

## RESEARCH ARTICLE

## Association of oxidative stress with microalbuminuria and eGFR in essential hypertension

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### Abstract

Target organ damage like left ventricular hypertrophy, renal injury and coronary artery disease stroke occurs in the course of hypertension. Although the prevalence of hypertension is high in India, the relationship between microalbuminuria, estimated glomerular filtration rate and oxidative stress is not studied together in essential hypertension. Hence, this study was designed to compare oxidative stress, microalbuminuria, eGFR in essential hypertension and controls and find the relationship with severity, duration of disease, body mass index (BMI) and age. A total of 100 hypertensive individuals (Age:  $47 \pm 10.2$  years; 68% males; BMI < 25; without diabetes mellitus and CVD) were included. 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. The estimated GFR was calculated by simplified modification of diet in renal disease study prediction equation and Cockcroft-Gault formula. Early morning urine sample was used to estimate microalbuminuria by immunoturbidometry. Data was analyzed by unpaired two-tailed student's 't' test, Pearson's correlation and linear regression. Systolic and diastolic blood pressure, lipid profile, serum creatinine, oxidant load, urinary microalbuminuria was significantly higher in hypertensive subjects ( $p < 0.001$ ). Total serum antioxidant capacity and estimated GFR was significantly lower in hypertensive subjects. An increase in oxidant load correlated significantly with a decrease in estimated GFR. A significant correlation existed between oxidative stress, microalbuminuria, severity, duration of hypertension, BMI and age. In hypertensive patients, an increased oxidative stress is associated with microalbuminuria and decreased eGFR. Early screening of hypertensive patients for microalbuminuria and insistent management of positive cases may reduce the load of chronic renal disease and cardiovascular diseases.

**Keywords:** Hypertension, microalbuminuria, body mass index, oxidative stress, immunoturbidometry.

### Introduction

Hypertension defined as raised blood pressure (systolic  $\geq 140$  and diastolic  $\geq 90$  mm of Hg) is a global problem affecting every socio-economic group of population (Park, 2009). It is an important public health concern since it is related with complications such as coronary artery disease, stroke, chronic renal disease, retinopathy, nephropathy and various other complications. Various studies have associated oxidative stress with the etiopathogenesis of hypertension induced renal dysfunction. Hypertension causes benign arterionephrosclerosis which leads to elevated levels of serum creatinine (Rodriguez-Iturbe *et al.*, 2004; Zuccala *et al.*, 2005). During normal physiological state, the oxygen free radicals known as reactive oxygen species (ROS) are involved in maintaining cellular equilibrium such as regulation of vascular tone, sensing of oxygen tension and signal transduction (Droge, 2002). Under standard homeostatic state, the organism is protected from the toxic effects of ROS induced lipid peroxidation by antioxidants; conversely, when these antioxidants are overwhelmed, the organism is said to be under oxidative stress (Droge, 2002).

Research suggests the correlation of oxidative stress in both experimental and human hypertension and renal dysfunction (Romero *et al.*, 1999; Wilcox, 2002). The role of oxidative stress in the development of hypertension includes both hemodynamic (vasoconstriction) and structural (vascular remodeling) mechanisms. ROS may initiate and sustain hypertension by numerous mechanisms such as destroying of endothelial derived vasodilator, nitric oxide by superoxide, generation of vasoconstrictive lipid peroxidation products, damage to endothelial cells, damage to vascular smooth muscles, augment in intracellular free calcium, amplified endothelial permeability, stimulation of inflammation and stimulation of growth signaling events (McIntyre *et al.*, 1999; Vaziri *et al.*, 2000; Zalba *et al.*, 2001; Cracowski *et al.*, 2002). Research has implied oxidative stress mediated inflammation and interstitial infiltration of immune cells cause renal cell damage and end organ injury of kidney in hypertension (Martindale and Holbrook, 2002). The association between arterial hypertension and renal disease is well established (Perera, 1955).

In recent years it has been established that even minor renal dysfunction lead to an enhanced cardiovascular risk and an increase in chronic renal failure due to hypertension (Henry *et al.*, 2002; Sarnak *et al.*, 2003). Essential hypertension is usually associated with cardiovascular risk factors such as age, obesity, dyslipidemia. Subtle end organ damage such as left ventricular hypertrophy, coronary artery disease, microalbuminuria and renal injury occurs early in course of hypertension. The most common cause of chronic renal disease is hypertensive nephropathy. Hypertension causes chronic renal ischemia leading to small and large vessel renovascular disease which might be left undiagnosed. Previous studies have documented that the high systemic blood pressure impairs renal glomerular apparatus leading to an increase in glomerular ultrafiltration of albumin (Pedrinelli *et al.*, 1999). Hypertension increases capillary pressure due to increased systemic perfusion pressure which may accelerate hyperfiltration, transcapillary macromolecular transport and damage the glomerular barrier which is composed of endothelial cell layer, glomerular basement membrane and podocyte foot processes.

Hypertension impairs glomerular permeability by various pathways such as diffusion through endothelial cell membranes, passage through intercellular junctions, transendothelial channels of organs and tissues with high permeability and the surface area products (Zuccala, 2005). A recent concept postulates that increased albumin leaks through exaggeratedly permeant glomeruli that implies the systemic damaging impact of subclinical atherogenesis, a process characterized by diffuse involvement of the entire vascular system (Ceriello, 2008). Though the prevalence of hypertension is high in India, the relationship between oxidative stress, microalbuminuria end organ damage in hypertension is yet to be elucidated. Hence, this study was designed to compare oxidative stress, microalbuminuria, eGFR in essential hypertension and controls and to find the relationship with severity, duration of disease, body mass index and age.

## Materials and methods

**Subjects and sample size:** Case-control study included 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. The prevalence of microalbuminuria in hypertension is 27% (Hitha *et al.*, 2008). Hence, with the confidence interval of 95% and allowable error of 30%, the sample size calculated using the formula,  $n=4pq/L^2$ , was 99.5 ie 100.

**Collection of data:** The case control study included 100 hypertensive patients and an equal number of age and sex matched healthy volunteers as controls.

Study sample consisted of 200 individuals; 100 hypertensive subjects (cases) with the mean age of  $47 \pm 10.2$  years, 68% male and an equal number of age and sex matched controls.

**Inclusion and exclusion criteria:** The inclusion criteria for the cases were diagnosed as essential hypertension with systolic blood pressure (SBP)  $\geq 140$  mm Hg and diastolic blood pressure (DBP)  $\geq 90$  mm Hg of either sex between the age group 30-90 years without any associated diseases like diabetes mellitus, cardiovascular, liver or renal disease and urinary tract infection. Patients on medication like steroids, OC pills, thyroxin and 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** (National blood pressure education programme, 2004): Each subject was seated in a quiet and comfortable position for 5 min, with feet on the floor and arm supported at heart level and then two readings of BP were measured on the right arm, 5 min 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 2 mm 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, 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 (Levey *et al.*, 1976) and Cockcroft-Gault (CG) formula (Cockcroft and Gault, 1976). The CG formula was corrected for body surface area of  $1.73 \text{ m}^2$ .

**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) (Benzie *et al.*, 1996). 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) (Halliwell and Gutteridge, 1999). Inter and intra assay coefficient of variation for FRAP were 3 and 1% respectively.

**Measurement of microalbuminuria in urine:** Five mL of first voided early morning sample of urine was collected. The patients were asked to avoid exercise or exertion prior to urine collection. In women, urine was collected during the non-menstrual phase of their cycles. Microalbuminuria was estimated by quantitative immunochemical and turbidometric method using kits from Tulip diagnostic (Turbilyte MA). The turbidity formed was measured at 340 nm. Reference cut off values of microalbumin in urine is 0-30 mg/L. Microalbuminuria is said to be present when the urine albumin is 30-300 mg/L.

**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.

**Results**

Demographic profile of the study subjects is depicted in Table 1. Cases included 68 males and 32 females with the male-to-female ratio of 2.1:1 and controls consisted of 65 male and 35 females with a male-to-female ratio of 1.9:1. SBP of cases (168.8 ± 13.6 mm Hg) is significantly higher (p value <0.001) and DBP 100.06 ± 11.9 mm Hg is also significantly higher (p value <0.002) than that of controls, with SBP 117.8 ± 4.87 mm Hg and DBP 80.34 ± 3.5 mm Hg (Table 2). There was no significant difference between the BMI of cases (24.34 ± 4.22) and that of controls (23.96 ± 3.74). Significant difference was not observed between the blood glucose of cases (106 ± 8.4 mg/dL) and that of controls (105 ± 6.6).

Table 1. Gender distribution of the study groups.

Sex	Cases	Controls
Males	68	65
Females	32	35
M:F ratio	2.1:1	1.9:1

Table 2. Comparison of clinical and laboratory data of hypertensive subjects and healthy controls.

Parameters	Hypertensive subjects	Healthy controls	p value
BMI (Kg/m <sup>2</sup> )	24.34 ± 4.22	23.96 ± 3.74	NS
SBP (mm Hg)	168.8 ± 13.6	117.8 ± 4.87	0.001
DBP (mm Hg)	100.06 ± 11.9	80.34 ± 3.5	0.002
Blood glucose (mg/dL)	106 ± 8.4	105 ± 6.6	NS
Total cholesterol (mg/dL)	211.14 ± 7.12	146.41 ± 0.74	0.001
Triglyceride (mg/dL)	169.18 ± 12.72	88.40 ± 20.22	0.000
HDL cholesterol (mg/dL)	37.07 ± 7.96	32.08 ± 2.59	NS
LDL cholesterol (mg/dL)	192.03 ± 15.86	130.03 ± 17.07	0.05
Serum creatinine (mg/dL)	1.28 ± 0.02	0.97 ± 0.10	0.002
eGFR (mL/min)	57.70 ± 23.19	94.17 ± 9.31	0.000
Microalbuminuria (mg/L)	79.72 ± 55.1	8.96 ± 2.27	0.000

Data is represented as mean ± SD; p value <0.001 and <0.05 are considered significant.

Fig. 1. Microalbuminuria (MA) in various age groups.

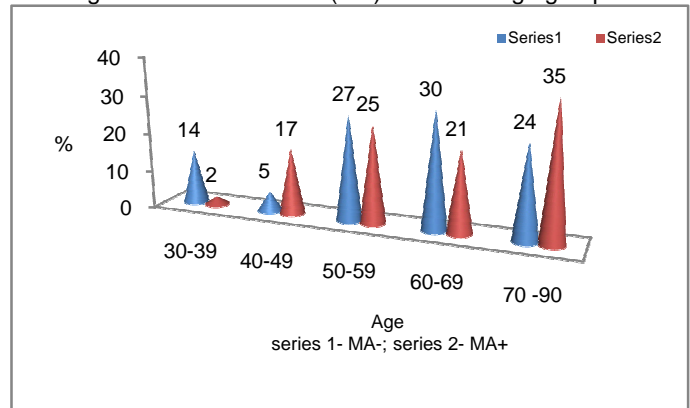


Fig. 2. Association of MA with duration of disease.

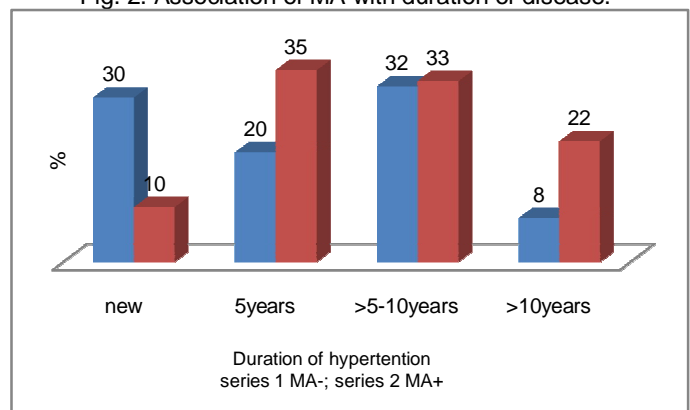


Fig. 3. Association of MA with severity of hypertension.

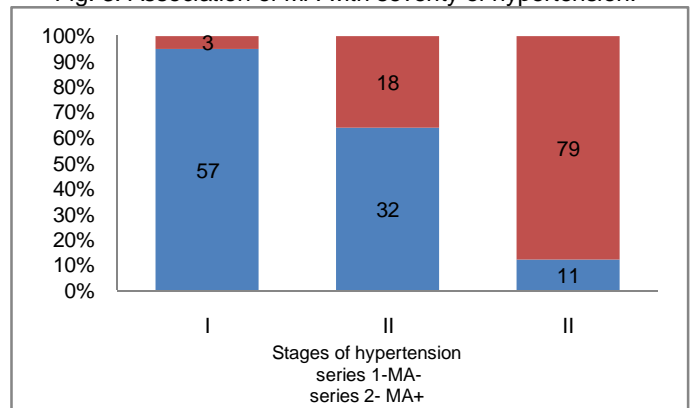


Table 3. Evaluation of oxidative stress in hypertensive cases and normotensive controls.

Parameters	Hypertensive cases	Normotensive controls
FOX2 (µmol/L) equivalent of hydrogen peroxide	14.50 ± 4.9*	4.43 ± 0.7
FRAP (µmol/L) equivalent of Ferrous Sulphate	98.97 ± 8.84*	420 ± 14.22

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

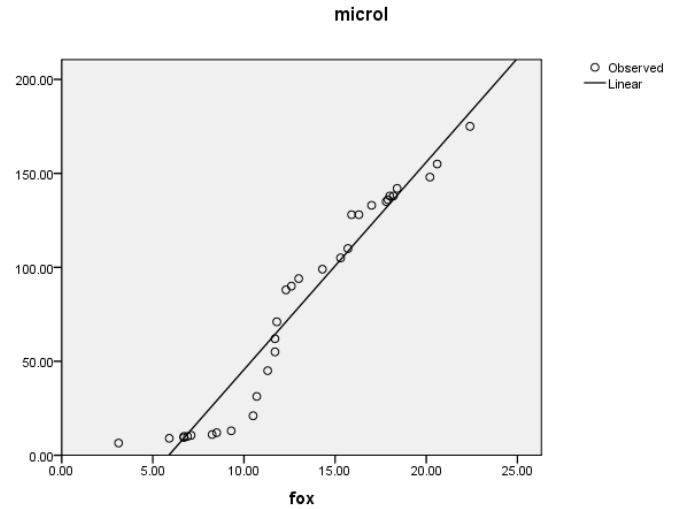
Table 4. Pearson's correlation between oxidative stress parameters and blood pressure, eGFR, microalbuminuria in hypertensive cases.

Parameters	FRAP (µmol/L) R <sup>2</sup> value; p value	FOX2 (µmol/L) R <sup>2</sup> value; p value
eGFR (mL/min)	0.266; 0.01	-0.54; 0.003
Microalbuminuria (mg/L)	-0.703; 0.000	0.850; 0.000
SBP (mm Hg)	-0.42 ; <0.005	0.55; <0.005
DBP (mm Hg)	-0.50; <0.005	0.65; <0.003

There was significant (p value 0.001) difference in the lipid profile between cases (total cholesterol: 211.14 ± 7.12 mg/dL; triglyceride: 169.18 ± 12.72 mg/dL; HDL cholesterol: 37.07 ± 7.96 mg/dL; LDLcholesterol: 192.03 ± 15.86 mg/dL) and controls (Table 2). The serum creatinine level of cases (1.28 ± 0.02 mg/dL) was significantly higher (p value 0.002) than controls (0.97 ± 0.1mg/dL). The eGFR of the cases (57.70 ± 23.19 mL/min) was significantly (p value <0.000) lower in comparison to the controls (94.17 ± 9.31 mL/min). Microalbuminuria levels were significantly higher (p value <0.000) in cases (79.72 ± 55.1) in comparison to controls (8.96 ± 2.27) (Table 2). Figure 1, 2 and 3 revealed the relation between microalbuminuria and age, duration of disease and severity of hypertension. We observed that incidence of microalbuminuria is associated with increase in age, longer duration of disease and increasing severity of hypertension.

There was 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 (Table 3). Serum oxidant load measured by FOX2 assay of cases is 14.50 ± 4.9 µmol/L equivalent of hydrogen peroxide and that of controls is 4.43 ± 0.7 µmol/L (p value 0.001). Serum total antioxidant level of the cases is 98.97 ± 8.84 µmol/L equivalent of ferrous sulphate and that of the controls is 420 ± 14.22 µmol/L. Table 4 depicts the correlation between the oxidative stress parameters, eGFR and microalbuminuria in the cases. Figure 4 depicts the significant linear regression between oxidant load and microalbuminuria in hypertensives (R<sup>2</sup> = 0.939 and p value 0.000).

Fig. 4. Linear regression depicting the relationship between microalbuminuria and oxidant load (R<sup>2</sup> = 0.939, p value 0.000).



### Discussion

Essential hypertension is a complex multifactorial syndrome characterized by an elevation in both systolic and diastolic blood pressure (SBP and DBP) than the normal physiological level. In our study, the SBP and DBP of the hypertensive subjects were significantly higher than that of controls. Confounding factors were minimized since all the participants were drawn from the same population. Most of the study participants had analogous diet and lifestyle with regard to their daily exercise patterns. Microalbuminuria, renal and vascular dysfunctions occur early in hypertension. The most common cause of chronic kidney disease is hypertensive nephropathy (Zuccala *et al.*, 2005).

Essential hypertension causes renal ischemia and nephrosclerosis, which leads to renal impairment (Rodriguez-Iturbe *et al.*, 2004). We observed a significant association of microalbuminuria with severity and duration of hypertension and also observed a significant correlation between microalbuminuria and oxidative stress parameters. The oxidative stress parameters were significantly increased in hypertensives than the controls. These findings are in agreement with previous research reports (Kedziora-Kornatowska *et al.*, 2004; Simic *et al.*, 2006). The correlation of blood pressure with oxidative stress parameters in hypertensive subjects implies that these parameters are involved in modulating the blood pressure. Hypertensive cases have exhibited a decreased total antioxidant status, which is in accord with the previous studies (Russo *et al.*, 1998; Kashyap *et al.*, 2005; Muda *et al.*, 2005; Moreno *et al.*, 2006). Supplementary evidence is the negative correlation between SBP and DBP with total antioxidant status assed by FRAP levels which indicate the importance of serum antioxidant status in blood pressure modulation.

In normotensives healthy controls there was no significant correlation between blood pressure and antioxidant status. This particular finding requires additional analysis. Recent studies have suggests that exposure to ROS increase the expression of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase (Talalay *et al.*, 2003; Bae *et al.*, 2004). Thus, the genes encoding these enzymes are coordinately regulated by antioxidant responsive elements (ARE) in their regulatory regions. This process occurs through the activation of transcription factor NF-E2-related factor 2 (Nrf2) (Lee and Johnson, 2004). The binding of Nrf2 to ARE regions in genes causes upregulation of the downstream genes which regulate the antioxidant activity of enzymes in response to ROS activity. It may be illustrated that this mechanism is activated 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 and is in concordance with our previous research (Roma *et al.*, 2013).

The increased oxidative stress in hypertensives may be due to their low antioxidant defense activity and an increased oxidant load. This derangement causes damage to various biomolecules in hypertensive patients. As a corollary, of increased oxidant load a reduction in endothelium-dependant vasodilation of vascular smooth muscles occurs in hypertensive patients (Lassegue and Griendling, 2004; Touyz and Schiffrin, 2004). This elevation 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 assessed and recognized the significant association between oxidative stress and inflammation in hypertensive patients resulting in renal damage (Dohi *et al.*, 2003; Fliser *et al.*, 2004). Our study has also revealed a significant correlation between increased oxidant load with eGFR in hypertensive patients. We also obtained a significant positive correlation between decreased antioxidant status and eGFR. Based on these observations, it may be recommended that increased oxidative stress constitutes a powerful factor for stimulates renal damage in hypertensive cases and it is in accord with previous studies (Clerk *et al.*, 1998; Droge, 2002). It has been experienced that an elevated oxidative stress in hypertensive cases activates various transcription factors such as NF- $\kappa$ B, activator protein-1, MAP kinases, p38 (Clerk *et al.*, 1998; Droge, 2002). These transcription factors initiate rapid-response pro-inflammatory genes resulting increased interstitial inflammation, increased apoptosis and damage of renal tissue in hypertensive subjects (Muller *et al.*, 2000; Mervaala *et al.*, 2003).

The significant negative correlation of oxidative stress with eGFR in hypertensive cases in our study reveals the association of oxidative stress with reduced renal functional status or the commencement of end organ damage. We observed a significant correlation between increased oxidative stress and microalbuminuria. Various studies have implied an association of microalbuminuria and coronary artery disease in hypertensives (Jensen *et al.*, 2000). Auxiliary, MA was associated with end organ damage, electrocardiographic changes (Pontremoli *et al.*, 1997). Recent research reports also indicate that age and microalbuminuria act as independent risk factors for the development of electrocardiographic changes. They indicated that elevated urinary albumin excretion is coupled with worse cardiovascular risk profile and is a coexistent indicator of early end organ damage (Pontremoli *et al.*, 1997; Jenson *et al.*, 2000).

### Conclusion

We suggest that microalbuminuria, eGFR and oxidative stress in essential hypertension increase the risk of end organ damage and cardiovascular disease. Hence, early screening of hypertensive patients for microalbuminuria, eGFR and aggressive management of positive cases may reduce the burden of chronic renal disease and cardiovascular disease.

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