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Role of Oxidative Stress in Glycated Hemoglobin among Chronic Kidney Disease (CKD) Patients in Sokoto

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Abstract:***Background:****Diabetes is one of the major risk factors of CKD and if not properly controlled, may hasten the progression of CKD to attain end stage renal disease (ESRD). As such the maintenance of glucose homeostasis is crucial in preventing pathological consequences that may result from hyperglycemia. Oxidative stress is known to play a crucial role in the pathogenesis of diabetes and its long term complications. This study examined the role of oxidative stress on the glycemic control of CKD patients in Sokoto.*

Methods:*Sixty seven CKD patients (age 47.17 ± 1.77) were studied. The CKD patients were grouped into stages based on their estimated glomerular filtration rate (eGFR) from plasma creatinine using the MDRD 4-variable equation. Plasma glucose and glycated hemoglobin (HbA1c) levels were determined as markers of glycemic control. Plasma levels of malondialdehyde (MDA) and antioxidant enzymes were determined as markers of oxidative stress.*

Results: *The glucose level decreased while the HbA1c significantly ($p< 0.05$) increased from stage 1 to 5 of the CKD. The levels of malondialdehyde significantly ($p<0.05$) increased as CKD advances, while the total antioxidant status (TAS), superoxide dismutase, catalase and glutathione peroxidase significantly ($p < 0.05$) decreased.Asignificant ($p <0.05$) positive correlation exist between MDA and HbA1c, along with a significant ($p < 0.05$) negative correlation between TAS and HbA1c.*

Conclusion:*The results of this study stressed the importance of glycated hemoglobin (HbA1c) as a better marker of glycemic control than glucose in CKD patients. Glycation of hemoglobin could be increased by the presence of oxidative stress in the CKD patients, even with a normal glycemic condition, thereby deteriorating their condition to ESRD.*

Keywords: *Chronic Kidney Disease, Diabetes, Glycated Hemoglobin, Oxidative stress, Malondialdehyde.*

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I. Introduction

Oxidative stress is a state of imbalance between excessive oxidant formation and lack of antioxidants as a defense mechanism (Balasubramanian, 2013; Kuo, 2010). Normally, reactive oxygen species (ROS) are countered by endogenous natural defenses known as antioxidants, and it is the imbalance between ROS and antioxidants which favours the increased accumulation of ROS that gives rise to oxidative stress. When ROS are made in excess, they react with various molecules such as lipids, carbohydrates, proteins and DNA, altering their structure and function (Cibulka and Racek, 2007; Klaunig *et al*., 1998).

Glycated hemoglobin (HbA1c) is a measure of an average endogenous exposure to glucose over a period of 2-3 months. Normally, when blood plasma glucose is elevated, there is non-enzymatic glycation of hemoglobin which increases as the glucose level increases. This alteration reflects the glycemic history over the previous 2-3 months, since the erythrocytes have an average life span of 120 days (Selvin *et al*., 2007: Braunwald *et al*., 2001; Murray *et al*., 2000).As such, glycated hemoglobin is used for the estimation of glucose control in subjects with diabetes which is a major risk factor of CKD.

CKD patients with known diabetes require rigorous glucose control to prevent end stage renal disease (ESRD). However, diabetes management in CKD population entails careful considerations due to the decrease in renal mass (Synder and Bernds, 2004).With CKD, there is decreased clearance of insulin from circulation, leading to prolonged circulating insulin and decreased insulin requirement in the CKD patients.

This study therefore aims to determine the role of oxidative stress in glycated hemoglobin levels among CKD patients.

II. Materials And Method

This study group consisted of 67 patients attending Nephrology Units of Usmanu Danfodiyo University Teaching Hospital (UDUTH) and Specialist Hospital, Sokoto. The consent of the patients were obtained and questionnaires administered as approved by the Ethical Committees of Usmanu Danfodiyo University Teaching Hospital Sokoto and Specialist Hospital Sokoto.

Blood samples were drawn from all the participants into vacutainer tubes with lithium heparin as the anticoagulant. The creatinine, glucose, glycated hemoglobin, malondialdehyde, total antioxidant status, superoxide dismutase, catalase and glutathione peroxidase were measured using kits according to the manufacturers' instructions.

III. Statistical Analysis

The results were expressed as Means ± SEM. Student's t-test was used to compare the healthy control and the CKD subjects. ANOVA was used to compare between the different stages and with the control with Dunnet post test. Pearson's correlation was used to determine the relations between the different variables. $P<0.05$ was considered as statistically significant. Statistics was by Graphpad instat3 version 3.02, USA.

IV. Results

Table 1 gives the characteristics of the CKD patients recruited in this study. The overall mean age of the CKD patients was not statistically different ($p>0.05$) from the healthy control subjects. The result also indicated significant increase ($p<0.05$) in creatinine level of the CKD patients and significant decrease in eGFR of the CKD subjects when compared to the healthy control. There was a significant increase ($p<0.05$) in glycated hemoglobin of the CKD subjects as compared to the healthy control subjects.

Table 1: Clinical Characteristics of CKD Patients in Sokoto

	CKD PATIENTS (n=67)	CONTROL (n=15)
Age (yrs)	47.47 ± 1.77	48.13 ± 5.26
Sex (M:F)	36 : 31	7 : 8
Creatinine (mg/dl)	3.55 ± 0.49*	0.79 ± 0.05
eGFR (ml/min/1.73m^2)	49.97 ± 4.69*	117.53 ± 7.99
Glucose (mmol/l)	5.61 ± 0.27*	4.53 ± 0.14
HbA1c (ng/ml)	45.32 ± 4.07*	25.72 ± 5.00

Values are mean ± standard error of means

The results of the plasma creatinine and eGFR of the CKD patients, at various stages of the disease are shown in Fig 1. There was steady increase ($p < 0.05$) in the mean concentrations of creatinine and consequent decrease ($p < 0.05$) in eGFR from stage 1 to stage 5 of the CKD patients as compared to the apparently healthy control subjects.

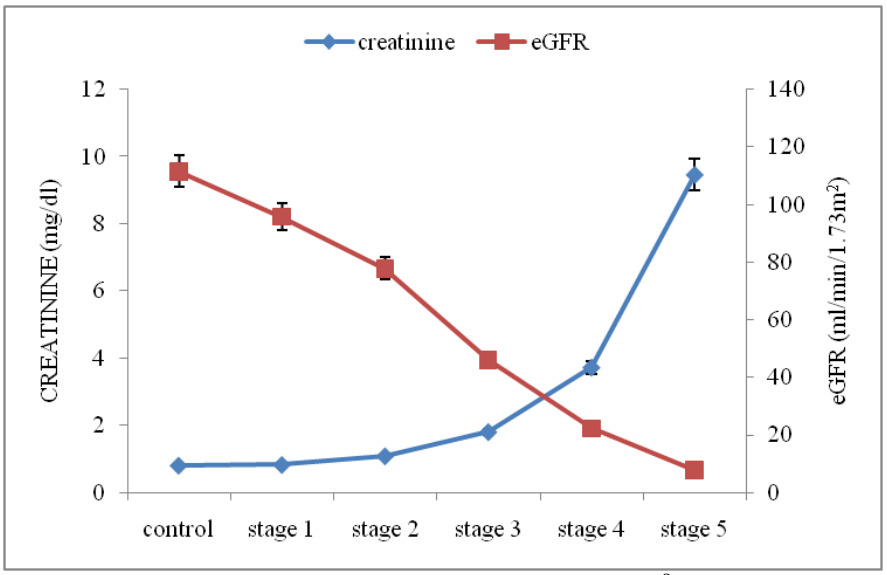

Fig 1: Plasma Levels of Creatinine (mg/ml) and eGFR (ml/min/1.73m^2) at Various Stages of CKD.

The results of the plasma malondialdehyde and antioxidant profiles of the CKD patients in Sokoto are presented in Table 2. The results indicated a significant ($p<0.05$) increase in the levels of malondialdehyde in the CKD subjects compared to apparently healthy control subjects. The patients had significantly ($p<0.05$) lower levels of total antioxidant status and glutathione peroxidase activity in comparison to apparently healthy control subjects.

Table 2: Plasma levels of malondialdehyde and antioxidant markers of CKD patients and apparently healthy control subjects in sokoto

	CKD PATIENTS (n=67)	CONTROL (n =15)	P-VALUE
Malondialdehyde (nmol/ml)	0.81 ± 0.04	0.52 ± 0.03	0.0001
Total Antioxidant Status (mmol/l)	6.11 ± 0.31	9.55 ± 0.93	0.0027
Superoxide Dismutase (U/ml)	142.74 ± 6.58	176.33 ± 20.77	0.1427
Catalase (U/ml)	2.47 ± 0.29	5.37 ± 1.57	0.0908
Glutathione Peroxidase (U/dl)	51.03 ± 2.06	66.68 ± 4.21	0.0031

Values are mean ± standard error of means

The result of the plasma levels of malondialdehyde of the CKD patients at various stages of the disease is presented in Fig 2. The result indicated progressive increase in the plasma levels of malondialdehyde at various stages of the disease.

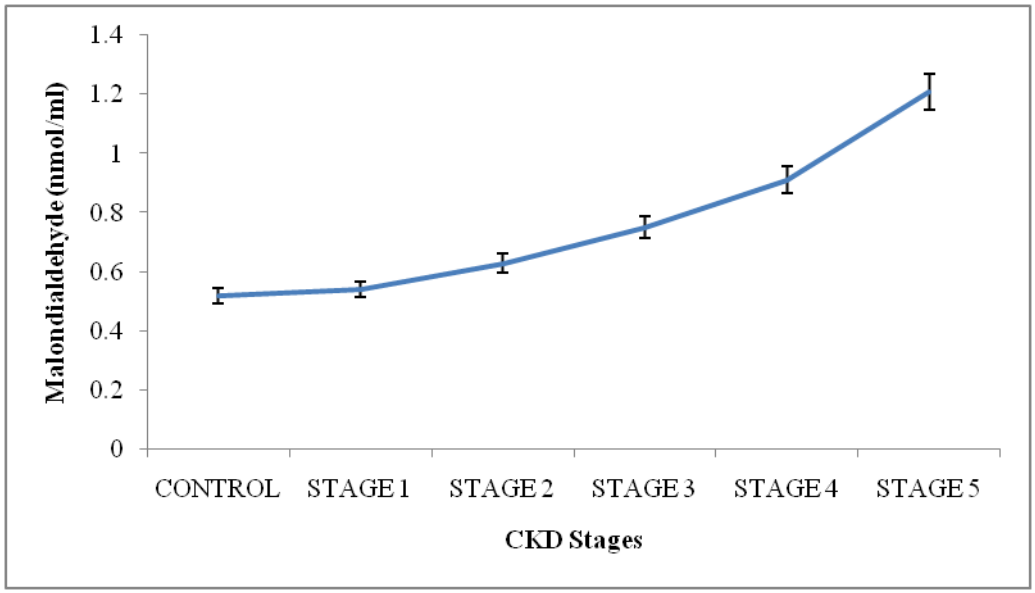

Fig 2: Plasma Level of Malondialdehyde from stage 1 to stage 5 of CKD

The results of the plasma antioxidant profile of the CKD patients at different stages of the disease are presented in Fig 3. There was steady decrease in all the antioxidant enzymes at different stages of the CKD when compared to the healthy control subjects. The patients at the advanced stages (stages 4 and 5) had significant ($p<0.05$) decreases in the concentrations of plasma total antioxidant status and glutathione peroxidase when compared to the apparently healthy controls.

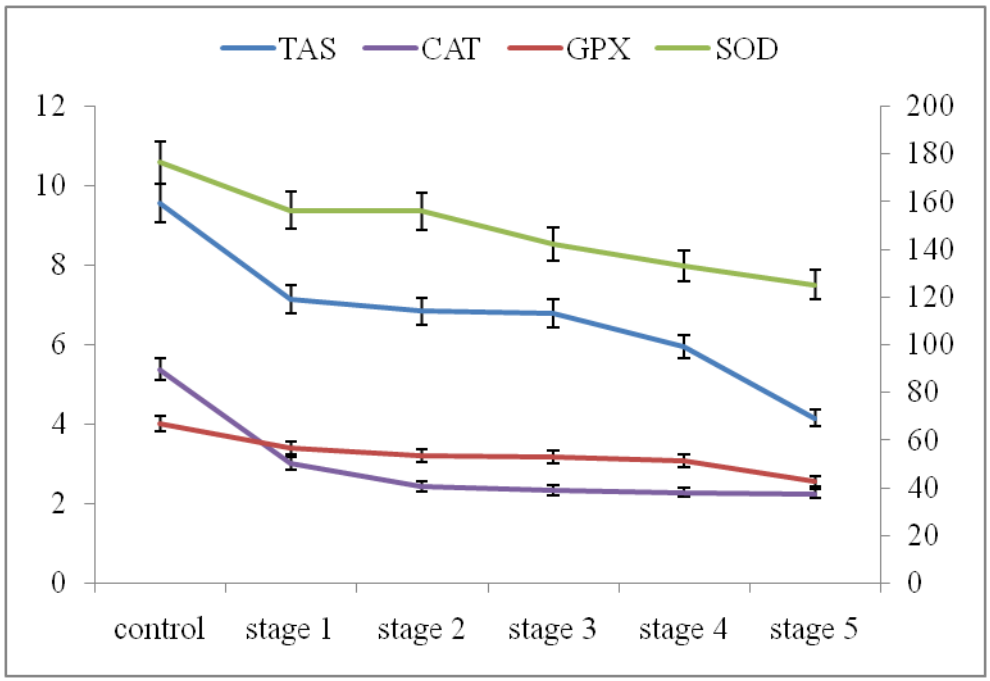

Fig 3: Plasma Levels of Antioxidants Biomarkers at Various Stages of CKD

TAS = Total Antioxidant Status. SOD = Superoxide Dismutase. CAT = Catalase. GPx = Glutathione Peroxidase.

The results of the plasma glucose and glycated hemoglobin at various stages of CKD are presented in Fig 4, the glucose levels slightly decreased ($p>0.05$) from stage 1 to a normal glucose level at stage 5. However, the HbA1c increased significantly ($p<0.05$) as CKD advances, confirming poor glycemic control.

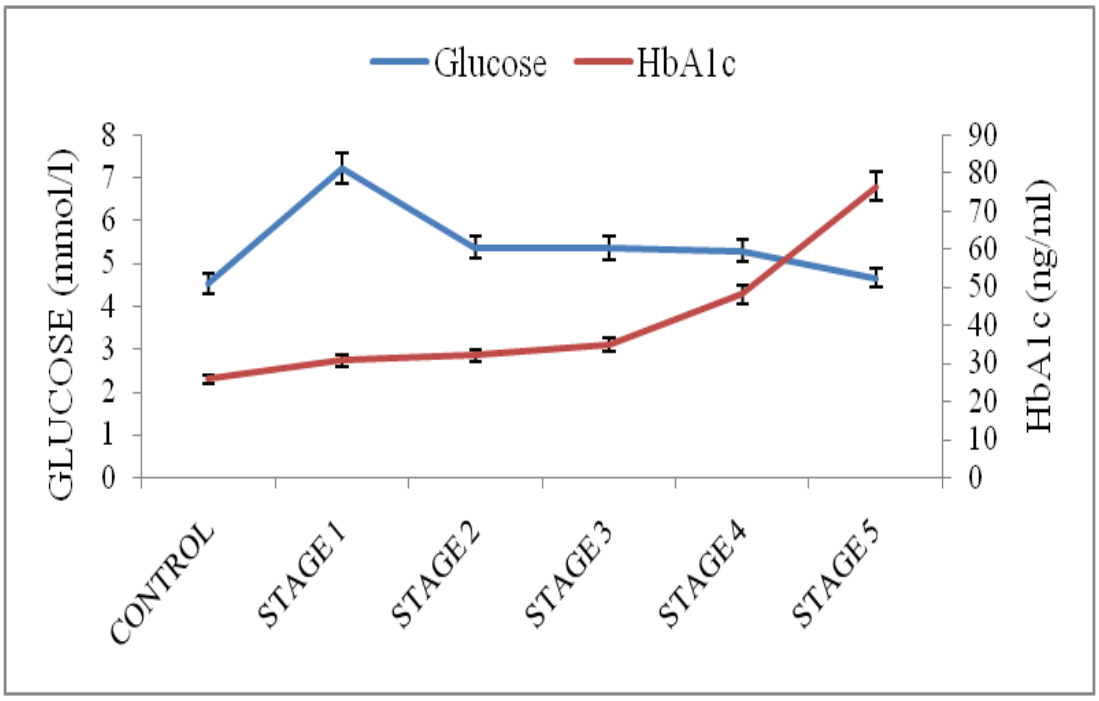

Fig 4: Plasma Levels of Glucose (mmol/l) and HbA1c (ng/ml) at Various Stages of CKD.

The correlation between HbA1c and MDA shows a significant (p = 0.0001) positive correlation coefficient as shown in Fig 5. In addition, the correlation between TAS and HbA1c shows a significant (p < 0.05) correlation coefficient as shown in Fig 6.

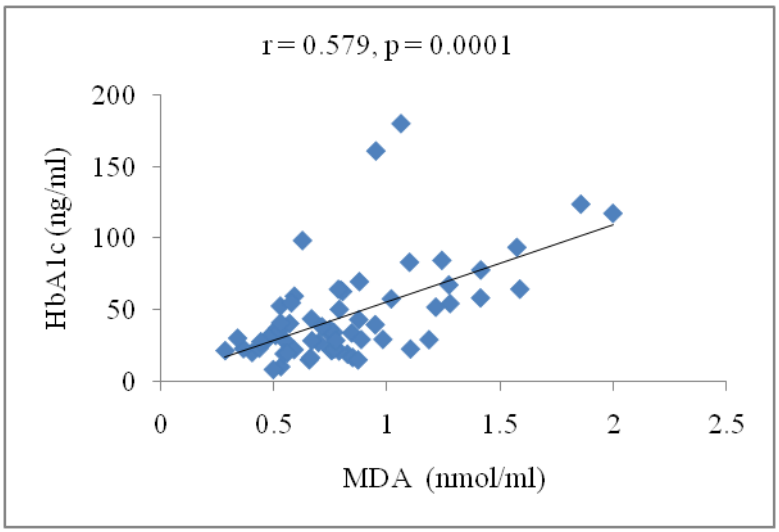

Fig 5: Correlation Graph of MDA and HbA1c Levels in CKD Patients

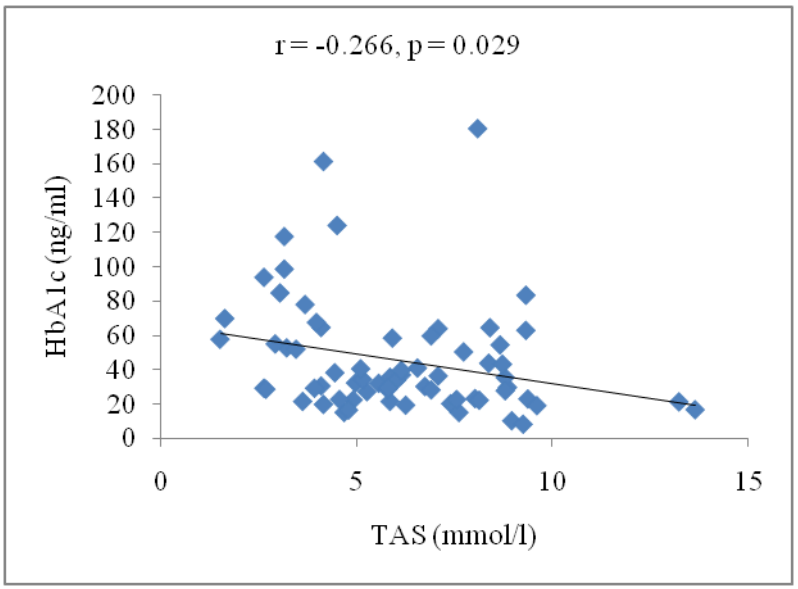

Fig 6: Correlation Graph of TAS and HbA1c Levels in CKD Patients

V. Discussion

Oxidative stress has been linked to various pathological conditions such as diabetes, anaemia, inflammation, infectious diseases and cardiovascular diseases. However, the role of oxidative stress in CKD patients is not yet fully elucidated. This study therefore aims to determine the role of oxidative stress in glycated hemoglobin among CKD patients in Sokoto.

In Table 1, the overall mean age of the CKD patients was 47.17 ± 1.77, the economically active group. This was found to be similar to the reports of Makusidi *et al.*,2015; Ulasi *et al.*, 2010 and Alebiosu *et al.*, 2006 in Nigeria and Eghan *et al.*, 2009 in Ghana, that CKD affects people in their productive years (sexual activity and economic productivity) thereby having a negative impact on the society, unlike the situation in many developed countries where the mean age is generally over 60 years (Stel *et al.*, 2009).

The sex distribution in Table 1 shows that CKD is more prevalent in men than in women. There was a preponderance of males (53.7%) to females (46.3%). This is comparable to similar studies in Nigeria by Ulasi and Chinwuba (2010) who showed a male preponderance of 65.3% vs 34.7%. The male predominance might be due to the fact that CKD and its risk factors such as hypertension, smoking and advanced age are common in

males than females. Difference in the health seeking behaviours of males and females might also play a role in the dominance of males among the CKD patients.

In this study, the patients were grouped into various stages of CKD based on their estimated GFR, using the 4-variable MDRD equation from plasma creatinine (NKF-KDOQI, 2000). As observed, the plasma creatinine level increased while the glomerular filtration rate was greatly reduced when compared to the healthy control (Table 1). The increasing plasma creatinine and consequent decreasing eGFR from stage 1 to 5, as shown in Fig 1, indicated the kidney status of these patients.

Oxidative stress is the result of an imbalance in pro-oxidants and antioxidants leading to the generation of toxic reactive oxygen and nitrogen species which could cause damage to important cellular components such as lipids, proteins and nucleic acids. In this study, there was highly significant increased levels of MDA, a marker of lipid peroxidation (Table 2) and decreased levels of antioxidants enzymes; total antioxidant status, superoxide dismutase, catalase and glutathione peroxidase (Table 2) when compared to the healthy control.

MDA (Malondialdehyde) is a low molecular weight hydro-soluble molecule, which can be cleared by the kidneys but is increased in cases of kidney dysfunction. This study showed the highest increase in MDA at stage 5 of CKD as compared to the other stages and the healthy control (Fig 2), probably due to the low glomerular filtration rate and the hemodialysis procedure. Also, the increased levels of MDA could lead to the production of more free radicals which could result in increased peroxidation of lipids and consequent overproduction of MDA. Moreso, the increased levels of the molecules at stage 1 shows oxidative stress could occur at early stages of the CKD.

It was also observed from this study that the antioxidant enzymes (TAS, SOD, CAT and GPx) decreased from stage 1 of the CKD to stage 5 (Fig 3). This has also clearly shown that oxidative stress occurs even in the early stages of CKD. The decreased levels of the total antioxidant enzymes (TAS) across the stages indicate the low antioxidant potential in CKD patients and increased risk of further complications. In addition, the decreased levels of superoxide dismutase as CKD advanced in stages suggest that accumulation of superoxide anion radical might be responsible for the increased lipid peroxidation (Tbahriti *et al.,* 2013). Glutathione peroxidase is responsible for the decomposition of lipid peroxide and protects the cell from the deleterious effects of peroxides while catalase converts H_2O_2 to harmless H_2O and O_2. However, these enzymes were found to be decreased when compared to the healthy control subjects and as CKD advances in stages, thereby contributing to the increased levels of oxidative stress.Treatment of CKD patients with hemodialysis or peritoneal dialysis has been suggested to contribute to oxidative stress and reduced antioxidant levels in CKD patients (Lim *et al.*, 2002; Witko-Sarsat *et al*., 1996).

Hemodialysis can expose patients to the risks associated with long term indwelling catheters, membrane bio-incompatibility, lack of ultrapure dialysis water, endotoxin leaks through back filtration and infections (Guo *et al.,* 2011; Opatrny, 2003). The interaction between dialysis membranes and blood can trigger the release of oxygen free radical and oxidizing agents such as superoxide anion, hydrogen peroxide, and myeloperoxidase. In turn, these molecules contribute to the oxidation of lipid products, proteins and nucleic acids.

According to Griendling *et al*. (1994), compromised antioxidant functions results in the well known cascade of hypoxic ischemic injury, inflammation, apoptosis and cell death. Witko-Sarsat *et a*l., (1998) studied the end-products of protein and carbohydrate oxidation, malondialdehyde and glutathione hydroperoxidase in 162 patients with eGFR ranging from 80 to 20 ml/min. They found that oxidative stress markers were higher in the CKD patients compared with controls, and correlated inversely with eGFR. Likewise, in 159 patients with stages 1 to 5 CKD, Yilmaz *et al.,* (2006) showed that the level of oxidative stress markers such as malondialdehyde and oxidized low density lipoprotein increased, whereas the antioxidant erythrocyte superoxide dismutase, glutathione peroxidase, plasma selenium, erythrocyte zinc and copper decreased as CKD advances in stage. These reports both support the findings in this study that there was an increased oxidative stress and decreased antioxidant activities in the CKD patients.

In this present study, it was observed the blood glucose level decreased as the kidney function deteriorates (Fig 4). This could be as a result of the level of circulating insulin among the CKD patients due to the reduced renal mass. However, in this study, an increased HbA1c was observed as CKD progresses (Fig 4) which could be due to the non-compliance of patients to medications over the 3 months period thereby reflecting the true glucose control. It could also be due to other factors that could increase the glycated hemoglobin levels such as oxidative stress present in the CKD patients, leading to increased glycation of hemoglobin. According to some reports, other factors that could increase glycated hemoglobin are reduced red blood cell life span, recent transfusion, accelerated erythropoiesis due to erythropoietin therapy, and metabolic acidosis (Ansari *et al.,* 2003; Joy *et al.,* 2002).

Increased oxidative stress could exacerbate the glycation of hemoglobin as shown by the highly significant positive correlation between MDA and HbA1c (Fig 5), as well as the significant negative correlation

between TAS and HbA1c (Fig 6). This has clearly shown that oxidative stress may enhance the glycation of hemoglobin in CKD patients, even in a normoglycemic condition.

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