Original Article

Evaluation of serum 8-isoprostane levels and arterial stiffness index in normotensive, prehypertensive and hypertensive subjects

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Objective: To compare serum levels of 8-isoprostane and arterial stiffness index (ASI) in normotensive, prehypertensive and hypertensive subjects and to ascertain its correlation with ASI and blood pressure.

Methodology: We studied a total of ninety male subjects ranging from 35-55 years of age. They were classified into normotensive, prehypertensive and hypertensive groups in light of JNC-8 Guidelines. Each group comprised of thirty subjects. Blood pressure was measured with mercury sphygmomanometer and digital volume pulse was recorded by photoplethysmography and ASI was calculated. Serum 8-isoprostane levels were measured by ELISA.

Results: Serum 8-isoprostane levels and ASI were significantly (0.0001) different amongst the three groups. The Post Hoc Tukey's Test revealed p-value of 0.006 between normotensive and prehypertensive groups and 0.0001 between prehypertensive and hypertensive as well as normotensive and hypertensive groups for ASI. Plasma 8-isoprostane levels were non-significant (0.113) between normotensive and prehypertensive groups while significant (0.0001) between prehypertensive and hypertensive as well as normotensive and hypertensive groups. In normotensive subjects, the relationship between serum 8-isoprostane levels was moderately (r=0.4) correlated with ASI while, it was relatively strongly related with ASI in prehypertensive (r = 0.5) and hypertensive (r = 0.5) subjects.

Conclusion: The oxidative stress is involved in the development and worsening of arterial stiffness in prehypertensive and hypertensive patients. (Rawal Med J 201;43:349-353).

Key words: Prehypertension; hypertension, arterial stiffness index, 8-isoprostane levels, oxidative stress.

INTRODUCTION

The oxidative stress leads to arterial stiffness and hypertension. When the free radicals formation surpasses the body antioxidant defense mechanism it results in activation of many undesirable pathways. The free radicals synthesize oxidized low-density lipoproteins and oxidized cholesterol, which are major culprits in vascular injury. The accumulation of these abnormal molecules results in atherosclerosis.[1,2] The increased stiffness and decreased elasticity of the vessel wall is the principal cause of increased pulse pressure and isolated systolic hypertension.[3] The increased stretch of an artery will relocate stress to the less distensible collagenous components of its wall and makes the stretched artery more stiffer leading to the worsening of hypertension.[4] Arterial stiffness is a cause rather than a consequence of hypertension and precede the development of hypertension in animal model.[5]

There is well documented evidence that the long term rise in blood pressure accelerates the on-going hyperplastic and hypertrophic changes in the arterial smooth muscle. Whenever there is long-term expansion of the arterial wall, it results in greater and greater recruitment of the inelastic collagen fibers resulting in reduction of its elasticity. The gradual elastic fibre degeneration is the major culprit leading to structural changes in the tunica media of the elastic arteries [6]. Arterial stiffness has been related with increased activity of Angiotensin-II which is responsible for raised blood pressure. It has been postulated that Angiotensin-II leads to reduced nitric oxide bioavailability, increased NADPH oxidase activity and increased production of reactive oxygen species which precipitates the

arterial stiffness and hypertension [7].

The reaction of arachidonic acid and free oxygen species yield 8-isoprostane by non-enzymatic breakdown of arachidonic acid, which has been observed both in vivo and in vitro studies. The chemical and structural properties of 8-isoprostane are highly specific and it is stable chemically. Hence, it is employed as a marker of oxidative stress both in vivo and in vitro.[8] The role that systemic arterial stiffness plays in the pathogenesis of hypertension and cardiovascular disease has generated great interest in defining basic mechanisms that stiffen the vascular wall, increase blood pressure, and contribute to the target organ damage with a hope that clarification of these mechanisms will allow for the development of more effective treatments.[9] This study was carried out to determine the role of oxidative stress in modulating the elasticity of vessel wall in prehypertensive and hypertensive subjects Our hypothesis is that oxidative stress contributes in stiffening of the vessel wall that may lead to the development of hypertension.

METHODOLOGY

This study was carried out at Department of Physiology in collaboration with Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College and its allied hospitals from December 2013 to Dec 2014. It was non-randomized case control study and included 90 male subjects aged between 35-55 years using non-probability, convenience sampling. Each group comprised of thirty subjects. The subjects were placed into various groups according to JNC-VIII report.[10] More than one thousand subjects were interviewed and those having fever, any allergic disease, or taking any kind of medications for at least last two weeks were excluded from the study. Those who had history of acute coronary syndrome, malignancy chronic inflammatory disease, diabetes, hypo or hyperthyroidism or any prolonged illness were also excluded. The study approved by post graduate board of studies Army Medical College and Ethical Review Committee, CREAM.

The weight, height, hip circumference and waist circumference were measured according to standard protocols. The body mass index (BMI) and Waist hip ratio (WHR) were calculated. Arterial blood pressure was measured by auscultatory method with the mercury sphygmomanometer. The random blood sugar was checked by the glucometer in order to screen for diabetes mellitus and those having random blood sugar above 140 mg/dl were excluded from the study. Digital Volume Pulse (DVP) was recorded by placing velcro scrap of photoplethysmograph on volar surface of middle finger via iWorx-214 physiological interface system and ASI was calculated. The LabScribe software was used to analyze data. By placing cursor on the two peaks of DVP, the reflection time was calculated. The ASI was calculated by the formulae [ASI = Height (meters)/ Reflection time (seconds)]. Arterial stiffness index score resulting by this simple technique is comparable to the arterial stiffness score determined by pulse-wave velocity, which is the unanimously agreed gold standard marker.[11] The samples were analyzed at CREAM Lab using 8-Isoprostane ELISA (Cayman chemical company USA; Item # 516351).

Statistical analysis was performed by using SPSS version 20. The study variables in the three groups were compared by one way ANOVA followed by Post-Hoc Tukey's test. In order to determine the correlation between 8-isoprostane, ASI and BP variables; Pearson's correlation coefficient was calculated. The p-value <0.05 was considered statistically significant.

RESULTS

The comparison of age, anthropometric indices, blood pressure variables and arterial stiffness index has been presented in Table 1. The groups were compared by one way ANOVA to study the difference in variables amongst the normotensive, prehypertensive and hypertensive groups. The age, waist circumference, waist hip ratio, the blood pressure variables and arterial stiffness index (ASI) were significantly different amongst the groups. In order to determine the difference of each variable between the two groups Post-Hoc Tukey's test was applied (Table 2).

Table 1. Comparison of anthropometric indices, blood pressure variables and arterial stiffness index among normotensive, prehypertensive and hypertensive groups by one-way ANOVA.

Variables	Group 1 Normotensive (n=30)	Group 2 Prehypertensive (n=30)	Group 3 Hypertensive (n=30)	p-value
Age (years)	40.10 ± 4.29	43.17 ± 5.17	47.90 ± 5.30	**0.0001**
BMI (Kg/m^2)	25.47 ± 2.78	25.77 ± 3.16	26.76 ± 3.01	0.223
WC (m)	0.90 ± 0.08	0.95 ± 0.09	0.98 ± 0.08	**0.003**
WHR	0.94 ± 0.56	0.999 ± 0.08	1.03 ±.09	**0.0001**
SBP (mm of Hg)	110.17 ± 5.89	129.93 ± 4.46	164.03 ± 11.63	**0.0001**
DBP(mm of Hg)	72.90 ± 5.87	86.20 ± 2.25	104.97 ± 11.17	**0.0001**
ASI (m/s)	6.67 ± 0.52	7.85 ± 0.62	12.19 ± 2.62	**0.0001**
Plasma 8-isoprostane (ng/ml)	**124 ± 22**	**147 ± 16**	**241 ± 54**	**0.0001**

[BMI: body mass index; WC: waist circumference; WHR: waist to hip ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure; ASI: arterial stiffness index; Kg: kilogram; m: meters; s: seconds]

Table 2. Comparison of anthropometric indices, blood pressure variables arterial stiffness index and plasma 8-isoprostane between the two groups by Post-Hoc Tukey's Test.

Variables	Normotensive vs. Prehypertensive	Normotensive vs. Hypertensive	Prehypertensive vs. Hypertensive
Age	**0.048**	**0.0001**	**0.001**
WC	0.057	**0.003**	0.523
WHR	**0.011**	**0.0001**	0.371
SBP	**0.0001**	**0.0001**	**0.0001**
DBP	**0.0001**	**0.0001**	**0.0001**
ASI	**0.006**	**0.0001**	**0.0001**
Plasma 8-isoprostane (ng/mL)	0.113	**0.0001**	**0.0001**

[WC: waist circumference; WHR: waist to hip ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure; ASI: arterial stiffness index]

In normotensive subjects, the relationship between plasma 8-isoprostane levels were correlated with ASI (r=0.4; p=0.04) while, it was relatively strongly related with ASI in prehypertensive (r=0.5; p=0.02) and hypertensive subjects (r=0.5; p=0.02). The plasma 8-isoprastane levels were correlated with systolic (r=0.58) and diastolic (r=0.32) blood pressure in hypertensive subjects but no such statistical relationship was seen amongst the normotensive and prehypertensive groups.

DISCUSSION

Oxidative stress is the hallmark of arterial stiffness leading to hypertension. There was a significant difference of ASI between the normotensive, prehypertensive and hypertensive subjects. The arterial stiffness index in our study was strongly correlated to the documented factors, which alter the stiffness of the vessel wall like age, blood pressure and measures of central obesity.[12]

In our study, there was a significant difference of plasma 8-isoprostane levels, between the normotensive, prehypertensive and hypertensive groups. In a study, by Rodrigo et al there was similar findings in normotensive and hypertensive subjects. In a study by Vassalle et al, on healthy controls vs. patients with coronary artery disease undergoing angioplasty there were significant differences seen in plasma 8-isoprostane levels in subjects with and without hypertension.[13,14]

In a study by Ward et al plasma 8-isoprostane levels were similar in normotensive and hypertensive subjects. This contrary finding may be due the difference in selection criteria of subjects in which both treated and untreated subjects were taken in the hypertensive group. The various antihypertensive drugs may have different influence in terms of improving the status of oxidative stress in hypertensive subjects. The lipid lowering drugs are believed to improve the oxidative stress.[15]

In each of group, correlation was observed between the plasma 8-isoprostane levels and ASI. These results were similar to the studies by Kim et al in

prehypertensive subjects where a positive correlation was observed between various markers of oxidative stress and arterial stiffness.[16] The correlation seen in hypertensive subjects is similar to the findings of other studies.[17-19]

There was no correlation between plasma 8-isoprostane and blood pressure when studied separately in normotensive and prehypertensive groups. These results are in agreement with findings of other studies.[18-20]. The findings of Rodrigo et al were different from ours where plasma 8-isoprostane levels were correlated with blood pressure in normotensive subjects as well.[13] This difference in findings may be incidental due to small sample size in both the studies, which needs to be confirmed at a cross-sectional study with large sample size in normotensive and prehypertensive subjects by controlling the confounding factors that alter the status of oxidative stress in normotensive healthy subjects. There was a strong correlation seen between the plasma 8-isoprostane and arterial stiffness index in each of normotensive, prehypertensive and hypertensive groups. It may be inferred from these findings that oxidative stress plays an important role in the initiation and development of arterial stiffness.

In our study, the plasma 8-isoprostane levels were not only associated with ASI in hypertensive subjects, but had strong correlation in prehypertensive and normotensive subjects. It may be concluded from these findings that oxidative stress may be involved in all stages from initiation to the full-blown development of the vascular stiffness. Our results showed that plasma 8-isoprostane levels were not correlated with blood pressure in normotensive and prehypertensive subjects. Therefore, in initial stages, the rise in arterial stiffness due to oxidative stress may be independent of the changes in blood pressure. It has been proposed that increased oxidative stress in the initial stages leads to the up regulation of the enzymes, which take up the majority of free radicals and protect from the extensive damage, which has been evident from the studies by Simic et al.[17-20] The positive correlation of arterial stifffness in normotensive and prehypertensive subjects is also supported by the findings of Pratico et al. that antioxidant supplementation (vitamin E) reduced the aortic leisions but had no effect on plasma cholesterol and blood pressure.[21]

The strong positive correlation of blood pressure with plasma 8-isoprostane levels in hypertensive subjects indicated that the increased oxidative stress might result in worsening of the hypertension.[18] Moreover, raised blood pressure may lead to increased synthesis of plasma 8-isoprostane due to mechanically stimulated free radical generation. Therefore cascade leads to further worsening the elasticity of vessel wall and hypertension in a cyclic self perpetuating manner.[13]

There has been conflicting evidence that oxidative stress is involved in hypertension or is the consequence of hypertension.[20] Our data suggest that oxidative stress contribute in the pathogenesis of hypertension by altering the stiffness of vessel wall which increases the peripheral vascular resistance and blood pressure. However, during the initial stages of hypertension, oxidative stress may be masked due to upregulation of antioxidant enzyme system. The effects of oxidative stress are expressed during the initial stages in the vessel wall leading to the arterial stiffness but magnitude of reduction in the compliance of the vessel wall might not be optimum enough to cause hypertension. So, it is suggested that ASI may be used as the marker to determine the effects of antihypertensive and antioxidant supplementation to determine their benificial effects in decreasing the stiffness of the vessel wall along with their effect on lowering the blood pressure.

CONCLUSION

The oxidative stress contributes in the process of stiffening of the vascular wall, which may precede the development of hypertension. The oxidative stress is involved in the development and worsening of arterial stiffness in prehypertensive and hypertensive patients. The strong association of serum 8-isoprostane levels with ASI and blood pressure observed in our study needs evaluation at large sample size and the potential role of antioxidants and antihypertensive drugs in reduction of ASI and blood pressure needs to be determined.

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REFERENCES

1. Kayama Y, Raaz U, Jagger A, Adam M, Schellinger I, Sakamoto M, et al. Diabetic Cardiovascular Disease Induced by Oxidative Stress. Int J Molecular Sci 2015;16:25234.
2. Virdis A, Dell'Agnello U, Taddei S. Impact of inflammation on vascular disease in hypertension. Maturitas 2014;78:179-83.
3. Segers P, Rietzschel ER, De Buyzere ML, Vermeersch SJ, De Bacquer D, Van Bortel LM, et al. Noninvasive (input) impedance, pulse wave velocity, and wave reflection in healthy middle-aged men and women. Hypertension 2007;49:1248-55.
4. Patel RS, Al Mheid I, Morris AA, Ahmed Y, Kavtaradze N, Ali S, et al. Oxidative stress is associated with impaired arterial elasticity. Atherosclerosis 2011;218:90-5.
5. Weisbrod RM, Shiang T, Al Sayah L, Fry JL, Bajpai S, Reinhart-King CA, et al. Arterial stiffening precedes systolic hypertension in diet-induced obesity. Hypertension 2013;62:1105-10.
6. Gkaliagkousi E, Douma S. The pathogenesis of arterial stiffness and its prognostic value in essential hypertension and cardiovascular diseases. Hippokratia 2009;13:70-5.
7. Lin L, Park S, Lakatta EG. RAGE signaling in inflammation and arterial aging. Frontiers Biosci 2009;14:1403.
8. Cracowski JL, Devillier P, Durand T, Stanke-Labesque F, Bessard G. Vascular biology of the isoprostanes. J Vascu Res 2001;38:93-103.
9. Mitchell GF. Arterial stiffness and hypertension. Hypertension 2014;64:13-8.
10. Armstrong C. JNC8 guidelines for the management of hypertension in adults. Am Fam Physician 2014;90:503-4.
11. Clarenbach CF, Stoewhas AC, van Gestel AJ, Latshang TD, Lo Cascio CM, Bloch KE, et al. Comparison of photoplethysmographic and arterial tonometry-derived indices of arterial stiffness. Hypertension Res 2012;35:228-33.
12. Millasseau SC, Kelly RP, Ritter JM, Chowienczyk PJ. Determination of age-related increases in large artery stiffness by digital pulse contour analysis. Clin Sci (Lond). 2002;103:371-7.
13. Rodrigo R, Prat H, Passalacqua W, Araya J, Guichard C, Bachler JP. Relationship between oxidative stress and essential hypertension. Hypertens Res 2007;30:1159-67.
14. Vassalle C, Botto N, Andreassi MG, Berti S, Biagini A. Evidence for enhanced 8-isoprostane plasma levels, as index of oxidative stress in vivo, in patients with coronary artery disease. Coron Artery Dis 2003;14:213-8.
15. Sugiyama M, Ohashi M, Takase H, Sato K, Ueda R, Dohi Y. Effects of atorvastatin on inflammation and oxidative stress. Heart Vessels 2005;20:133-6.
16. Kim M, Jung S, Kim SY, Lee SH, Lee JH. Prehypertension-associated elevation in circulating lysophosphatidlycholines, Lp-PLA2 activity, and oxidative stress. PLoS One. 2014;9(5):e96735.
17. Simic DV, Mimic-Oka J, Pljesa-Ercegovac M, Savic-Radojevic A, Opacic M, Matic D, et al. Byproducts of oxidative protein damage and antioxidant enzyme activities in plasma of patients with different degrees of essential hypertension. J Human Hypertension 2006;20:149-55.
18. Klima L, Kawecka-Jaszcz K, Stolarz-Skrzypek K, Menne J, Fijorek K, Olszanecka A, et al. Structure and function of large arteries in hypertension in relation to oxidative stress markers. Kardiologia Polska 2013;71:917-23.
19. de Faria AP, Fontana V, Modolo R, Barbaro NR, Sabbatini AR, Pansani IF, et al. Plasma 8-isoprostane levels are associated with endothelial dysfunction in resistant hypertension. Clin Chim Acta 2014;433:179-83.
20. Cracowski JL, Baguet JP, Ormezzano O, Bessard J, Stanke-Labesque F, Bessard G, et al. Lipid peroxidation is not increased in patients with untreated mild-to-moderate hypertension. Hypertension 2003;41:286-8.
21. Pratico D, Tangirala RK, Rader DJ, Rokach J, FitzGerald GA. Vitamin E suppresses isoprostane generation in vivo and reduces atherosclerosis in ApoE-deficient mice. Nature Med 1998;4:1189-92.