



Contents lists available at ScienceDirect

## Asian Pacific Journal of Tropical Disease

journal homepage: [www.elsevier.com/locate/apjtd](http://www.elsevier.com/locate/apjtd)

Document heading

doi: 10.1016/S2222-1808(14)60465-5

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# Correlation of serum paraoxonase activities in known cases of 130 elderly hypertensive South Asian aged 56–64 years – a hospital based study

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## PEER REVIEW

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## Comments

This is a good study in which the author tried to fill the missing links between PON1, hypertension and antioxidants. The results are interesting. Over all the paper is an informative and open avenue for future studies based on PON1.

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## ABSTRACT

**Objective:** To evaluate paraoxonase activity, antioxidant status and lipid peroxidation in hypertensive participants and to address the hypothesis that oxidative modifications of lipids due to hypertension can cause changes in serum paraoxonase activities.

**Methods:** The serum paraoxonase activities, antioxidants and lipid peroxidation were determined in 130 hypertensive participants and 130 age–sexes matched normotensive healthy volunteers served as control. Serum paraoxonase activities were measured by enzymatic kit. The glutathione peroxidase, superoxide dismutase and catalase activity were determined by standard methods. Malondialdehyde was measured by thiobarbituric acid reaction. Conjugated diene level was measured by Recknagel and Glende method. Serum uric acid, total bilirubin, serum albumin, serum ascorbic acid and lipid profile were analyzed by standard methods.

**Results:** Total cholesterol, triglycerides, low–density lipoprotein cholesterol were significantly higher and high–density lipoprotein cholesterol were significantly lower in hypertensive patients when compared to normotensive healthy controls. The superoxide dismutase, glutathione peroxidase and catalase were significantly lower in hypertensive when compared with normotensive. Similar findings were observed in the levels of albumin, uric acid, bilirubin and ascorbic acid when hypertensives were compared with normotensive. The oxidative stress indicators namely malondialdehyde and conjugated diene were significantly higher and paraoxonase activity were significantly lower in hypertensive.

**Conclusions:** Our study concludes that paraoxonase activities are bound to alter in hypertension which is caused due to interplay of several confounding factors namely oxidative stress, increased oxidized low–density lipoprotein and depletion of antioxidants.

## KEYWORDS

Hypertension, Paraoxonase, Antioxidants, Oxidative Stress, South Asian

## 1. Introduction

Essential hypertension, or hypertension of unknown cause, accounts for invariably more than 90% of diagnosed cases of hypertension[1]. Among the enlisted risk factors for cardiovascular diseases, hypertension is one of them[2]. In addition, hypertension is associated with subclinical vascular impairment as endothelial dysfunction and as an early marker of atherosclerosis[3].

Various underlying mechanisms have been put forward

over the years in the causation of hypertension. It could be vasoconstrictive mechanisms among them, the sympathetic nervous system, the endothelin system, the vasopressin system and more recently the reactive oxygen species which is suggestive in the development of experimental or human hypertension[4]. Yet another cause is increased vascular oxidative stress which could be involved in the pathogenesis of hypertension, a major risk factor for cardiovascular disease mortality[5]. Oxidative stress occurs when there is an imbalance in the interplay between

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Foundation Project: Supported by a grant from Confederation of Epidemiological Associations registered under Govt of Kerala (Grant No. 30–955/2013CEA).

Article history:

Received 30 Nov 2013

Received in revised form 2 Dec, 2nd revised form 10 Dec, 3rd revised form 17 Dec 2013

Accepted 10 Jan 2014

Available online 28 Jan 2014

reactive oxygen species and the antioxidant defense systems whereby the oxidants super cedes the antioxidants[6]. One of the most important oxidative processes is oxidation of lipids and lipoproteins, namely oxidized low-density lipoprotein (Ox-LDL)[7]. Ox-LDL is a major cause of vessel wall injury and atherosclerosis[8]. Ox-LDL has a prominent role in the pathogenesis of atherosclerosis, and the elevation of ox-LDL levels in atherosclerotic plaques is an important event in the development of atherosclerosis[9]. It also induces foam cell formation from macrophages that plays a key role in early atherogenesis[10]. Ox-LDL may also be involved in atherogenesis by inducing smooth muscle cell proliferation and smooth muscle foam cell generation[11]. Under oxidative stress, not only LDL, but other serum lipids are exposed to oxidation[12]. High-density lipoprotein (HDL) is one of the most important independent protective factors for the arteriosclerosis which underlies coronary heart disease[13]. Paraoxonase (PON1), an enzyme well known for three activities, namely paraoxonase, arylesterase and diazoxonase, is a calcium-dependent esterase with 354 amino acids residue which is found exclusively associated with HDL in serum. It protects LDL from oxidative stress by destroying biologically active phospholipids[14]. The verdict is justified by developing PON knockout mouse model that demonstrated greater susceptibility of the lipoproteins to oxidation[15]. In humans, PON is an independent, genetic risk factor for coronary artery disease[16,17], and low PON1 activities are observed in atherosclerotic and hypercholesterolemic patients[16,18–20]. Numerous cohort studies and clinical trials have confirmed the association between a low HDL-cholesterol concentration and increased risk of coronary heart diseases[21,22].

As it is well established PON1 activity is decreased in dyslipidemia accompanied by higher ox-LDL levels[23] which is also observed in hypertensive patients. So the current study was designed to measure the PON1 activity in hypertensive, along with their serum antioxidants, lipid peroxides and lipid profile and the findings were compared with normotensive subjects, to establish their relationship. Also, the study addressed the hypothesis that oxidative modifications of lipids due to hypertension can cause changes in serum paraoxonase activities.

## 2. Materials and methods

A total of 130 hypertensive patients and 130 age-sex matched normotensive healthy volunteers were selected for this study. The study was conducted for a period of three years from September 2007 to August 2010. The design of this study was pre-approved by the institutional ethical committee board of the Institution and informed consent was obtained from the patients and controls.

Biochemical parameters and other parameters such as smoking habits, systolic and diastolic blood pressure and

family history were recorded.

### 2.1. Diagnostic criteria of patients

All the patients had their blood pressure measured using standard mercury manometer. At least two readings at 5-min intervals as per World Health Organization guidelines were recorded[24]. If high blood pressure ( $\geq 140/90$  mmHg) was noted, a third reading was taken after 30 min. The lowest of the three readings was taken as blood pressure. Thus the patients were diagnosed as hypertensive.

### 2.2. Exclusion criteria

Patients with diabetes mellitus, renal insufficiency, hepatic disease or taking lipid lowering drugs or antioxidant vitamin supplements were excluded.

Venous blood was collected after overnight fast of 12 h and ethylene diamine tetraacetic acid was added and samples were processed for lipid profiles.

Blood collection and biochemical methods used: 10 mL of blood was collected after overnight fasting in different containers.

### 2.3. Preparation of erythrocytes for antioxidants studies

A total of 5 mL of blood was taken. Red cells were washed 3–4 times with ice-cold normal saline and used for estimation of glutathione peroxidase, superoxide dismutase and catalase.

#### 2.3.1. Glutathione peroxidase (GPx)

The GPx activity was determined by the procedure of Paglia and Valentine[25]. Briefly, the oxidized glutathione produced during GPx enzyme reaction was immediately reduced by nicotinamide adenine dinucleotide phosphate and glutathione reductase. Therefore, the rate of nicotinamide adenine dinucleotide phosphate consumption was monitored as a measure of formation of oxidized glutathione. Results were expressed as units of GPx per gram of hemoglobin.

#### 2.3.2. Superoxide dismutase (SOD)

Superoxide dismutase enzyme activity was measured by SOD assay kit using rate of inhibition of 2-(4-indophenyl)-(4-Nitrophenol)-5-phenyltetrazolium chloride reduction method modified method of Sun *et al.* using assay Ransod kit SD 125, Randox Lab[26]. One unit of SOD activity was defined as the amount of protein that inhibits the rate of inhibition of 2-(4-indophenyl)-(4-Nitrophenol)-5-phenyltetrazolium chloride reduction by 50%. Enzyme activity was expressed as unit per gram hemoglobin (U/gHb). Hemoglobin was measured by Drabkin's method.

#### 2.3.4. Catalase

Catalase activity was measured spectrophotometrically

as described by Beutler<sup>[27]</sup> and Beutler *et al*<sup>[28]</sup>. One unit of enzyme activity was expressed as micromole hydrogen peroxide decomposed per min per gram hemoglobin.

#### 2.4. Serum for lipid profile, paraoxonase activity, lipid peroxides, conjugated dienes measurement and endogenous antioxidants

Remaining blood was taken and serum was separated. Serum was used for determination of lipid profile, albumin, uric acid, bilirubin, malondialdehyde and conjugated dienes.

##### 2.4.1. Lipid profile

(Total cholesterol, triglycerides, and HDL-cholesterol) were analyzed enzymatically using kit obtained from (Randox Laboratories Limited, Crumlin, UK). Plasma LDL-cholesterol was determined from the values of total cholesterol and HDL-cholesterol using the following formulae:

$$\text{LDL-C} = \frac{\text{TC} - \text{TG}}{5 - \text{HDL-C}}$$

##### 2.4.2. For PON1

Activity studies blood samples were collected in patients once confirmed with raised blood pressure. Only hypertensive patients were included in the study as patients. The assay kit manufactured from Zeptometrix Corporation 872 Main street, Buffalo New York 14202 (ZMC catalogue 0801199)<sup>[29,30]</sup>. The assay is based on the principle; PON1 catalyzes the cleavage of phenyl acetate resulting in the phenol. The rate of formation of phenol was measured by monitoring the increase in absorbance at 270 nm at 25 °C. One unit of arylesterase activity is equal to 1 μmol/L of phenol formed per minute. The activity is expressed in kU/L, based on the extinction coefficient of phenol of 1310 mol/L<sup>-1</sup>·cm<sup>-1</sup> at 270 nm, pH 8.0, and 25 °C. Blank samples containing water are used to correct for non-enzymatic hydrolysis.

##### 2.4.3. Thiobarbituric acid reactive substances

Malondialdehyde (MDA) levels were estimated by thiobarbituric acid reaction<sup>[31]</sup>. Using 40% trichloroacetic acid, proteins were precipitated from 0.5 mL serum, and precipitated proteins were incubated with thiobarbituric acid reagent in a boiling water bath for 1 h. After bringing down to room temperature, the colored complex formed was measured using spectrophotometer at 532 nm. 1,1,2,3-tetraethoxypropane (1 nmol/L) was used as a standard for MDA estimation. Concentrations were expressed in nmol/L.

##### 2.4.4. Conjugated dienes (CD)

CD levels were measured by the method of Recknagel and Glende with little modification<sup>[32]</sup>. Briefly, the principle of the assay is based on with the rearrangement of double bonds in polyunsaturated fatty acids leading to the formation of DC, which absorb light at 233 nm. The oxidation index of

the lipid sample at 233 nm and 215 nm is computed which reflect the diene content and the extent of peroxidation. The lipid peroxidation products measured in serum were treated with antioxidant butylated hydroxytoluene twice, immediately after obtaining and before adding the test reagents to suppress artifactual changes during handling and assay procedures. The first stage of lipid peroxidation consists of the molecular rearrangement of the double bonds in polyunsaturated fatty acids residues of lipids, which leads to CD formation and conversion of CD in hydroperoxide. Serum was chosen to avoid possible influences of substances required for plasma preparation. Serum sample (150 μL) and (150 μL) of 0.9% NaCl (reagent blank contains only isotonic saline) were incubated at 37 °C for 25 min. About 0.25% butylated hydroxyl Toluene (150 μL) was added and the lipids were extracted by heptane/isopropanol (1:1). Then samples were acidified by 5 mol/L HCl and extracted by cold heptane (1600 μL). After centrifugation for 5 min at 3000 r/min, the absorbance of heptane fraction were measured spectrophotometrically at absorbance maximum between 220 nm and 250 nm. The amount of hydroperoxides produced was calculated using molar coefficient of 2.52×10<sup>4</sup> m<sup>-1</sup>.

#### 2.5. Other assays

For estimation of other biochemical parameters, standardized reagents and chemicals of analytical grade were obtained from Sigma-Aldrich Company, New Delhi, India. Serum uric acid was estimated by the method of Brown based on the development of a blue color due to tungsten blue as phosphotungstic acid is reduced by uric acid in alkaline medium<sup>[33]</sup>. Serum total bilirubin was estimated by the method of Jendrasik and Grof<sup>[34]</sup>, serum albumin by bromocresol green dye binding method<sup>[35]</sup>, and serum ascorbic acid by the method of Roe and Kuether<sup>[36]</sup>.

#### 2.6. Statistical analysis

Data on lipid profile and PON1 activity was entered in Microsoft Excel for windows 2007. The mean±SD was obtained using excel software. The two-sample-*t*-test value was obtained between the patients and the control. The distribution of '*t*'- probability was calculated depending on '*n*' and significance of test was obtained. For *P* value <0.001 was considered as highly significant.

### 3. Results

Total cholesterol, triglycerides, LDL-cholesterol were higher in hypertensive patients when compared to normotensive healthy controls (Table 1) (*P*<0.001). The HDL-C levels were significantly lowered in hypertensive when compared with normotensive. Also, significant

differences were observed in total cholesterol, triglycerides, LDL-cholesterol in hypertensive when compared with normotensive healthy controls ( $P<0.001$ ).

**Table 1**

Lipid profile and blood pressure in hypertensive and normotensive.

Variables	Study subjects (n=260)		
	NT (n=130)	HT (n=130)	P value (95%CI)
Age yrs	61.93±3.82	61.72±2.94	0.0039 (61.20–62.23)
Total cholesterol <sup>†</sup>	154.32±13.69	195.31±17.21	<0.001 (192.26–198.35)
HDL-Cholesterol <sup>†</sup>	51.29±6.32	36.82±5.67	<0.001 (35.81–37.82)
Triglycerides <sup>†</sup>	119.73±12.37	146.73±17.37	<0.001 (143.66–149.79)
LDL-Cholesterol <sup>†</sup>	102.39±11.43	132.81±13.51	<0.001 (130.42–135.19)
Systolic blood pressure <sup>††</sup>	117.82±5.37	141.32±12.37	<0.001 (139.13–143.50)
Diastolic blood pressure <sup>††</sup>	76.86±6.78	95.42±7.91	<0.001 (94.02–96.81)

All values are expressed as mean±SD; values in the parenthesis indicate the number of subjects. <sup>†</sup>: mg%; <sup>††</sup>: mm/Hg; NT: Normotensive; HT: Hypertensive.

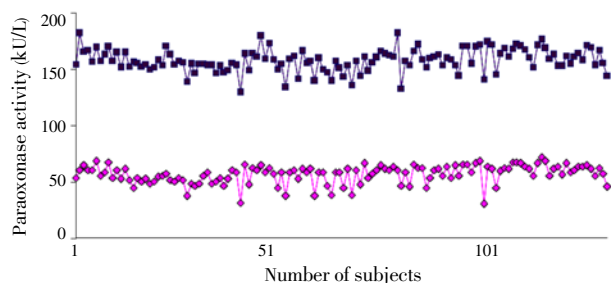
The SOD, GPx and catalase were significantly lower (Table 2) ( $P<0.001$ ) in hypertensive when compared with the observations among controls. Similar findings were observed in the levels of albumin, uric acid, bilirubin and ascorbic acid (Table 2) ( $P<0.001$ ) when hypertensive were compared with normotensive. The oxidative stress indicators namely MDA and CD were significantly higher and paraoxonase activity were significantly lower in hypertensive when compared with normotensive indicating the extent of free radicals generated in hypertensive (Table 2, Figures 1 and 2) ( $P<0.0001$ ).

**Table 2**

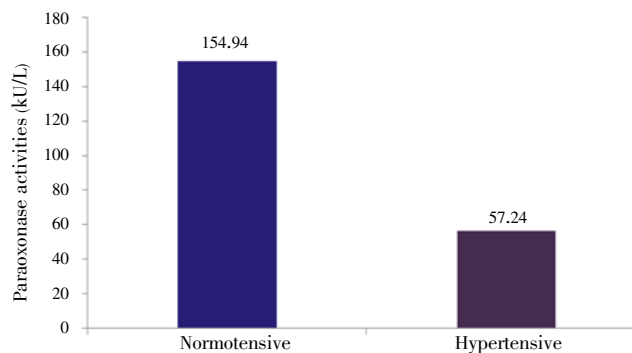
Antioxidant status, oxidative stress and paraoxonase activities in hypertensive and normotensive.

Variables	Study subjects (n=260)		
	NT (n=130)	HT (n=130)	P value (95%CI)
SOD (U/gHb)	1893.23±46.62	826.49±12.86	<0.001 (806.54–846.43)
GPx (U/gHb)	65.28±7.93	37.29±6.52	<0.001 (36.13–38.44)
Catalase (k/gHb)	274.28±22.73	198.35±28.63	<0.001 (193.29–203.40)
Serum albumin <sup>††</sup>	4.32±0.63	4.94±0.46	<0.01 (4.85–5.02)
Serum uric acid <sup>†</sup>	5.27±1.83	4.86±0.93	<0.001 (4.69–5.02)
Serum bilirubin <sup>†</sup>	0.88±0.27	0.63±0.12	<0.01 (0.60–0.65)
Serum ascorbic acid <sup>†</sup>	4.63±1.66	2.98±0.76	<0.001 (2.84–3.11)
MDA (nmol/L)	4.31±1.29	15.67±2.97	<0.0001 (15.14–16.19)
Conjugated diene(μmol/L)	22.46±4.62	66.72±6.71	<0.0001 (65.53–67.90)
Paraoxonase (kU/L)	154.94±15.69	57.24±8.09	<0.0001 (55.94–58.79)

All values are expressed as mean±SD; values in the parenthesis indicate the number of subjects. <sup>†</sup>: (mg%); <sup>††</sup>: (g%); NT: Normotensive; HT: Hypertensive.



**Figure 1.** Paraoxonase activity in normotensive (■) vs. hypertensive (◆) subjects.



**Figure 2.** Comparison of paraoxonase activities in normotensive vs. hypertensive subjects. P value is significant ( $P<0.0001$ ) between hypertensive and normotensive.

#### 4. Discussion

Hypertension is widely established as a primary risk factor for atherosclerosis and cardiovascular disease. Increased oxidative stress is one of the principal mechanisms by which it may exert its pathological influence[37]. Earlier studies conducted provide supportive arguments for PON as an antioxidant function[38–40], which has anti-atherogenic potential[41]. In this context, the results of the current study are agreeable with the hypothesis oxidative modifications due to hypertension causes changes in serum PON activity there by accelerating the atherogenic process. Hypertension are also associated with lower serum levels of HDL concentrations hence could explain alterations in PON activities. Antioxidants and free radicals could conceivably protect PON through augmentation of the overall antioxidant capacity, therefore the result of the current study showed significant differences among hypertensive patients when compared to normotensive controls.

The evident question is whether the observations are of relevance to the occurrence of hypertension. Hypertension has such a powerful impact on the risk of cardiovascular disease in which it is difficult to dissociate a risk factor that may interact with it. The rationale was that if serum PON were of relevance, lower levels could be observed in more severe cases of hypertensive patients. The analysis demonstrated that enzyme activities and concentrations were significantly lowered in hypertensive patients.

More important, perhaps, we demonstrate that differences in PON concentrations, on the order of those observed in the present study can influence the ability of HDL to protect LDL from oxidation. Thus, incremental increases in HDL PON are associated with incremental decreases in the level of LDL hydroperoxides generated under oxidization conditions.

*In vitro* studies are providing a wealth of data on the functions of PON, but observations that concern the clinical consequences of modifications to serum PON are less abundant. A limited number of studies have reported lower PON activities in pathologies associated with a higher risk of vascular disease. PON has also been identified as a genetic risk factor for vascular disease[42]. The current

study observed the association between blood pressure, a prooxidant phenomenon with a demonstrated inhibitory effect on PON, and serum PON activities and concentrations and its concentrations remain unaltered in normotensive controls. Our data also indicate that lower serum PON levels are associated with increased severity of hypertension and reduced capacity to protect LDL from oxidation. They are consistent with the hypothesis that hypertension modifies serum PON such that there is an increased risk of coronary artery disease, which may be due to a diminished capacity to protect lipoproteins from oxidative stress.

Research into paraoxonases has flourished over the last decade. It seems now evident that PON1 has the ability to degrade lipid peroxides in lipoproteins and in cells, and that plays a protective role against oxidative stress and inflammation, which are key processes involved in the pathophysiology of atherosclerosis and hypertension. In future, PON1 measurement can be included to the battery of routine analysis in clinical biochemistry laboratories.

Based on the observations, our study concludes that paraoxonase activities are bound to alter in hypertension which is caused due to interplay of several confounding factors namely oxidative stress, increased ox-LDL and depletion of antioxidants.

Though the observation made in the current study can not draw a definitive conclusion, due to inadequacy in sample size which is just 130. To validate the findings of the current study, multicenter study with large sample size should be carried out to support the findings of the current observation from this study.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Comments

#### Background

Hypertension is the most common disorder of the world and one of the major risk factors for cardiovascular disease. Several etiological factors are responsible for hypertension but the major one is the formation ox-LDL which can be due to altered levels of antioxidant/oxidants balance. Paraoxonase is the most extensively studied enzyme which is associated with antioxidant activity with HDL-cholesterol the so called good cholesterol. If the circulating ox-LDL is limited, the chances of hypertension can be alleviated.

#### Research frontiers

The study is first conducted based on the correlation of PON1, antioxidants and hypertension. The current study tries to fill the missing links between PON1, hypertension and antioxidants.

#### Related reports

Earlier studies have reported on the hypertension subjects with metabolic syndrome. This current study is designed to conjoin the relation between hypertension, PON1 and antioxidants.

#### Innovations & breakthroughs

This is the first study done on essential hypertension subjects with its association of PON1 and other variables which could limit the occurrence of hypertension.

#### Applications

The timely measurement of PON1 activity would help to decrease the incidence of coronary syndrome as hypertension is one of the conventional risk factor of cardiovascular disorder.

#### Peer review

This is an good study in which the author tried to fill the missing links between PON1, hypertension and antioxidants. The results are interesting. Over all the paper is an informative and open avenue for future studies based on PON1.

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