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Original Article

Study of Inflammatory Marker IL-6 and Sympathetic Activity Among WHR Matched Prehypertensive & Normotensive Males

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Abstract

Background: Evidence suggests that Prehypertension is an inflammatory process and cardiovascular damage due to the inflammation is initiated by sympathetic activity. Therefore this study was conducted with the objective to measure IL-6 and sympathetic function in Prehypertensive.

Material and methods: A Case-control study was conducted on 40 prehypertensive (as per JNC VII criteria) male subjects between 20 to 40 years. Equal numbers of age and WHR matched normotensive males were taken for comparison. They were investigated for serum IL6 and sympathetic reactivity by sustained hand grip (SHG) test.

Results: SerumIL-6 level was higher in prehypertensive than in normotensive, though insignificantly (p=0.17). Significantly higher resting heart rate (RHR) (p=.001) and significantly lower change in diastolic blood pressure (Δ DBP, p=0.000) following SHG test were observed in prehypertensive. No significant association was observed between IL-6 and ΔDBP in Prehypertensive.

Conclusion:.Although the sympathetic reactivity is elevated in prehypertensive, its contribution in initiation of inflammatory process in prehypertensive was not observed in our study.

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Introduction

Prehypertension is a category designated by the seventh Report of the Joint National Committee on Prevention Detection, Evaluation, and Treatment of

High Blood Pressure (JNC VII) in 2003(1). It is estimated that about 37% of the prehypertensive below the age of 65 years are likely to become hypertensive over a period of 4 years (2). Individuals with prehypertension also have high risk of target organ damage, cardiovascular related morbidity and mortality (3). The prevalence of prehypertension is increasing worldwide and in developing country like India the cross sectional surveys have reported prehypertension in 46.6% of men and 49.8% of women (4).

Although several factors contribute to the pathogenesis of elevated blood pressure including genetic predisposition, diet, high salt intake, obesity, insulin resistance, rennin-angiotensin system, and sympathetic nervous system (5), evidence suggests the immunological and inflammatory pathways to be the final common pathway in pathophysiology of elevated Blood Pressure (6). Since the prehypertension is a fore runner of hypertension several of the pathophysiological mechanisms that play role in development of hypertension are likely to be deranged in pre-hypertensive subjects including an increase in sympathetic nervous system activity and deranged inflammatory pathways. In most subjects with elevated blood pressure up-regulation of sympathetic nerve activity (7) with exaggerated generalized sympathetic efferent to end organs like kidney & heart (8) contributes to increased mortality and morbidity (9). There is increasing evidence that cardiovascular damage caused by excessive stimulation of the sympathetic nervous system and rennin angiotensin system and their receptors (á and â-adrenergic and angiotensin 2 AT1 receptors respectively) is mediated through a pro-inflammatory activation of the immune system (10). Abdominal obesity is also one of the independent risk factors for cardiovascular diseases and is associated with concomitant release of proinflammatory cytokines (11). Therefore this study was planned with the aim of assessing the level of inflammatory marker interleukin-6 and sympathetic activity in non- obese prehypertensive.

Materials and Methods

This case-control study was conducted at the department of Physiology, Swami Rama Himalayan University after obtaining approval from the Institutional Ethical Committee of Himalayan Institute of Medical Sciences. A sample size of 38 in each group was calculated based on the formula for difference in mean of inflammatory marker IL-6 (12) with the power of 80% and an alpha error of 0.05.

Screening of a representative group of male residents, employees and students of the university between 20 to 40 years was carried out. Blood pressure was taken in the resting condition three times with a gap of 3 minutes as per the standardized protocol (1) and those with systolic blood pressure 120-139 mm Hg and (or) diastolic blood pressure 80-89 mm Hg were recruited for the study after obtaining their informed, written consent.

The recruited subjects were asked to report to the department of Physiology between 9:00 am and 10:00 am in all working days. The subjects were measured for waist circumference and hip circumference using standard procedure (13) and WHR was calculated. 40age and WHR matched (±1SD) normotensive were also recruited for comparison. Volunteers were interviewed for recording of demographic indices including name, age, address, occupation and relevant history of present illness, past illness, personal history, drug history, family history, and history of allergic reactions. The following two groups were formed according to the JNC VII classification (1), and their inclusion and exclusion criteriawere as follows.

1. Normotensive Group (n=40)

   Inclusion Criteria

   - Apparently healthy subjects with Blood Pressure systolic < 120 mm Hg and diastolic < 80 mm Hg (per JNC VII criteria)

2. Prehypertensive group (n=40)

   Inclusion Criteria

   - Apparently healthy subjects with the Blood Pressure-systolic 120-139 mm Hg and/or a

diastolic 80-89 mm Hg (as per JNC VII criteria).

Exclusion criteria were common for both Normotensive and Prehypertensive groups and consisted of history of: coronary artery disease, kidney disease, hepatic disease, chronic pulmonary obstructive disease, inflammatory bowel disease, autoimmune disease, diabetes mellitus, osteoarthritis, postural orthostatic tachycardia syndrome, orthostatic hypotension, and chronic skin infection. Chronic smokers and chronic alcoholics were also excluded. Clinical examination was carried out to rule out any active or chronic infection. Recruitment of the study subjects were continued till the total sample in each group was collected.

Selected volunteers were instructed to come on next visit following a good night rest and a light breakfast. They were also instructed to avoid caffeinated drinks and any kind of heavy exercise during last 24 hours.

Following Baseline parameters were then measured in both the groups

- Anthropometric indices measurement.

  Standing height:

  Height was measured in centimeters (cm), without shoes and with light clothes against the wall mounted measuring tape in cm to the nearest of 1 cm.

  Weight was measured in kg nearest to 100 gm by using Krups weighing machine.

- Cardio vascular parameters: Basal systolic and diastolic blood pressure, resting heart rate were recorded using automated BP apparatus (model no. EW 254 DC6V) in a sitting position after rest of at least 15 minutes. Recording was done at an ambient temperature of 25^{O}C.

- Experimental procedure for SHG test was used to assess reactivity of sympathetic nervous system. Procedure for the same was explained to the subject to remove any apprehension. Baseline BP and HR were recorded in sitting position. The subject was asked to hold the dynamometer in the non-dominant hand, with the arm by the side of the body, with the pointer on the dial adjusted at zero and the base rested on the palm. Before the test Maximum Voluntary Contraction (MVC) was recorded. The subject was instructed to squeeze the dynamometer with maximum isometric effort for at least 5 seconds and the result was recorded, no other body movement was allowed. After this the subject was asked to press handgrip dynamometer at 30% of MVC for 3 minutes. The alteration in the BP and HR just before the release of hand grip was taken as the index of response to hand-grip test.

- Test for Inflammatory markers.

  Blood sampling for serum interleukin 6 (IL6) was done in the morning hours after taking all aseptic precautions. The samples were collected from cubital vein in a plain yellow top vaccutainer for estimation of IL6. The freshly collected samples were centrifuged for 15 minutes at 3000 RMP; serum was separated and stored in a separate container at -70C° until further analysis. Quantitative measurements of IL6 were performed by immunoassay method; using Diaclone IL-6 kit in Bio Rad i Mark micro plate reader within two months from sample collection.

Statistical analysis:

Statistical analysis was done using the software SPSS (Statistical Package for the Social Sciences) version 20.0 for Windows. Descriptive summary statistics were presented as Mean±SD for continuous variables. Baseline characteristics, variables of

sympathetic reactivity and inflammatory markers of normotensive and pre-hypertensive groups were compared using unpaired t-test. The Pearson correlation coefficient test was used to analyze relation of IL-6 with sympathetic nervous system reactivity. Logistic Regression analysis was carried out to find the % of accuracy in predicting the prehypertension and odds of prediction by different variables.

P value <0.05 was considered statistically significant.

Results

Table I shows comparison of demographic & anthropometric parameters among normotensive and prehypertensive. Difference in age, height, weight and WHR among two groups was insignificant.

TABLE I : Demographic & anthropometric parameters among prehypertensive and normotensive male adults.

Parameters	*Prehypertensive (n=40)*	*Normotensive (n=40)*	*P value*
Age (yrs)	30.6±7.2	30.2±7	0.79
Height (cm)	169.7±5.2	170.59±6.7	0.52
Weight (kg)	72.9±11.8	68.4±12.5	0.095
WHR (ratio)	0.92±0.05	0.92±0.09	0.91

Data presented as Mean±Standard deviation (SD); p value < 0.05 is significant; WHR: waist hip ratio; t-test.

Table II shows baseline cardiovascular parameters and parameters of sympathetic activity in prehypertensive and normotensive groups. There is significant increase is resting heart rate of prehypertensive subjects. The change in diastolic blood pressure following SHG test was significantly lower in pre-hypertensive as compared to normotensives. These findings show that rise in DBP declines as blood pressure increases which indirectly shows an already raised sympathetic activity among prehypertensive as compared to normotensive subjects. It also shows that higher mean value of inflammatory markers IL-6 is observed in prehypertensive as compared to normotensive, though the difference is not statistically significant.

TABLE II : Comparison of Cardiovascular, Sympathetic system activity and IL-6 levels between normotensive and prehypertensive groups.

Parameters	*Prehypertensive (n=40)*	*Normotensive (n=40)*	*P value*
Cardiovascular parameters			
RHR beats/min	74.28±8.59	68.45±6.53	0.001
SBP mm Hg Baseline	129.7±4.48	113.1±3.97	0.001
DBP mm Hg Baseline	81.43±5.9	72±5.28	0.001
SHG parameters : resting DBP was recorded before the SHG test was used			
Resting DBP (mmHg)	81.03±6.31	70.55±5.76	0.000
Maximum DBP (mmHg)	97.2±12.045	97.18±12.87	0.993
ΔDBP (mmHg)	16.13±11.69	26.50±10.61	0.000
Inflammatory Marker			
IL-6pg/ml	1.85±0.86	1.57±0.93	0.17

Data is presented as Mean±Standard deviation (SD); p value < 0.05 is considered statistically significant; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; LF: low frequency; HF: high frequency; IL-6: interleukin 6; p value < 0.05 is considered statistically significant; t test.

In this study both Systolic and Diastolic blood pressures showed a strong positive relationship with resting heart rate (r=0.371, p=0.001; r=0.369, p=0.001 respectively) and negative relationship with sympathetic system reactivity (ΔDBP, r=-0.303, p=0.006; r=-0.344, p=0.002 respectively) in overall volunteers. IL-6 was positively but insignificantly related to both Systolic and Diastolic blood pressure. Possible explanation to this can be insufficient rise in IL-6 levels to elicit the significant correlation in this range of blood pressure.

Table III : positive correlation between ΔDBP and

TABLE III : Correlation between parameters of sympathetic system activity and inflammation in prehypertensive.

	RHR beats/ min	*SBP mmHg*	*DBP mmHg*	*IL-6 pg/ml*	*ΔDBP mmHg*
RHR beats/min	1	.000 (.998)	.064 (.697)	.008 (.963)	.140 (.389)
SBP mmHg		1	.062 (.705)	.072 (.658)	.099 (.545)
DBP mmHg			1	.020 (.904)	–.259 (.106)
IL-6 pg/ml				1	.311 (.050)
ΔDBP mmHg					1

Values are presented as: r (p-value).

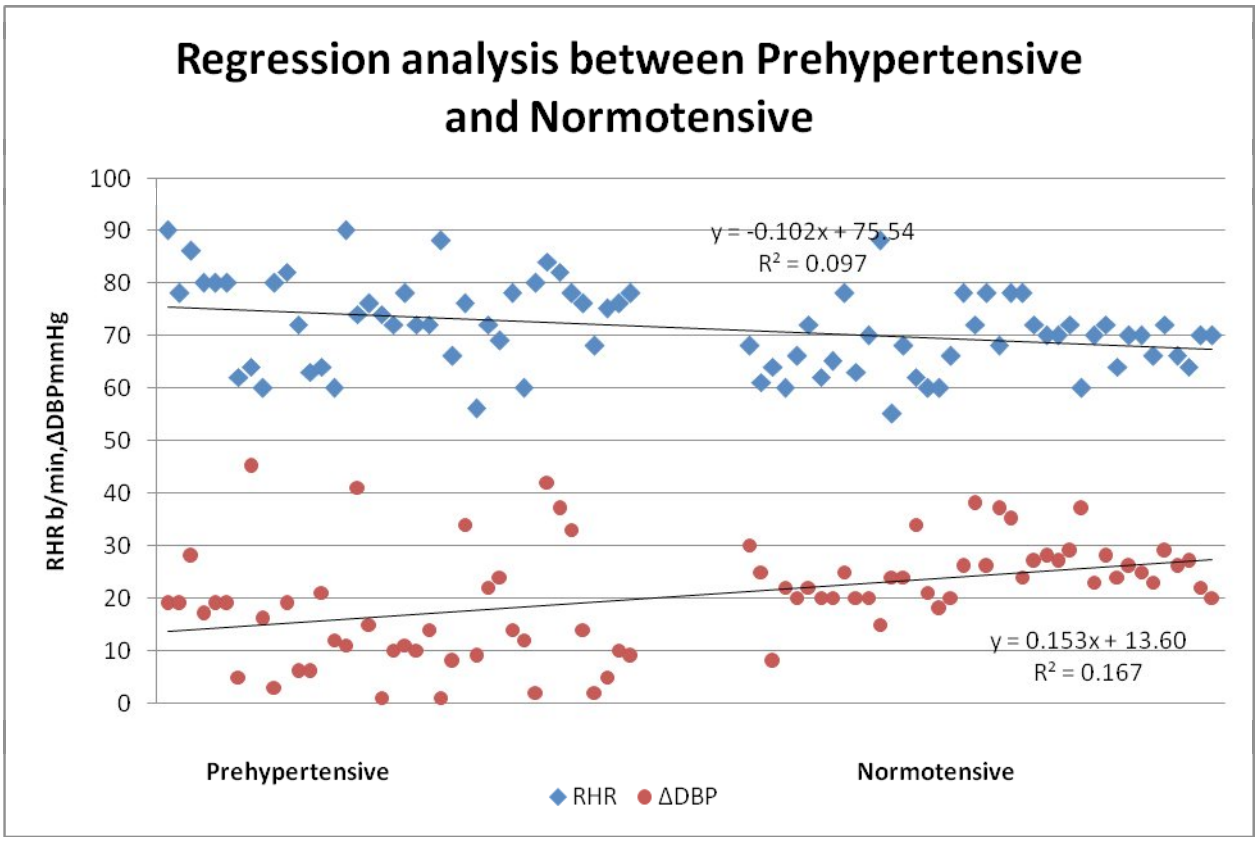

Fig. 1 : Regression analysis of variables significantly different between prehypertensive and normotensive.

inflammatory marker IL-6 was found to be non significant.

Fig. 1 83% prediction probability of developing prehypertension could be explained by resting heart rate and change in DBP after controlling for age, weight, WHR. Binary logistic regression analysis observed significant contribution by RHR (-0.103; p=.009) and sympathetic reactivity represented by change in DBP (0.152; p=.001) to predict the development of prehypertension.

Discussion

The current study investigates association of inflammatory marker interleukin-6 with sympathetic reactivity in prehypertensive male subjects of 20 to 40 years of age. The groups were matchedfor age and WHR (±1SD) and no significant difference in height & weight was observed between the groups suggesting that the groups were homogenous with respect to height & weight (Table I).

In resting conditions heart rate is mainly dependant on the vagal tone. But the increase in heart rate in prehypertensive subjects can be attributed to both: increase in sympathetic and decrease in parasympathetic tone (7). In the present study a significantly increase in resting heart rate was observed in the prehypertensive subjects as compared to normotensive (74.28±8.59Vs68.45±6.53), (p=0.001). Significantly higher heart rates in prehypertensive, as compared to normotensive, were also observed by studies done by GK Pal et al (14), Thiyagarajan et al. (12) and Maple M Fung et al. (15).

Evidence suggests that elevated blood pressure is initiated and maintained by increased sympathetic tone, generated by RVLM and influenced by cortical and hypothalamic impulses along with several blood borne gases and chemicals including cytokines (8). It has been demonstrated that elevated sympathetic drive is not only essential for hypertensive patients, but also for milder stages of disease process as

well for high normal normotensive (16) and thus supported the hypothesis that the sympathetic drive is a major factor in the development of high blood pressure.

Isometric exercise induces somatosensory reflex and is often used to assess the impaired autonomic sympathetic response by sustained hand grip (SHG) test (17) in the form of a lower rise in DBP in subjects with impaired autonomic response (18).

Our study observed a lesser increase in the diastolic blood pressure following SHG test in prehypertensive as compared to normotensive and the difference was statistically significant (Table II). The findings are similar to observations by M Bhondage et al. with lesser response of change in DBP to isometric hand grip in prehypertensive but only in subjects with BMI ≥23, but not obese. This was explained by the authors as reduced increase in peripheral response in this group (19). It can also be said that as baseline diastolic blood pressure was already on a higher side in prehypertensives, it did not increase above a certain value under further stressful conditions of isometric exercise, thus limiting the difference between the baseline and the maximum diastolic value (20).

Role of inflammation in pathogenesis of elevated blood pressure is gaining more and more support with demonstration of different inflammatory and immune markers being elevated in subjects with prehypertension and hypertension (21).

In the present study we have observed higher mean value of inflammatory marker IL-6 in prehypertensive as compared to normotensive, though the difference was not statistically significant (>0.05; Table II). In a study done by R Thiyagarajan et al. a significant increase in the values of inflammatory markers including IL-6 was observed in prehypertensive in comparison to normotensive subjects (12). Similar findings of statistically significant increased levels of IL-6 in both hypertensive and prehypertensive groups in comparison to normotensive were observed in another study (21). In the studies done by M Fung et al and Kim et al prehypertensive subjects showed significantly increased levels of several other inflammatory markers along with IL-6 as compared to normotensive controls (15, 22). The insignificant difference in IL-6 levels between prehypertensive and normotensive observed in our study can be attributed to small sample size.

In this study both Systolic and Diastolic blood pressures showed a strong positive relationship with resting heart rate and negative relationship with sympathetic system reactivity (ΔDBP). Similar strong, positive relationship between resting heart rate and systolic and diastolic blood pressures was observed in the Study by Farinaro E et al. in a cohort of hypertensive (23). In a large French population of 100,000 subjects, heart rate was positively associated with blood pressure, with 21.3% of hypertensive men having HR >/= 85 beats/min as compared to only 4.0% of normotensive men. Those with mild hypertension also showed tachycardia compared to normotensive (24). Tachycardia may be an important step during development of hypertension (25). Minimal change in DBP with increase in blood pressure, following SHG test indicated towards imbalance in sympathetic response in prehypertensive subjects (17, 18) as does raised RHR (26). This suggests that basal sympathetic nervous system activity is increased in prehypertensive. At the same time responsiveness of sympathetic nervous system to sympathetic stimuli (SHG test) is decreased as seen by significant decrease in “DBP in comparison to normotensive. But “DBP was insignificantly related to the inflammatory marker IL-6 in prehypertensive group showing that no relationship between sympathetic activity and inflammation could be established, when examined by SHG test, RHR and IL-6 levels.

When parameters were analyzed to predict the development of prehypertension, 86% of prediction probability can be explained by resting heart rate (β=-.108; p=.011) and change in DBP(β=.140; p=.001) taken together after controlling for age and WHR variables (Fig. 1). In a HARVEST study it was observed that in prehypertensive heart rate and heart rate changes during the first 6 months of follow-up are independent predictors of subsequent increase of systolic and diastolic blood pressure (27) and association of high HR and increased BP is stronger

in individuals with elevated sympathetic activity (28). Our study is also supported by a study by R H Chaney et al, which observed that the best prediction for development of high BP was by the interaction between resting diastolic BP × handgrip diastolic BP (29).

Resting heart rate was elevated in prehypertensive as compared to age and WHR matched normotensive with significantly impaired sympathetic reactivity to SHG in them. Though the level of inflammatory marker IL-6 were elevated (although insignificant) in prehypertensive no significant relation to sympathetic activity (∆DBP, RHR) was observed.

Limitations:

Sample size taken was small so larger study is recommended to see the significant difference in the groups.

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