National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTICLE

A study of serum nitric oxide levels and pulse pressure in aging

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Received: April 18, 2018; **Accepted:** May 03, 2018

ABSTRACT

Background: Nitric oxide (NO) is one of the major signaling messengers in the cardiovascular system. A sufficient level of endothelial NO is necessary to preserve normal vascular physiology and is one of the regulators of pulse pressure. Aging is linked to a gradual decrease of NO activity in the vessel wall. Hence, we tried to find a relationship between serum NO levels and pulse pressure in aging. **Aims and Objective:** The aim and objective of this study are to estimate serum NO levels and pulse pressure in normal aging. **Materials and Methods:** A total of 90 healthy male subjects aged between 70 and 80 otals were randomly selected. Fasting blood samples of the subjects were obtained for the estimation of serum NO levels (Griess method). NO concentration is indirectly measured by means of accurately quantifying the levels of nitrite (NO_2^-). The resting pulse pressure of the subjects was recorded on 3 consecutive days, and the average of the three values was recorded. **Results:** The mean pulse pressure of the study population was 60.6 ± 6.2 mm Hg, and the mean serum NO level was 27.9 ± 9.9 (μM). A negative correlation was observed between serum NO levels and pulse pressure. The Pearson's coefficient of correlation was $r = -0.398$ which is statistically significant at the 0.05 level (one-tailed). **Conclusion:** In our study population of 90 urban dwelling elderly men (mean age 74 ± 3 years), we observed that higher levels of serum NO levels were associated with lower pulse pressures.

KEY WORDS: Nitric Oxide; Pulse Pressure; Nitrite (NO_2^-); Griess Method

INTRODUCTION

Nitric oxide (NO) is one of the major signaling messengers in the cardiovascular system. NO plays a crucial role as an intermediary of vasodilatation in blood vessels.[1] It is stimulated by numerous substances. After its production by the endothelial NO synthase, NO is released from the endothelial cells to the tunica media which lies adjacent to it and leads to phosphorylation of various proteins that cause relaxation of the vascular smooth muscle. This vasodilator action of NO is indispensable for the control of blood flow and blood pressure.[2]

NO acts as a highly reactive free radical. It rapidly diffuses into the smooth muscle cells of the blood vessel and acts on soluble guanylate cyclase. NO stimulates the soluble guanylate cyclase to produce the second messenger cyclic 3',5' guanosine monophosphate from guanosine triphosphate (GTP). Cyclic guanosine monophosphate activates cyclic nucleotide-dependent protein kinase G (PKG or cGKI). PKG phosphorylates a number of proteins regulating calcium concentrations, calcium sensitization, hyperpolarization of the cell through potassium channels, and dynamic alterations in actin and myosin that results in vascular smooth muscle relaxation.[1,2]

The pulse pressure is calculated as the difference between systolic and diastolic pressure. It is affected by the cardiac

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Website: www.njppp.com	Quick Response code
DOI: 10.5455/njppp.2018.8.0416903052018	

output and arterial stiffness. Pulse pressure is in fact considered as a significant indicator of vascular stiffness, which was first documented by Bramwell and Hill in the year 1922. They proved that the difference between systolic and diastolic pressure, i.e. the pulse pressure is positively associated with the stiffness of the arteries.[3]

Both systolic and diastolic blood pressures tend to increase with age. However, after the age of 60, there is usually no added increment in the diastolic blood pressure, whereas this is not the case with systolic pressure which can increase. Thus, as age increases, there is a widening of pulse pressure.[4,5]

Pulse pressure can be easily calculated using the regular sphygmomanometer. It is accepted as the most simple approach in measuring arterial stiffness and can be recorded in outpatient settings without difficulty.[5] Several longitudinal investigations declare pulse pressure to be a valuable indicator of cardiovascular risk. Figures of the Framingham study have shown that, in individuals more than 50 years of age, pulse pressure is an excellent marker of coronary artery disease risk in hypertensive patients when compared to only systolic or diastolic pressure.[6]

NO being the prime mediator of vascular elasticity, we ventured on finding whether any relationship exists between serum levels of NO and pulse pressure.

MATERIALS AND METHODS

The study was conducted after obtaining the approval of the institutional ethics committee. A total of 90 healthy male subjects aged between 70 and 80 years were randomly selected from urban population of Chennai.

Subjects with a history of cigarette smoking (for the preceding 10 years), average blood pressure more than 140/90 mm Hg, hypercholesterolemia, Type 2 diabetes, ischemic heart disease, major cardiac arrhythmia (atrial fibrillation and second-degree heart block), valvular heart disease excluding mild valvular insufficiency, cerebrovascular accidents, chronic respiratory illness, neurological disease, thyroid disease or other endocrine disorders, psychiatric illness, and substance abuse were excluded from the study. Subjects with a body mass index of more than 30 and patients on cardiovascular drugs were also excluded from the study. We explained the scope and details of the study to the subjects. The subjects underwent routine clinical examination and biochemical tests to satisfy the selection criteria. The resting blood pressure of the subjects was recorded on 3 consecutive days, and the average of the three values was recorded. The pulse pressure was calculated as the difference between systolic and diastolic pressure.

Fasting blood samples of the subjects were obtained for the estimation of NO levels. Fasting blood samples were obtained under strict aseptic precautions, by venepuncture of the antecubital vein. The serum was separated and stored in the deep freezer at −20°C. The NO estimation is on the basis of diazotization (Griess method) technique and is capable of measuring *in vitro* concentration of NO. This estimation facilitates to overcome technical hitches in detecting NO attributable to the brief half-life of almost 5 sec. NO concentration is indirectly measured by means of accurately quantifying the levels of nitrite (NO_2^-) and the derivative of NO in live tissues. The chemical basis of the test is a color change that takes place when the compound naphthyl ethylenediamine is added to the offshoot of the reaction amid sulfanilamide and nitrite.

RESULTS

The mean pulse pressure of the study population was 60.6 ± 6.2 mm Hg, and the mean serum NO level was 27.9 ± 9.9 (μM). A negative correlation was observed between serum NO levels and pulse pressure. The Pearson's coefficient of correlation was $r = -0.398$ which is statistically significant at the 0.05 level (one-tailed) [Figure 1 and Table 1].

DISCUSSION

The present study was aimed to assess the vascular health through aging and pulse pressure in relation to endothelial

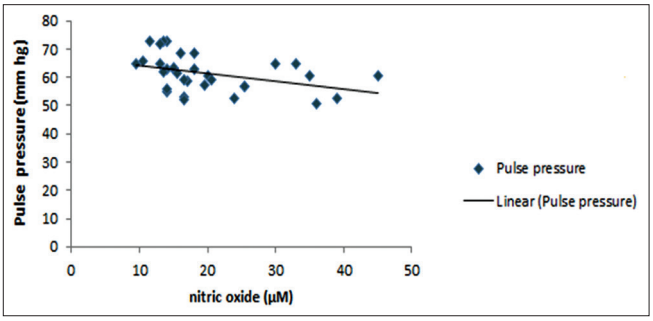

Figure 1: Correlation between serum nitric oxide levels and pulse pressure

Table 1: Correlation between serum NO levels and pulse pressure

	NO	Pulse pressure
Pearson correction		
NO	1.000	−0.398
Pulse pressure	−0.398*	1.000
Sig (1-tailed)		
NO		0.011
Pulse pressure	0.011	
n		
NO	33	33
Pulse pressure	33	33

*Correlation is statistically significant at the 0.05 level (one-tailed).
NO: Nitric oxide

functions among the general healthy population. Our study population consisted of 90 urban dwelling elderly men (mean age 74 ± 3 years). The mean pulse pressure of the study population was 60.6 ± 6.2 mm Hg, and the mean serum NO level was 27.9 ± 9.9 (μM). A significant negative correlation was observed between serum NO levels and pulse pressure. We observed that higher serum NO levels were associated with lower pulse pressure.

Our results are also supported by the study of Yoshihiro Miyamoto and Yoshihiko Saito who strongly suggested that variations in NO metabolism are concerned with the increase of arterial pulse pressure in endothelial NO synthase gene knockout mice.[7] Yet, another study also correlates with our findings which implies that decreased arterial compliance in the medium-sized blood vessels is the principal means of blunting of baroreceptor sensitivity with increasing age.[8] Likewise, an age-associated decrease in the arterial compliance can affect optimal cardiac function by escalating the impedance to ejection of the left ventricle.[8,9] Our study is supported by the findings of Zanzinger which explains that NO is such a powerful controller of blood pressure that even a momentary inhibition of this protective mechanism produces large increase in the pulse pressure. NO is also associated with normal arterial function, and its deficiency is shown to be an important cause of hypertension.[10,11] Nevertheless, alterations in arterial compliance can also occur within a short interval of time, by means of variations of the sympathetic tone of vascular smooth muscle in the tunica media of the blood vessel wall.[12] It has been proved that NO exerts a sympatholytic effect by antagonizing the effects of the sympathetic system on the vascular tone.[13]

Our present study was done on healthy elderly males without any comorbidities. Our aim was to identify the role of NO on the alteration of vascular function related only to aging. Females were not added as subjects to avoid any variations in the levels of NO or endothelial changes that were related to gender. Pulse pressure was selected as an indicator, as its recording was a non-invasive, quick, cost-effective, simple technique but well established as an indicator of arterial stiffness. However, further prospective studies on interventions for improving NO levels such as physical activity, diet, and aerobic training could enhance the value of this study.

CONCLUSION

Arterial stiffness is one of the major indicators of endothelial functions and it is age dependent. Pulse pressure may be considered as a potential indicator of age-related alteration of vascular stiffness. Endothelial-derived serum NO plays as an important role as a mediator to influence vascular stiffness in the process of aging. Decreased bioavailability of NO, in resistance and conduit arteries, is characterized as an endothelial dysfunction and is a predictor of cardiovascular risk and outcome.[14-16] Understanding of these mechanisms in depth may help to explore new avenues on our knowledge in vascular sciences and prevention of age-related cardiovascular risks.

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How to cite this article: Udayakumar RK, Muthupandian A. A study of serum nitric oxide levels and pulse pressure in aging. Natl J Physiol Pharm Pharmacol 2018;8(9):1262-1264.

Source of Support: Nil, **Conflict of Interest:** None declared.