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Research Article

Evaluation of the Levels of some Inflammatory Markers in Patients Undergoing Haemodialysis Management

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Abstract
The rising incidence of kidney diseases and concern for management outcomes utilizing Haemodialysis was a driving force for this research. We evaluated the levels of some inflammatory markers. Utilizing biochemical methods specifically spectrophotometric and immunoassays. We determined the levels of oxidative stress markets Total Antioxidant Capacity, Malondialydehyde, Sodium Dismutase, Glutathione Peroxidase, Human Interleukins 6 (lL-6) along with immunoglobulin G and M and Troponin from 150 samples. The samples consist of a cohort of 50 Normal control, 50 pre-dialysis and 50 post-dialysis subjects. The result obtained were analyzed using descriptive statistics, one-way analysis of variance and post hoc Tukey HSD test via IBM SPSS version 27, with significance set at $P < 0.05$. Result showed a pattern in which among the oxidative stress marker parameters SOD was significantly increased. Troponin was significantly raised in dialysis patients when compared to controls. The result also shows a reduction in the concentration of IGg and IGm buttressing immunocompromised state. The need for consistent monitoring and evaluation has been elucidated by these results.

Keywords: Inflammatory, Marker, Renal Failure, Haemodialysis, Management

Introduction
The kidney plays a general and specific function which includes homeostasis, acid-based balance, secretion; excretion, regulation of osmolality and blood pressure regulation. The production of hormones such as erythropoietin and prostaglandins are also within the ambits of its function. A dysfunctional state of the kidney elicits several complications that result from mild, chronic to end-stage kidney failure. Kidney diseases are rampant with increasing morbidity and mortality.

Haemodialysis is the process of cleaning blood outside the body which involves taking blood from blood vessels and conveying it through a synthetic filter called a dialyzer. It is then cleaned and returned to the body. The dialyzer is considered as an artificial kidney. The process is under the control of dialysis machine that pumps the blood around a circuit, adds an anticoagulant and modulates the cleaning process. Haemodialysis is one of the renal replacement therapies [1]. The application of this techniques plays a vital role in the process for the extracorporeal removal of waste products such as urea, creatinine and other nitrogenous products and free water from the blood when the kidneys are impaired.

The underlying principle behind haemodialysis is the diffusion of solutes through a semipermeable membrane. The kidney as an important organ receives one quarter of the total blood flow. However, in the state of deranged function the kidney becomes a target of persistent chronic inflammation due to impaired antioxidant and anti-inflammatory defenses and detoxification. Arising from this is an increase of inflammatory factor production and impaired proximal tubular clearance, blood concentrations of acute phase protein (CRP and fibrinogen) and pro-inflammatory cytokines (lL-1β, lL-6 and TNF-α) increase progressively as renal function depreciates [2].

It has earlier been noted that long-term maintenance haemodialysis reduces blood levels of inflammatory factors (including lL-2, lL-6) and high sensitivity C-Reactive protein (hs-CRP) in patients with renal failure. Inflammation is still the dominant factor for cardiac insufficiency that occurs at the onset of dialysis [3]. Chronic renal failure is accompanied by oxidative stress (Himmelfarband) Hakim; 2003; Galle, 2001) which consist in the damage of biological structure by reactive oxygen species due to their excessive generation of an impaired efficiency of antioxidant defense mechanisms [4].

Kidney problem have unique impact on individuals and society. They can lead to decreased kidney function, result in effects like fluid retention, electrolyte imbalances, and cardiovascular disease. Moreover, ongoing management of renal disease such as dialysis or kidney transplantation, can impose a reasonable financial burden on the health care.

According to the World Health Organization (WHO) renal disease account for 4.5% of all disability adjusted life. End-stage Kidney Disease (ESKD) which is the last stage of chronic kidney disease is now rampant. When the glomerulus is unable to function properly with a glomerular filtration rate of less than 15 millimeters per minute, there is a major health challenge. In the work, we evaluated levels of some inflammatory markers in patients undergoing haemodialysis management.

MATERIAL AND METHOD
This research work was carried out in Port Harcourt, Rivers State of Nigeria. A total of 150 samples were used for the work consisting of 50 sam-

ples from apparently healthy subjects which constituted the control, 50 samples from pre-dialysis cases and 50 samples from post-dialysis cases. Both males and females were included in all groups after obtaining their consent.

Parameters evaluated were Total Antioxidants capacity, Superoxide Dismutase, Glutathione Peroxidase, Malondialydehyde (MDA) and Human Interleukin 6 (lL-6).

Total Antioxidant Capacity was determined spectrophotometrically with chromogenic reagent and measured at 450mm.

Superoxide Dismutase (SOD) was determined using auto-oxidation method.

Malondialdehyde (MDA) was determined colometrically by using the method of.

The method of Rotruck et al., 1973 was utilized in the determination of Glutathione Peroxidase.

Human Interleukin (lL-6) was assayed using ELISA, K-Assay, a product of Kamiya Biochemical Company. The method using serum employs the quantitative sandwich enzyme immunoassay technique where antibody specific for lL-6 has been pre-coated onto a microplate. Calibrators and samples were pipetted into the wells and any lL-6 present is bound by the immobilized antibody. After removing any unbound substance, a biotin-conjugated antibody specific for lL-6 is added to the wells. Final measurement is taken at 450mm.

RESULTS

The outcome of results of parameters analyzed after subjecting them to statistical analysis are shown in Tables below.

Table 1 presents the comparison of Mean and Standard deviation of the oxidative stress markers Tachykinin 2 (TAC 2), Malondialdehyde (MDA), Superoxide dismutase (SODs) and glutathione peroxidase (GPx) among the study participants using one-way Analysis of Variance (ANOVA) and Post Hoc Tukey HSD tests.

Table 1: Comparison of the Mean and Standard deviation of oxidative stress markers among the study subjects

Groups	N	TAC (iu/L) Mean ± SD	MDA (iu/L) Mean ± SD	SOD (ui/L) Mean ± SD	GPx (iu/L) Mean ± SD	lL-6 (pg/ml) Mean ± SD
Control (c)	50	55.28 ± 17.09	2.93 ± 1.49	53.14 ± 10.33	375.49 ± 12.23	35.16 ± 8.67
Test A	50	70.10 ± 46.03	3.46 ± 1.76	72.19 ± 22.30	375.49 ± 190.12	40.53 ± 31.03
Test B	50	62.23 ± 46.03	3.46 ± 1.76	76.69 ± 23.61	316.50 ± 244.08	79.51 ± 65.48
F-Value		1.82	2.17	20.15	1.06	19.55
P-Value		0.17	0.12	< 0.01	0.35	< 0.01
Remarks		NSS	NSS	SS	NSS	SS
Post Hoc						
T_1 VS T_2		NSS (0.14)	NSS (0.29)	SS (<0.01)	NSS (0.96)	NSS (0.81)
T_1 VS T_2		NSS (0.65)	NSS (0.12)	SS (<0.01)	NSS (.0.35)	SS (<0.01)
T_2 VS T_3		NSS (0.57)	NSS (0.89)	NSS (0.49)	NSS (0.51)	SS (<0.01)

P < 0.05 = Statistically significant
C = Control (Normal subject without CKD (T_1)
T_2 = Test A (CKD subjects before dialysis) (T_2)
T_3 = Test B (CKD subjects after dialysis) (T_3)
SS = Statistically Significant
NSS = Not Statistically Significant
TAC 2 = Tachykinin 2, MDA = Malondialdehyde, SOD_3 = Superoxide
GPx = Glutathione Peroxide, lL-6 = Interleukin 6

Table 2: Comparison of the Mean and Standard Deviation of Immunoglobulin and Troponin among the Study Subjects

Groups	N	IgG (g/L) Mean ± SD	IgM (g/L) Mean ± SD	Troponin (mg/L) Mean ± SD
Control (C)	50	873.66 ± 100.33	258.79 ± 38.47	0.56 ± 0.03
Test A	50	890.79 ± 114.35	320.02 ± 55.20	1.06 ± 0.57
Test B	50	920.42 ± 103.73	330.06 ± 53.00	1.21 ± 073
F-Value		2.45	30.31	7.22
P-Value		0.09	< 0.01	< 0.01
Remark				
T_1 V5 T_2		NSS (0.70)	SS (< 0.01)	SS (0.01)
T_1 V5 T_2		NSS (0.08)	SS (< 0.01)	SS (< 0.01)
T_2 V5 T_3		NSS (0.35)	NSS (0.57)	NSS (0.65)

$P < 0.05$	=	Statistically Significant
T_1 (CKD)	=	Control (C) Normal subjects without
T_2	=	Test A (CKD subjects before dialysis)
T_3	=	Test B (CKD subjects after dialysis)
IgG	=	Immunoglobulin G
IgM	=	Immunoglobulin M
Trn-1	=	Troponin 2
SS	=	Statistically Significant
NSS	=	Not Statistically Significant

Using Post Hoc Tukey Honest Significant Difference (HSD) test, statistically significant increased level of Troponin 1 was observed in test A and test B groups when compared with control group whereas there was no statistically significant difference between the two test groups (see Table 2). Comparison of the values obtained among the oxidative stress markers shows that the Mean + Standard deviation of SOD_3 for control group was 53.14 + 10.33, that for test group A was 72.19 + 22.30 while that for Test Group B was 76.69 + 23.61 with F=20.15 and P-Value < 0.01, there was statistically significant increased level of SOD_3 in the Test A and Test B groups when compared with the control group (see Table 1).

DISCUSSION

This study evaluated the levels of inflammatory biomarkers in subjects with chronic kidney disease who are being managed by haemodialysis and normal subjects without the disease.

The age distribution of participants ranged from 30 to 70 years with most of them falling within 50-59 age brackets.

Our observation in this work shows that Malondialdehyde (MDA) which serves as an indicator of lipid peroxidation did not exhibit a statistically significant difference across the various groups that were evaluated. This is indicative of the fact that the level of oxidative stress as measured by MDA may not exhibit significant variation between patients undergoing dialysis and healthy control subjects. This does not support earlier findings of [5], who had earlier documented a significant increase in MDA levels among haemodialysis patients.

Tachykinin 2 (TAC 2) identified as antioxidant enzyme demonstrated no statistical significance when compared with the control group with the pre-dialysis and post-dialysis suggestive of the fact that TAC 2 may not exert influence on dialysis as they are mainly linked to neurogenic inflammation and the modulation of pain [6]. There is currently a dearth of literature allowing for direct comparison and this elicits a gap.

There was marked elevation of SOD_3 in both patients in pre and post phases of haemodialysis. Our findings here negate earlier report of [7], that earlier documented a reduction in superoxide dismutase (SOD). The findings here were also at variance in the case of Glutathione Peroxidase (GPx) as reported by [8], which documented a notable reduction in GPx levels in haemodialysis patients when compared to control subjects.

Our findings were in agreement with the studies carried out by [9], on Troponin 1. The elevation of Troponin 1 levels observed in patients with chronic kidney disease (CKD) an be due to persistent cardiovascular stress, myocardial injury and subclinical cardiac ischaemia that are frequently linked to CKD. However, the work of [10], was at variance with our findings in this current research.

The levels of Interleukin-6 (lL-6) were observed to be significantly elevated in the post dialysis phase among patients with renal failure when compared with control group and pre-dialysis phase. No notable distinction was illustrated between the control group and the pre-dialysis cohort. The elevation of lL-6 following dialysis may be due to the activation of immune cells induced by bio-incompatible dialysis membranes alongside the presence of uremic toxins that exacerbate the release of pro-inflammatory cytokines. Our findings agree with those of [11], who documented elevated lL-6 in patients on haemodialysis.

We documented results on immunoglobulins that reflect variation in pre and post dialysis subjects in values higher than control. This finding is in concordance with those of [12-15].

CONCLUSION

Elevated troponin 1 in both pre and post-dialysis groups is indicative of ongoing myocardial stress underscoring the cardiovascular risk in CKD. The need for continuous monitoring of the kidney, immunologic, oxidative stress and cardiac markers are suggested for effective management.

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