Original Article

# Assessment of Oxidative Stress Markers Related to Atherosclerosis in Pre-Hypertensive Women

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## Abstract

***Background:*** *Lipid peroxidation is a free radical-generating process which occurs on every membranous structure of the cell. Free radicals are known to be involved in a number of human pathologies including atherosclerosis. The purpose of this cross-sectional study was to examine the association between pre-hypertension status and oxidative stress markers [total antioxidant capacity (TAC) and malonedialdehyde (MDA) levels] in a random sample of cardiovascular disease-free women.*

***Methods:*** *In this study, 160 women of 20-45 years of age were randomly selected. General information data were gathered from each sample using questionnaires and face-to-face interviews. Blood pressure (BP) was measured for each subject. Body weight, height, and waist and hip circumferences were measured and body mass index (BMI) 120*

*and waist-to-hip ratio (WHR) were calculated for each subject. Venous blood samples were drawn from the subjects, and plasma was separated. In this study, the oxidative stress status was assessed by measuring the concentrations of plasma MDA and TAC levels.*

***Results:*** *Our results show that both systolic and diastolic blood pressures were inversely correlated with TAC ($p<0.01$) and positively correlated with MDA levels ($p<0.01$). Particularly, compared to the normotensive subjects, the pre-hypertensives had 19% lower TAC ($p<0.05$) and 22% higher MDA levels ($p<0.01$), after correcting for multiple comparisons and adjusting for age, body mass index, waist-to-hip ratio, and other potential confounders.*

***Conclusion:*** *Our findings revealed an association between pre-hypertension and oxidative stress markers linked to atherosclerosis process. Thus, the identification of the underlying molecular mechanisms in pre-hypertension, which seem to include oxidative stress, may serve as an important lead for developing potentially new treatment modalities in this group of patients at risk for future cardiovascular complications.*

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***Keywords:*** *Pre-hypertension • Oxidative stress • Atherosclerosis • Women*

## Introduction

Hypertension is considered a state of oxidative stress that can contribute to the development of atherosclerosis[1] and other hypertension-induced organ damage.[2] Excessive production of reactive oxygen species (ROS), outstripping antioxidant defense mechanisms, has been implicated in pathophysiologic conditions that impact on the cardiovascular system.[3] Several mechanisms have been suggested to describe how elevated blood pressure might confer the

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increased risk of coronary artery disease and stroke. Among them, structural and functional changes of the arterial wall, increased oxidative stress, and accelerated atherogenesis have been implicated in hypertension.[4-5] However, the question of whether hypertension or oxidative stress is the primary event remains still unanswered. Recently, the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure has suggested a new classification for borderline blood pressure levels, the "pre-hypertension". This "new" category between normal blood pressure and established hypertension includes a population at high risk for developing hypertension and in which lifestyle modifications are needed.[6]

The determination of antioxidative capacity is now considered as a tool in the medical diagnosis and treatment of several diseases, including cardiovascular disease, cancer, diabetes mellitus, and aging.[7] Total antioxidant capacity (TAC) considers the cumulative action of all the antioxidants present in plasma and body fluids and provides an integrated parameter rather than the simple sum of measurable antioxidants. There is now a wide rang of evidence indicating the importance of TAC in plasma and modification during oxidative stress development, as well as its feasibility as a tool for investigating the association between diet and oxidative stress.[8] In addition, lipid peroxidation and oxidative conversion of low density lipoproteins (LDL) are now considered to be a key event in the biological process that initiates and accelerates the development of the early atherosclerotic lesion, the fatty streak.[9] The presence of any relationship between pre-hypertension status and oxidation, linking pre-hypertension with atherosclerosis process directly, has not been thoroughly investigated. Furthermore, this stage of pre-hypertension can be an ideal model for studying the early metabolic manifestations related to elevated blood pressure levels without the confounding effects mediated by other risk factors commonly clustering with advanced stages of hypertension.

The purpose of this study was to investigate whether pre-hypertension status had any relationship with the levels of oxidative stress markers. We studied the association between pre-hypertension status and oxidative stress markers by measuring the concentrations of plasma malondialdehyde (MDA) and TAC in a random sample of cardiovascular disease-free women.

## *Methods*

The subjects included in this study were selected from women under the cover of rural health centers of the Kerman Province, Iran. In this study, 160 women between 20 and 45 years old were randomly selected. Power analysis showed that the pre-specified number of participants is adequate to evaluate two-sided standardized differences between the subgroups of the study and the investigated parameters greater than 0.5, achieving statistical power>0.80 at <0.05 probability level (p-Value). Pregnant and lactating women and subjects with history of cancer, cardiovascular disease, diabetes, and renal or liver diseases and those taking vitamin or mineral supplements were excluded.

Informed written consent was obtained from the subjects before entering the study. The data collecting form included demographic characteristics (age, number of pregnancies, and education), detailed medical history, and lifestyle habits such as smoking status and physical activity. Body weight was measured to the nearest 0.1Kg while subjects were wearing neither shoes nor clothes. Body height was also measured to the nearest 1cm while subjects were not wearing shoes, in the standing position. Body mass index (BMI) was measured as weight (in kilogram) divided by height (in meters-squared). Obesity was defined as BMI >29.9 $Kg/m^2$.[10] To calculate waist-to-hip ratio (WHR), the waist circumference was measured in a horizontal plane at the level of the high point of the iliac crest to the nearest 0.1 cm. Hip circumference was measured in a horizontal plane at the maximum extension of the buttocks. Abdominal obesity was defined as WHR>0.8.[11]

Blood pressure (BP) was measured with a mercury sphygmomanometer, with the subject in the sitting position after 15-min of rest in a quiet environment, in accordance with the recommendations of the British Hypertension Society. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were averaged by using three readings measured at 5-min intervals. Differences of <5mmHg were allowed. The study participants were divided into three groups according to their average SBP and DBP levels. Subjects whose average BP levels were greater than or equal to 140 mmHg/90mmHg or were on antihypertensive medication or had been diagnosed with hypertension but were untreated were classified as hypertensives. Participants who had mean systolic/diastolic blood pressures within the range of 120-139mmHg/80-89mmHg and had never been told that they had high BP levels were defined as pre-hypertensives in accordance with the Seventh Report of the Joint National Committee on the Prevention Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7).[6] Fifty-three women were recruited as a pre-hypertensive group (Case group) and 75 healthy, normotensive women were selected as a control group.

Venus blood samples were obtained from the median cubital vein and collected into standard tubes containing ethylene diamine tetra acetic acid (EDTA). The blood samples were centrifuged at 3000rpm for 15-min at 4°C, and plasma was separated to assay MDA and TAC concentrations. The plasma MDA concentration was assayed by measuring thiobarbituric acid reactive substances (TBARS) according to the Satoh method.[12] The pink chromogen produced by the reaction of thiobarbituric acid with MDA was measured at 530nm. Plasma TAC levels were determined by the colorimetric assay using 2, 2'-Azino-di-[3-ethylbenzthiazoline sulphonate] (ABTS).[13] The assay relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS to $ABTS^{\bullet+}$ by a peroxidase.

The amount of $ABTS^{\bullet+}$ produced can be monitored by reading the absorbance at 600 nm. The intra- and inter-assay coefficients of variation of TAC and MDA did not exceed 4 and 7%, respectively.

Continuous variables are presented as mean values±standard deviation (SD), while categorical variables are presented as absolute and relative frequencies. Associations between categorical variables were tested by the use of contingency tables and the calculation of Chi-squared test. Pearson's correlation coefficient was applied to test the relationships between oxidative stress markers and BP levels or other normally distributed continuous variables. Comparisons between continuous variables across the hypertension groups or other categorical variables were performed by the calculation of multi-way analysis of covariance, after controlling for equality of variances and adjusting for the potential confounding effect of several covariates. Furthermore, multiple linear regression models were applied to evaluate the association between MDA and TAC and the hypertension status of the participants, after controlling for various potential confounders. The results from the regression models are presented as beta-coefficients and standard error of the coefficient. Normality tests were applied using the Kolmogorov-Semirnov criterion. We also calculated $R^2$ in order to find how well each fitted model predicted the dependent variables. Finally, a logistic regression analysis evaluated the odds (likelihood) of having the oxidative stress markers at the extremes tertiles according to the hypertension status of the participants. Two-tailed values of $p<0.05$ were considered as statistically significant. SPSS version 12.5 (Statistical Package for Social Sciences, SPSS Inc, Chicago, IL. USA) software was used for all the statistical calculations.

## *Results*

We observed that 53 (33.1%) and 32 (20%) participants were pre-hypertensive and hypertensive, respectively. The demographic and anthropometric characteristics of the participants in terms of blood pressure status are shown in Table 1.

Normotensive women were at a significantly younger age as compared to pre-hypertensive and hypertensives (p=0.001). Hypertensive and pre-hypertensive participants were more frequently obese and abdominally obese as compared to normotensive (p=0.002).

Total antioxidant capacity was inversely correlated with SBP and DBP (r=–0.21, p<0.001 and r=–0.18, p<0.001, respectively). In addition, TAC levels were inversely associated with age (r=–0.19, p<0.01), BMI (r=–0.24, p<0.001), number of pregnancies (r=–0.16, p<0.01), and WHR (r=–0.26, p<0.001). In addition, no significant relationships were observed between TAC levels with physical activity and education status.

Regarding MDA levels, we found that it was positively correlated with SBP and DBP (r=0.24, p<0.001 and r=0.18, p<0.001, respectively), age (r=0.15, p<0.01), BMI (r=0.22, p<0.001), and WHR (r=0.24, p<0.001). As expected, an inverse relationship was observed between MDA and TAC levels (r=–0.18, p<0.001). Figure1 illustrates the previous relationships. An association was found between hypertension status, TAC, and MDA levels. As we can see from the descriptive results presented in Table 2, pre-hypertensive women had 19% lower TAC (p<0.05) and 22% higher MDA levels (p<0.01) compared to normotensives. Moreover, differences were also observed between hypertension and pre-hypertension status (Table 2).

We then evaluated the association between oxidative stress indices and hypertension status after taking into account the potential confounding effect of age, number of pregnancies, BMI, WHR, and physical activity. We observed that the aforementioned relationships remained significant even after adjusting for the previous characteristics of the participants (Table 3). Furthermore, MDA levels were positively associated with age (p=0.02), number of pregnancies (p=0.04), BMI (p=0.003), and WHR (p=0.001), while TAC levels were inversely correlated with the number of pregnancies (p=0.02). No significant relationships were found between TAC levels and age, physical activity, BMI, and WHR.

Table 1. Demographic and anthropometric characteristics of the participants in terms of blood pressure status (Data are presented as mean±SD)

| Variables | Normotensive (n=75) | Pre-hypertensive (n=53) | Hypertensive (n=32) | p-Value† |
|---|---|---|---|---|
| Age (yr) | 31±11 | 36±12 | 42±3* | 0.001 |
| Education status (years of school) | 7±4 | 7±3 | 6±3 | 0.321 |
| Physical inactivity (%) | 48 | 47 | 51 | 0.247 |
| Body mass index (Kg/m$^2$) | 24±4 | 26±5* | 29±4** | 0.001 |
| Obesity (%) | 9 | 12* | 16** | 0.002 |
| Waist-to-hip ratio | 0.74±0.06 | 0.82 ± 0.08 | 0.91±0.07* | 0.030 |
| Abdominal obesity (%) | 11 | 14* | 19* | 0.002 |
| Number of pregnancies | 3.4±2.1 | 3.8±3.1 | 4.2±3.7* | 0.031 |

† p-Value derived from one-way ANOVA was used to evaluate differences in the investigated variables and blood pressure.
* p<0.05 from the post hoc comparisons of the investigated variables between pre-hypertensive or hypertensive subjects and normotensives
** p<0.01 from the post hoc comparisons of the investigated variables between pre-hypertensive or hypertensive subjects and normotensives

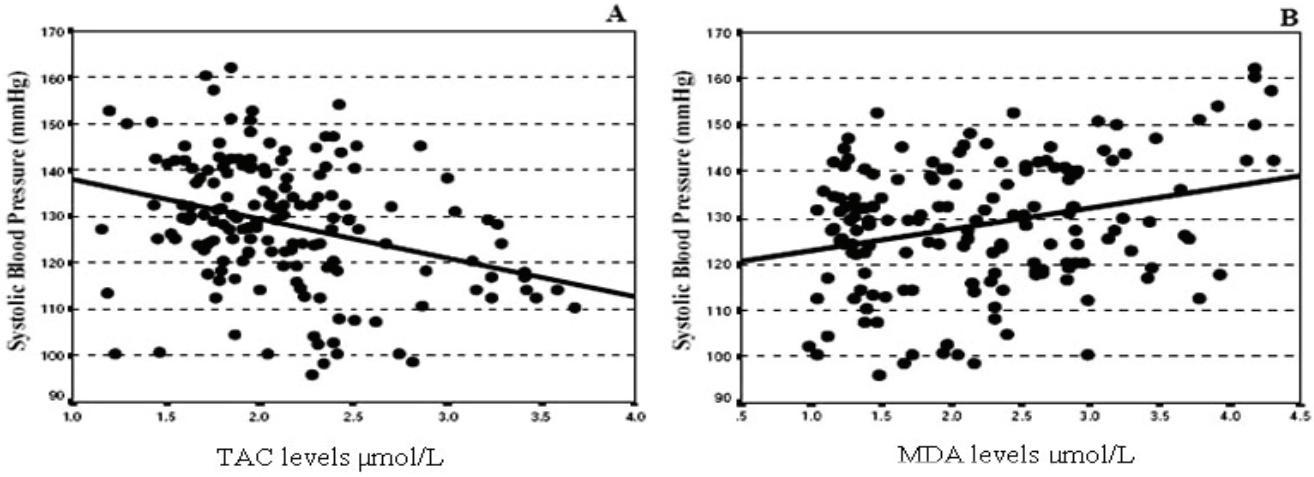


Figure 1. Correlation between systolic blood pressure and total antioxidant capacity (TAC) levels (r=–0.21, p<0.001) (A) and malonedialdehyde (MDA) levels(r=0.24,p<0.001) (B)

Table 2. Oxidative stress markers and blood pressure status (Data are presented as mean±SD)

| Oxidative stress markers | Normotensive (n=75) | Pre-hypertensive (n=53) | Hypertensive (n=32) | p-Value† |
|---|---|---|---|---|
| MDA (µmol/L) | 1.84±0.64 | 2.38±0.79**, § | 3.41±0.96** | <0.001 |
| TAC (µmol/L) | 3.56±0.84 | 2.89±0.57* | 1.98±0.39** | <0.001 |

† p-Values were calculated from the multi-way ANOVA after adjusting for age
* p<0.05 from the post hoc comparisons between pre-hypertensive or hypertensive subjects and normotensives
** p<0.01 from the post hoc comparisons between pre-hypertensive or hypertensive subjects and normotensives
§ p<0.01 from the post hoc comparisons between pre-hypertensive and hypertensive subjects
MDA, Malonedialdehyde; TAC, Total antioxidant capacity

Table 3. Results from multiple linear regression analysis, evaluating the association between plasma TAC and MDA levels (dependent) and hypertension status (independent), after controlling for various potential confounders

| Model for | Beta-coefficient ± SE | p-Value |
|---|---|---|
| **MDA levels** | | |
| Constant of the model | 47±7 | |
| Pre-hypertension vs. normal | 7.2±2.3 | 0.02 |
| Hypertension vs. normal | 10.4±2.7 | 0.03 |
| Age (per year) | 0.58±0.04 | 0.02 |
| Physical activity (yes vs. no) | -0.29±0.06 | NS |
| Number of pregnancies | 2.1±0.8 | 0.04 |
| Body mass index (Kg/m$^2$) | 3.6±1.2 | 0.003 |
| Waist-to-hip ratio | 2.6±0.4 | 0.001 |
| Adjusted $R^2$ with/without hypertension status | 12% / 8% | |
| **TAC levels** | | |
| Constant of the model | 85±19 | |
| Pre-hypertension vs. normal | -10.8±3.8 | 0.02 |
| Hypertension vs. normal | -14.6±4.2 | 0.004 |
| Age (per year) | -0.26±0.07 | N.S |
| Physical activity (yes vs. no) | 3.8±0.2 | N.S |
| Number of pregnancies | -0.42±0.03 | 0.02 |
| Body mass index (Kg/m$^2$) | -0.81±0.2 | N.S |
| Waist-to-hip ratio | -0.74±0.4 | N.S |
| Adjusted $R^2$ with/without hypertension status | 11% / 6% | |

TAC, Total antioxidant capacity; MDA, Malonedialdehyde; vs, Versus; NS, Non-significant

## Discussion

Lipid peroxidation is a free radical-generating process which occurs on every membranous structure of the cell. Free radicals are known to be involved in a number of human pathologies including atherosclerosis.[14] The findings that show TAC levels are lower and MDA levels are higher in pre-hypertensive women compared to normotensive group may state a hypothesis about the direct relationship between borderline blood pressure levels and oxidation process.

Despite the well-established association between atherosclerosis and low density lipoprotein (LDL)-cholesterol, it was not realized, until 1984, that the oxidation of LDL represents a biological modification giving rise to an LDL particle that supports foam cell formation. According to this hypothesis, oxidized LDL contributes to atherogenesis by aiding the recruitment of circulating monocytes into intima space, inhibiting the ability of resident macrophages to leave the intima, enhancing the rate of lipoprotein leading to foam cell formation and being cytotoxic leading to a loss of endothelium integrity.[9,15-16] Previous studies have suggested that hypertension may be related to increased lipid peroxidation.[5,17] In our study, we found that pre-hypertension was associated with 22% higher levels of MDA, as a marker of lipid peroxidation, compared to normal blood pressure levels, after taking into account the potential confounding effect of various factors. This finding comes in accordance with the findings of Toikka et al., who illustrated evidence of sub-clinical atherosclerosis, by measuring the intima-media thickness of the carotid and brachial arteries and increased oxidative stress levels in healthy men with borderline hypertension.[18] Some experimental studies suggesting the importance of pressure changes on the arterial wall in the development of atherosclerosis and lipoprotein oxidation have provided insight into the possible mechanisms by which blood pressure elevation increases lipid peroxidation levels. Among them, Meyer et al. induced luminal pressure on the rabbit aorta in vitro and observed that this stretching increased the uptake of LDL into the arterial wall.[19] This theory may offer a mechanistic explanation of how slightly elevated blood pressure levels enhance the oxidation process, except for inflammation.[20] Furthermore, according to the Seventh Report of the National Joint Committee, even slightly elevated blood pressure levels increase cardiovascular risk; beginning at a systolic/diastolic blood pressure level of 115mmHg/75mmHg and the risk of cardiovascular disease doubles with each increment of 20mmHg/10mmHg.[6] This study associated with both systolic and diastolic blood pressure levels, while TAC is inversely correlated with blood pressure levels. This linear association may explain how slight elevations of blood pressure levels increase the oxidation process. Our results were in agreement with those of Lip et al. and Ward et al., who found increased MDA levels in hypertensive subjects.[21-22] Similarly, Russo et al. in their study of patients with essential hypertension reported increased MDA levels.[23] In addition, human studies have reported that pre-hypertensives males and females have 31% higher C-reactive protein, 32% higher tumor necrosis factor α, 9% higher homocystein levels, and a 10% higher white blood cell counts, compared to normotensives.[20] This observation indicates that in the pre-hypertension category, which is created for stratification for the management of the cardiovascular risk according to the coexistence of other cardiovascular risk factors, inflammation plays a significant role.

One of the unresolved questions in hypertension research is whether the elevation of blood pressure per se constitutes the sole risk factor for cardiovascular complications. Indeed, a variety of clinical and experimental observations suggest that pathogenic cofactors are present.[24] During the last years, there has been increasing evidence that reactive oxygen species such as superoxide, hydrogen peroxide, and hydroxyl radicals may play a role in the development of organ damage associated with cardiovascular disease in general and hypertension in particular.[25-26] The endothelium has been shown to beget elevations of superoxide levels resulting either from an increase in xanthine oxidase activity or from a decrease in the production of nitric oxide.[27] Isolated neutrophils from hypertensives have been reported to produce higher than normal levels of superoxide.[28] It is known that antioxidants prevent ROS from causing damage to cells and tissues. Furthermore, sufficient levels of antioxidants protect plasma levels of hydrogen peroxide from becoming elevated.[28-29] Additionally, Lacy et al. illustrated that plasma hydrogen peroxide production is genetically oriented, while even normotensives with a positive family history of hypertension show higher plasma production of hydrogen peroxide than do normotensives, without a family history of hypertension.[30] Furthermore, some prominent risk factors associated with cardiovascular disease, like obesity and increased age[4] have been shown to be associated with decreased antioxidant activity in the kidney, brain, and heart.[31-32] In this study, we also found a positive correlation between MDA levels and pre-hypertension status, as well as between age and BMI, suggesting that aging and obesity might have a significant impact on the oxidation process and pre-hypertension status. We revealed that even slightly elevated blood pressure levels, in the stage of pre-hypertension, are associated with significant lower antioxidant capacity, independent of the coexistence of other atherogenic risk factors.

The limitation of this study is that we could not assess metabolic syndrome status and nutrient intakes (including antioxidant nutrients and electrolytes) of the participants. Moreover, the cross-sectional design of the study did not allow us to conclude causal relationships. Further studies are needed in order to refuse or confirm the stated hypothesis of a direct relationship between pre-hypertension condition and oxidation promoting to sub-clinical atherosclerosis disease. Thus, the identification of the underlying molecular mechanisms in pre-hypertension, which seem to include

oxidative stress, may serve as an important lead for developing potentially new treatment modalities in this group of patients who are at risk of future cardiovascular complications.

## Conclusion

We revealed an association between pre-hypertension status and oxidative markers among cardiovascular disease-free women, independently of other coexisting risk factors or unhealthy lifestyle behaviors. This evidence may suggest that excessive production of oxidative markers could be an early event in the pathogenesis of hypertension, preceding excess rise in blood pressure levels and could also be an element that contributes to vascular injury. However, the opposite relationship (i.e. elevated blood pressure levels could lead to excessive production of oxidative stress) also be supported. Thus, the identification of the underlying molecular mechanisms in pre-hypertension, that seems to include oxidation process, may serve as an important lead for developing potentially new treatment modalities in this group of patients at risk for future cardiovascular complications.

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