Cardiovascular Modulation, Oxidative Stress, and Cardiovascular Risk Factors in Prehypertensive Subjects: Cross-Sectional Study

Ramkumar Thiyagarajan,1 Pravati Pal,1 Gopal Krishna Pal,1 Senthil Kumar Subramanian,1 Zachariah Bobby,2 Ashok Kumar Das,3 and Madanmohan Trakroo4

BACKGROUND
Hypertension, one of the modifiable risk factors for cardiovascular disease (CVD), is known to be associated with increased oxidative stress and reduced cardiac modulation. Similar to hypertension, prehypertension is associated with increased risk of adverse cardiovascular (CV) events. We planned this study to find the association between prehypertension, cardiac modulation, oxidative stress, and associated CV risk factors.

METHODS
We recruited 178 subjects through hypertension screening camps conducted in Puducherry, India. Subjects were grouped into prehypertensive (n = 97) and normotensive (n = 81) groups. They were further subdivided, based on age, as young (20–39 years) and middle-aged (40–60 years) adults. We measured basal physiological parameters, heart rate variability, oxidative stress (thiobarbituric acid reactive substance and total antioxidant capacity (TAC)), and CV risk factors.

RESULTS
We found significant increase in oxidative stress in prehypertensive subjects of both age groups but the cardiac modulation decreased significantly in young prehypertensive subjects when compared with normotensive subjects. Correlation of TAC with root mean square of the sum of successive R wave to R wave (RR) interval differences (RMSSD), a cardiac modulation parameter (r = 0.437; P < 0.001), and mean arterial pressure (MAP) (r = −0.318; P < 0.001) was significant even after adjusting for CV risk factors. The correlation between MAP and RMSSD (r = 0.199; P = 0.009) was reduced after adjusting for CV risk factors.

CONCLUSIONS
Prehypertension in young adults is associated with increased oxidative stress and altered cardiac modulation. The risk factors for CVDs in prehypertensive young adults were found to be equivalent to that of middle-aged adults who are in the twilight zone for developing CV dysfunctions.

Keywords: blood pressure; cardiovascular risk factors; heart rate variability; hypertension; oxidative stress; prehypertension.

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The Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure in its 7th report introduced the term “prehypertension” for those with systolic blood pressure (BP) ranging 120–139 mm Hg or diastolic BP ranging 80–90 mm Hg.1 This definition is based on the increase in risk of cardiovascular (CV) complications associated with the level of BP, which was previously considered to be normal.1 The prevalence of prehypertension, hypertension, and CV diseases (CVDs) are increasing in developing countries.2 Prevalence of prehypertension in South India has been found to be 47%.2

The autonomic nervous modulation of cardiac function is measured using a noninvasive technique, heart rate variability (HRV), which measures the variation in R wave to R wave interval (RR). In HRV analysis, the successive RR interval differences can be produced only by the parasympathetic system, as vagal response occurs in 400 ms of application of stimulus.3,4 This beat-to-beat control of cardiac function by the vagus nerve is considered cardiac modulation. Root mean square of the sum of successive RR interval differences (RMSSD) has been documented to be one of the important measures of cardiac modulation.3 Reduced cardiac modulation is an important marker for predicting future CVDs and morbidity,5–8,9 which is reported to be associated with premature aging,7 prehypertension,10,11 and hypertension.12,13

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Oxidative stress is produced by an imbalance between the reactive oxygen species and the antioxidants in the biological system. Thiobarbituric acid reactive substance (TBARS), an important marker of oxidative degradation of lipids, and the total antioxidant capacity (TAC), an aggregate of all antioxidants present in biological fluid are used to assess the magnitude of oxidative stress, which has been observed to be associated with prehypertension, hypertension, CVDs, and aging. Further oxidative stress has been implicated in the progression of prehypertension to hypertension.

Age is known to affect CV autonomic function and oxidative stress. As age increases, cardiovagal modulation decreases and oxidative stress increases. Presence of any 2 major risk factors, such as hypertension, diabetes, increased cholesterol, or smoking in middle-age, increases the lifetime risk for CVDs. Several studies have demonstrated clustering of risk factors in young adults that can lead to CVDs in later life.

Previous studies have elucidated the association between HRV and oxidative stress in essential hypertension. However, there is a paucity of literature on the relation between cardiovagal modulation, oxidative stress, prehypertension, and associated CV risk factors. Therefore, in this study, we have assessed the association of cardiovagal modulation, oxidative stress, and CV risk factors in young and middle-aged prehypertensive subjects.

METHODS

Subject recruitment

This study was conducted in the autonomic function testing (AFT) lab, Department of Physiology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India, after getting approval from the JIPMER Scientific Advisory Committee and Institute Human Ethics Committee.

Subjects were recruited from the community by 4 hypertension screening camps conducted in Puducherry, India, during the period of March 2011 to December 2011. A total of 524 volunteers consented for the screening program. In the camps, BP was recorded 3 times with 5-minute intervals between each recording, using an automatic BP monitor (CH432B, Citizen Systems Japan Co., Ltd, Tanashi, Tokyo, Japan) after 5 minutes of rest in the sitting position. The average of these 3 recordings was considered the final reading. Out of 524 volunteers, 294 were recruited for the study after considering the inclusion (systolic BP < 140 mm Hg, diastolic BP < 90 mm Hg, aged 20–60 years) and exclusion (history of chronic illness, CVDs, diabetes, primary autonomic insufficiency, or kidney diseases; sports person; under medication for prehypertension and chronic illness) criteria. The study protocol was explained to the subjects, and written informed consent was obtained before their participation in the study.

Laboratory measurements

Subjects were requested to report to the AFT lab between 7:00 AM and 9:00 AM. Out of 294 subjects recruited in the screening camp, 116 subjects dropped out. Thus, 178 subjects constituted the final study sample size. They were instructed to avoid consuming alcohol a day before and abstain from cigarette smoking for at least 30 minutes before the recordings in the lab. BP was recorded once again in the lab with the same instrument used in camp in the sitting position after 5 minutes of rest. Two recordings were taken with a 5-minute interval between readings, and the average of the recordings was considered for categorizing the subjects, as depicted in Figure 1.

CV risk factors. Waist circumference was measured midway between the top of the iliac crest and the lower costal border. Body mass index was calculated based on the weight and height. The Global Physical Activity Questionnaire (GPAQ) was used to assess the physical activity of the subjects, which was quantified as metabolic equivalents. A minimum of 1 cigarette per day and intake of at least 1 alcoholic drink (90–100 ml) per day was considered as history of smoking and alcohol intake, respectively, and the family history of hypertension and diabetes were also recorded (first-degree relatives). Fasting plasma glucose, total cholesterol, triglycerides, and high-, low- and very low-density lipoprotein cholesterol were analyzed using fully automated clinical chemistry analyzer (AU400; Mishima Olympus Co., Ltd, Shinjuku, Tokyo, Japan).

Short-term heart rate variability. Lead II electrocardiogram and respiration were recorded in a dimly lighted room with the room temperature of 24–26 °C. Analogue signals were digitized using a 16-bit data acquisition system (LabChart; AD Instruments, Bella Vista, Sydney, Australia), and the sampling rate was kept at 500 Hz. The trend in RR interval variation was analyzed using HRV software (version 1.1.; Biomedical Signal Analysis Group, University of Kuopio, Kuopio, Northern Savonia, Finland). Frequency spectral components were classified based on their range of area under the power spectrum. Low frequency power (LF) between 0.04 to 0.15 Hz, representing the contribution from sympathetic and parasympathetic systems, high frequency power (HF) between 0.16 to 0.4 Hz, representing the contribution from parasympathetic system, and the ratio of LF/HF, representing the balance between the sympathetic and parasympathetic systems, were calculated. Time domain components such as SD of RR intervals recorded for 5 minutes (SDNN), representing the contribution from both sympathetic and parasympathetic systems, and RMSSD, adjacent RR intervals differing more than 50 ms (NN50), and NN50 divided by the total number of RR intervals in percentage (pNN50), all representing parasympathetic modulation, were calculated.

The time and frequency domain components that represented parasympathetic modulation alone were considered as cardiovagal modulation parameters.

Oxidative stress parameters. TBARS and TAC were measured using an enzyme-linked immunosorbent assay kit according to the manufacturer’s instructions (Cayman Chemical Company, Ann Arbor, Michigan, USA).
Data analysis

Continuous data were expressed as mean ± SD, and categorical data were expressed as frequencies. Frequency distributions between the groups were compared using the χ² test. Comparisons of continuous data between groups were done by one-way analysis of variance for parametric data and Wilcoxon signed-rank test for nonparametric data. HRV components such as LF and HF power were natural log transformed and denoted as ln(LF) and ln(HF), respectively, for statistical analysis. For correlation and regression analysis, the entire cohort (n = 178) was considered together. In the correlation and regression analysis, RMSSD was considered to represent cardiac modulation. The association between the parameters was analyzed using Spearman rank 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., Chicago, Illinois, USA). P < 0.05 was considered statistically significant.

RESULTS

Distribution of demographic profile

More of the prehypertensive subjects were middle-aged adults (n = 62) than young adults (n = 35). In prehypertensive subjects, the male/female ratio was 2.18 for young adults and 1.21 for middle-aged adults (Table 1). Family history of hypertension was equally distributed between normotensive and prehypertensive young adults (30.43% and 34.28%) and middle-aged adults (37.14% and 33.87%).

Comparison between normotensive subjects and prehypertensive subjects of both age groups

Basal physiological and heart rate variability parameters. Subjects with prehypertension were compared with those with normotension of the same age group. There was no significant difference in basal heart rate between normotensive and prehypertensive subjects.
of both age groups. The resting cardiovagal modulation parameters expressed as frequency domain indices (In(HF), Hfnu) and time domain indices (RMSSD, NN50, and pNN50) decreased significantly in prehypertensive young adults compared with normotensive young adults, whereas, no significant difference was observed between prehypertensive and normotensive middle-aged adults (Table 2). The LF/HF ratio, the indicator of sympathovagal balance, was significantly high in prehypertensive young adults compared with normotensive young adults, whereas no significant difference was observed between normotensive and prehypertensive middle-aged adults (Table 2).

CV risk factors and oxidative stress parameters. In this study, men with a history of smoking and alcohol intake were found to be equally distributed between the normotensive and prehypertensive groups of both age groups, and there were no women with smoking and alcohol intake history. Body mass index, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol levels were comparable between normotensive and prehypertensive subjects of both age groups. There was a significant increase in waist circumference, fasting plasma glucose, and triglycerides in prehypertensive young adults compared with normotensive young adults, whereas no such difference was observed between prehypertensive and normotensive middle-aged adults (Tables 1 and 3). The oxidative stress parameters TBARS and TAC were significantly deranged in both young and middle-aged prehypertensive subjects compared with normotensive subjects in the same age group (Table 3).

Prehypertension in young adults

Resting basal heart rate, cardiovagal modulation parameters, oxidative stress parameters, and CV risk factors in young adults with prehypertension were found to be comparable with those of middle-aged normotensive adults.

Association between BP, oxidative stress, and cardiovagal modulation

Correlation analysis revealed a direct correlation between MAP and TBARS ($r = 0.411; P < 0.001$) and indirect correlation between MAP, cardiovagal modulation (RMSSD) ($r = -0.301; P < 0.001$), and TAC ($r = -0.403; P < 0.001$) (Figures 2 and 3). After fixing CV risk factors (age, waist circumference, physical activity, fasting plasma glucose, total cholesterol, and triglycerides), MAP showed reduced but significant correlation with RMSSD, TBARS, and TAC ($r = 0.199, P = 0.009; r = 0.335, P < 0.001; and r = -0.318, P < 0.001$, respectively).

Correlation analysis revealed an indirect correlation between RMSSD and TBARS ($r = -0.484; P < 0.001$) and direct correlation between RMSSD and TAC ($r = 0.601; P < 0.001$) (Figure 4). The correlation of TBARS and TAC with RMSSD remained high ($r = -0.311, P < 0.001$; and $r = 0.437, P < 0.001$, respectively) even after adjusting for the CV risk factors.

By linear regression analysis, TBARS and TAC explained 23.2% of the variance in MAP and 36.8% of the variance in RMSSD (Table 4).

DISCUSSION

The major finding of this study is that young adults with prehypertension had decreased cardiovagal modulation,
Table 2. Comparison of supine short-term heart rate variability among normotensive and prehypertensive young and middle-aged adults

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normotension</th>
<th>Prehypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young adult (n = 46)</td>
<td>Middle-aged adult (n = 35)</td>
</tr>
<tr>
<td>In(LF)</td>
<td>5.31±0.84</td>
<td>4.78±0.83</td>
</tr>
<tr>
<td>ln(HF)</td>
<td>5.74±0.85</td>
<td>4.76±0.90</td>
</tr>
<tr>
<td>LF/HF ratio</td>
<td>0.81±0.59</td>
<td>1.37±1.27</td>
</tr>
<tr>
<td>LF nu</td>
<td>40.13±14.83</td>
<td>49.87±16.28</td>
</tr>
<tr>
<td>HF nu</td>
<td>59.87±14.83</td>
<td>50.13±16.28</td>
</tr>
<tr>
<td>SDNN, ms</td>
<td>45.96±17.45</td>
<td>32.60±10.28</td>
</tr>
<tr>
<td>RMSSD, ms</td>
<td>47.47±21.74</td>
<td>30.94±10.70</td>
</tr>
<tr>
<td>NN50, count</td>
<td>88.96±60.84</td>
<td>35.86±32.39</td>
</tr>
<tr>
<td>pNN50, %</td>
<td>27.15±20.20</td>
<td>11.38±11.00</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD.
Abbreviations: bpm, beats per minute; HF, high frequency spectral power; HR, heart rate; LF, low frequency spectral power; NN, interval between R to R wave; NN50, consecutive R to R interval differs more than 50 ms; nu, normalized units; pNN50, NN50 count divided by total number of R to R interval; RMSSD, root mean of sum of squares of successive RR interval differences. *P value < 0.05 considered statistically significant.

Table 3. Comparison of biochemical parameters among normotensive and prehypertensive young and middle-aged adults

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normotension</th>
<th>Prehypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young adult (n = 46)</td>
<td>Middle-aged adult (n = 35)</td>
</tr>
<tr>
<td>TBARS, μmol/L</td>
<td>2.91±0.64</td>
<td>3.16±0.33</td>
</tr>
<tr>
<td>TAC, μmol/L</td>
<td>2.47±0.58</td>
<td>1.77±0.66</td>
</tr>
<tr>
<td>FPG, mg/dl</td>
<td>82.33±8.64</td>
<td>88.91±9.79</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>167.78±30.31</td>
<td>176.89±28.26</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>102.54±28.33</td>
<td>118.91±31.31</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>37.83±6.42</td>
<td>40.63±8.19</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>109.45±25.62</td>
<td>112.47±22.35</td>
</tr>
<tr>
<td>VLDL-C, mg/dl</td>
<td>20.51±5.67</td>
<td>23.78±6.26</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD or frequency.
Abbreviations: TAC, total antioxidant capacity; TBARS, thioarbituric acid reactive substance; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol. *P value < 0.05 considered statistically significant.

Reduced cardiovascular modulation is an important marker for prediction of future CV morbidity.7 The findings of this study revealed decreased cardiovascular modulation in prehypertensive young adults. Decrease in cardiovascular modulation is known to be associated independently with CV risk factors such as high blood pressure, waist circumference, physical inactivity, fasting plasma glucose, total cholesterol, triglycerides, and age.8–12 In our study, CV risk factors also differed significantly between prehypertensive and normotensive young adults. To determine the exact association between MAP (prehypertension) and cardiovascular modulation (RMSSD), CV risk factors were fixed, and we found a weak correlation between prehypertension and cardiovascular modulation. This indicates that the influence of prehypertension on cardiovascular modulation is less or vice versa. The findings of increased oxidative stress parameters in both young and middle-aged prehypertensive subjects and the strong correlation of oxidative stress with prehypertension after adjusting for confounding CV risk factors are consistent with the reports of previous studies.16 The association between oxidative stress and prehypertension alone may not, however, be adequate to conclude their causal relationship. Nevertheless, previous studies have demonstrated that hypertension per se can increase oxidative stress33 or vice versa.34
Figure 2. Association between mean arterial pressure and cardiovagal modulation ($r = -0.301; P < 0.001$). RMSSD, root mean of sum of squares of successive RR interval differences.

Figure 3. Association between mean arterial pressure and oxidative stress parameters thiobarbituric acid reactive substance (TBARS) ($r = 0.411; P < 0.001$) and total antioxidant capacity (TAC) ($r = -0.403; P < 0.001$).

Figure 4. Association between cardiovagal modulation (root mean of sum of squares of successive RR interval differences (RMSSD)) and oxidative stress parameters (TBARS) ($r = -0.484; P < 0.001$) and total antioxidant capacity (TAC) ($r = 0.601; P < 0.001$).
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**Table 4.** Contribution of independent variables (oxidative stress parameters) in the variance of dependent variables (mean arterial pressure and cardiovagal modulation) by linear regression

<table>
<thead>
<tr>
<th>Association between oxidative stress parameters and mean arterial pressure ($R^2 = 0.232$)</th>
<th>$\beta$ coefficient ± SE</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiobarbituric acid reactive substance</td>
<td>$4.64\pm1.17$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total antioxidant capacity</td>
<td>$-3.36\pm0.89$</td>
<td>&lt;0.001</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Association between oxidative stress parameters and cardiovagal modulation RMSSD ($R^2 = 0.368$)</th>
<th>$\beta$ coefficient ± SE</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiobarbituric acid reactive substance</td>
<td>$-8.27\pm2.12$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total antioxidant capacity</td>
<td>$11.03\pm1.61$</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviation: RMSSD, root mean of sum of squares of successive RR interval differences.

In this study, a strong correlation between oxidative stress and cardiovagal modulation and prehypertension and a weak correlation between cardiovagal modulation and prehypertension led us to hypothesize that oxidative stress may play a pivotal role in the pathogenesis of both prehypertension and altered cardiovagal modulation. Conduction of longitudinal studies in future may elucidate the fundamental relationship between oxidative stress, cardiovagal modulation, and prehypertension.

The report of the Framingham heart study has revealed the presence of multiple risk factors in middle age that increase the lifetime risk for CVD for the remaining years and reduces longevity by 10 years. In this study, we found that prehypertensive young adults tend to have a similar trend of oxidative stress, altered cardiovagal modulation, and CV risk factors as observed in middle-aged adults. This indicates that the presence of prehypertension and associated CV risk factors in young adults predispose them to the risk for adverse CV events. Nonetheless, prehypertension and CV risk factor assessment in young adults is often ignored, and therefore the CV complications that keep accumulating with increasing age will result in the development of other comorbid conditions.

One limitation of this study was that there were more male subjects in the young prehypertension group than the other groups, which could have influenced our observations because young men are more prone to develop prehypertension than young women of peer age group. Additionally, BP was measured on 2 separate occasions, once at screening camp and the other time at the lab, but the categorization of the subjects was based on the lab recording only.

Prehypertension in young adults is associated with increased oxidative stress and altered cardiovagal modulation. The risk factors for CVDs in prehypertensive young adults were found to be similar to those of middle-aged adults who are in the twilight zone for development of CV dysfunctions.

**DISCLOSURE**

The authors declared no conflict of interest.

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