0.11
Systolic Blood Pressure	0.89	-0.24

Diastolic Blood Pressure	0.23	0.24

Conflicts of interest

We declare there is no conflict of interest.

Ethical consideration.

Ethical approval was obtained before commencement of this research and all participants gave written consent to participate.