Original Research Article

Association of nitrotyrosine with oxidised LDL, C- reactive protein and malondialdehyde in prehypertensive and hypertensive patients

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Abstract

Background: Hypertension is a major risk factor for coronary artery diseases (CAD), chronic kidney diseases, peripheral arterial disease and other vascular complications. Oxidative stress plays a pivotal role in the development of CAD. So the assessment of oxidantmediated biomolecule are useful to predict clinical outcome. Nitric oxide derived oxidants interacts with superoxide anion to form peroxynitrite. Peroxynitrite is an effective oxidant which promotes nitration of protein tyrosine residues to form nitrotyrosine. So assessment of oxidative stress biomarkers in prehypertension and hypertension subjects useful for early diagnosis vascular complications and the process of low systemic inflammation is involved in pathophysiology of prehypertension. **Objectives:** The present study was to evaluate Nitrotyrosine levels in Prehypertensive and hypertensive cases compared with healthy subjects and to correlate these levels with Oxidised LDL , C- Reactive protein and Malondialdhehyde **Materials and Methods:** Sixty prehypertensive and sixty hypertensive subjects with 35 to 50 years age group were selected according to JNC- for this study and 60 healthy age matched subjects were selected as a controls. Serum Nitrotyrosine , Oxidised LDL, CRP was estimated by ELISA, malondialdehyde (MDA) was assessed by Thiobarbituric Acid Reactive Substances (TBARS) method and routine investigations were carried out by ERBA EM-360 fully automated analyzer. **Results:** Serum Nitrotyrosine, Ox LDL, CRP, Malondialdehyde levels were significantly increased in hypertensive subjects compared with prehypertensive subjects and there was also significant difference between healthy subjects. Serum Nitrotyrosine levels positively correlated with Ox LDL, CRP , Malondialdehyde, Triglycerides, LDL and negative correlation with HDL cholesterol levels. **Conclusion:** Serum nitrotyrosine might be potent oxidative stress biomarker in prehypertension and hypertension. Nityrotyrosine is considered as diagnostic marker for assessment coronary artery disease and vascular complications in hypertension. Regular monitoring and maintaining within normal range might be useful for reduction of cardiovascular morbidity and mortality in hypertensive patients.

Key Words: Hypertension, Nitrotyrosine, Oxidised LDL (Ox LDL), Malondialdehyde (MDA), C- reactive protein (CRP)

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INTRODUCTION

Hypertension is most important risk factor for coronary artery diseases (CAD), vascular complications develops because of genetic, anatomical, adaptive neural, endocrine, humoral, ,hemodynamic and environmental factors [1,2]. Oxidative stress plays a pivotal role in the pathogenesis of hypertension [3,4]. Oxidative stress is mainly due to excess levels of oxidants over antioxidants with increased production of reactive oxygen species (ROS), reactive nitrogen species (RNS) and decreased bioavailability of nitric oxide (NO) levels [5-7]. Nitrotyrosine is a potent oxidant biomarker of peroxynitrite ($ONOO^-$), reactive oxidative, nitrosative species which derives from in vivo reaction of superoxide anion and nitric oxide (NO) [8-10]. The production of free oxygen radicals are increased during reperfusion with prolonged ischemia and this subsequently increases formation of peroxynitrite [11,12]. Peroxynitrite oxidizes thiols or thioethers to nitrate tyrosine residues and

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oxidizes guanosine, resulting in oxidative damage to proteins, lipids, carbohydrates, DNA, and sulfhydryl groups and initiate lipid peroxidation [13].Lipoproteins play a very crucial role in atherogenesis. Serum low density lipoprotein cholesterol (LDL-C) firmly established risk factor for CAD [14]. LDL is a metabolic end product for apolipoprotein B (apoB), which circulates within the vascular compartments including subendothelial space, until removed by high-affinity apoB receptor-mediated endocytosis. Oxidation of LDL reacts with reactive oxygen species (ROS) which chemically damages LDL, enhancing lipid peroxidation (15). Ox -LDL molecule also increases the uptake of lipid products by macrophages leading to cholesterol accumulation and subsequent foam cell formation [16]. Ox-LDL directly delivers various lipid peroxides and hydroperoxide compounds. These compounds which act as cytotoxins, monocyte chemoattractants , macrophages and inhibitors of macrophage movement [17]. C-reactive protein (CRP) is a short pentraxin belonging to the highly conserved family of calcium-dependent ligand-binding plasma protein , non-specific marker for inflammation which is produced by the liver, and is known to play a central role in the aetiopathogenesis of arterial atherothrombosis [18,19]. So , in this view the objective of the present study was to evaluate serum Nitrotyrosine levels in prehypertensive and hypertensive subjects and its association with Ox-LDL, CRP and generalized lipid peroxidation marker malondialdehyde.

MATERIALS AND METHODS

Sixty pre hypertensive and sixty hypertensive subjects of both sexes aged between 35-50 years according to JNC-8 (Eighth Joint National Committee) guidelines attending Department of General Medicine, Nimra Institute of Medical sciences, Jupudi, Andhra pradesh state, India were selected for present study. The study was approved by Institutional Human ethics committee (IHEC) and informed consent was obtained from each subject before sample collection and general examination and experiments were performed in accordance with Helsinki declaration of 1975. Pre hypertensive subjects- systolic blood pressure (120-139) or diastolic blood pressure (80-89). Hypertensive subjects - systolic blood pressure (≥140) or diastolic blood pressure (≥90). The general characteristics age, gender, height, body weight, waist and hip circumferences were collected. Diabetes mellitus , cardiovascular diseases, renal impairment, liver dysfunction, thyroid disorders history of acute myocardial infarction, stroke, and peripheral vascular disease, chronic alcoholics, smokers, pregnant women and patients on antioxidant medication are excluded from the study. Sixty healthy sex and age matched subjects were selected as controls.

Blood pressure Measurement: Blood pressure was measured by Mercury Sphygmomanometer (Diamond, Mumbai, India) with the patients in a sitting position, legs uncrossed. After 5 minutes of rest in the sitting position, BP was measured on both arms and the higher of the two is taken into consideration. Based on the average of two or more properly measured, seated BP readings on each of two or more office visits.

Biochemical analysis: Fasting venous blood samples were obtained from the study subjects and centrifuged at 3000 rpm for 15 min. Routine laboratory investigations were performed immediately using autoanalyser and aliquots were stored at −80 °C for further estimation of serum nitrotyrosine, Ox-LDL levels. Glucose, serum cholesterol , triglycerides , HDL cholesterol , LDL cholesterol , Urea , Creatinine, Uric acid, Bilirubin , Total protein , Albumin , Globulin , Alanine aminotransferase, Asparatate amino transferase, Alkaline phosphatase were assessed by standardized protocols using ERBA EM-360 fully automated analyzer . Serum Nitrotyrosine (Sincere Biotech; Ltd, Beijing, China) , Ox –LDL (Elabscience Ltd, Wuhan, China) assessed by Enzyme Linked Immuno Sorbent Assay (ELISA). C-reactive protein was assessed by turbilatex method, malondialdehyde (MDA) assessed by Thiobarbituric Acid Reactive Substances (TBARS) method (20)

Statistical analysis: Statistical analysis carried out with SPSS 25.0 software and values were expressed as mean ± standard deviation, p value < 0.05 was considered as statistical significant. The Pearson correlation test was used for correlation analysis.

RESULTS

Table 1: Comparison of Age, BMI, Waist hip ratio, systolic and diastolic blood pressure in controls, Prehypertensive and Hypertensive subjects

Parameters	Controls (n=60)	Prehypertension subjects (n=60)	Hypertension subjects (n=60)
Age	37.9±4.2	38.1±.5.3	39.4±5.5
Body mass index	23.2±1.5	28.8±1.3 ^{a*}	29.06±2.85 $^{a\#,b*}$
Waist/Hip ratio	0.91±0.06	0.94±0.08 $^{a\#}$	0.99±0.03 $^{b*,c\#}$
Systolic BP (mm Hg)	113.4±5.4	136.2±5.5 ^{a*}	184.0±12.1 ^{b*,c*}
Diastolic (mm Hg)	73.4±3.1	88.1±4.1 ^{a*}	104.8±7.6 ^{b*,c*}

Data are expressed as mean ±SD, *p<0.001 ,#p<0.05 was considered statistically significant; a= comparison between Controls and Prehypertension subjects; b=comparison between Controls and Hypertension subjects; c=comparison between Prehypertension and Hypertension subjects

Table 2: Comparison of fasting plasma glucose, Lipid profile, Liver profile parameters in controls, prehypertensive and Hypertensive subjects

Parameters	Controls (n=60)	Prehypertension subjects (n=60)	Hypertension subjects (n=60)
FPG(mg/dl)	86.5±9.8	89.3±10.8	90.1±15.4
Serum cholesterol (mg/dl)	167.3±8.6	215.1±19.4 a*	230.7±14.7 b*,c#
Serum Triglycerides (mg/dl)	110.6±19.2	172.8±16.8 a*	199.9±20.6 b*,c#
HDL cholesterol (mg/dl)	44.1±5.4	40.5±5.6 a#	37.3±6.7 b*,c*
LDL cholesterol (mg/dl)	119.6±10.7	145.8±18.7 a*	157.1±16.9 b*,c#
Total Bilirubin(mg/dl)	0.78±0.09	0.79±0.05	0.81±0.07
Direct Bilirubin(mg/dl)	0.2±0.04	0.19±0.06	0.20±0.07
AST (IU/L)	28.6±3.5	27.8±8.2	29.3±7.6
ALT (IU/L)	29.4±3.9	28.5±5.7	30.5±5.7
ALP(IU/L)	98.6±14.1	99.2±16.7	98.2±12.9
Total protein(gm/dl)	7.5±0.5	7.3±0.7	6.9±1.4
Albumin(gm/dl)	3.9±0.3	3.8±0.7	3.5±0.4
Globulin(gm/dl)	3.2±0.4	3.3±0.6	3.2±0.7

Data are expressed as mean ±SD, *p<0.001,#p<0.05 was considered statistically significant.
a= comparison between Control and Prehypertension subjects; b=comparison between Control and Hypertension subjects; c=comparison between Prehypertension and Hypertension subjects

Table 3: Comparison Urea, Creatinine, Nitrotyrosine, OX-LDL, CRPand MDA levels in controls, prehypertensive and Hypertensive subjects

Parameters	Controls (n=60)	Prehypertension subjects (n=60)	Hypertension subjects (n=60)
Serum urea(mg/dl)	23.7±5.4	24.8±7.4	25.3±6.9
Serum creatinine(mg/dl)	0.73 ±0.1	0.79±0.3	0.82±0.3
Nitrotyrosine (µmol/l)	0.24±0.03	0.32±0.06 a*	0.44±0.09 a*,b*
OX-LDL (U/L)	81.5±10.26	92.5±9.4 a*	104±11.45 a*,b*
C RP (mg/L)	1.6±0.2	3.6±1.4 a*	6.4±2.8 a*,b**
MDA (µ mol/L)	1.67±0.97	4.2±1.1 a*	7.67±1.5 a*,b**

Data are expressed as mean ±SD, *p<0.001,#p<0.05 was considered statistically significant.
a= comparison between Control and Prehypertension subjects; b=comparison between Control and Hypertension subjects; c=comparison between Prehypertension and Hypertension subjects

Table 4: Correlation between Serum Nitrotyrosine andmeasured parameters in Prehypertensive patients and Hypertensive subjects

Parameters	Prehypertension (Correlation Coefficient-r)	Hypertension (Correlation Coefficient-r)
Ox-LDL	0.343*	0.487**
CRP	0.267*	0.408**
MDA	0.397**	0.523**
Cholesterol	0.287	0.323*
TGL	0.393*	0.427*
HDL	-0.198	-0.245
LDL	0.421**	0.532**
BMI	0.297*	0.321*
Waist /Hip ratio	0.267	0.312*
Systolic BP	0.145	0.267
Diastolic BP	0.124	0.321*

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

Oxidative stress more potentiating risk factor for hypertension. ROS such as superoxide anion (O2−•), hydrogen peroxide (H_2O_2), and hydroxyl anion (OH−•) are reactive byproducts of normal products of cellular metabolism [21,22] .ROS also formed by depletion of nitric oxide synthases (NOS) l-arginine or the cofactor tetrahydrobiopterin or partial inhibition of NOS by antagonists such as asymmetric dimethylarginine [23,24] . In this study we have observed body mass index and (BMI) and Waist hip ratio were significantly increased in hypertensive subjects compared with prehypertensive subjects and further there was also significant difference with control subjects. Oxidative stress plays major role in

development of obesity [25]. Chronic inflammation, elevated triglyglyceride, LDL levels leads to cause excess weight and endothelial dysfunction [26, 27]. Obesity induced oxidative stress leads development of various pathological complications such as diabetes, sleep disorders, reproduction, cardiovascular complications, rheumatological problems[28]. The present study showed increased total cholesterol, triglycerides, LDL cholesterol and decreased HDL cholesterol levels in hypertensive patients compared with prehypertensive and also significant difference with healthy controls as reported earlier studies [29,30,31] .In the present study we observed Nitrotyrosine levels were significantly increased in Prehypertensive, hypertensive subjects compared with healthy volunteers and also significant variation observed in between prehypertensive and hypertensive subjects. Oxidative stress promotes production of reactive carbonyl compounds and lipoperoxides. Reactive oxygen species (ROS) reacts with nitric oxide (NO) producing cytotoxic reactive nitrogen species capable of nitrating proteins and damaging other molecules [32]. Peroxynitrite, formed by the reaction between superoxides and nitric oxide (NO), modifies tyrosine in proteins to form nitrotyrosine, and this stable end-product is involved in the inactivation of mitochondrial and cytosolic proteins, resulting in cellular dysfunction or damage of cellular constituents [33,34]. It has been proposed that endothelial dysfunction associated with hypertension might be caused by augmented production of peroxynitrate associated oxidative and nitrosative effects on several targets, such as sulfydryl groups and aromatic rings of proteins, cell membrane lipids and nucleic acids [35]. The present study showed Ox-LDL levels were significantly increased in Prehypertensive, hypertensive subjects compared with healthy volunteers and also significant variation observed in between prehypertensive and hypertensive subjects.Ox-LDL to determine the contribution of oxidative stress to arterial stiffness as reported earlier studies [36,37]. It is a major risk factor for early ventricular remodeling and cardiac dysfunction [38,39]. The present study also exhibits Nitrotyrosine levels positively correlated with OX-LDL levels. Ox-LDL is a marker of oxidative stress, which in its turn is the leads ROS mediated myocardial damage . Earlier studies reported as oxidative stress in “ischemia-reperfusion injury, catecholamine cardiomyopathy relates to sudden massive oxidative stress. ROS and RNS production, which exerts a direct inhibitory effect on myocardial function through persistent cellular loss of potassium, depletion of high-energy phosphates, elevated intracellular calcium concentration, loss of systolic force development, a progressive increase in diastolic tension, depressed metabolic function, and arrhythmias ,membrane lipid peroxidation and protein oxidation, disturbances in calcium homeostasis disturbance leads to mycocardial contractile abnormalities [40,41]. In the present study it has been observed that CRP and MDA levels are significantly increased in Hypertensive groups compared to control subjects and also shows positive correlation with nitrotyrosine. Experimental studies have shown that short periods of ischemia followed by reperfusion elicit a cascade of proinflammatory reactions that include production of oxygen-derived free radicals, activation of the complement system, adherence of neutrophils to the coronary endothelium, leukocyte mediated injury of the myocardial cells, production of cytokines and acute phase proteins [42,43]. CRP has also been implicated as a mediator of vascular remodeling in response to injury and cardiac remodeling in response to vascular inflammatory pathology [44].

CONCLUSION

Nityrotyrosine is considered as diagnostic marker for assessment coronary artery disease and vascular complications in hypertension. Regular monitoring and maintaining within normal range might be useful for reduction of cardiovascular morbidity and mortality in hypertensive patients.

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