

## **Correlative association of Interleukin-6 with malondialdehyde in prehypertensive and hypertensive subjects**

\*Vijaya Bhaskar.M<sup>1</sup>, Prabhakara Rao.P<sup>2</sup>, Balu Mahendran.K<sup>3</sup>

1. Professor & Head, Department Of Biochemistry, Mamata Medical College, Khammam, Telangana State, India

2. Associate professor, Sri Venkateswara Medical College, Tirupati, Andhra Pradesh, India.

3. Ph.D. Scholar, Department of Biochemistry, Rajah Muthiah Medical College & Hospital, Annamalai University, Annamalainagar, Tamil Nadu, India

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### **Abstract**

**Background:** Hypertension is a major health burden throughout the world because of its high prevalence and its association with increased risk for coronary artery disease (CAD). Advances in the diagnosis and treatment of hypertension have played a major role in recent dramatic declines in coronary artery disease and stroke mortality in developing countries. Inflammatory process is the important mechanism in the pathophysiology of hypertension and Coronary artery disease. The identification of these inflammatory markers leading to the activation of inflammation should contribute to the development of specific therapeutic approaches to treat hypertension.

**Aim:** The present study was conducted to assess Interleukin-6 (IL-6) levels a marker of systemic inflammation in newly diagnosed prehypertensive and hypertensive patients, its association with malondialdehyde a generalized lipid peroxidation marker and serum lipids.

**Materials and methods:** Seventy hypertensive patients in the age group of 30 to 50 years were selected for this study and 36 healthy age matched subjects were selected as a control group. Serum IL-6 was assessed by ELISA, malondialdehyde (MDA) was assessed by Thiobarbituric Acid Reactive Substances (TBARS) method and routine investigations were done by ERBA EM-360 fully automated analyzer.

**Results:** The serum IL-6 was significantly elevated in hypertensive subjects compared with controls, and there was also significant difference between prehypertension and hypertension subjects. Serum IL-6 levels were positively correlated with malondialdehyde, Cholesterol, TGL, LDL and negatively correlated with HDL.

**Conclusion:** Interleukin-6 levels are elevated in prehypertension and hypertension subjects compared with controls. The elevation of IL-6 levels in prehypertension indicates the process of low systemic inflammation is involved in pathophysiology of prehypertension itself. Development of specific therapeutic strategies and lifestyle measures should be initiated early to reduce cardiovascular morbidity and mortality in hypertensive patients.

**Keywords:** Hypertension, Interleukin-6(IL-6), Malondialdehyde (MDA)

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### **I. Introduction**

Hypertension is a major health burden throughout the world because of its high prevalence and its association with increased risk of coronary artery diseases (CAD). Advances in the diagnosis and treatment of hypertension have played a major role in recent dramatic declines in CAD and stroke mortality in developing countries. The prevalence of hypertension in the last decade has increased from 2% to 25% among urban residents and from 2% to 15% among the rural residents of India [1]. An elevated arterial pressure is asymptomatic, readily detectable and usually easily treatable often leads to lethal complications, if left untreated. Despite the widely recognized dangers of uncontrolled hypertension, the disease remains inadequately treated, as a consequence, cardiovascular risk remains high among the majority of hypertensive patients [2, 3]. Since the last decade has shown an increase in the relevance of inflammation and its mediators in vascular biology, the role of inflammation during atherogenesis is now a matter of intense investigation [4]. Basic science studies proved that elements belonging to both innate and adaptive immunity can be involved in the formation, progression and complication of atherosclerosis in hypertensive patients [5, 6]. Hypertension acts as a major determinant of endothelial dysfunction and vascular damage, promoting inflammatory activation of endothelial cells, recruitment of inflammatory cells in the arterial wall and activation of vascular resident elements. The link between hypertension and inflammation emerges from the recent studies showing that circulating inflammatory molecules, such as interleukin-6 (IL-6) and C-reactive protein (CRP) are increased in hypertensive patients and their levels predict the onset of hypertension [7]. IL-6 is a proinflammatory cytokine produced by the activated macrophages, endothelial cells and vascular smooth muscle cells and is capable of promoting the secretion of other cytokines. To promote atherogenesis, IL-6 is reported to stimulate monocyte

chemo attractant protein-1 secretion from macrophages and is associated with the increased expression of cell adhesion molecules [8, 9, 10]. Additionally, IL- 6 has also been reported as a stimulator of the proliferation and migration of vascular smooth muscle cells [11]. So the objective of this study was to evaluate serum IL-6 level in prehypertensive and hypertensive subjects, its association with malondialdehyde a generalized lipid peroxidation marker and serum lipids.

## II. Materials And Methods

A total of 70 freshly diagnosed hypertensive patients of both sexes aged between 30-50 years attending Department of Medicine, Mamata Medical College, Khammam, Telangana state, India were selected for our study after approval of Institutional Human ethics committee. The included hypertensive patients were categorized into two groups according to Eighth Joint National Committee guidelines (JNC-8) .Group I (pre hypertension) - 35 patients with systolic blood pressure (120-139) or diastolic blood pressure (80-89). Group II (Hypertension) - 35 patients with systolic blood pressure ( $\geq 140$ ) or diastolic blood pressure ( $\geq 90$ ). The clinical characteristics of patients (age, gender, height, body weight, duration of disease, waist and hip circumferences) were collected. We excluded the patients based on the following criteria: subjects with diabetes mellitus, other cardiovascular diseases, renal dysfunction, chronic alcoholics, smokers, pregnant women and patients on medication such as antioxidant supplements and lipid lowering drugs. Thirty six healthy sex and age matched subjects were selected as controls.

**Biochemical analysis:** Fasting blood samples were obtained from the subjects. Blood samples were centrifuged at  $2000 \times g$  for 10 min. Samples were analyzed for glucose, lipid profile (Total Cholesterol, HDL, triglycerides), urea, creatinine using by ERBA EM-360 fully automated analyzer. Serum IL-6 was assessed by Enzyme Linked Immuno Sorbent Assay (ELISA).Serum malondialdehyde (MDA) was estimated by Thiobarbituric Acid Reactive Substances (TBARS) method [12].Serum cholesterol estimated by Cholesterol oxidase/ Peroxidase (CHOD/POD) method, triglycerides estimated by Glycerol phosphate oxidase/ Peroxidase (GPO/POD), HDL cholesterol estimated by Direct Enzymatic method and LDL cholesterol was calculated by using Friedwald formula.

**Statistical analysis:** Statistical analysis were carried out with SPSS 20.0. Values were expressed as mean  $\pm$  standard deviation, p value  $< 0.05$  was considered statistically significant. Normally distributed data were analyzed by using one-way ANOVA. The Pearson correlation test was used for correlation analysis.

## III. Results

**Table 1: Baseline parameters in controls and Hypertensive subjects.**

Parameters	Controls (n=36)	Group I Prehypertension (n=35)	Group-II Hypertension (n=35)
Age	40.4 $\pm$ 5.2	40.8 $\pm$ 4.6	39.8 $\pm$ 4.4
Body mass index	24.0 $\pm$ 1.3	25.8 $\pm$ 3.1 <sup>a*</sup>	26.06 $\pm$ 2.7 <sup>b**</sup>
Waist/Hip ratio	0.90 $\pm$ 0.05	0.92 $\pm$ 0.06	0.93 $\pm$ 0.04 <sup>b*</sup>
Males (%)	82.8	85.7	88.5
Females (%)	17.2	14.3	11.5
Systolic BP (mm Hg)	111.4 $\pm$ 5.6	131.2 $\pm$ 5.8 <sup>a**</sup>	152.0 $\pm$ 11.9 <sup>b**,c**</sup>
Diastolic (mm Hg)	73.4 $\pm$ 3.1	85.1 $\pm$ 4.1 <sup>a**</sup>	99.8 $\pm$ 5.6 <sup>b**,c**</sup>

Data are expressed as mean  $\pm$ SD, \*\*p<0.001,\*p<0.05 was considered statistically significant.

a= comparison between Control and Prehypertension subjects

b=comparison between Control and Hypertension subjects

c=comparison between Prehypertension and Hypertension subjects

**Table 2: FPG, Lipid profile, Urea, Creatinine, Malondialdehyde and IL-6 Levels in controls and Hypertensive subjects**

Parameters	Controls (n=36)	Group I Prehypertension (n=35)	Group-II Hypertension (n=35)
FPG(mg/dl)	84.0±7.0	86.3±10.2	88.1±13.4
Serum cholesterol (mg/dl)	166.3±8.6	193.1±16.4 <sup>a**</sup>	200.7±15.7 <sup>b**c*</sup>
Serum Triglycerides (mg/dl)	100.6±14.5	145.8±46.4 <sup>a**</sup>	147.9±44.6 <sup>b**</sup>
HDL cholesterol (mg/dl)	43.09±3.1	39.5±2.6 <sup>a**</sup>	39.3±2.4 <sup>b**</sup>
LDL cholesterol (mg/dl)	116.6±9.6	134.8±24.2 <sup>a**</sup>	146.1±22.4 <sup>b**c*</sup>
Serum Urea(mg/dl)	24.7±4.3	25.6±6.2	27.1±8.2
Serum Creatinine(mg/dl)	0.6±0.19	0.8±0.25 <sup>a**</sup>	0.9±0.21 <sup>b**</sup>
Malondialdehyde (µ mol/L)	1.68±0.51	2.96±0.44 <sup>a**</sup>	3.34±0.76 <sup>b**c*</sup>
Interleukin-6 (pg/ml)	0.93±0.26	2.16±0.66 <sup>a**</sup>	3.54±0.8 <sup>b**c**</sup>

Data are expressed as mean ±SD, \*\*p<0.001, \*p<0.05 was considered statistically significant.

a= comparison between Control and Prehypertension subjects

b=comparison between Control and Hypertension subjects

c=comparison between Prehypertension and Hypertension subjects

**Table 3: Correlation between Serum IL-6 &measured parameters in Hypertensive subjects**

Parameters	Correlation Coefficient(r)
Malondialdehyde	0.623**
Cholesterol	0.577**
TGL	0.475**
HDL	-0.422**
LDL	0.562**
BMI	0.317**
Waist /Hip ratio	0.185
Systolic BP	0.826**
Diastolic BP	0.841**

\*\*Correlation is significant at the 0.01 level (2-tailed).

#### IV. Discussion

There are continuing challenges in various aspects of hypertension research. A major problem and societal challenge is the rapidly increasing prevalence of hypertension and contributing to widespread cardiovascular morbidity and mortality in India. Hypertension is associated with inflammation; however, whether inflammation is a cause or effect of hypertension is not well understood. The purpose of this study was to explore IL-6 levels in hypertensive patients compared with healthy individuals and to find out its association with malondialdehyde and serum lipids. In the present study, we observed that serum IL-6 levels was significantly increased in both prehypertensive and hypertensive patients, and it also had significant positive correlation with malondialdehyde. There are a number of studies which have examined the association of IL-6 with CAD and their risk factors and those which found a significant association are numerous. IL-6 is identified as a potential predictor of future cerebrovascular events in patients with cardiovascular risk factors, including hypertension, diabetes mellitus, hyperlipidemia, history of smoking, arteriosclerosis (transient ischemic attack), stroke, CHD, or peripheral artery disease (PAD) [13]. Ridker et al., [14] in a 6-year follow-up study of healthy middle-aged men, have reported that baseline IL-6 levels of > 2.28 pg/ml are associated with 2.3 fold increased risk of future myocardial infarction. IL-6 is released in response to acute infections, chronic inflammation, obesity, and physiological stress. Increased IL-6 levels initiate the synthesis of acute phase reactants in the liver, endothelial activation, increased coagulation, stimulation of the hypothalamic hypophyseal-adrenal axis, and promotion of lymphocyte proliferation and differentiation [15].

Lipid peroxidation and lipid-derived oxidized products have been implicated in the pathogenesis of a variety of human diseases [16]. There is greater than normal lipid peroxidation and an imbalance in antioxidant status, suggesting that oxidative stress is important in the pathogenesis of hypertension [17]. Inflammation and oxidative stress thereby act as cooperative and synergistic partners in the pathogenesis of hypertension. As mentioned, inflammation is the primary immune response to eliminate pathogens or to repair tissue damage. Innate immune cells, such as neutrophils and macrophages, produce reactive oxygen species (ROS) such as superoxide and hydrogen peroxide in order to kill pathogens [18]. Nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase is a major source of ROS in immune cells and also in the vasculature [19]. Inflammatory processes continue until the pathogens are destroyed or the tissue repair process has been

completed. However, sustained inflammation can lead to an overproduction of ROS. Oxidative stress (defined as an imbalance between the production and breakdown of ROS) is a major cause of endothelial dysfunction, primarily through reducing NO bioavailability via the direct chemical reaction of superoxide with NO, resulting in the formation of peroxynitrite [20]. The reaction between superoxide and NO is faster than the breakdown of superoxide via superoxide dismutase [21, 22]. Furthermore, peroxynitrite formation may result in further impairment of NO levels and enhanced oxidative stress by inhibiting eNOS activity through oxidation of 4-tetrahydrobiopterin (BH4), a cofactor of eNOS. This leads to eNOS uncoupling, where eNOS produces superoxide instead of NO [23]. Excessive ROS levels can also induce cellular damage by interacting with DNA, lipids, and proteins which may further impair vascular structure and function [24].

In addition our study also exhibits disturbances in lipid profile as in the earlier studies. IL-6 levels shows strong positive correlation between malondialdehyde, BMI, total cholesterol, LDL, TGL and negative correlation with HDL. Dyslipidemia, one of the strong predictors of cardiovascular disease, causes endothelial damage and loss of physiological vasomotor activity [25-28]. The decrease in HDL-C could stimulate compensatory changes, as synthesis and accumulation of phospholipid-rich VLDL which binds bacterial products and other toxic substances, resulting in hypertriglyceridemia. The final consequence is an increased accumulation of cholesterol in cells. When the compensatory response (inflammation) is not able to repair injury, it turns into a harmful reaction, and the lipid changes will become chronic, either by repeated or overwhelming stimulus, enhancing the formation of atherosclerotic lesions [29]. In this regard, the current data support for the hypothesis that, all these factors are contributing elevated IL-6 levels and it has been regarded as an index of progressive coronary heart diseases in hypertensive patients.

Interleukin-6 levels are elevated in prehypertension and hypertension subjects compared with controls. The elevation of IL-6 levels in prehypertension indicates the process of low systemic inflammation is involved in pathophysiology of prehypertension itself. Development of specific therapeutic strategies and lifestyle measures should be initiated early to reduce cardiovascular morbidity and mortality in hypertensive patients.

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