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Biochemistry

A Correlative Study of Uric Acid, Malondialdehyde with Vitamin C Level in Essential Hypertension

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Original Research Article

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Abstract: 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 Hypertension (HTN) is a major modifiable risk factor for Cardio Vascular Disease. Also HTN produces imbalance between pro oxidants and antioxidants leading to increased oxidative stress, in turn leads to Atherosclerosis and Dyslipidemia. 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. 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 Malon di aldhyde (MDA) were estimated manually by Ester Bauer and SteinBerg method. Anti-oxidant parameters (i.e) Plasma vitamin C was estimated manually by Roe and Kuether method. 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 wall5. So, the aim of this study is to assess the level of oxidative stress parameters, with antioxidants parameter vitamin C and correlating the results with hypertension by analyzing selected samples through suitable methods 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. 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 Hypertesion.

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

INTRODUCTION

Hypertension is one of the leading causes of the global burden of disease. Hypertension doubles the risk of the cardio vascular diseases including coronary heart disease, congestive heart failure, ischemic and hemorrhagic stroke. HTN is the leading cause of morbidity and mortality in the industrial world as well as becoming a modern epidemic in the developing countries.

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 [1,9,10]. 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.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[2,6]. To assess the lipid peroxidation the breakdown products of lipid peroxides in plasma, the malondialdehyde (MDA) is measured. When the oxidative stress increases in hypertension the anti-oxidant defense mechanism in the body is decreased[3-5]. Vitamin C is a well-known antioxidant that has been shown to efficiently scavenge free oxygen radicals[7]. The overall goal of treating hypertension is to reduce HTN associated cardiovascular and renal morbidity and mortality.

AIMS AND OBJECTIVES

- To assess the level of oxidative stress markers, the serum uric acid and MDAwith anti-oxidants, plasma vit C level.
- To correlate the oxidative stress markers with the antioxidant level 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.

MATERIALS & 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 –manually by Esterbauer and Steinberg method 1989.
- Plasma Vit C –manually by Roe &Kutherford.

ASSAY OF MALONDIALDEHYDE

(Esterbauer and Steinberg method 1989)[7]

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

- A 7.6mM solution of 1-methly-2phenylindole (M.P.I) was prepared immediately prior to use in 33% methanol in acetonitrile.
- 650µl aliquot of M.P.I was placed in each test tube to which was added 200µl of plasma.
- 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.
- After incubation the tubes were chilled on ice bath and spun at 10,000 rpm for 5 minutes to remove debris.
- The absorbance at 586nm was measured and subtracted from the blank value obtained by replacing plasma with water.
- 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

$$\text{Plasma malondialdehyde (µmol/L)} = \frac{Abs\ S}{Abs\ T} \times \text{conc. of Std. (µmol/L)}$$

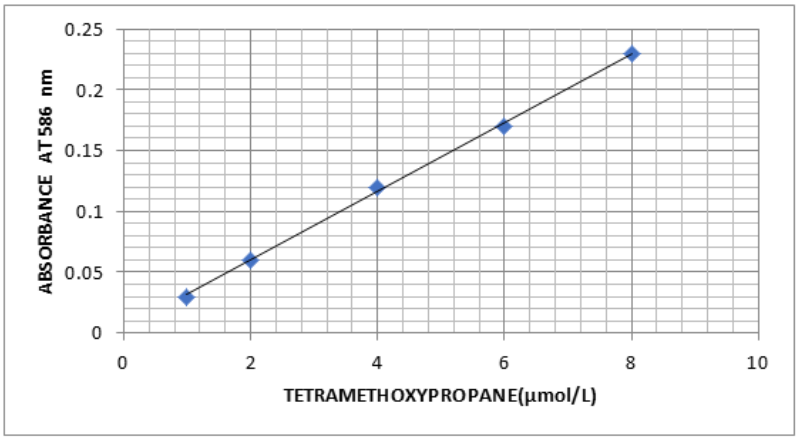

Fig-1: Standard Curve for Malondialdehyde

Estimation of Ascorbic Acid in Plasma [11]

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

- TCA 6%
- 2, 4-DNPH.
- Acid washed norit.
- 85% H_2SO_4

Procedure

To 6ml of 6% TCA in a centrifuge tube, add 2ml of whole blood 0r 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_2SO_4 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 0f DNPH to the blank. Mix well. The contents of both tubes while still in iced H_2O. Remove it after 30 mts read in the colorimeter at 540nm.

REFERENCE VALUE

Normal plasma values 0.6-1.6 mg/dl.

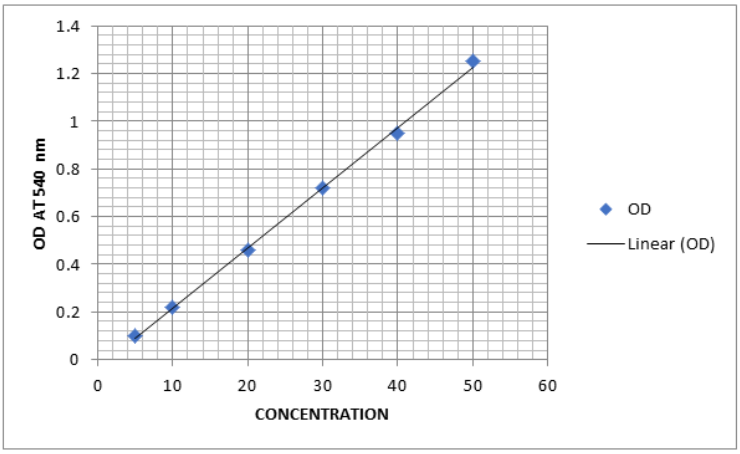

Fig-2: Standard curve for plasma vitamin c

RESULTS AND ANALYSIS

The present study was carried out in the department of biochemistry, Vinayaka mission's medical college & hospital, karaikal. The biochemical investigations were done on both cases and controls. The results obtained are presented as follows:

Table-1: Routine Biochemical parameters in Hypertensives and Controls

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)

This table 1 show, 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.

Table-2: Lipid Profile in Hypertensives and Controls

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*

This table 2, 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 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.

Table-3: Correlation of Serum Uric Acid with Plasma MDA in the Group Studied

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

In table 3, 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.

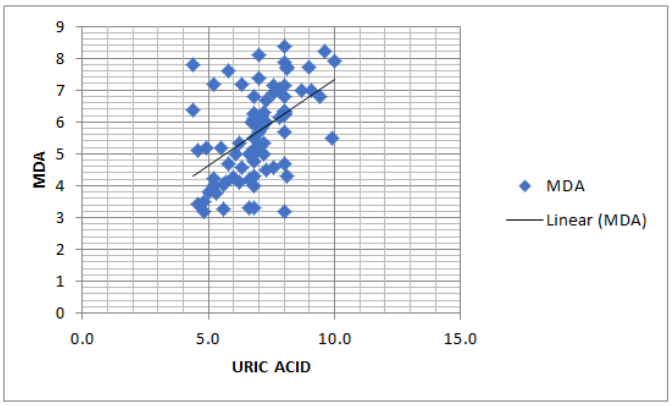

Fig-3: Correlation of Serum Uric Acid with Plasma MDA in Cases

Table-4: Correlation of Serum Uric Acid with Vit C in the Subjects Studied

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

This table 4 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.

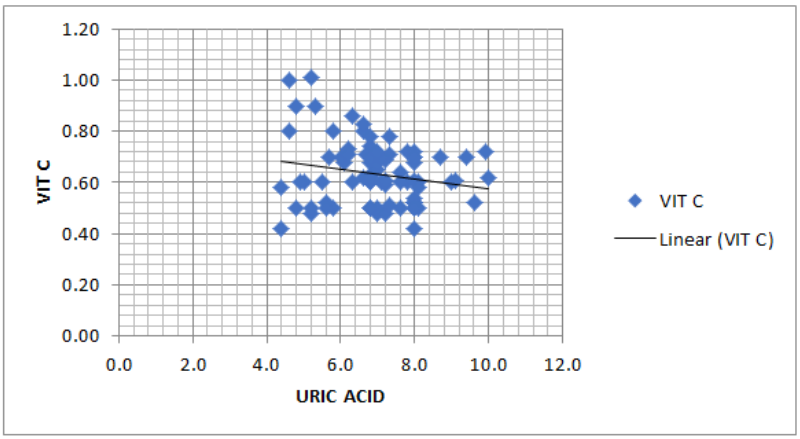

Fig-4: Correlation of Serum Uric Acid With Vit C In Cases

Table-5: Correlation of Serum Uric Acid with Lipid Profile in the Subjects Studied

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

In table 5 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 and the lipid profile showed positive correlaton and was significantly significant in cases.

This table 6, shows the correlation of plasma MDA with plasma Vit C in both group.

Table-6: Comparison of Plasma MDA with Vit C in the Subjects studied

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

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.

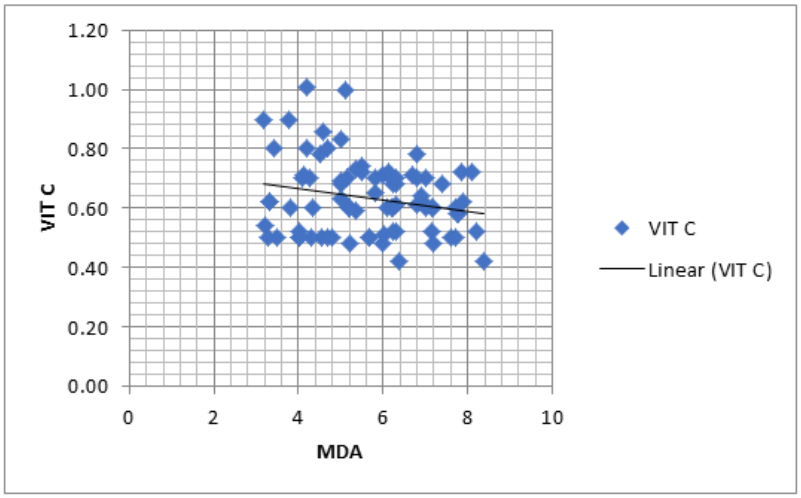

Fig-5: Correlation of Plasma MDA with Vit C in the Cases

CONCLUSIONS AND SUGGESTIONS

In this study we found high serum uric acid, lipid peroxidation, dyslipidemia with declined plasma vitamin C level. The serum uric acid reflected the renal status as well as behaves as a pro-oxidant in excess[3]. It was also correlated with lipid profile and lipid peroxidation. The increased serum uric acid, lipid peroxidation along with reduced anti-oxidant levels were observed in persons with significant modifying risk factors like dietary pattern and obesity in essential hypertension [2,6]. 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[10].

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