ASSOCIATION OF LOWER ANTIOXIDANT STATUS WITH ESTIMATED GFR IN HYPERTENSIVES WITH PRESERVED RENAL FUNCTION

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ABSTRACT: BACKGROUND AND OBJECTIVES: Renal dysfunction is end organ damage in hypertension. We investigated the association of oxidative stress with estimated glomerular filtration rate in hypertension. **METHODS**: A total of 116 hypertensive individuals (age 47 ± 10.2 years; 68% males; BMI < 25; without diabetes mellitus, CVD) were included. All the hypertensive subjects were albustix negative. Fasting blood glucose, lipid profile, urea and creatinine was estimated. Serum oxidant load was estimated by ferrous oxidation products in xylenol orange version 2(FOX2) and antioxidant power of serum was estimated by ferric reducing capacity, FRAP assay. Data is represented as mean± SD. Data was analysed by unpaired two-tailed students t test, Pearson's correlation and linear regression. The estimated GFR was calculated by simplified modification of diet in renal disease study prediction equation and Cockroft- Gault formula. **RESULT**: Systolic and diastolic blood pressure, lipid profile, serum creatinine and oxidant load was significantly higher in hypertensive subjects ($p < 0.001$). Total Antioxidant status and estimated GFR was significantly lower in hypertensive subjects. A significant negative correlation was present between oxidative stress and eGFR. **INTERPRETATION AND CONCLUSION**: Among hypertensive subjects with preserved renal function increased oxidative stress is associated with decreased eGFR. Whether estimation of oxidative stress and adjuvant antioxidant therapy are better prognostic markers requires further research.

KEY WORDS: estimated GFR, hypertension, oxidative stress

INTRODUCTION: Hypertension (systolic blood pressure ≥140 and diastolic blood pressure ≥90 mm Hg mercury) is the most the most common cardiovascular disorder affecting every socio economic group of population (1). It is a major public health concern as it is associated with coronary artery disease, stroke, chronic renal disease and various other vascular complications (2). Recent studies have associated oxidative stress with the pathophysiology of hypertension induced renal dysfunction. Benign arterionephrosclerosis occurring in hypertensive patients leads to a mild to moderate increase in serum creatinine (2, 3). Normally the oxygen free radicals called as reactive oxygen species (ROS) are involved in maintaining cellular

physiological equilibrium such as regulation of vascular tone, sensing of oxygen tension and signal transduction (4). Under normal physiological conditions the organism is protected from the toxic effects of ROS induced lipid peroxidation by antioxidants; however, when these antioxidants are overwhelmed, the organism is said to be under oxidative stress (4). Evidence suggests the association of oxidative stress in both experimental and human hypertension and renal dysfunction (5, 6). The role of oxidative stress in the pathogenesis of hypertension involves both hemodynamic (vasoconstriction) and structural (vascular remodeling) mechanisms (7-10). ROS may cause and maintain hypertension by various mechanisms such as quenching of vasodilator nitric oxide by superoxide, generation of vasoconstrictive lipid peroxidation products, damage to endothelial cells, damage to vascular smooth muscles, increase in intracellular free calcium, increased endothelial permeability, stimulation of inflammation and stimulation of growth signaling events (7- 10). Studies suggest oxidative stress mediated inflammation and interstitial infiltration of immune cells cause renal cell injury and end organ damage of kidney in hypertension (11). The association between arterial hypertension and renal disease as a end organ damage is well established (12). In recent years it has been demonstrated that even minor renal dysfunction entail an enhanced cardiovascular risk and an increase in chronic renal failure due to hypertension (13, 14). The best measure of overall renal function is to measure the glomerular filtration rate (GFR). It is normally around 100ml/min so that the result roughly indicates the percentage of normal renal function. Measurement of GFR is difficult but it can be estimated (eGFR) by laboratory from serum creatinine, gender, body surface area and age. Nevertheless, the available data are not conclusive and the relationship between oxidative stress, hypertension and renal dysfunction in humans remains to be elucidated. The purpose of the present study was to (a) investigate the association between hypertension, oxidative stress and estimated glomerular filtration rate (eGFR) in hypertensive subjects with preserved renal function and volunteer normotensive persons, (b) to find a correlation between serum oxidant load and serum total antioxidant status with eGFR.

MATERIAL AND METHODS: This case-control study included 116 hypertensive patients attending the Medicine outpatient department of MKCG Medical College, Berhampur. The study protocol was approved by the Institutional Ethical Committee. Informed consent was obtained from all the study participants. Study sample consisted of 232 individuals; 116 hypertensive subjects (cases) with the mean age of 47±10.2 years, 68% male and having a body mass index (BMI) less than 25 and an equal number of age and sex matched controls.

The inclusion criteria for the cases were diagnosed essential hypertension with systolic blood pressure (SBP) >140 mm Hg and diastolic blood pressure (DBP)> 90mm Hg of either sex between the age group 30-65 years with a BMI <25 and without any associated diseases like diabetes mellitus, cardiovascular, liver or renal disease. Patients on medication like steroids, OC pills, thyroxin, HRT were excluded. Criteria for controls were age and sex matched healthy normotensive individuals without H/O of hypertension.

The physiological parameters at the time of admission such as age, height, weight, duration of disease and blood pressure (BP) were recorded.

MEASUREMENT OF BLOOD PRESSURE (15): Each subject was seated in a quiet and comfortable position for five minutes, with feet on the floor and arm supported at heart level and then two readings of BP were measured on the right arm, five minutes apart with a mercury sphygmomanometer (cuff size 12.5 X 40 cm) with auscultatory method of BP measurement. BP

readings were confirmed in the contralateral arm at the same time. The SBP and DBP were read to the nearest 2mm Hg. First and fifth phases of Korotkoff's sounds were taken as criteria for SBP and DBP respectively. The average of the two consecutive readings was recorded.

MEASUREMENT OF BIOCHEMICAL PARAMETERS: All the biochemical parameters were estimated in the clinical biochemistry laboratory at the Regional Diagnostic Centre of MKCG Medical College. Fasting venous sample was collected and the biochemical parameters were measured by using commercial kits adapted to EM360 Erba Transasia Autoanalyser. Glucose was estimated using glucose oxidase peroxidase method (Siemens), lipid profile parameters such as total cholesterol, triglycerides, HDL-Cholesterol was measured using kits from Erba diagnostics, Germany. LDL-Cholesterol was calculated using Freidewalds equation. Serum creatinine was measured using kits from Erba diagnostics, Germany. The estimated glomerular filtration rate was calculated by two different methods: simplified MDRD Study prediction equation (16) and Cockcroft-Gault (CG) formula (17). The CG formula was corrected for body surface area of 1.73 m^2 .

MEASUREMENT OF OXIDATIVE STRESS PARAMETERS: The oxidative stress was evaluated by estimating the amount of oxidant load of lipid peroxides was determined by ferrous oxidation products in xylenol orange assay in conjunction with triphenylphosphine version 2 (FOX2 assay) (18). The inter assay and intra assay coefficient of variation for FOX2 were 4.9% and 2.7% respectively. Antioxidant power of serum was measured by ferric reducing ability of serum (FRAP assay) (19). The inter assay and intra assay coefficient of variation for FRAP were 3.0% and 1.0%, respectively.

STATISTICAL ANALYSIS: Data is expressed as mean ± standard deviation (SD). The data was analyzed by student's t test for unpaired data. Correlation was derived by Pearson's correlation analysis. A p value < 0.05 was considered significant. Statistical analysis was done using SPSS version 16 software.

RESULTS: Demographic profile of the study subjects is depicted in Table 1 and Figure 1. Cases included 79 males and 37 females with the male-to-female ratio of 2.13:1 and controls consisted of 65 male and 51 females with a male-to-female ratio of 1.27:1. Cases had a mean age of 47±10.2 years compared to the mean age 44±7.8 years of controls (table 1).

Clinical and laboratory data as represented in Table 2. SBP of cases 164.5±13.6mm Hg is significantly higher (p value <0.000) and DBP 99.06±11.9mm Hg is significantly higher (p value <0.001) than that of controls, with SBP 118.7±4.87mm Hg and DBP 82.34±3.5mm Hg. There was no significant difference between the BMI of cases (24.3±4.2) and that of controls (24.06±3.7). Significant difference was not observed between the blood glucose of cases (107±6.4 mg/dl) and that of controls (101±8.6). There was significant (p value 0.001) difference in the lipid profile between cases (total cholesterol-201.14±6.12 mg/dl; triglyceride-159.18±11.7 mg/dl; HDL cholesterol-36.07±6.9 mg/dl; LDLcholesterol-182.03±14.86 mg/dl) and controls (total cholesterol-147.41±1.74mg/dl; triglyceride-89.40±22.2mg/dl; HDL cholesterol-31.08±3.59 mg/dl; LDLcholesterol-132.03±18.07 mg/dl). The serum creatinine level of cases (1.18±0.04mg/dl)was significantly higher (p value 0.001) than controls (0.99±0.1mg/dl). The eGFR of the cases (58.70±24.19ml/min) was significantly lower in comparison to the controls (94.18±10.31ml/min) as represented in Table 2.

Table 3 and Figure 2 shows there is a significant increase in the oxidant load and a significant decrease in the total antioxidant power of the serum of hypertensive patients as

compared to the normotensive controls. The serum oxidant load measured by FOX2 assay of cases is 13.0±4.9µmol/L equivalent of hydrogen peroxide and that of controls is 4.33±1.7µmol/L (p value 0.001). The serum total antioxidant level of the cases is 99.87±7.48µmol/L equivalent of Ferrous Sulphate and that of the controls is 423±15.23µmol/L. Pearson's correlation revealed a significant negative correlation between oxidant load and antioxidant status with R^2=-0.797 and p value=0.000.

Table 4 and Figure 3 depict the correlation between the oxidative stress parameters and eGFR of the cases. Figure 4 shows the linear regression between oxidative stress parameters and eGFR in hypertensive cases. Linear regression shows that an increased antioxidant capacity of serum corresponds to increased eGFR and a high oxidant load predicts a decrease in eGFR.

DISCUSSION: Hypertension is a complex multifactorial disease of blood pressure regulation characterized by an increase in both systolic and diastolic blood pressure (SBP and DBP) than the optimal level. In the present study, the SBP and DBP of the cases were significantly higher than that of controls. Confounding factors which might influence the study parameters were minimized since all the participants were drawn from the same population. Almost all the study participants had similar diet and lifestyle with regard to their daily exercise patterns.

The findings of the present study demonstrate a strong association between blood pressure and oxidative stress parameters. The increased oxidative stress parameter levels observed in the hypertensive cases of our study is consistent with the findings of several previous studies (20-21). However, in certain studies no significant association was found between hypertension and oxidative stress, either due to the fact that these studies were conducted in the early stages of the disease or with patients receiving statins medication, which is an interfering factor of oxidative stress (22, 23, and 24). The correlation of blood pressure with oxidative stress parameters in hypertensive subjects suggests that these parameters have a blood pressure modulating effect. Hypertensive cases showed an impaired total antioxidant status, which is in agreement with the previous studies (25-28). Further, the negative correlation between SBP and DBP with total antioxidant status assed by FRAP levels suggests the importance of serum antioxidant status in blood pressure modulation. In normotensives subjects there was no significant correlation between blood pressure and antioxidant status. This finding requires special analysis. Many recent studies have documented that exposure to ROS increase the expression of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase (29, 30). Thus, genes encoding these enzymes are coordinately regulated by antioxidant responsive elements (ARE) in their regulatory regions, a process which occurs through the activation of transcription factor NF-E2-related factor 2 (Nrf2) (31). The binding of Nrf2 to ARE regions in genes causes up-regulation of the downstream genes which regulate the antioxidant activity of enzymes in response to ROS activity. It may be noted that this mechanism is triggered in most hypertensive patients in response to their increased oxidant load as compared to normotensives. The significant negative correlation between SBP vs FRAPS and DBP vs FRAP in hypertensive cases in our study strongly indicates the low antioxidant status of the patients leading to oxidative stress.

The low oxidative stress in hypertensives may be due to their decreased antioxidant defense activity and an increased oxidant load. This derangement leads to damage to various biomolecules in hypertensive patients. As a consequence, of increased oxidant load a reduction in endothelium- dependant vasodilation of vascular smooth muscles occurs in hypertensive patients (32, 33). This increase in blood pressure contributes to an increase in ROS and oxidant

load, thereby enhancing the ROS- mediated hypertension through a complex interdependent cycle.

Previous studies have investigated and established significant association between oxidative stress and inflammation in hypertensive patients resulting in renal damage (34, 35). Our study, also demonstrated a significant correlation between increased oxidant load with eGFR in hypertensive patients. We also observed a significant positive correlation between decreased antioxidant status and eGFR. Based on these observations, it may be suggested that increased oxidative stress constitutes a powerful factor for promoting renal damage in hypertensive cases and it is in concordance with previous studies (36, 37). It has been observed that an increased oxidative stress in hypertensive cases activates various transcription factors such as NF-kB, activator protein-1, MAP kinases, p38 (36, 37, and 38). These transcription factors lead to rapid-response proinflammatory genes resulting increased interstitial inflammation, increased apoptosis and damage of renal tissue in hypertensives (39, 40). The significant negative correlation of oxidative stress with eGFR in hypertensive cases in our study indicates the association of oxidative stress with reduced renal functional status or the commencement of end organ damage.

The uniqueness of this study is that the hypertensive cases included although exhibited an increased serum creatinine level, had preserved renal function as implied by the negative albustix test done to detect urine albuminuria. However, our findings indicate the importance of oxidative stress parameters and eGFR in detecting early renal damage in hypertensive patients.

In conclusion, we suggest that antioxidant therapy may be included in adjunct with antihypertensive therapy to reduce oxidative stress induced end organ renal damage.

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Table 1 Age and gender distribution of the study group		
	Cases	controls
Males	79	65
Females	37	51
M:F ratio	2.13:1	1.27:1
Age	47±10.2 years	44±7.8 years

Table 2 Clinical and Laboratory Data of hypertensive cases and controls

Parameters	Hypertensive cases	Normotensive controls	p value
BMI (Kg/m^2)	24.3±4.2	24.06±3.7	NS
SBP (mm Hg)	164.5±13.6	118.7±4.87	0.001
DBP (mm Hg)	99.06±11.9	82.34±3.5	0.002
Blood glucose (mg/dl)	107±6.4	101±8.6	NS
Total cholesterol (mg/dl)	201.14±6.12	147.41±1.74	0.001
Triglyceride (mg/dl)	159.18±11.72	89.40±22.22	0.000
HDL cholesterol (mg/dl)	36.07±6.96	31.08±3.59	NS
LDL cholesterol (mg/dl)	182.03±14.86	132.03±18.07	0.05
Serum creatinine (mg/dl)	1.18±0.04	0.99±0.11	0.002
eGFR (ml/min)	58.70±24.19	94.18±10.31	0.000

Data is represented as mean±SD and analysed by unpaired student's t test. Pvalue <0.001 and <0.05 are considered significant.

Table 3 Comparison of oxidative stress parameters between hypertensive cases and controls

Parameter	Hypertensive cases	Normotensive controls
FOX2 (µmol/L)	13.0±4.9*	4.33±1.7
FRAP (µmol/L)	99.87±7.48*	423±15.23

Data is represented as mean±SD and analyzed by unpaired student's t test. *Significant with p value <0.001 as compared to controls.

Table 4 Correlation between oxidative stress parameters with blood pressure and eGFR in hypertensive cases

Parameters	FRAP µmol/L R^2 value; p value	FOX2 µmol/L R^2 value; p value
eGFR ml/min	0.276; 0.02	-0.53; 0.003
SBP mm Hg	-0.44 ; <0.005	0.52; <0.005
DBP mm Hg	-0.49; <0.005	0.55; <0.003

Figure 1 Gender distribution of the study group

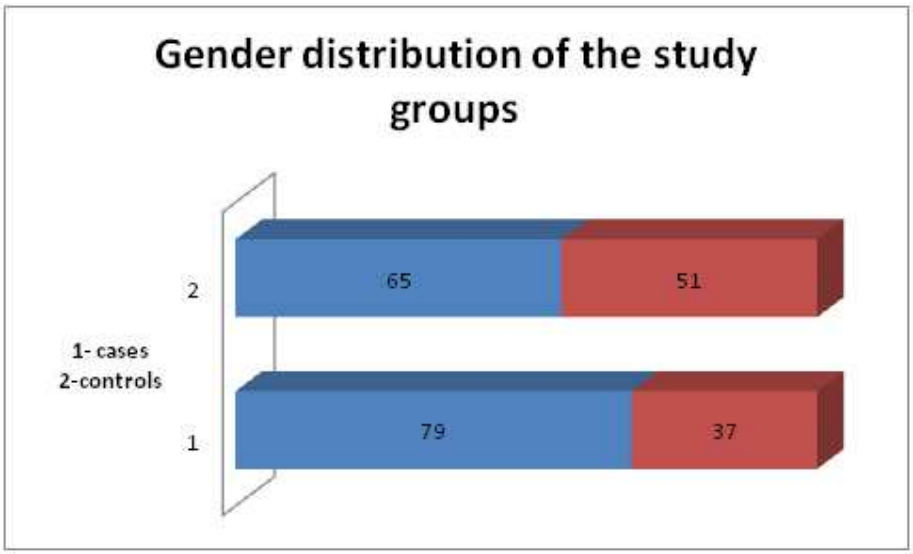
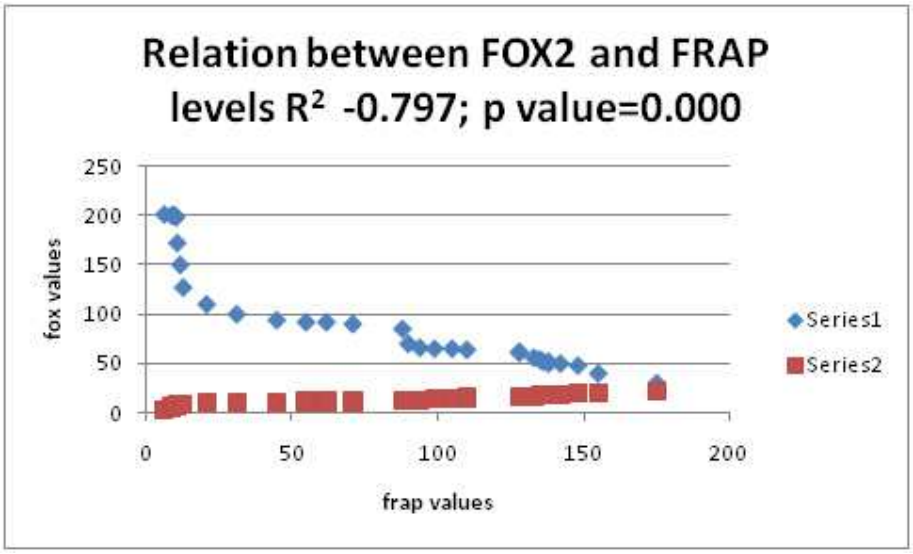
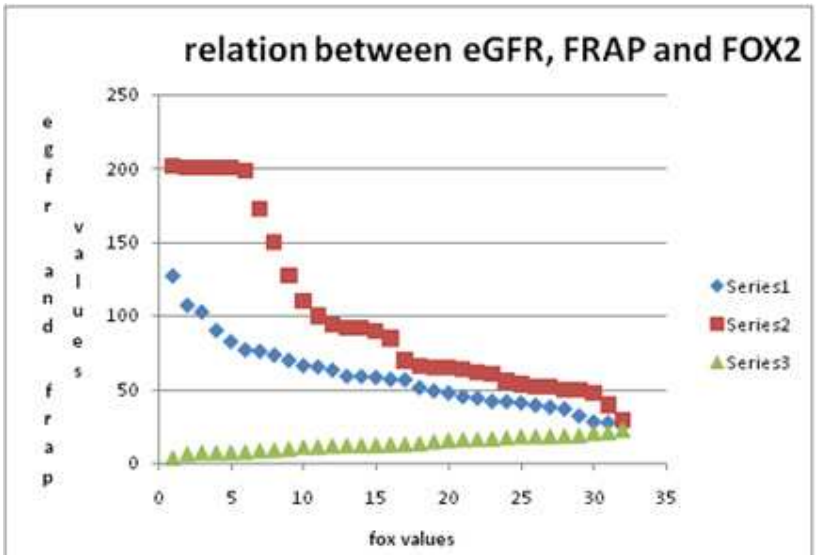


Figure 2 Relation of oxidant load and antioxidant status of the serum of hypertentive cases



Series 1 depicts decreasing antioxidant (frap) levels and Series 2 shows increasing oxidant (fox_2) levels. An increasing oxidant load corresponds to a decrease in antioxidant status leading to oxidative stress in hypertensive cases.

Figure 3 Correlation of oxidative stress parameters- FOX2, FRAP with eGFR



Series 1-eGFR, Series 2-FRAP, Series 3-FOX2. The graph shows negative correlation of FOX2 (increased oxidant load) with eGFR and positive correlation of eGFR with FRAP (antioxidant status) values.

Figure 4 A shows linear regression of eGFR with FRAP

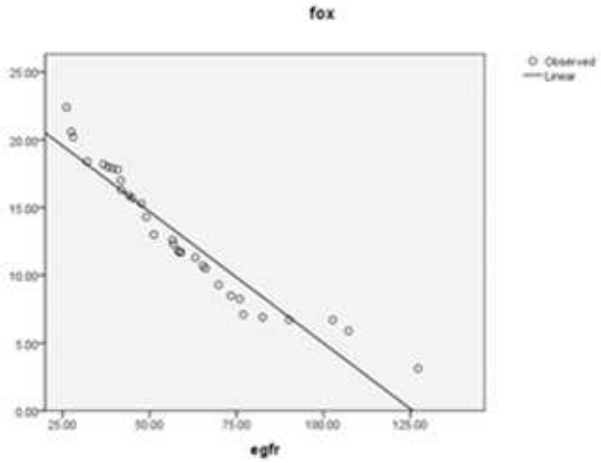


Figure 4 B shows linear regression of eGFR with FOX2