

# Markers of Inflammation and Insulin Resistance in Age and Body Mass Index Matched Subjects with Prehypertension and Normotension

Ramkumar Thiyagarajan<sup>a</sup>, Pravati Pal<sup>a</sup>, Gopal Krushna Pal<sup>a</sup>, Senthil Kumar Subramanian<sup>b</sup>, Madanmohan Trakroo<sup>c</sup>, Zachariah Bobby<sup>d</sup> and Ashok Kumar Das<sup>e</sup>

<sup>a</sup> Department of Physiology, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India.

<sup>b</sup> Department of Physiology, Sri Venkateshwaraa Medical College Hospital and Research Centre, Puducherry, India.

<sup>c</sup> Department of Physiology, Mahatma Gandhi Medical College & Research Institute, Puducherry, India.

<sup>d</sup> Department of Biochemistry, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India.

<sup>e</sup> Department of Endocrinology, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India.

**Abstract-** Background: Hypertension, an important risk factor for cardiovascular disease (CVD), accounts for 57% and 24% of deaths due to stroke and coronary artery disease, respectively. Even blood pressure (BP) in prehypertension category (systolic BP 120-139 mm Hg and/or diastolic BP 80-89 mm Hg) hold 3 times more risk for CVD than normal BP (systolic BP <120 mm Hg and diastolic BP <80 mm Hg). We sought to compare the markers of inflammation and insulin resistance in age and body mass index (BMI) matched prehypertensive and normotensive subjects, and to evaluate the association of prehypertension BP status with markers of inflammation and insulin resistance.

Methods: A total of 572 participants in the age group 20-60 years of both gender without any known CVD from the community were recruited (Aug 2010 to Dec 2011). After considering the BP, inclusion and exclusion criteria and written informed consent, a total of 186 participants were grouped into prehypertensives (n=104) and normotensives (n = 82). We have measured basal physiological parameters, insulin resistance (HOMA-IR) and markers of inflammation, such as, C-reactive protein, tumor necrosis factor- $\alpha$  and interleukin-6.

Results: Markers of inflammation and insulin resistance were significantly elevated in prehypertensive subjects as compared to that of age and BMI matched normotensive subjects. Cardiovascular risk factors significantly reduced the association of BP with insulin resistance, but not with inflammatory markers. Regression analysis revealed the better relationship between inflammatory markers (CRP and TNF- $\alpha$ ) and prehypertension BP status.

Conclusion: Although, insulin resistance and inflammatory markers were elevated in prehypertensive subjects as compared to age and BMI matched normotensive subjects, the inflammatory markers alone significantly associated with BP status, independent of cardiovascular risk factor.

**Index Terms-** Prehypertension, insulin resistance and inflammatory markers

## I. INTRODUCTION

Worldwide, cardiovascular disease (CVD) is the foremost cause of morbidity and mortality. Hypertension is one of

the major contributor or modifiable risk factors for CVD mortality and morbidity (1). In India, hypertension accounts for 57% of stroke deaths and 24% of coronary heart disease deaths (2). Globally, it is affecting approximately 26.4% of the adult population and accounting for 13.5% (approximately 7.1 million) of global deaths, and its occurrence is expected to be more than 1.5 billion by 2025 (3, 4).

After considering the increasing evidence of CVD and its strong association with BP, even starting from the BP range of 115/75 mm Hg (5). Joint national committee on detection, evaluation, prevention and treatment of high blood pressure (JNC 7) emphasized a new BP category, prehypertension, BP range of 120/80 mm Hg to 139/89 mm Hg (6).

Insulin resistance, a resistance to insulin stimulated glucose uptake in insulin-dependent cells is the primary underlying defect, associated with CVD (7). Xu et al., proposed that the insulin resistance plays an important role in the metabolic abnormalities of hypertension (8). Nonetheless, prehypertensive subjects with insulin resistance also present with accentuated cardiovascular risk profile (9). Homeostasis model assessment of insulin resistance (HOMA-IR) is accepted as a reliable tool in the assessment of insulin resistance even before the clinical diagnosis of diabetes, applied to quantify insulin resistance in subjects with or without glucose intolerance (10).

Inflammation is defined as body's natural defense against invading pathogens by producing various inflammatory proteins. Excess or chronic expression of pro-inflammatory cytokines can lead to the development of tissue injury and damage, as is evident in atherosclerosis (11, 12). Preston et al., demonstrated increased plasma levels of the primary inflammatory cytokine TNF- $\alpha$ , IL-6, and CRP in hypertension (13). Previous studies also observed elevated inflammatory marker in prehypertensive subjects as compared to normotensive subjects (14, 15) and a strong association between prehypertension and inflammatory markers (14).

Of note, worldwide, conventional cardiovascular risk factors, such as ageing, high cholesterol, overweight or obese, smoking, and physical inactivity in association with BP contribute about 80-90% of ischaemic heart disease and 70-75% of stroke (16). Especially, the obesity and advancing age play an important role in the process of insulin resistance and inflammation (17, 18),

and also contribute in the association between BP and insulin resistance (19).

Therefore, in the present study, we evaluated the markers of inflammation and insulin resistance in prehypertensive subjects and compared it with the age and BMI matched normotensive subjects, and the association between inflammation, insulin resistance and prehypertension BP status.

## II. METHODOLOGY

### 2.1 Study approval:

The study has been approved by JIPMER scientific advisory committee and JIPMER ethics committee for human studies. The present study (cross-sectional) data is a part of the Ph.D., thesis work, thus the study protocol was also approved by the panel of Ph.D., doctoral committee.

### 2.2 Subjects recruitment:

After the approval from the human ethics committee, we have conducted community-based hypertension screening camps in Puducherry, India, between August 2010 and December 2011, to recruit study subjects. During the camp, after the period of comfortable rest, BP was recorded three times in the sitting posture with five minute interval between the recordings (by the same observer), using digital automatic BP monitor (CH432B, Citizen Systems, Japan Co., Ltd, Japan). The average of these three recordings was considered as the final reading. Based on the BP and medical history, a total of 572 were recruited for the study after considering the inclusion (systolic BP < 140 mm Hg, diastolic BP < 90 mm Hg, aged 20–60 years) and the exclusion (history of chronic illness, CVDs, diabetes, primary autonomic insufficiency, or kidney diseases; sports person; under medication for prehypertension and chronic illness). The study protocol was explained to the individuals, and written informed consent was attained before their participation in this study.

### 2.3 Laboratory measurements:

The volunteers were requested to report to Autonomic lab, department of Physiology, JIPMER, Puducherry between 07.00 a.m. to 09.00 a.m., after overnight fasting. The BP was recorded once again in the lab by the same observer with the same instrument used in camp, in sitting position after comfortable rest. Two readings were taken with 5 minute interval and the average of the readings was considered for categorizing the volunteers. A total of 82 normotensives (BP: <120/80 mm Hg) and 104 prehypertensives (BP: 120-139 and/or 80-89 mm Hg) were included in the study after obtaining the written informed consent. The details of subject recruitment and categorization are depicted in Figure 1.

#### 2.3.1 Anthropometric measurements:

Anthropometric measurements: Body mass index was calculated using body weight and height of the individual.

#### 2.3.2 Biochemical tests:

Fasting plasma glucose (FPG) was assessed by glucose oxidase-peroxidase method (Genuine Biosystem, India). Plasma insulin was measured using chemiluminescence immunoassay (Siemens, USA). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated based on the FPG and insulin values, i.e., fasting insulin (mU/L) X [fasting glucose (mmol/L)/22.5], high score denotes reduced insulin sensitivity i.e., insulin resistance (20). Inflammatory markers: hs-CRP

(DBC, Canada), IL-6 and TNF- $\alpha$  (Orgenium, Finland) were measured using ELISA kit, and the manufacturer's instructions were followed.

### 1.4 Data analysis:

Continuous data were presented as mean  $\pm$  SD, and the categorical data as frequencies. Comparison of continuous data between groups were done by Student t test. Between groups difference in frequency were compared using the  $\chi^2$  test. Entire cohort (n=186) was used for the correlation and regression analysis. The association between the parameters was analyzed using Pearson correlation. The contribution of the independent variable on the variance of dependent variable was assessed using linear regression. Data analysis was performed with Statistical Package for Social Sciences version 19.0 for Windows (SPSS Inc., USA).  $P < 0.05$  was considered as statistically significant.

## III. RESULTS

In total, tests performed on 186 participants (82 normotensives and 104 prehypertensive subjects), after considering the inclusion, exclusion criteria, and obtaining written informed consent. The participants recruited in the age group of 20 to 60 years. Mean age of the subjects recruited were similar between normotensive and prehypertensive group, 41 and 43 respectively. The BMI also did not differ significantly between normotension and prehypertension group. Difference in the distribution of gender was statistically significant between normotension and prehypertension group, i.e., more male subjects (67.31%) recruited in prehypertension group, because men are prone to develop prehypertension than women of peer age group (21), Table 1.

Family history of diabetes was equally distributed between normotensive and prehypertensive subjects (27% vs 30%), but the family history of hypertension between the groups were statistically different between the groups (26% vs 45%). The 5% and 9% of the subjects in normotension and prehypertension group, respectively, did not know about their family history of diabetes and family history of hypertension. The history of smoking and alcohol intake were not significantly different between normotension and prehypertension.

The mean systolic and diastolic BP of normotensive subjects were 109 mm Hg and 73 mm Hg, respectively. The detail of BP, mean arterial pressure and heart rate of both the groups are presented in Table 1. The markers of inflammation (CRP, TNF- $\alpha$  and IL-6) and insulin resistance were significantly elevated in prehypertensive subjects as compared to that of normotensive subjects, but the level of insulin was not statistically different between the groups.

Entire cohort was used for the correlation and regression analysis. The BP (both systolic and diastolic BP) showed a significant direct association with insulin resistance and markers of inflammation (CRP, TNF- $\alpha$  and IL-6), Table 2. This association remained robust after adjusted for cardiovascular risk factors like, age, family history of hypertension, BMI, smoking habit and fasting plasma glucose, but not with insulin resistance. The association between heart rate and inflammatory markers, except with CRP, and insulin resistance were also reduced

significantly when cardiovascular risk factors were fixed, Table 3.

By linear regression analysis, markers of inflammation and insulin resistance explained 28.6% of the variance in the presence of prehypertension BP status, i.e., normotension vs prehypertension, but the CRP and TNF- $\alpha$  alone significantly contributed in the variance, Table 4.

#### IV. DISCUSSION

In this study of age and BMI matched subjects with normotension and prehypertension, we observed elevated inflammatory markers and insulin resistance in subjects with prehypertension as compared to that of normotensive counterparts. The direct association between BP (both systolic and diastolic BP) and inflammatory markers was robust even after adjusted for cardiovascular risk factors, but not with the state of insulin resistance. Regression analysis further revealed the significant contribution of inflammatory markers in the variance of prehypertension BP status.

Prehypertensive subjects hold more than 3 fold risk for developing hypertension and CVD in the future when compared to normotensive subjects (22) and at the same time the level of BP is directly correlated with mortality (23). Meta-analysis of 12 studies, including a total of over five lakh participants, demonstrated that prehypertension was associated with an increased risk of stroke (24). Studies have also demonstrated subclinical atherosclerosis and target organ damage in prehypertensive subjects (25).

Of note, the exact cause of high BP is not clear. This cross-sectional data analysis also could not reveal the cause-effect relationship of increasing BP with inflammation and insulin resistance, i.e., whether the increased in inflammatory markers or insulin resistance state caused the elevation in BP or the increase in BP with other risk factors preceded the alteration in the markers of inflammation and insulin resistance. An epidemiological study by Reaven et al., stated that the individuals with insulin resistance has increased chance for developing hypertension (26). A study by Blake et al., revealed the importance of inflammatory markers as an independent risk factors for the development of hypertension (27).

In the present study, we observed elevated inflammatory markers in prehypertensive subjects as compared to that of normotensive subjects. This is in accordance with a study conducted in the ATTICA region on 3,042 prehypertensive subjects without clinical CVD (14). Numerous epidemiological studies demonstrated elevated inflammatory proteins in patients with CVD and even in healthy subjects (28, 29). We also detected a strong positive association between BP and inflammatory markers, and the association remained robust after adjusted for cardiovascular risk factors like, age, smoking habit, BMI and fasting plasma glucose. NHANES III study also found independent relationship between inflammatory proteins and BP (15). Our observation indicates that the prehypertension might be a proinflammatory condition, progress to subclinical atherosclerosis.

Similar to the inflammatory markers in prehypertension, we observed increased insulin resistance in prehypertensive subjects as compared to that of normotensive subjects. Previous studies

also demonstrated the presence of increased insulin resistance in prehypertensive subjects as compared to normotensive subjects and their accentuated cardiovascular risk (9, 30, 31). Nonetheless, the positive association between insulin resistance and BP was not significant after adjusted for cardiovascular risk factors. In agreement, previous studies also demonstrated the association between BP and insulin resistance and the significant contribution of cardiovascular risk factors (31, 32). But, a study by Hwu et al., demonstrated the association between BP and insulin resistance, independent of cardiovascular risk factors (33).

The existence of increased insulin resistance (18%), and markers of inflammation, such as, CRP, TNF- $\alpha$  and IL-6 by 48%, 30% and 24%, respectively, in prehypertensive subjects as compared to that of age and BMI matched normotensive subjects, indicates that the subjects with prehypertension present with a state of inflammation and insulin resistance. Thus, this study highlight the importance of prehypertension BP category, because the increased inflammatory markers and insulin resistance present in prehypertensive subjects are well known for its association with the development of CVD. The strong association between BP and inflammation and the significant contribution of inflammatory markers (CRP and TNF- $\alpha$ ) on the variance of prehypertension BP status, indicates the importance of a state of inflammation in individuals with prehypertension. Further, the influence of cardiovascular risk factors on the association of BP with insulin resistance and inflammatory markers, emphasizes the importance of targeting lifestyle factors in the reduction of BP and metabolic derangements, so as to reduce the occurrence of CVD.

#### V. LIMITATIONS

The cause-effect relationship between BP, insulin resistance and markers of inflammation cannot be imputed, because the present study is cross-sectional. Longitudinal studies with more number of subjects can reveal the exact association. 24 hours ambulatory BP monitoring could have added more information in the clinical setting about the BP status of the subjects.

#### VI. CONCLUSION

The presence of elevated inflammatory markers and insulin resistance in prehypertensive subjects, and the strong association of inflammatory markers with BP status, independent of other comorbid risk factors like, age, BMI, smoking habit and fasting plasma glucose designates their higher risk for conversion to hypertension, development of subclinical atherosclerosis and CVDs in future. By considering the influence of risk factors on the association of BP with insulin resistance and inflammation, it is advisable to introduce lifestyle interventions right from the stage of prehypertension for delaying the disease progression and the incidence of CVD. This may obviate the need for expensive and complicated therapies.



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#### AUTHORS

**First Author** – Ramkumar Thiyagarajan, M.Sc., Ph.D., Scholar, Department of Physiology, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India.

**Second Author** – Pravati Pal, M.D., Professor, Department of Physiology, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India.

**Third Author** – Gopal Krushna Pal, M.D., Professor & Head, Department of Physiology, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India.

**Fourth Author** – Senthil Kumar Subramanian, M.D., Assistant Professor, Sri Venkateshwaraa Medical College Hospital and Research Centre, Puducherry, India.

**Fifth Author** – Madanmohan Trakroo, M.D., D.Sc., Professor & Head, Mahatma Gandhi Medical College & Research Institute, Puducherry, India.

**Sixth Author** – Zachariah Bobby, Ph.D., Professor, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India.

**Seventh Author** – Ashok Kumar Das, M.D., Ph.D., Former Senior Professor of Medicine & Former Professor & Head of

Endocrinology, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India.

**Correspondence Author** – Dr. Pravati Pal, Professor, Department of Physiology, Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER), Puducherry, India. PIN: 605 006. E-mail: [drpravatipal@gmail.com](mailto:drpravatipal@gmail.com); [ramkumar.t@jipmer.edu.in](mailto:ramkumar.t@jipmer.edu.in).

**Table 1.** Comparison of demographic profile, anthropometric measurements, blood pressure, markers of inflammation and insulin resistance between normotensive and prehypertensive subjects.

Parameters/ Group	Normotension (n=82)	Prehypertension (n=104)
Age (year)	41.23 ± 8.51	43.17 ± 9.02
Gender distribution (Male/ Female)	39/43	70/34 **
Body mass index (kg/m <sup>2</sup> )	25.32 ± 4.01	26.16 ± 3.53
Smoking habit (Yes/ No)	10/72	22/82
Alcohol intake (Yes/ No)	09/73	22/82
Systolic blood pressure (mm Hg)	109.07 ± 6.71	126.43 ± 6.64 ***
Diastolic blood pressure (mm Hg)	72.54 ± 5.64	84.36 ± 4.17***
Heart rate (beats/min)	71.83 ± 7.28	74.50 ± 10.10*
Mean arterial pressure (mm Hg)	84.72 ± 5.26	98.38 ± 4.00***
Fasting plasma glucose (mg/dL)	86.73 ± 11.91	92.72 ± 15.39**
Fasting plasma insulin (µU/L)	13.75 ± 7.66	15.45 ± 5.93
HOMA-IR	3.02 ± 1.82	3.57 ± 1.51*
High sensitive C-reactive protein (µg/mL)	3.95 ± 2.13	5.86 ± 1.75***
Tumor necrosis factor-α (pg/mL)	61.72 ± 19.09	80.31 ± 15.23***
Interleukin-6 (µg/mL)	19.33 ± 6.67	23.91 ± 7.81 ***

Data are expressed as mean ± SD or frequency. Abbreviations: HOMA-IR, homeostatic model assessment-insulin resistance.  $P < 0.05$ , considered statistically significant.

\* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ , difference between normotension and prehypertension

**Table 2.** Correlation between blood pressure, insulin resistance and markers of inflammation

Parameters	IR	Hs-CRP	TNF-α	IL-6
Systolic blood pressure	0.311 ***	0.504***	0.492***	0.297***
Diastolic blood pressure	0.285***	0.464***	0.456***	0.343***
Heart rate	0.224**	0.237**	0.159*	0.157*
Mean arterial pressure	0.317***	0.504***	0.498***	0.338***

Abbreviations: hs-CRP, high sensitive C-reactive protein; IR, insulin resistance; IL-6, interleukin 6; TNF-α, tumor necrosis factor-alpha.  $P < 0.05$ , considered statistically significant. \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$

**Table 3.** Correlation between blood pressure, insulin resistance and markers of inflammation after adjusting for age, smoking habit, body mass index, family history and fasting plasma glucose.

Parameters	IR	Hs-CRP	TNF-α	IL-6
Systolic blood pressure	0.093	0.342***	0.339***	0.201**
Diastolic blood pressure	0.107	0.354***	0.333***	0.236**
Heart rate	0.133	0.206**	0.043	0.114
Mean arterial pressure	0.108	0.372***	0.359***	0.238**

Abbreviations: hs-CRP, high sensitive C-reactive protein; IR, insulin resistance; IL-6, interleukin 6; TNF-α, tumor necrosis factor-alpha.  $P < 0.05$ , considered statistically significant. \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$

**Table 4.** Contribution of independent variables (inflammatory markers) in the variance of dependent variables (prehypertension BP status and insulin resistance) by linear regression

Relationship between markers of inflammation and prehypertension BP status ( $R^2 = 0.286$ )		
	$\beta$ co-efficient $\pm$ SE	p Value
Hs- C-reactive protein ( $\mu\text{g/mL}$ )	$0.065 \pm 0.017$	<0.001
Tumor necrosis factor- $\alpha$ (pg/mL)	$0.009 \pm 0.002$	<0.001
Interleukin-6 ( $\mu\text{g/mL}$ )	$-0.001 \pm 0.005$	0.781
Insulin resistance	$-0.004 \pm 0.02$	0.855

Abbreviations: BP, blood pressure; SE, standard error.  $AP < 0.05$ , considered statistically significant.

**Figure 1.** Participant recruitment and categorization in to groups

