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OXIDATIVE STRESS IN HYPERTENSION AND OTHER METABOLIC DISORDERS IN NORTH INDIAN PATIENTS

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ABSTRACT

Background: Oxidative stress is enhanced in hypertension and participates in the mechanisms of vascular injury. The study aims to determine oxidative stress status in patients of hypertension and hypertension with diabetes or obesity or both.

Methods: This prospective study was conducted on 34 patients with hypertension and 32 age matched control. The TAS, TOS and OSI were determined by novel automatic colorimetric methods from blood plasma.

Results: The risk factors like obesity, higher BSA and diabetes were found significantly associated with hypertension. Plasma TOS and OSI were significantly higher while level of TAS was lower in hypertension than in normal control subjects. Multivariate and ROC curve analysis suggested, a strong association between hypertension and higher TOS level (>8 μmol H_2O_2/L) [*P= 0.009*, Relative Risk (RR) =6.885, 95% CI=1.939-95.512] & obesity (BMI≥25) [*P= 0.001*, Relative Risk (RR) =10.210, 95% CI=3.815-267.220]. The area under the ROC curve was 0.763 (SE 0.06) with 95% CI=0.642-0.884 and P<0.001. The oxidative stress was found to be greater when hypertension was associated with obesity, diabetes or both.

Conclusion: Hypertension with addition to other metabolic conditions like diabetes, obesity or both implicit an additive effect on oxidative stress. The only remedy apart from early diagnosis is opting for a more natural lifestyle that will affect energy equilibrium and prove to be a viable option for prevention in hypertension.

KEYWORDS: Antioxidants, Diabetes, Hypertension, Obesity, Oxidative Stress

INTRODUCTION

Worldwide prevalence estimates for hypertension may be as 1 billion individuals, and approximately 7.1 million deaths per year may be attributable to hypertension [1]. Oxidative stress is enhanced in hypertension, atherosclerosis, and other forms of cardiovascular disease and participates in the mechanisms of vascular injury. Oxidative stress is induced through Reactive oxygen species (ROS) that include $\cdot O2^-$, H2O2, $\cdot$OH, HOCl and the reactive nitrogen species (RNS) like nitric oxide (NO) and peroxynitrite ($ONOO^-$). ROS and RNS are usually highly regulated and function as part of the intracellular signaling mechanisms of cells [2][3]. In hypertension, atherosclerosis, coronary artery disease (CAD), heart failure, diabetes, and other contexts of vascular damage, increased ROS production leads to endothelial dysfunction, enhanced contractility and growth of vascular smooth muscle cells (VSMCs), lipid peroxidation, inflammation, and

increased deposition of extracellular matrix proteins. Markers of systemic oxidative stress are increased in both experimental and human hypertension [4][5].

It was known that oxidants were increased and antioxidants were decreased, and as a result of these, oxidative/antioxidative balance shifted to oxidative side in patients with Hypertension/CAD [6]. Furthermore, previous studies showed that plasma antioxidant capacity was significantly reduced in patients with CAD as compared with healthy subjects but the Total Antioxidant Status (TAS) level was not found to be an independent risk factor [7]. Several large studies have reported on the associations between antioxidants and cardiovascular disease [8], myocardial infarction [9] and mortality [10], fewer studies have focused on the relationship between antioxidant measures in hypertension with associated metabolic disorders.

The dynamic distribution of different antioxidants in various biological samples and their potential interactions make it difficult to measure each antioxidant separately, and such measurements are also unlikely to represent the total antioxidant substances (TAS) in the body or total oxidant status (TOS). However implication of total oxidant/antioxidant status on hypertension and its interactive disease (obesity/diabetes) are yet to be studied. The purpose of the current study was to investigate the trend of TOS, TAS and OSI value on hypertension and its interactive disease group.

MATERIAL AND METHODS

Patients and Sample Specimens

The study was carried out at the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. The study protocol was approved by the Institutional Ethics Committee. A series of 34 patients with hypertension and 32 age matched control were treated at Department of Medicine, Sir Sundar Lal Hospital, Banaras Hindu University, India during the period from 2012 to 2014. All adult patients with hypertension reporting to Medicine OPD were screened to rule out secondary hypertension, or organ failures, presence of neoplastic disease, chronic inflammatory or chronic neurological diseases. Patients were screened for co-morbidities. Total 40 patients were enrolled out of which 34 patients completed the study protocol; 32 age matched healthy adults were taken as control subjects.

Hypertension was defined as a diastolic blood pressure ≥90 mmHg, systolic blood pressure ≥140 mmHg or self-reported use of an antihypertensive drug. Diabetes mellitus was diagnosed if the fasting plasma glucose concentration was ≥110 mg/dl on two separate occasions or if the patient was treated with insulin or oral hypoglycemic agents. Smokers were defined as those who had been smoking regularly (smoking daily for at least 1 year) until admission.

Estimation of Body Mass Index (BMI)

Body Mass Index (BMI) was calculated by weight (kg)/ {height (m)}^{2} at the time of hospital admission for treatment and the above criteria were used to categorize patients.

According to WHO, the BMI cut-off in Europeans for overweight (≥25.0 kg/m^2) and obesity (≥30.0 kg/m^2) are higher than Asian-pacific region. Steering committee (WHO Western Pacific Region 2000, the international association for the study of obesity and international obesity task force) recommended the cutoff for overweight (≥23.0 kg/m^2) and obesity (≥25.0 kg/m^2) for Asians [11][12].

Estimation of Body Surface Area (BSA)

Body Surface Area was calculated from the formula of DuBois and DuBois [13]:

$$BSA = 0.20247 \times (W^{0.425} \times H^{0.725})$$

Where the weight (W) is in kilograms and the height (H) is in meters.

Blood Samples

In all patients routine biochemical test were carried out to screen for blood glucose, renal function test, liver function test and complete blood count. Venous blood was withdrawn into citrated tubes. One milliliter of blood was centrifuged at 3000 rpm for 10 min to separate plasma. The plasma samples were stored at −80°C until analysis of total antioxidant status (TAS), total oxidant status (TOS), & oxidative stress index (OSI) were performed.

Measurement of the Total Oxidant Status of Blood Plasma (TOS)

The total oxidant status of the blood plasma was measured using a novel automated colorimetric measurement method for TOS developed by Erel [14]. In this method Oxidants present in the sample oxidize the ferrousion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Equivalent/L).

Measurement of the Total Antioxidant Status of Blood Plasma (TAS)

The total antioxidant status of the blood plasma was measured using a novel automated colorimetric measurement method for TAS developed by Erel [15]. In this method the reduced ABTS [2,2'-azino-bis(3-ethylbenzothiazo-line-6-sulfonic acid)] molecule is oxidized to $ABTS^{\cdot+}$ using hydrogen peroxide alone in acidic medium (the acetate buffer 30 mmol/l pH 3.6). In the acetate buffer solution, the concentrate (deep green) $ABTS^{\cdot+}$ molecules stay more stable for a long time. While it is diluted with a more concentrated acetate buffer solution at high pH values (the acetate buffer 0.4 mol/l pH 5.8), the color is spontaneously and slowly bleached. Antioxidants present in the sample accelerate the bleaching rate to a degree proportional to their concentrations. This reaction can be monitored spectrophotometrically and the bleaching rate is inversely related with TAC of the sample. The reaction rate is calibrated with Trolox, which is widely used as a traditional standard for TAC measurement assays. The assay results are expressed in mmol Trolox equivalent/l and the precision of this assay was excellent.

Determination of Oxidative Stress Index (Osi)

The percent ratio of TOS to TAS was accepted as the oxidative stress index (OSI). The OSI value was calculated according to the following formula [16]:

OSI (arbitrary unit) = [TOS (μmol H_2O_2 Eq/L)/TAS (mmol Trolox Eq/L)] ×100.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows version 16.0 (SPSS, Chicago, IL, USA). The chi-square test was used to compare categorical variables between groups. The independent sample T-test and Mann Whitney-U tests were used to compare continuous variables between the two groups. Multivariate logistic regression & ROC curve analysis was performed to evaluate the association of hypertension with TOS, TAS, OSI and diabetes.

A two-sided p value < 0.05 was considered statistically significant.

RESULTS

Demographic Presentation

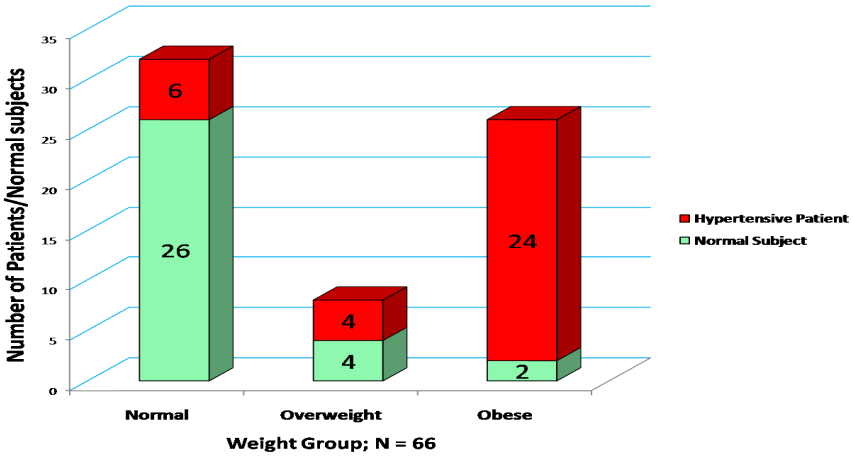
Demographic and clinical data of patients with hypertension and controls are shown in Table 1. The mean age of hypertensive patients and control was 58.71±12.37 years and 55.16±8.08 years respectively. This study showed a significant positive association between hypertension with BMI, Body Surface Area (BSA) and diabetes while factors like gender, residence, educational status, addiction of tobacco and nature of diet did not show any significant change between these two groups.

Table 1: Demographic and Clinical Characteristic of Patients with Hypertension

Variables		Hypertensive Patients (N=34)	Normal Subject (N=32)	*P* Value
Age (Years)	Mean±SD	58.71±12.37	55.16±8.08	0.175
BMI (Kg/M^2)	Mean±SD	25.58±3.55	19.86±2.40	0.000
BSA	Mean±SD	1.67±0.18	1.51±0.15	0.000
Gender	Male (%)	18 (52.9%)	18 (56.2%)	0.810
	Female (%)	16 (47.1%)	14 (43.8%)	
Residence	Rural (%)	22 (64.7%)	24 (75.0%)	0.428
	Urban (%)	12 (35.3%)	8 (25.0%)	
Educational Status	Illiterate (%)	17 (50.0%)	12 (37.5%)	0.744
	Primary (%)	3 (8.8%)	2 (6.2%)	
	Middle (%)	6 (17.6%)	6 (18.8%)	
	HS/Intermediate (%)	6 (17.6%)	8 (25.0%)	
	UG/PG (%)	2 (5.9%)	4 (12.5%)	
Addiction to Tobacco	Yes (%)	19 (55.9%)	16 (50%)	0.805
	No (%)	15 (44.1%)	16 (50%)	
Diabetes	Yes (%)	14 (41.2%)	2 (6.2%)	0.001
	No (%)	20 (58.8%)	30 (93.8%)	
Nature of Diet	Vegetarian (%)	13 (38.2%)	16 (50%)	0.457
	Non-Vegetarian (%)	21 (61.8%)	16 (50%)	

BMI: Body Mass Index, **BSA**: Body Surface Area, **SD**: Standard Deviation, **HS**: High School, **UG**: Undergraduate, **PG**: Postgraduate

BMI elevation was more frequently observed in hypertensive patients (Figure 1). A comparative view reveals that out of 66 subjects, in subjects having normal BMI (<22.99 Kg/M^2), only 6 out of 26 (23.07 %) were hypertensive; whereas subjects in overweight (BMI=23.0-24.99 Kg/M^2) and obese (BMI≤25.0 Kg/M^2), 4 out of 8 (50%) overweight and 24 out of 26 (92.3%) obese were found hypertensive respectively. Out of total hypertensive patients (N = 34), 24 patients were found obese, 14 were diabetic and 12 were both obese as well as diabetic.



Normal (Underweight +Normal ; BMI=<22.99), Overweight (BMI=23-24.99), Obese (Obese I + Obese II; BMI ≥25.00)

Figure 1: Association of Weight Group with Hypertensive and Normal Subject

Association of Oxidative Stress Parameters with Study Group

Oxidative stress parameters with study group are listed in Table 2. Significant positive associations were found between hypertensive patients with TOS, TAS and OSI. Plasma TOS and OSI are higher in hypertension than in normal control [9.56±1.40 vs. 6.65±0.96, 95% CI = 2.322 ─ 3.507, p<0.001; 1.15±0.42 vs. 0.62±0.19, 95% CI = 0.372 ─ 0.697, p<0.001, respectively]. Plasma TAS level in hypertension was lower than in normal control [0.89±0.18 vs. 1.11±0.16, 95% CI = -0.305 ─ -0.138, p<0.001].

Table 2: Association of Oxidative Stress Parameters with Study Group

Variables		Hypertensive Patients (34)	Normal Subject (32)	95% Confidence Interval of the Difference		*P* Value
				Lower	Upper	
TOS (µM)	Mean±SD	9.56±1.40	6.65±0.96	2.322	3.507	0.000
TAS (mM)	Mean±SD	0.89±0.18	1.11±0.16	-0.305	-0.138	0.000
OSI (AU)	Mean±SD	1.15±0.42	0.62±0.19	0.372	0.697	0.000

TOS: Total Oxidant Status, **TAS**: Total Antioxidant Status, **OSI**: Oxidative Stress Index, **AU**: Arbitrary Unit

Association of Study Group with TOS, TAS, BMI and Diabetes through Logistic Regression and ROC Curve Analyses

Multivariate analysis (Table 3) suggested a strong association between hypertension and parameters like TOS, TAS, BMI and diabetes. The hypertension was found significantly associated with higher TOS level (>8 µmol H_2O_2/L) [*P= 0.009*, Relative Risk (RR) = 6.885, 95% CI=1.939-95.512] & obesity (BMI≥25)[*P= 0.001*, Relative Risk (RR)= 10.210, 95% CI=3.815-267.220] in comparison to lower TOS (0-8 µmol H_2O_2/L) and normal BMI respectively. Lower TAS value (<1 mmol Trolox equivalent/L) had been found 32% increased relative risk of hypertension while diabetes had 1.25 fold, but these parameters was not up to significance.

ROC curve analysis (Figure 2) estimates sensitivity and specificity for predicted probability to test variables (TOS, TAS, BMI, Diabetes) with hypertension. The area under the ROC curve was 0.763 (SE 0.06) with 95% confidence interval 0.642-0.884 and P<0.001.

Table 3: Multivariate Logistic Regression Analysis of Hypertension with TOS, TAS, BMI and Diabetes

Variables		Relative Risk (RR)	95% Confidence Interval		P Value
			Lower	Upper	
TOS	>8 µmol H_2O_2/L	6.885	1.939	96.512	**0.009**
	0-8 µmol H_2O_2/L	1	-	-	
TAS	>1 mmol Trolox equivalent/L	0.679	0.077	2.840	0.410
	0-1 mmol Trolox equivalent/L	1	-	-	
BMI	Obese	10.210	3.815	267.220	**0.001**
	Overweight	3.377	0.867	84.677	0.066
	Normal	-	-	-	
Diabetes	Yes	1.251	0.309	73.189	0.263
	No	1	-	-	

TOS: Total Oxidant Status, **TAS**: Total Antioxidant Status, **BMI**: Body Mass Index

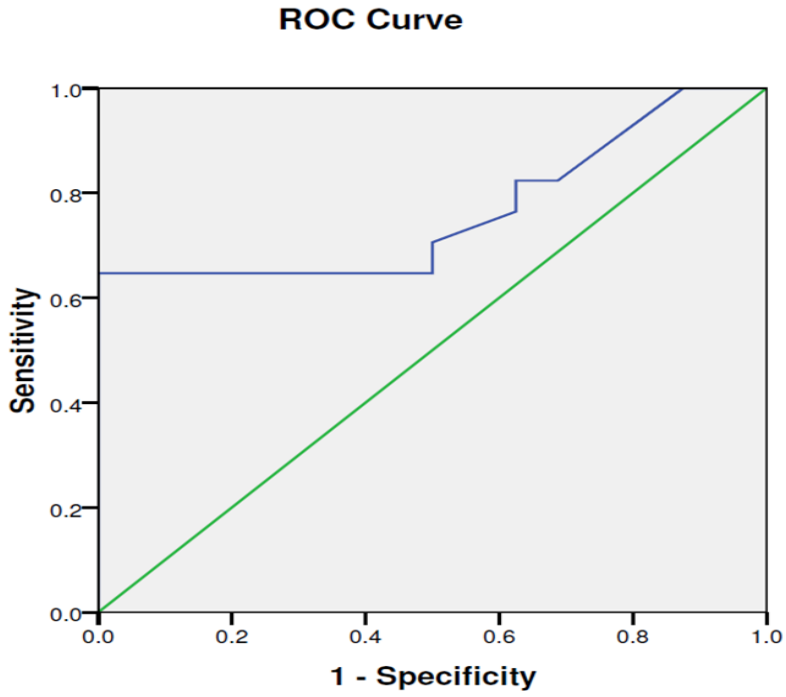


Figure 2: Receiver-Operating Characteristic Curve for Hypertension by Predicted Probability to Test Variables (TOS, TAS, BMI & Diabetes)

Effect of Oxidative Stress (TOS, TAS & OSI) on Hypertension & its Interactive Disease

Association of TOS, TAS & OSI with hypertension and interactive disease is listed in Table 4 and representation of oxidative stress parameters with all groups are plotted in figure 3. There was a significant association between disease groups and oxidative stress that persisted in ANNOVA analysis, suggesting a significant association of oxidative stress with the hypertension (TOS=9.56±1.39, TAS=0.89±0.17 & OSI=1.15±0.41), hypertension + obesity (TOS=9.81±1.42, TAS=0.86±0.18 & OSI=1.22±0.43), hypertension + diabetes (TOS=10.47±1.03, TAS=0.77±0.16 & OSI=1.43±0.40) and hypertension + obesity + diabetes (TOS=10.71±0.90, TAS=0.73±0.14 & OSI=1.52±0.37).

Table 4: Representation of Oxidative Stress Parameter with Hypertension and its Interactive Diseases

Variable		TOS (Mmol H_2O_2/L)		TAS (Mmol Trolox Equivalent/L)		OSI (AU)	
		Mean±SD	*P Value*	Mean±SD	*P Value*	Mean±SD	*P Value*
Hypertension	**Yes (N=34)**	9.56±1.39,	*0.000*	0.89±0.17	*0.000*	1.15±0.41	*0.000*
	No (N=32)	6.64±0.95		1.11±0.15		0.61±0.19	
Hypertension with Obesity	**Yes (N=24)**	9.81±1.42	*0.000*	0.86±0.18	*0.000*	1.22±0.43	*0.000*
	No (N=42)	7.19±1.41		1.07±0.16		0.70±28	
Hypertension with Diabetes	**Yes (N=14)**	10.47±1.03	*0.000*	0.77±0.16	*0.000*	1.43±0.40	*0.000*
	No (N=52)	7.52±1.55		1.06±0.16		0.74±0.28	
Hypertension with Diabetes & Obesity	**Yes (N=12)**	10.71±0.90	*0.000*	0.73±0.14	*0.000*	1.52±0.37	*0.000*
	No (N=54)	7.57±1.54		1.05±0.16		0.75±0.28	

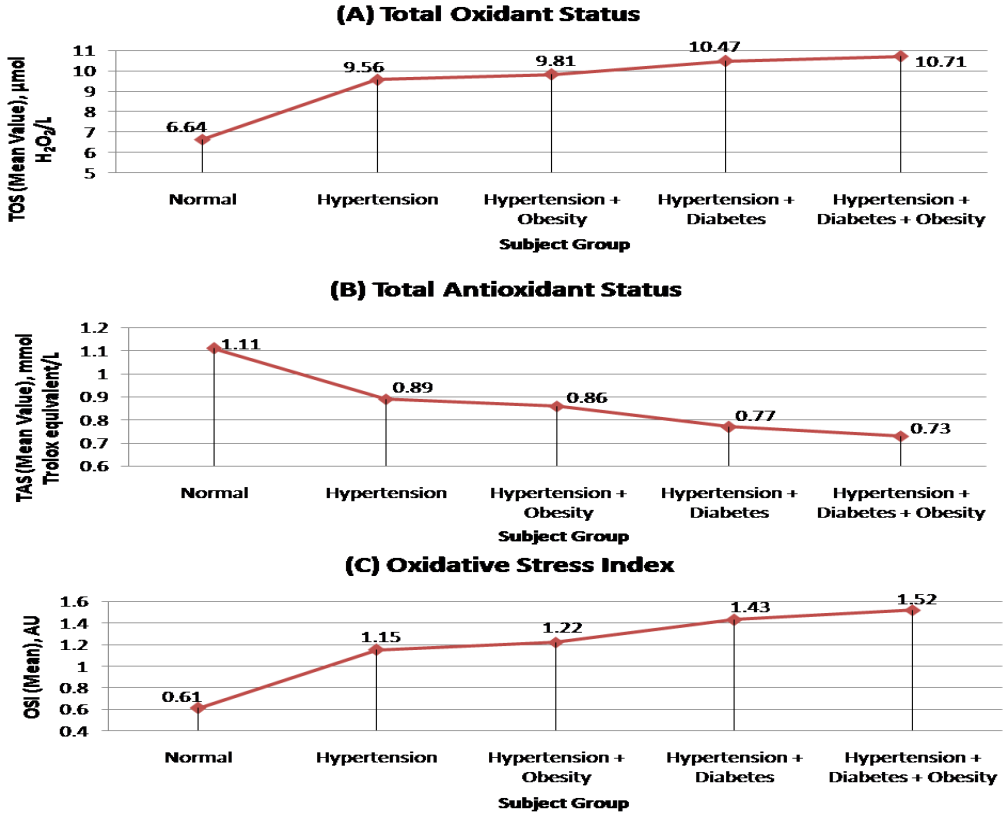


Figure 3: Representation of Oxidative Stress Parameter Plot with Normal Subject and Interactive Disease

DISCUSSIONS

There is no doubt that an imbalance of the cellular redox status is associated with the pathogenesis of cell/organ dysfunction in some diseases. Several studies mostly performed on single parameters associated with oxidative stress or antioxidative potential during the course of illness. However, up to now, no single parameter can be recommended as gold standard to define the oxidative status of patients. Therefore, a cluster of parameters representative of the antioxidative potential and of oxidized biomolecules should be analyzed. However the relationship between oxidative status with hypertension and additive diseases like obesity, diabetes or both has not been clearly identified and generally viewed, yet.

Present study analyzed comparison of cases and controls with demographic factors (Table 1). Body Mass Index (BMI) and diabetes were ascertained significant association with risk of hypertension. This observation is similar to the findings of others researchers that say, hypertension which co-exists with type 2 diabetes in about 40% at age 45 rises to 60% at age 75 years[17]. On these bases someone diagnosed with type 2 diabetes in middle age (40 – 60 years) stands to lose as much as 10 years of their life expectancy [17][18][19][20]. The risk of death among individuals with diabetes mellitus is almost twice that of individuals without diabetes of similar age [21]. The findings of this study confirmed that diabetes and hypertension are now associated with increased oxidative stress and also the higher BMI in hypertensive patients is associated with greater oxidative stress. This could possibly be due to sedentary life style, resulting from decreased exercises as the individuals increase in age [22, 23].

We found decreased plasma TAS levels and increased plasma TOS levels in patients with Hypertension (Table 3). Therefore, it is thought that Hypertension may be related with insufficient antioxidant capacity and excessive ROS generation which contributed to pathogenesis of the disease in Hypertensive patients. Oxidative stress, which often arises as a result of an imbalance in the human oxidative/antioxidative status, has been implicated in aging and a number of diseases such as cancer, atherosclerosis, rheumatoid arthritis, osteoarthritis, fibromyalgia, and osteoporosis [24][25][26]. A number of reports in the literature implicate excessive oxidative stress and/or inadequate antioxidant defenses in the pathogenesis of cardiovascular risk and disease [6][27][28][29]. In addition to traditional risk factors, oxidative stress has been regarded as one of the most important contributors to the progression of atherosclerosis [30][31].

Table 4 and figure 2 shows multivariate logistic regression analysis and ROC curve of different independent parameters (TOS, TAS, BMI and diabetes) with hypertension. Higher TOS level (>8 μmol H_2O_2/L) has been found significantly elevated relative risk of hypertension while ROC curve analysis also suggested significant association of predictive value for independent parameters with hypertension. In some other study hypertensive condition has been found to produce increased blood lipid peroxidation values and lower total antioxidant capacity (TAC) levels [32][33]; a fact that was supported by the present study.

The last finding of present study is a positive accelerated and significant association of TOS, TAS & OSI with hypertensive and associated disorders (obesity & diabetes) as depicted in Table 5, figure 3. As the hypertension become more associated with other metabolic disorders like obesity & diabetes, oxidant level increases and antioxidant level decrease with additive effect due to probable compensating mechanisms. Series of clinical and experimental studies have also shown that oxidative stress, through free radical generation, plays a major role in the onset of diabetes [34] and hypertension [35]. The deleterious effect of which can be prevented by antioxidants [36][37]. But the effectiveness of antioxidants enzymes in scavenging free radicals depends on their antioxidant cofactors.

CONCLUSIONS

The present study shows oxidative stress is significantly increased in patients with hypertension & when hypertension coexisted with diabetes, obesity or both the level of oxidative stress additively increase and antioxidant level regressively decrease. Hence, dietary supplements with antihypertensive, antioxidant and related anti-inflammatory effects may present a novel strategy of controlling and reducing complications.

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CONFLICT OF INTEREST STATEMENT

Accordingly there is no-conflict of interest arising whatsoever with this article. There is neither medical writing nor editorial assistance was used for the preparation of the article. The authors declare that all of them have made substantial contribution towards the writing of this article.

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