

## A Study Of Oxidative Stress Parameters And Body Mass Index with Antioxidant Levels In Essential Hypertension

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**Abstract:** Hypertension (HTN) is a major modifiable risk factor for Cardio Vascular Disease. Incidence of HTN has been increasing in India between 3rd and 6th decades of life. 90-95% of the HTN falls under the primary or essential or idiopathic type and the cause is unknown. It is suggested that the pathophysiology might result from the interactions between genetic and the environmental factors.

Also HTN produces imbalance between pro oxidants and antioxidants leading to increased oxidative stress, in turn leads to Atherosclerosis and Dyslipidemia. So, the aim of this study is to assess the level of oxidative stress parameters, body mass index (BMI) and antioxidants parameters and correlating the results with hypertension by analyzing selected samples through suitable methods. For the said purpose, a study of 80 hypertensive subjects and 51 non hypertensive and healthy subjects was carried out. Blood investigations in terms of Sugar, Urea, Creatinine, Lipid Profile and Uric Acid were estimated by standard kit method. Oxidative stress parameters (i.e) Plasma Malondialdehyde (MDA) were estimated manually by Ester Bauer and Steinberg method. Anti oxidant parameters (i.e) Plasma Nitric Oxide was estimated manually by modified Ding et al, using Greess Reagent, and Plasma Vitamin C was estimated manually by Roe and Kuether method. It is proven from the study that the oxidative stress parameters are at a higher level and the antioxidant parameters are at a lower level in the samples examined. It is also suggested that the anti oxidant supplementation may enhance the scavenging of free radicals and prevent the further complications. So this paper aims at exploring the pattern or relationship between the oxidative stress parameters and antioxidants parameters with the Hypertension.

**Keywords:** HTN, Oxidative stress, antioxidants, BMI, Ester Bauer and Stein Berg method, manual method.

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### I. Introduction

Hypertension is the most common of the cardio vascular diseases which is the leading cause of morbidity and mortality in the industrial world as well as becoming a modern epidemic in the developing countries. Blood pressure (BP) is a powerful cardiovascular (CV) risk factor that acts on the arterial wall and is responsible in part for various CV events, such as cerebrovascular accidents and ischemic heart disease. The estimated total number of adults with hypertension in 2000 was 972 million. By 2025, the number of people with hypertension will increase by about 60% to a total of 1.56 billion as the proportion of elderly people will increase significantly.<sup>1</sup>

Other reasons are the continuing population increase and changes in lifestyle, which includes a diet rich in sugar and high-fat processed foods and sedentary behavior. Since the proportion of hypertensive people will increase dramatically worldwide, the prevention, detection, treatment and control of this condition should be a top priority.<sup>2</sup>.

- In search for a causative factor for essential hypertension, the life style changes and obesity could contribute the increase of oxidative stress markers such as uric acid and lipid peroxidation.<sup>3,4</sup> To assess the lipid peroxidation the breakdown products of lipid peroxides in plasma, the malondialdehyde (MDA) is measured.<sup>7</sup> When the oxidative stress increases in hypertension the anti oxidant defense mechanism in the body is decreased.<sup>9,10,13</sup> Vitamin C is a well-known antioxidant that has been shown to efficiently scavenge free oxygen radicals.<sup>6</sup> NO can inhibit the oxidation of free fatty acids and lipoprotein molecules and thus this mechanism bears a clinical importance<sup>5</sup>. The overall goal of treating hypertension is to reduce HTN associated cardiovascular and renal morbidity and mortality.<sup>2</sup>

## II. Aims and Objectives

Assess the status of oxidative stress parameter, the serum uric acid and MDA, and the level of anti oxidants, plasma vit C and plasma nitric oxide.

To correlate the oxidative stress parameters and BMI with the antioxidant levels in essential hypertension in comparison to healthy controls.

- To recommend the preventive measures with respect to oxidative stress and to improve the antioxidants level for preventing the complications.

## III. Material & Methods

The study was carried out in eighty cases of essential hypertension aged between 31-69 years and fifty one age and sex matched healthy normotensive controls. Patients were diagnosed as cases of essential hypertension and following investigations were done.

Routine blood tests were done by standard kit methods.

- Fasting Blood sugar,
- Serum Lipid profile,
- Serum urea,
- Serum creatinine and
- serum uric acid
- Plasma MDA –Esterbauer and Steinberg method 1989.
- Plasma Vit C –Roe &Kutherford.
- Plasma Nitric oxide –Griess reagent method. and also BMI was calculated.

### ASSAY OF MALONDIALDEHYDE

(Esterbauer and Steinberg method 1989)<sup>7</sup>

**Principle**-This method is based on the fact that lipid peroxides condense with 1 methyl-2 phenyl Indole (M.P.I) under acidic conditions resulting in the formation of chromophore. To determine specifically lipid peroxides in serum or plasma they are precipitated along with serum or plasma proteins to remove water soluble MPI reactive substance. The level of lipid peroxide is expressed in terms of MDA, which is unstable. Tetramethoxy propane, which is converted quantitatively to MDA in the reaction procedure, is used as standard. The chromophore formed during reaction has absorbance maximum at 586nm.

### Procedure

1. A 7.6mM solution of 1-methyl-2-phenylindole (M.P.I) was prepared immediately prior to use in 33% methanol in acetonitrile.
2. 650µl aliquot of M.P.I was placed in each test tube to which was added 200µl of plasma.
3. The test tubes were mixed and 150µl of 10 M HCL was added. After mixing once more, the tubes were sealed and incubated for 60 minutes at 45°C.
4. After incubation the tubes were chilled on ice bath and spun at 10,000 rpm for 5 minutes to remove debris.
5. The absorbance at 586nm was measured and subtracted from the blank value obtained by replacing plasma with water.
6. A calibration graph was prepared using 2µmol/L, 4µmol/L, 6µmol/L,8µmol/L, of 1,1,3,3, tetra methoxypropane in 20mm Tris HCL buffer, PH 7.4.

### Calculation

Plasma malondialdehyde (µmol/L) =  $\frac{AbsS}{AbsT} \times \text{conc. of Std. (µmol/L)}$

### Estimation Of Nitric Oxide Assay<sup>11</sup>

Modified Ding et al 1998

**Principle**-Griess reaction was used to measure the amount of nitrates and Nitrites produced (an indicator of NO production). The Nitric oxide thus produced reacts with the mixture of sulphanilamide & naphthylene diamide dihydrochloride to produce a colour complex which is measured in the spectrophotometer at 570 nm.

### Reagents

1. 1% sulphanilamide : 1 gram of dry sulphanilamide powder was dissolved in 100 ml of 2.5% phosphoric acid or 10 mg of sulphanilamide powder was dissolved in 1 ml of 2.5% phosphoric acid.
2. 0.1% naphthylene diamide dihydrochloride : 0.1gm of the dry powder was dissolved in 100 ml of 2.5% phosphoric acid or 1 mg of dry powder was dissolved in 1ml of 2.5% phosphoric acid.

The above two reagents were prepared fresh before performing the test.

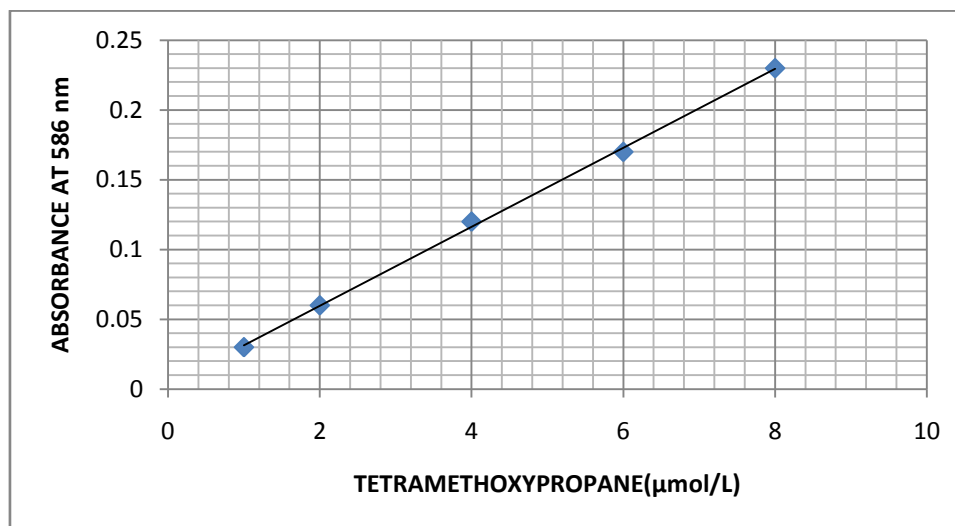
3. 2.5% phosphoric acid: 1.47ml of phosphoric acid was made upto 100ml by distilled water.

4. Nitrite stock standard(1000 $\mu$ mol per litre): 0.007 gm of nitrite powder was dissolved in one litre of distilled water.

The solution should be refrigerated to prevent oxidation

5. Nitrite substock(100  $\mu$ mol per litre): 1ml of stock standard was made upto 10 ml by distilled water.

By serial dilution of the substock we get different concentration ranging from 0 to 100 $\mu$ mol per litre.



**Figure 1** - Standard Curve for Malondialdehyde

#### Procedure

0.5 ml of the serum was taken & treated with 0.5 ml of 1:1 mixture of 1% sulphanilamide in 2.5% phosphoric acid & 1% Naphthylene diamide dihydrochloride in 2.5% phosphoric acid. The solution was allowed to stay at room temperature for 10 min. The intensity of pink colour was measured spectrophotometrically at 570nm.

#### Estimation of Ascorbic Acid In Plasma (Roe And Kuether, 1943; Roe, 1961<sup>12</sup>)

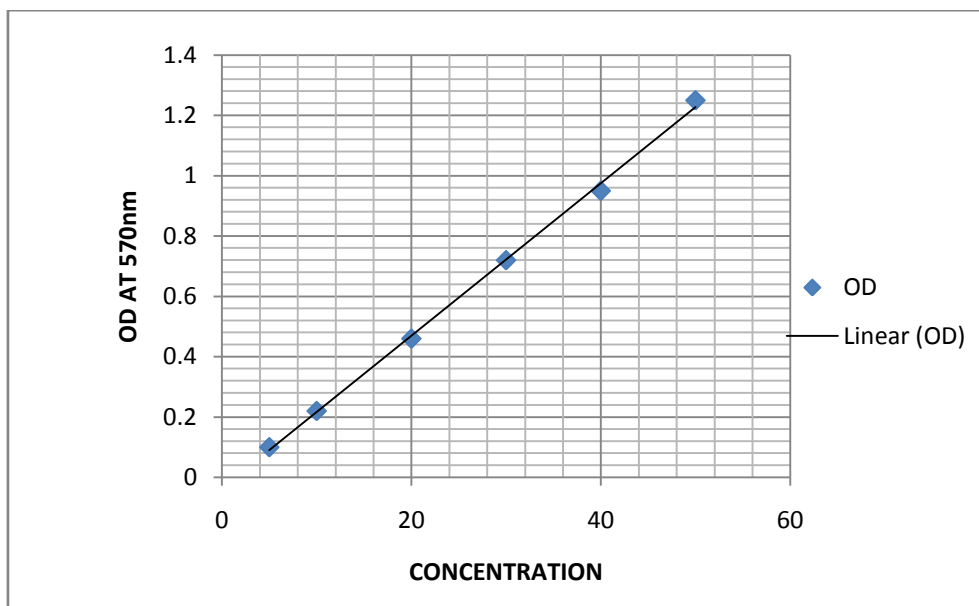
**Principle**-The Ascorbic acid is converted to dehydro ascorbic acid by shaking with norit and this is then coupled with 2,4 - DNPH in presence of thiourea as a mild reducing agent. Sulphuric acid then converts the DNPH hydrozone into a red compound which is assayed colorimetrically.

#### Reagents:

1. TCA 6%
2. 2, 4-DNPH.
3. Acid washed norit.
4. 85% H<sub>2</sub>SO<sub>4</sub>

#### Procedure

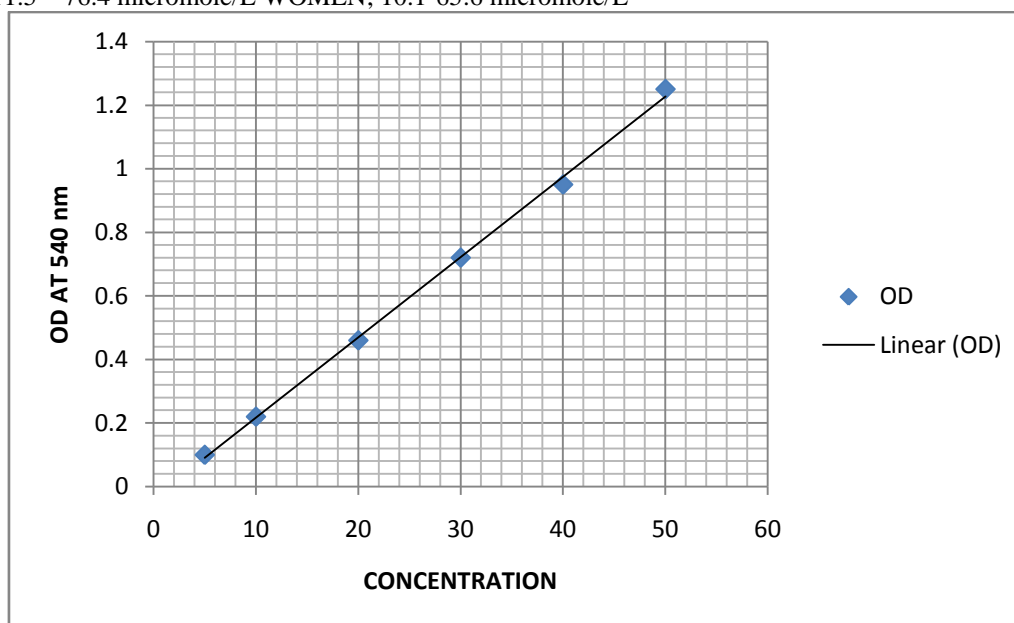
To 6ml of 6% TCA in a centrifuge tube, add 2ml of whole blood Or plasma slowly with constant stirring to produce a fine suspension. Stand for 5 minutes. Centrifuge and then add 0.3 gm of acid washed norit to the supernatant fluid. Cork & shake vigorously. Filter. This converts ascorbic acid to dehydro ascorbic acid. Measure out 2ml of the filtrate in to each of two test tubes. Keep 1 tube as blank, add to the other .5 ml of 2,4-DNPH reagent. Place in a water bath at 37°c for 3 hrs. Remove and place both test & blank tubes in ice cold water and add slowly 2.5 ml of 85% H<sub>2</sub>SO<sub>4</sub> drop by drop and taking about half a mt to do so, so that there is no appreciable rise in temperature. Finally add 0.5 ml of DNPH to the blank. Mix well. The contents of both tubes while still in iced H<sub>2</sub>O. Remove it after 30 mts read in the colorimeter at 540nm.



**Figure 2 - Standard Curve for Plasma Nitric Acid**

**REFERENCE VALUE:<sup>8</sup>**

MEN: 11.5 – 76.4 micromole/L WOMEN; 10.1-65.6 micromole/L



**Figure 3 - Standard curve for plasma vitamin c**

**REFERENCE VALUE**

Normal plasma values 0.6-1.6 mg/dl.

**IV. Results And Analysis**

The present study was carried out in the department of biochemistry, Vinayaka missions medical college & hospital, karaikal.. The biochemical investigations were done on both cases and controls.

The results obtained are presented as follows:

**TABLE-1: GENDER WISE DISTRIBUTION IN THE SUBJECTS STUDIED:**

GROUPS	MALE	MEDIAN AGE	FEMALE
HYPERTENSIVE CASES(n=80)	49(61%)	48	31(39%)
CONTROLS (n=51)	30(59%)	46	21(41%)

In table 1 shows 80 hypertensive subjects were studied against 51 apparently healthy, normotensive subjects. The above table shows in both the subjects, males were predominant. The mean age of hypertensives was around 48years. The mean age for controls was 43 years.

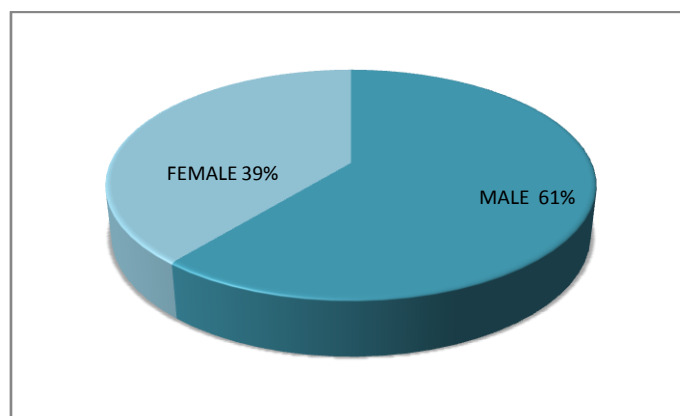


Figure 1-Genderwise distribution of cases

Parameters	Hypertensive CASES(n=80)	Controls (n=51)	t value	p value
Fasting Blood Sugar (mg/dl)	82.2 ± 8.7 (68-104)	79.7 ± 7.4 (68-93)	0.08	0.936(NS)
Serum Urea (mg/dl)	27.1 ± 6.6 (16-42)	24.6 ± 5.3 (15-34)	0.015	0.98(NS)
Serum Creatinine (mg/dl)	0.9 ± 0.1 (0.7-1.2)	0.8 ± 0.1 (0.7-1.1)	0.01	0.99(NS)

**Table-2:** Routine Biochemical parameters in Hypertensives and Controls:

This table 2 shows, the routine biochemical parameters in both groups studied. The fasting blood sugar, serum urea and creatinine in hypertensive cases and controls were observed within normal reference range and were not statistically significant ruling out diabetes, renal pathology.

Classification	Cases(n=80)
Pre-Hypertension (SBP120-139, DBP-80-89)	6(8%)
Stage I (SBP140-159, DBP 90-99)	49(61%)
StageII (SBP≥160, DBP≥100)	25(31%)

**Table-3:** Distribution of cases based on classification of Hypertension:

In table 3 study, hypertensive cases were classified based on JNC-7 classification. Out of 80 hypertensive cases, almost more than half of the cases (61%) were in stage II hypertension.

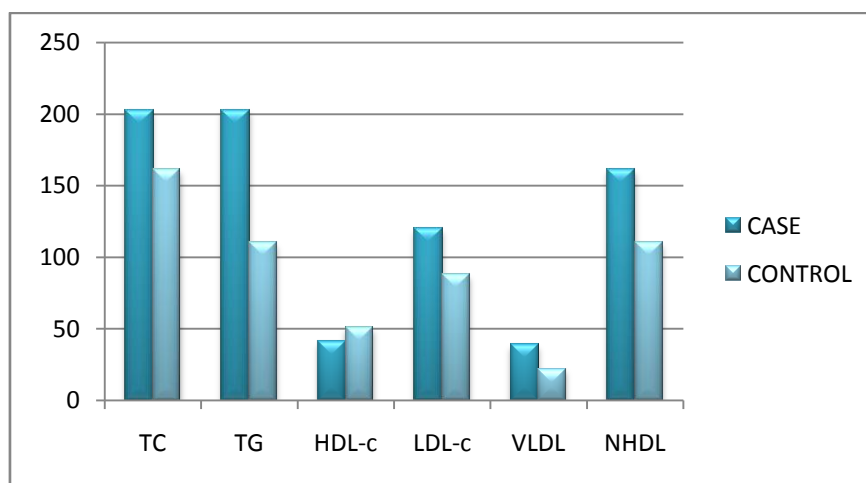
Parameters (mg/dl)	Hypertensives(n=80) Mean±SD	Controls(n=51) Mean±SD	t value	p value
TC(mg/dl)	203.1 ± 29.1 (150-285)	161.9 ± 24.6 (108-200)	2.421	0.01
TG(mg/dl)	203.2 ± 45.1 (128-340)	110.4± 27.6 (58-182)	2.400	0.01*
HDL-c(mg/dl)	41.3 ± 5.5 (25-50)	51.3 ± 7.1 (32-63)	3.296	0.001*
LDL-c(mg/dl)	120.8 ± 28.1 (54-198)	88.5 ± 24.8 (33-134)	4.529	0.0001*
VLDL(mg/dl)	39.1 ± 9.0 (26-69)	22.1 ± 5.5 (12-36)	9.87	0.0001*
N.HDL-c (mg/dl)	161.9 ± 28.8 (111-240)	110.6 ± 26.3 (53-161)	2.776	0.006*

**Table- 4** - Lipid Profile In Hypertensives and Controls

This table 4 shows the serum lipid profile in both the Subjects. In the hypertensive cases, the serum TC, TG, LDL-c, VLDL-c and N.HDL-c were definitely on the higher side as compared to the controls who were in the normal reference range. The difference of these two subjects were statistically significant with p value 0.01, 0.01, 0.0001, 0.0001 and 0.006 respectively. The serum HDL-c was declined in cases as compared to controls and was statistically significant with p value of 0.001.

The serum lipid profile in the cases were markedly elevated compared to the controls. The serum TC at  $203.1 \pm 29.1$  is marginally above the upper reference limit signifying tendency of hypertensives to be hypercholesterolemic.

As regards, the serum TG, the hypertensives are distinctly hypertriglyceridemic at  $203.2 \pm 45.1$  which is much above the upper reference limit. The HDL-c in hypertensive group is below the lower reference limit. The other lipid profile LDL-c, VLDL are distinctly elevated.



**Figure 2-** Lipid Profile In Hypertensives and Controls

Parameters	Hypertensive Cases(n=80)	Control Groups(n=51)	t value	p value
TC:HDL-c Mean± SD	5.0 ± 1.6 (2.6-8.8)	2.2 ± 0.7 (1.0-4.7)	8.321	0.0001
LDL-c:HDL-c Mean± SD	3.0 ± 0.9 (1.4-5.6)	1.7 ± 0.7 (0.6-4.1)	5.036	0.0001

**Table-5 -** Lipid Risk Ratios in Hypertensives and Controls

This table 5 shows that in cases the lipid risk ratio and TC:HDL-c were definitely elevated than the normal reference range as compared to controls and the difference is extremely significant at p value of 0.0001. The LDL: HDL-C were also observed in the higher range expecting the cases to a higher cardio vascular disease risk as compared to controls and the difference is extremely significant at p value of 0.0001. In control groups(51), the lipid risk ratios were within the normal reference range.

Parameters	Hypertensive cases(n=80)	Control groups(n=51)	t value	p value
TC:HDL-c Mean± SD	5.0 ± 1.6 (2.6-8.8)	2.2 ± 0.7 (1.0-4.7)	8.321	0.0001
LDL-c:HDL-c Mean± SD	3.0 ± 0.9 (1.4-5.6)	1.7 ± 0.7 (0.6-4.1)	5.036	0.0001

**Table 6 -** Lipid Risk Ratios in Hypertensives and Controls:

This table 6 shows that in cases the lipid risk ratio and TC:HDL-c were definitely elevated than the normal reference range as compared to controls and the difference is extremely significant at p value of 0.0001. The LDL: HDL-C were also observed in the higher range expecting the cases to a higher cardio vascular disease risk as compared to controls and the difference is extremely significant at p value of 0.0001. In control groups(51), the lipid risk ratios were within the normal reference range.

Group BMI (kg/m <sup>2</sup> )	S. Uric Acid Mean± SD (mg/dl)	Plasma Mda Mean±SD (µmol/l)	Plasma Vit C Mean±SD (mg/dl)	Plasma Nitric Oxide Mean±SD (µmol/l)
18-23	6.9±1.3	5.7±1.6	0.7±0.2	38.0±4.9
23-25	6.1±1.0	4.8±1.3	0.7±0.1	37.5±4.1
25-28.5	7.1±1.4	5.8±1.4	0.6±0.1	37.1±9.0
28.5-32.5	7.1±1.1	5.9±1.3	0.6±0.1	35.6±5.9
32.5-37.5	7.7±0.4	6.2±0.2	0.6±0.1	32.6±7.5

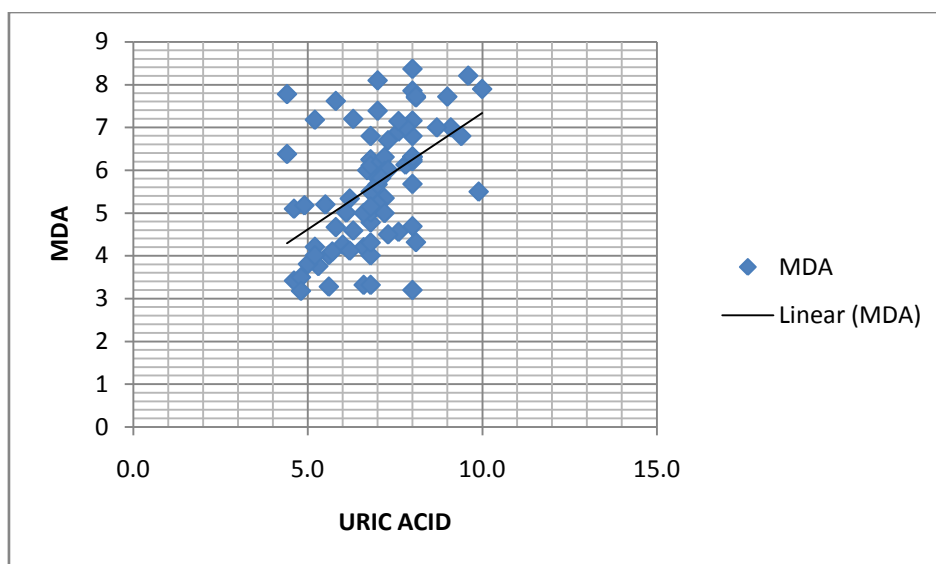
**Table 7 -** A study of Oxidants & Antioxidant parameters based on their BMI

In table 7, the oxidative stress parameter and anti oxidant levels were studied based on their BMI. The serum uric acid & the plasma MDA in the hypertensive cases were gradually elevated as the BMI increases. The antioxidant plasma vit C & plasma nitric oxide were shown the gradual decline as the BMI increases. From this study it was observed that the oxidative stress parameters were markedly elevated in class III obese subjects of the cases and there was no such distinct decrease in the anti oxidant levels.

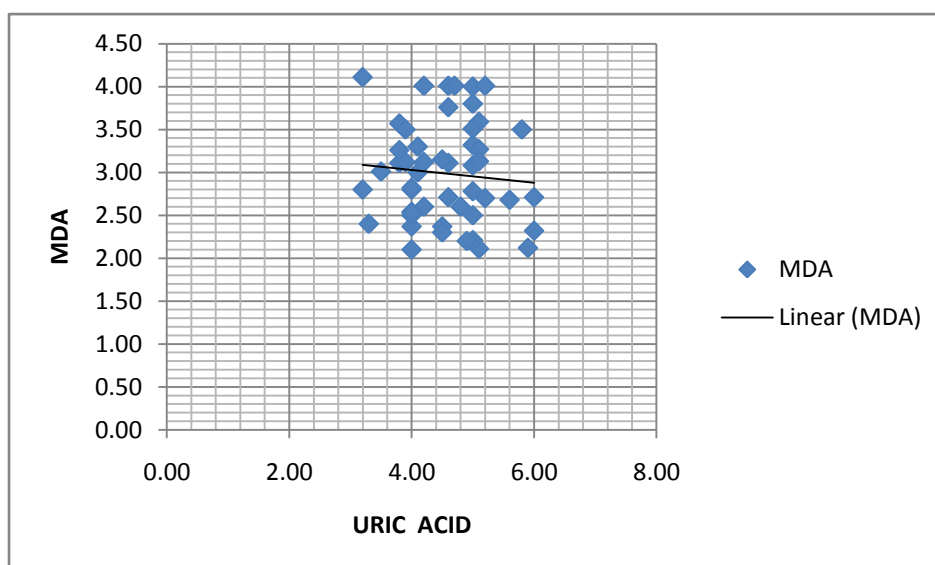
Group	Serum Uric Acid (mg/dl)	Plasma MDA (μmol/l)	r Value	p Value
CASES(n=80)	6.9±1.3	5.7 ± 1.4	+0.495	0.0001
CONTROLS(n=51)	4.6±0.6	3.0 ± 0.6	-0.088	0.31

**Table 8:** Correlation of Serum Uric Acid with Plasma MDA in the Group Studied

In table 8, the serum uric acid is positively correlated to the MDA, the marker of lipid peroxidation more pronounced in hypertension. The serum uric acid in cases were showing positive correlation and was extremely statistically significant with p value of 0.0001. The controls were not showing any significant correlation.



**Figure 3 -** Correlation of Serum Uric Acid with Plasma MDA in Cases



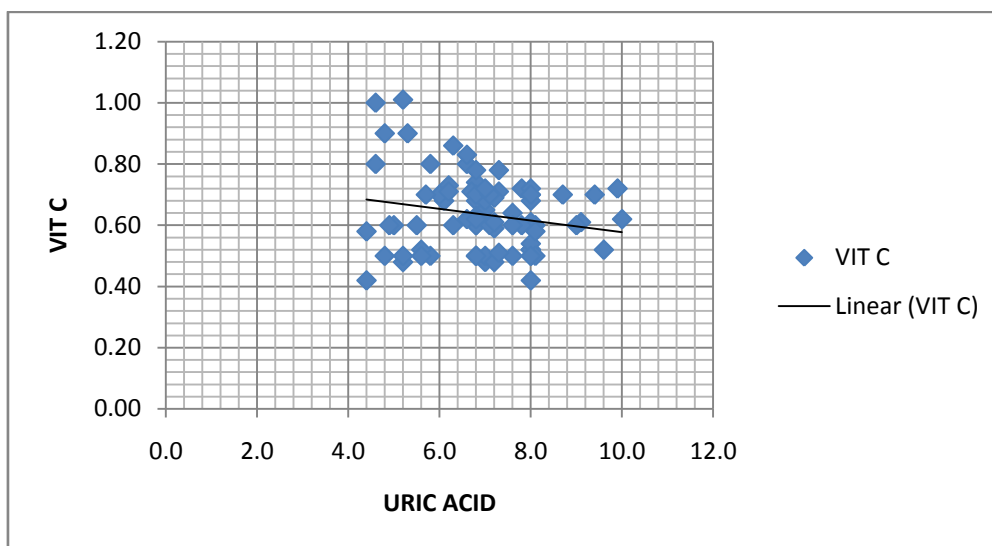
**Figure4 -** Correlation of Serum Uric Acid with Plasma MDA in Controls

Group	SERUM Uric Acid MEAN±sd (mg/dl)	Plasma Vit C Mean±sd (mg/dl)	r Value	p Value
CASES(n=80)	6.9±1.3	0.7±0.2	-0.38	0.0005
CONTROLS (n=51)	4.6±0.6	1.0 ± 0.2	0.1	0.37

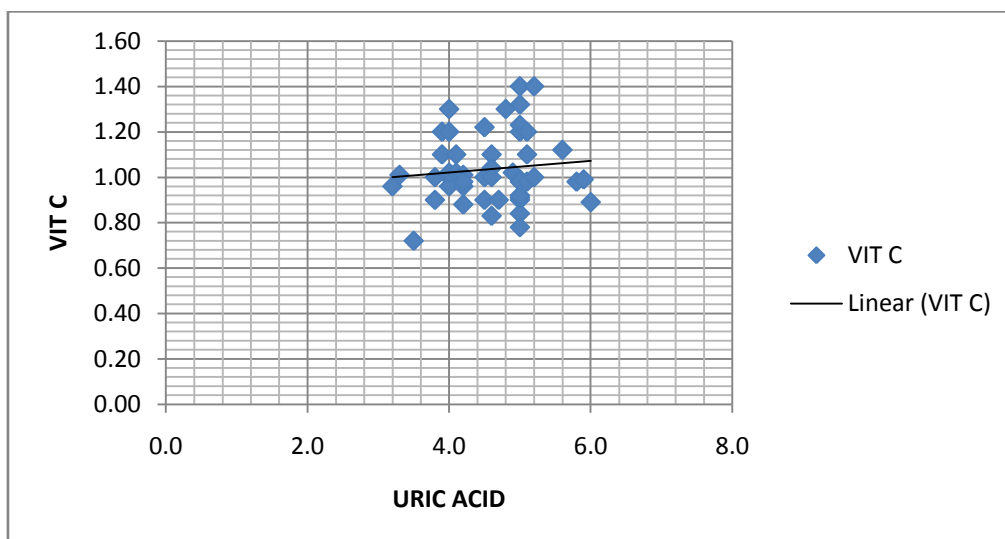
**Table 9-** Correlation Of Serum Uric Acid with Vit C in the Subjects Studied

This table 9 shows that the oxidative parameter the serum uric acid was negatively correlated the anti oxidant vit C and the difference was statistically significant at p value of 0.0005.

In controls, there was no correlation and the difference was not significant.



**Figure 5 - Correlation Of Serum Uric Acid With Vit C In Cases**



**Figure 6 - Correlation of Serum Uric Acid with Vit C in Controls**

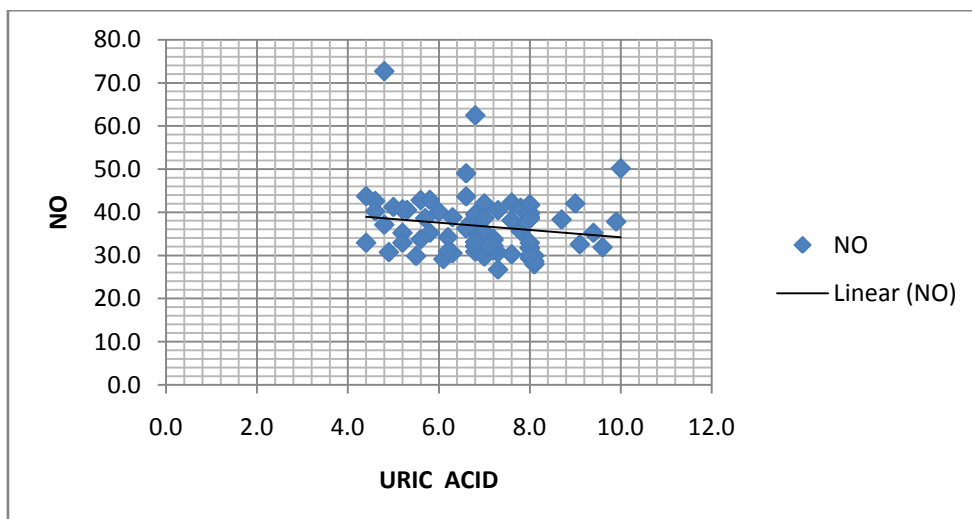
Group	Serum Uric Acid	Plasma Nitric Oxide	r Value	p Value
Cases(n=80)	6.9±1.3	36.8 ±7.0	-0.155	0.07
Controls(n=51)	4.6±0.6	77.0 ± 14.4	-0.064	0.5

**Table 10 - Correlation of Serum Uric Acid with Plasma Nitric Oxide**

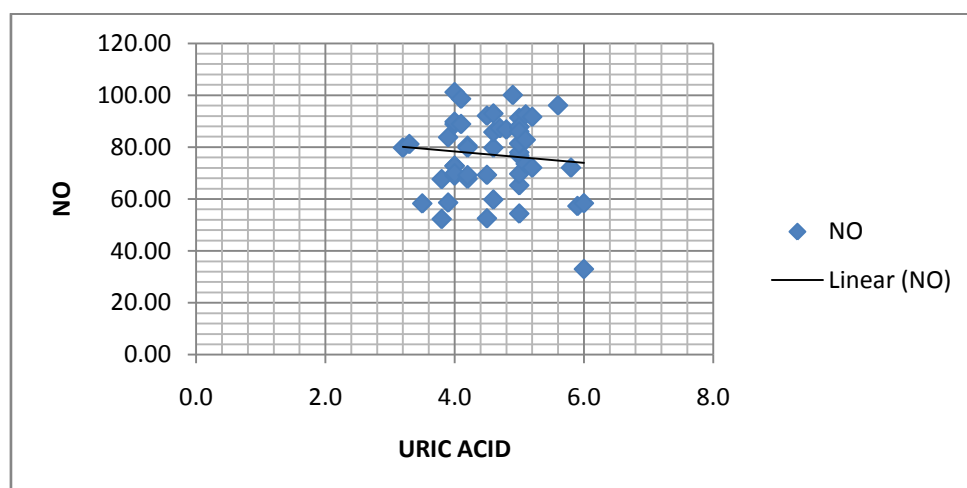
This table 10 shows that the correlation of the oxidative stress parameter the serum uric acid with the antioxidant the plasma nitric oxide.

The serum uric acid was negatively correlated with plasma nitric oxide and the difference was showing insignificant.





**Figure 7** Correlation of Serum Uric Acid with Plasma Nitric Oxide in Cases



**Figure 8-** Correlation of Serum Uric Acid with Plasma Nitric Oxide in Controls

Parameters	r Value	p Value
TC	+0.424	0.0001
TG	+0.145	0.09
HDL-c	+0.175	0.04
LDL-c	+0.369	0.0001
VLDL-c	+0.184	0.03
N.HDL-c	+0.395	0.0001

**Table 11** - Correlation of Serum Uric Acid with Lipid Profile in the Subjects Studied

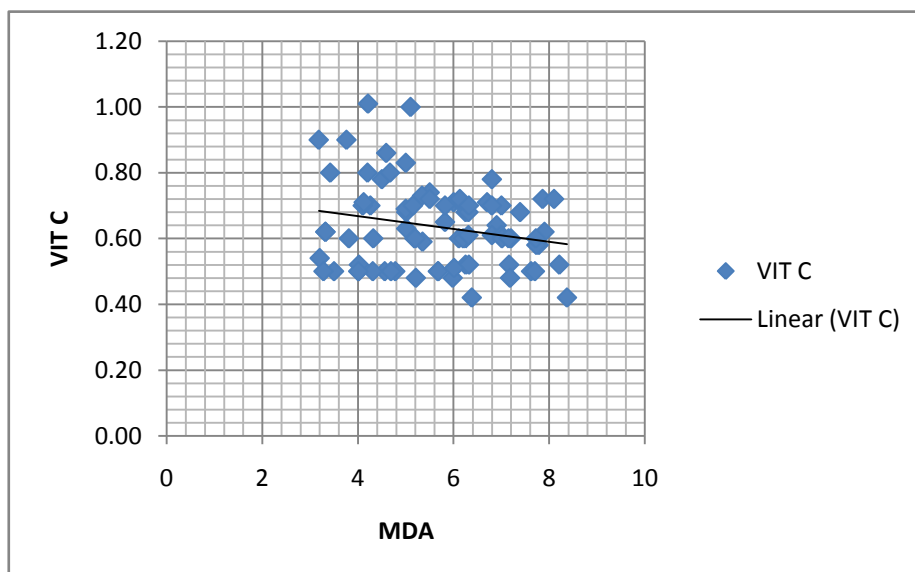
In table 11 shows, the serum uric acid is elevated in essential hypertension. In essential hypertension the serum lipid profile fractions are elevated causing dyslipidaemia. So the serum uric acid was positively correlated to the serum lipid profile. The serum TC was showing positive correlation with serum uric acid and showed statistically significant with p value of 0.0001. The TG was shown positive correlation but was not statistically significant with serum uric acid.

The other lipid profile fractions, the LDL-c, VLDL-c and N.HDL-c were constantly elevated and was shown positive correlation with serum uric acid and was statistically significant with p value 0.0001, 0.03 and 0.0001 respectively. It is observed from this study that the hypertension produces hypercholesterolemia, hypertriglyceridemia and elevated LDL-c, VLDL-c and N.HDL-

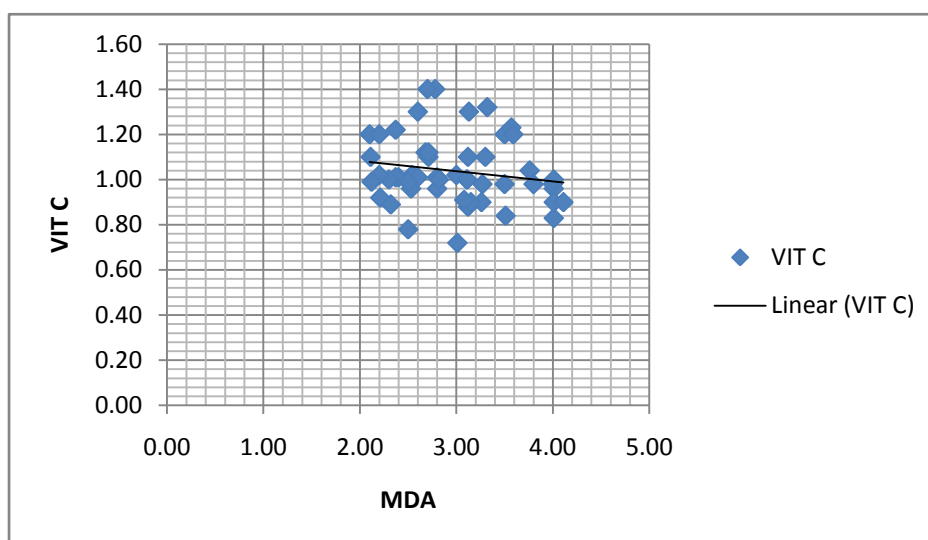
Group	Plasma MDA Mean±SD mg/dl	Plasma Vit C Mean±SD (mg/dl)	r Value	p Value
CASES(n=80)	5.7 ± 1.4	0.6 ± 0.2	-0.376	0.0006*
CONTROLS (n=51)	3.0 ± 0.6	1.0 ± 0.2	-0.2	0.233

**Table-12** - Correlation of Plasma MDA with Vit C in the Subjects studied

This table 12 shows the correlation of plasma MDA with plasma Vit C in both group. The oxidative stress parameter plasma MDA was negatively correlated with the anti oxidant plasma vit c and the difference was statistically significant at p value of 0.0006. The control group was negatively correlated and was insignificant. It was observed that the oxidative stress parameter, MDA increased, the anti oxidant level is decreased in hypertension.



**Figure 9:** Correlation of Plasma MDA with Vit C in the Cases

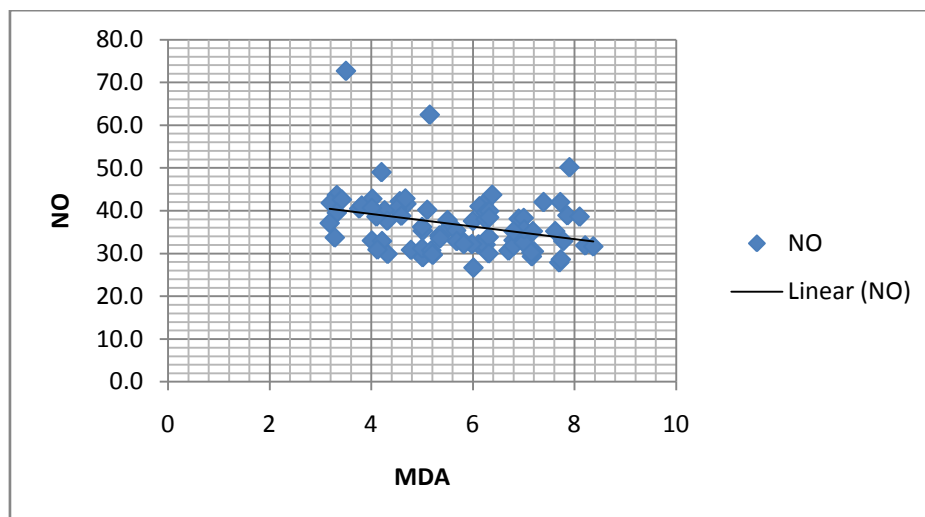


**Figure 10-** Correlation of Plasma MDA with Vit C in the Controls

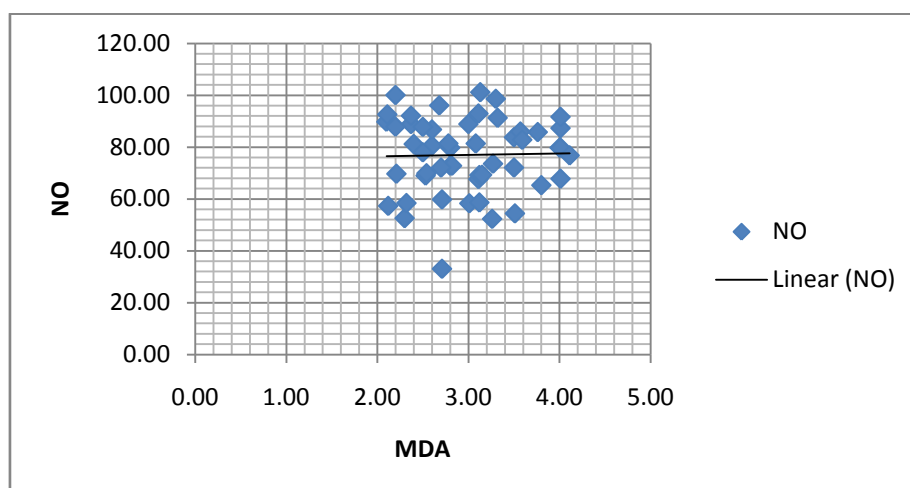
Group	Plasma MDA Mean±SD ( $\mu\text{mol/l}$ )	Plasma Nitric Oxide Mean±SD ( $\mu\text{mol/l}$ )	r VALUE	p VALUE
Cases(n=80)	5.7 ± 1.4	36.8 ± 7.0	-0.294	0.008*
Controls (n=51)	3.0 ± 0.6	77.0 ± 14.4	0.023	0.87

**Table 13 -** Correlation of Plasma MDA with Plasma Nitric Oxide

In table 13, the correlation of oxidative parameter the plasma MDA and anti oxidant plasma nitric oxide was studied. The plasma MDA was negatively correlated to plasma nitric oxide and the difference was statistically significant at p value of 0.008.



**Figure 11:** Correlation Of Plasma Mda With Plasma Nitric Oxide In Cases



**Figure 12:** Correlation Of Plasma MDA With Plasma Nitric Oxide In Controls

### V. Conclusions And Suggestions

In this study we found high serum uric acid, lipid peroxidation, dyslipidemia and increased body mass index(BMI) with declined plasma vitamin C, plasma nitric oxide levels. The serum uric acid reflected the renal status as well as behaves as a pro-oxidant in excess.<sup>3</sup> It was also correlated with lipid profile and lipid peroxidation. The increased serum uric acid, lipid peroxidation along with increased body mass index (BMI) and reduced anti oxidant levels were observed in persons with significant modifying risk factors like dietary pattern and obesity in essential hypertension<sup>13</sup>. So early intervention can be made by modifying the life style, dietary habits and antioxidants supplementations and drugs aimed at treatment for hypertension as well as to prevent the complications.

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