Paraoxonase 1 Activities and Lipid Parameters in Hypertension and Their Association with Chronic Alcoholism

Sudeep Kumar,[1] Jyoti Batra,[2] Sumesh Prasad Sah,[1] Dinesh Kumar,[1] Shahid Iqbal,[1] Manisha Arora,[3] Shivani Sharma[4]

[1]Muzaffarnagar Medical College, Muzaffarnagar, Uttar Pradesh, India
[2]Santosh Medical College & Hospital, Ghaziabad, Uttar Pradesh, India
[3]Department of Metabolism & Nutrition, Medical University of the Americas, St. Kitts & Nevis
[4]Department of Physiology, Medical University of the Americas, St. Kitts & Nevis

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Abstract

Objective: To determine lipid profile, antioxidant vitamin (E and C), and malondialdehyde (MDA), and superoxide dismutase (SOD) levels, as well as paraoxonase 1 (PON 1) activities in alcoholic hypertensive patients.

Methods: Five hundred subjects were selected for this study, consisting of 250 normal healthy individuals and 250 alcoholic hypertensive subjects. Total cholesterol, triglyceride, and HDL levels were measured using the enzymatic method while the LDL and VLDL levels were calculated by Friedwald equation. The MDA level was measured using thiobarbiturate (TBA), and the Vitamin E and C levels were measured using the enzymatic method. The SOD and PON 1 activities were measured using phenyl acetate as the substrate.

Results: Total cholesterol, triglycerides, LDL, VLDL, and MDA levels were found to be significantly high, while the HDL, Vitamin E, and Vitamin C levels decreased among the alcoholic hypertensive subjects as compared to the control. Furthermore, significant decreases in SOD and PON 1 activities were also found among the alcoholic hypertensive subjects as compared to control.

Conclusion: Alterations in lipid parameters, antioxidant vitamin levels, MDA level, SOD activities, and PON 1 activities are associated with hypertension that may be enhanced by alcohol intake, which may lead to the development cardiovascular diseases.

Keywords: Alcoholism, hypertension, MDA, lipid parameter, antioxidants vitamins (E and C)

Introduction

Alcohol abuse leads to the buildup of fat in the liver. In alcoholics, significant amounts of alcohol disrupt various metabolic processes in the liver, resulting in the formation of reactive oxygen species (ROS). In mammalian tissues, free radicals are produced in both healthy and pathological situations. In chronic alcoholism, free radicals create adverse impacts and creating oxidative damages, which is a shift in the oxidant-antioxidant balance.[1]

Chronic liver disease is the tenth leading cause of mortality in adults, with alcoholic cirrhosis accounting for over 40% of cirrhosis-related deaths. Three enzymes, i.e., Alcohol Dehydrogenase (ADH), Cytochrome P-4502E1 (CYP2E1), and mitochondrial catalase, involve in metabolising alcohol in the liver. Heavy drinkers have steatosis in 90 percent to 100 percent of cases; alcoholic hepatitis in 10% to 35% of cases; and alcoholic cirrhosis in 8% to

Correspondence:
Sumesh Prasad Sah
Muzaffarnagar Medical College Campus, Muzaffarnagar, UP, India
e-mail: sumesh.sah@gmail.com

20% of cases. Alcoholic fatty liver eventually leads to alcoholic hepatitis, cirrhosis, and liver failure.[2]

The enzyme paraoxonase, or known as the PON, has both paraoxonase and aryl esterase activities. Aromatic carboxylic acid esters and certain organophosphorus insecticides, particularly paraoxon and nerve gasses, are hydrolyzed. The PON1, PON2, and PON3 are members of the PON gene family, which are found on chromosome 7q21.3–22.1. PON1 is primarily produced in the liver and is firmly linked to HDL, protecting both LDL and HDL against lipid peroxidation (LPO).[3]

Paraoxonase 1 (PON1) activity varies up to 40-fold between individuals[4], and is regulated by genetic, developmental, environmental, and pathologic factors.[5,6] Low PON1 and Arylesterase (AE) activities have been linked to a number of health problems.[7] With the liver damages produced by high alcohol use, it has been hypothesized that excessive alcohol consumption would result in a decrease in serum PON1 and AE activities, as already observed in a several studies.[8]

The excess synthesis of the peroxides and free radicals causes oxidative stress, which is described as an imbalance between oxidants and reductants within the body. Oxidative stress is linked to an increase in the generation of oxidizing species or a considerable chemical reduction in the efficiency of antioxidants and antioxidant enzymes. The LPO is a free radical-related process that can be damaging if it goes unchecked, as the self-enhancing process will disrupt the membranes, lipids, and other cell components.[9] Thus, the presence of LPO in the blood can help determine the prognosis of non-alcoholic fatty liver disease (NAFLD) patients. Studies on PON 1 activities in hypertension and its association with chronic alcoholism have been limited; hence, the aim of this study was to evaluate the effect of chronic alcohol intake on lipid parameters, oxidative stress, and paraoxonase 1 activities in hypertensive subjects.

Methods

The present cross-sectional study was carried out at the Department of Biochemistry, Santosh Medical College and Hospital Ghaziabad and Department of Biochemistry, Muzaffarnagar Medical College and Hospital, Muzaffarnagar. This study was approved by the Institutional Ethical Committee and informed consent was obtained from the subjects prior to study. The minimum sample size has been calculated using the following sample size formula:

$n=z^2pq/d^2$
Where z=1.96 at 95 % confidence interval,
p=0.20 and q=1-p=0.80,
d=absolute error 5%
$n= (1.96)^2\times0.20\times0.80/(0.05)^2$
$=245.86\approx246$

In this study, 500 subjects were included, where 250 were subjects with alcoholism who were already diagnosed as hypertensive and 250 normal and healthy controls. All subjects were male aged 30-60 years old.

Patients with pulmonary tuberculosis, gout and arthritis, hepatic disease, renal disease, acute or chronic inflammatory illness, Type 2 diabetes mellitus, prolonged illness receiving medicines known to alter glucose and lipid metabolism, cardiovascular disease, and who were not willing to give consent for the study were excluded from the study.

The subjects were requested individually for overnight fasting. Blood samples were drawn with the help of disposable syringe and collected in clean vials. The serum was separated and lipid profile was done on fresh serum and remaining serum sample was kept in small fractions at -20^0C. Total cholesterol and HDL cholesterol were measured by CHOD-PAP method, triglyceride by GPO-PAP method, and the level of LDL-c and VLDL-c were calculated by Friedwald Equation. For the MDA, the measurement was performed using a chemical method using thiobarbituric acid (TBA), while the SOD was measured by modified Marklund and Marklund method by pyrogallol. Vitamin E and C were determined by chemical methods. The activity of PON 1 was measured by chemically using phenyl acetate as the substrate. Statistical analysis was performed to determine the differences between controls and study subjects using the Student's t- test in the SPSS package for windows. Data were expressed as mean ± SD, with $p<0.05$ considered as highly significant.

Results

The difference between alcoholic hypertensive subjects and control subjects were statistically significant. Alcoholic hypertensive patients were showed significant increase in CHO, TG, LDL and VLDL ($p<0.001$) and significantly decrease in HDL ($p<0.0001$) as compared to healthy control. The level of antioxidant vitamins (E and C) were found significantly lower ($p<0.001$) and MDA ($p<0.001$) was

Table 1 Demographical Parameters in Studied Subjects

Variable	Control	Hypertensive with Alcoholics	p-Value
Age (Years)	42.93 ± 6.32	42.99 ± 6.53	>0.05 NS
SBP (mm of Hg)	113.86 ± 4.99	160.15 ± 6.47	<0.001 S
DBP (mm of Hg)	81.42 ± 3.82	100.15 ± 3.66	<0.001 S
BMI* (kg/m^2)	24.92 ± 3.29	27.71 ± 3.17	<0.001 S
WHR**	0.80 ± .058	1.08 ± 0.11	<0.001 S

SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: basic metabolic index; WHR: waist to height ratio; S: statistically significant; NS: statistically non-significant

found to be significantly higher in alcoholic hypertensive subjects as compared to normal healthy Individuals. The superoxide dismutase and paraoxonase 1 activities were found to be reduced in alcoholic hypertensive subjects as compared to normal healthy controls (Table 2). Upon correlation analysis, a significant and negative correlation of MDA with SOD and PON 1 and a significant positive correlation of SOD and PON 1 were observed (Table 3).

Discussion

Essential hypertension are often accompanied by multiple metabolic abnormalities and is associated with increased production of ROS predisposing to increase in lipid peroxidation which is a marker for cellular damage. MDA can exacerbate the actions of superoxide ions by impairing endothelium-dependent relaxation and propagation of lipid peroxidation by a chain reaction in membranes.

In this study, increased BMI and Waist/Hip Ratio (WHR) were observed in hypertensive subjects with alcoholics compared to controls. As BMI and WHR being the markers of general obesity and central obesity, increased BMI and WHR in hypertensive subjects with alcoholics predispose these subjects to an increased risk for CVD.

Table 2 Biochemical Parameters in Studied Subjects

Variable	Control	Hypertensive with Alcoholics	p-Value
Total Cholesterol (mg/dL)	197.75 ± 23.21	280.52 ± 31.64	<0.001
Triglycerides (mg/dL)	125.93 ± 18.72	239.18 ± 46.16	<0.001
HDL- Cholesterol (mg/dL)	48.82 ± 7.68	32.24 ± 4.62	<0.001
LDL-Cholesterol (mg/dL)	123.75 ± 21.18	200.45 ± 30.18	<0.001
VLDL-Cholesterol (mg/dL)	25.19 ± 3.74	47.84 ± 9.23	<0.001
MDA (nmol/mL)	2.92 ± 0.45	5.05 ± 1.41	<0.001
PON 1 (U/mL)	67.61 ± 7.45	47.21 ± 13.45	<0.001
SOD (U/mL)	10.88 ± 1.87	7.63 ± 1.65	<0.001
Vitamin-E (mg/dL)	1.76 ± 0.25	1.30 ± 0.12	<0.001
Vitamin-C (mg/dL)	1.57 ± 0.18	1.19 ± 0.092	<0.001

MDA: malondialdehyde; PON 1: paraoxonase 1; SOD: superoxide dismutase

Table 3 Correlation Coefficient Among Parameters in Hypertensive Subjects with Alcoholism

Variable	PON1	SOD	Vitamin E	Vitamin C
MDA	r=-0.740 p<0.001	r=-0.620 p<0.001	r=-0.244 p<0.01	r=-0.512 p<0.001

In this study, significant increase in CHO, TG, LDL-c, VLDL-c and significant decrease in HDL-cholesterol were observed in alcoholic hypertensive subjects as compared to normal healthy individuals. A study by Paneri *et al.*[10] has concluded that there is rise in MDA level in alcoholic hypertensive individuals as compared to normal controls. Decrease in the serum HDL and TAC is also observed in alcoholic individuals when compared to normal controls. Serum total cholesterol, TG, LDL, VLDL were also found to be elevated in the study group when compared to the normal controls. Chen *et al.*[11] found significant increase in total cholesterol, triglycerides, LDL-c and VLDL-c in heavy alcohol consuming individuals. Consumption greater than 50 g/ day significantly reduced the risk of developing low levels of HDL-c, but elevated the risks of developing high levels of cholesterol.

Increased plasma total cholesterol levels, which are known to be associated with decreased LDL receptor gene expression and protein abundance in the liver, may be a causing factor for the change. Chronic alcohol exposure may activates down-regulating the activation of a signaling enzyme that is known to be associated with decreased LDL receptor expression in hepatocytes, suggesting that multiple mechanisms are involved in alcohol-induced down-regulation of LDL receptor.

This study revealed a significant increase in MDA levels associated with high alcohol consumption when compared to control. These results are in consistent with Deshpande *et al*.[12] who concluded that increase in MDA levels are related to the alcohol consumption and that may be associated with pathogenesis and progression of liver disease. A study by Tan *et al.*[13] reported the same results. An increased MDA level inactivates the antioxidant enzyme (SOD) in untreated hypertension 14 causing damage to various proteins that may also the cause for reduced enzymatic activity of SOD.[15]

The body produces its own antioxidants as a protective mechanism against oxidative stress. Vitamin-like beta carotene, ascorbic acid, antioxidants enzyme like glutathione peroxidase, Vitamin E, catalase and superoxide dismutase are natural antioxidants, which maintain the balance between oxidants and antioxidants.

As discussed earlier, antioxidant vitamin (E and C) decreases the superoxide anion level in the presence of superoxide dismutase in a healthy individual. In hypertension, it was found in the present study that there is a direct correlation between the activity of superoxide dismutase (SOD) and the levels of Vitamin E and ascorbic acid.

The significant rise in the levels of reactive oxygen species and decrease antioxidant enzyme may be explained as the combat mechanism showing a relationship between oxidant and antioxidant that has been further raised in alcoholic with hypertensive subjects.

This study observed a significant decrease in PON 1 activity in hypertensive alcoholic patients as compared to control. A positive correlation was also seen between antioxidant vitamin (E and C) and PON 1. Individuals carrying low PON 1 activity may have a higher risk for CVD. A case-control study has shown that the reduced PON 1 activity is very common in CHD patients.[16]

Previous studies, including this study, have suggested that PON1 provides the protection for LDL and HDL oxidation and PON1 confers antioxidant activity on HDL. Despite the fact that the susceptibility of HDL to lipid peroxidation and the antioxidant effect of HDL were not measured in our population, the increased risk of CVD in subjects carrying low PON 1 activity could be attributed to the acceleration of atherosclerotic process in these subjects, i.e., an increased susceptibility of LDL to oxidation, a reduction in the antioxidant effect of HDL, and an alteration of their functionality.[17]

Serum PON 1 level declines in various types of liver diseases. PON1, in association with HDL in the circulation, protects LDL from peroxidation. The lowering of PON 1 may be due to peroxidative changes that occur in the hepatocytes. A fall in serum PON 1 could be taken as a manifestation of the different liver function tests i.e., synthesis, detoxication, and secretion.

Correlation studies have found that MDA was positively and significantly correlated with TC, where as it significantly negatively correlates with HDL, PON1, SOD, Vit E, and Vit C. However, PON1 was significantly and negatively correlated with TC and MDA where as it was positively correlated with HDL, SOD, Vit E and C.

Furthermore, SOD was significantly and negatively correlates with TC and MDA. Yet, it positively correlates with HDL, PON1, Vit E and C in hypertensive subjects with alcoholism. Moreover, Vitamin E significantly and negatively correlates with TC and MDA and positively correlates with HDL, PON1, SOD and Vit C. Similarly, Vitamin C significantly and negatively correlates with TC and MDA and positively correlates with HDL, PON1, SOD and

Vit E in hypertensive subjects with alcoholism.

This study showed a negative correlation between MDA, vitamin C, vitamin-E, SOD, and PON1, and positive correlation between PON1 and antioxidants (SOD, Vitamin-E & C). Results of present study demonstrate that serum PON1 activity measurement may add a significant contribution to cardiac markers. Therefore, it is concluded that the decreased activity of PON1 and HDL-c levels may contribute to the risk of atherosclerosis via alteration in oxidized-LDL and oxidative stress.

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