** ORIGINAL ARTICLE: **

Paraoxonase-1 Enzyme Activity and Oxidative Status in Pulmonary Hypertension

**Original Article**

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**Abstract:**

**Objective:** Oxidative stress has been considered to be one of the main causes for the development of pulmonary hypertension (PH) via leading alteration of pulmonary vasomotor tone induced by hypoxia. The aim of this study is to determine the serum paraoxonase-1 enzyme (PON-1) activity, arylesterase activity, the antioxidant-oxidant status in patients with PH and to compare with healthy controls. **Material and Methods:** Thirty five healthy individuals (mean age 45.7±5.9 years) as a control group and thirty eight patients (mean age 46.5±12.6 years) with a diagnosis of PH were included in the study. Serum PON-1 and arylesterase activity, the total antioxidative capacity of plasma (TAC) and total oxidant status (TOS) were measured by using colorimetric methods. The Oxidative Stress Index (OSI) was calculated as TOS/TAC×100. **Results:** Serum PON1 activity is significantly lower in PH patients when compared with healthy controls (p=0.001). The serum arylesterase activity and TAC, TOS and OSI status were similar in both groups. There is inverse correlation between serum PON1 activity and NYHA functional capacity (r:-0.649 p=0.001). Furthermore, PON1 activity of study patients are similar in the PH subgroups. Serum activity of PON1 was found to be the only independent parameter for the presence of PH in binary logistic regression analysis (OR 0.984, 95 % CI 0.977-0.992, p=0.001). Eight patients died follow up period (27.6±14.5 months) and none of the parameters including PON1 were associated with mortality. **Conclusion:** Serum PON1 activity of PH patients is lower than healthy population, but does not predict mortality.

**Keywords:** Oxidative stress, paraoxonase enzyme, pulmonary hypertension, arylesterase

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**Introduction**

Pulmonary hypertension (PH) is defined as a mean pulmonary arterial pressure ≥ 25 mm Hg at rest that is identified by right heart catheterization and pulmonary vascular resistance (PVR) > 3 Wood units. There are five groups of PH encompassing, Pulmonary arterial hypertension (PAH, Group 1); Left heart disease (group 2); Lung diseases (group 3); Chronic tromboembolic pulmonary disease (group 4); PH with unclear multifactorial mechanisms (group 5). PAH represents a group of diseases with similar pathophysiological consequences and includes connective tissue disease, congenital heart disease, HIV, sestozomyosis or PH due to drugs and toxins. PAH is a rare but serious clinical issue which is characterized with increased PVR, right ventricular dysfunction and shortened survival. The pathogenesis of PH is multifactorial. Changes in pulmonary vasculature and increased PVR may be associated with different mechanisms such as --

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vasoconstriction, proliferative and obstructive remodeling of the pulmonary vascular wall, inflammation of the pulmonary vascular wall, shear stress, pseudo-hypoxia, thrombosis and plexiform lesion formation. Oxidative stress (OS) is an imbalance between oxidants (reactive oxygen and nitrogen species) and antioxidants that may affect lipids, proteins and physiologic functions of DNA. It has been considered to be one of the main causes of molecular damage to cellular and tissue structures and implicated in the development of PH via leading to alteration of pulmonary vasomotor tone induced by hypoxia. Serum paraoxonase (PON) is a calcium-dependent esterase synthesized by the liver, bound exclusively to high-density lipoprotein (HDL) in plasma and is associated with apolipoprotein A1 (Apo A1). PON1 has been demonstrated to preserve HDL function and to protect low-density lipoprotein (LDL) from oxidative modification by hydrolyzing lipid peroxides; therefore, it can reduce the risk of development of atherosclerosis via exerting antioxidant and anti-atherogenic effects. PON1 enzyme has three activities; paraoxonase, arylesterase and diazoxonase. Reduced serum PON1 activity was reported in patients with clinically manifest atherosclerosis and in those with increased risk for atherosclerosis such as familial hypercholesterolemia and diabetes mellitus. Serum PON-1 activity, arylesterase (ARLS) activity and oxidative status has not been studied in patients with PH. The aim of this study is to determine the serum PON1 activity, ARLS activity, total antioxidative capacity (TAC), total oxidant status (TOS) and oxidative stress index (TOS/TACx100) of the patients with PH and to compare with healthy controls.

Material and Methods
All of the patients were included in the study group after the routine diagnostic tests for PH including right heart catheterization according to 2015 European Society of Cardiology (ESC) guidelines (1). The following criteria were used as inclusion criteria: A diagnosis of any of the following: idiopathic pulmonary arterial hypertension (IPAH), PAH associated with congenital systemic to pulmonary shunts (operated or not), PH due to chronic thromboembolic pulmonary hypertension (CTEP), PH associated with connective tissue disorders, and associated with other causes in clinical classification class I. The study was approved by the ethical committee of the medical faculty and written informed consent was obtained from all of the study participants.

Blood sampling and laboratory measurements
Serum samples were taken after fasting 12 hours. Blood samples were centrifuged 3000 rpm x10 minutes. Than, all samples were stored at -20 C°. The levels of uric acid, (Konelab Commercial Kits), PON1, TOS, TAS and ARLS (Rel assay diagnostics kits-Gaziantep Turkey) were measured using Konelab Biochemistry Analyzer (Thermo Electron Corporation, Vantaa-Finland). Serum NT-proBNP levels were measured chemiluminometric method (Immulate 1000 Immunassay System, Siemens Diagnostic, Deerfiled, USA). Oxidative Stress Index (OSI) was calculated as TOS/TAS x100 and reported as OSI units.

Statistical analyses
Statistical analysis were obtained using the ready-to-use program of SPSS (Statistical Package for Social Sciences) for Windows version 12 (Chicago Illinois, USA). Results were expressed as mean ± standard deviation for continuous data or as percentages and numbers for categorical data. Continuous variables with normal distribution and unequal distribution were analyzed with independent Student’s t test and Mann–Whitney U-test respectively. Chi-square and Fisher’s Exact test were performed for nominal variables. Spearman correlation analysis was used to evaluate the relationship between variables. Binary logistic regression analysis was utilized for multivariate analysis. To determine the test performance of PON1 as a predictor of pulmonary hypertension, the area under the receiver-operating characteristic curve was used. An optimal cut-off level was calculated from analyses of the receiver-operating characteristic curve. p <0.05 value was defined as statistically significant.

Results
The study group consisted of 38 consecutive patients (21 female, 17 male, mean age 46.5±12.6 years) with a diagnosis of PH: Of these, 14 had IPAH, 14 had PAH associated with congenital systemic to
pulmonary shunts (5 of these patients had previous surgery), 2 patients had PAH associated with scleroderma and 8 had PH due to CTEPH. There were 35 (23 female, 12 male, mean age 45.7±5.9 years) healthy individuals in our control group. There were no significant differences between the study group and the control group in terms of age (PH: 46.5±12.6, controls 45.7±5.9, p=0.71) or gender and female/male ratio (for PAH: 21 female and 17 male and for control group: 23/12, p=0.36). Six minutes walking test (6MWT), NT-proBNP measurements and right heart catheterization was not performed in the control group.

The clinical characteristics of the study patients are given in table 1. The results of the serum levels of the biochemical parameters of our study was given in table 2. Serum PON1 activity was significantly lower in PH patients when compared with healthy controls (p=0.001). However, the serum ARLS activity and TAC, TOS and OSI status were similar in both groups. The serum PON1 activity of study patients were similar in the specific groups of PH including the IPAH, congenital systemic to pulmonary shunts, previously corrected congenital systemic to pulmonary shunts, and PH due to CTEPH (p=0.32).

Analysis of the receiver-operating characteristic (ROC) curve for PON-1 as a predictor for the PH showed an area under the curve of 0.86 (95% confidence interval 0.765 to 0.934, p<0.001). Using an optimized cut-off level at 182 pg/ml derived from the ROC curve for PON-1 as a predictor for the PH with 68.4 % sensitivity and 97.1 % specificity. In spearman correlation analysis, PON-1 activity exhibited inverse correlation between serum PON1 activity and NYHA functional capacity (FC) (r: -0.649, p=0.001) whereas uric asit was positively correlated with NYHA FC (r:440 p=0.01). In addition, correlation analysis also revealed that, 6MWT was negatively correlated with NT-proBNP (r: -0.482, p=0.007) and positively correlated with TAC (r:0.45, p=0.005). Factors independently associated with PH were analyzed with use of binary logistic regression analysis that took into account age, gender, and serum activity of PON-1, TAC, TOS and ARLS. The serum activity of PON1 was found to be the only factor to be associated with presence of PH (OR 0.984, 95 % CI 0.977-0.992, p=0.001).

In PH group the mean follow-up time was 27.6±14.5 months (range 6-62 months). 8 patient died in this time interval. 5 of these patients were IPAH, 2 of them were CTEPH and one was ventricular septal defect with Eisenmenger physiology. Only ventricular septal defect patient was in functional class 2 and the others were in either functional class 3 or 4. Two of them were on monotherapy (bosentan), 2 of them were on two drugs (one was on bosentan plus sildenafil and one was on bosentan plus Inhaled iloprost) and 3 of them were on triple therapy (bosentan plus sildenafil plus Inhaled iloprost). The patient with ventricular septal defect was not on specific therapy because she preferred to became pregnant despite advices against pregnancy. 6 of the patients died because of progressive right heart failure, 2 of them was a sudden death, one died in the pulmonary thromboendarterectomy operation and one died 24 hours after giving a birth. The clinical characteristics and biochemical values of the PAH patients with or without death were given in table 3. There were no differences in the study parameters. However, serum NT-proBNP values and serum uric acid levels were significantly higher and 6 MWT was significantly lower in non-survivors when compared with survivors. However, none of the parameters including PON1 activity, serum NT-proBNP, and uric acid predicted death in PH in multivariant analysis (p>0.05).

**Discussion**

In this study, we found that serum PON1 activity of patients with PH is lower than PON1 activity of healthy population. In addition, serum PON1 activity was not different in the subgroups of PAH patients and was not found to be related with mortality. The other parameters of oxidation which are ARLS, TAC, TOS, and OSI, were similar in PH patients and healthy population. We also found that serum NT-proBNP values were significantly higher in PH patients who died when compared with surviving PH patients in nearly two and a half years mean follow-up time.

Oxidative Stress (OS) has been considered to be one of pathophysiological causes of molecular damage to cellular and tissue structures and implicated in the development of PH via leading to alteration of pulmonary vasomotor tone induced by hypoxia.3-5 OS is an imbalance due to increased reactive oxygen or nitrogen species (ROS or RNS) with or without decreased antioxidant defense, can lead to cell damage by inhibition of protein, lipid, and DNA physiologic functions.2,5,11,12 So, oxidative damage occurs when the production of ROS exceeds the scavenger capacity of the anti-oxidant mechanisms of the cell.

PON1 is one of the endogenous free-radical scavenging systems in human body.11 Reduced serum PON1 activity was reported in patients with clinically manifest atherosclerosis and in those with...
increased risk for atherosclerosis such as familial hypercholesterolemia, coronary artery disease, diabetes mellitus, diet, cigarette use, pregnancy and obesity.\textsuperscript{6,8,11} In addition, the serum PON1 activity has been shown to be significantly reduced in inflammatory conditions including rheumatoid arthritis, ulcerative colitis and Behcet’s disease.\textsuperscript{4,15} Thus, increased OS is associated with reduced serum PON1 activity.\textsuperscript{8,16}

In our study, we found that, serum PON1 activity is reduced in PH patients but it was not different in PH subgroups. This finding may indicate that serum PON1 activity may reflect the inflammatory condition that ensues after the disease exists. In addition, although we found decreased PON1 activity in PH group, the ARLS activity was similar in the PH patients and controls. The PON-1 activity has traditionally been measured by quantifying enzymatic hydrolysis rates of two known in vitro substrates with functional activities named paraoxonase and ARLS activities.\textsuperscript{17,18} Although it was reported that both PON1 activity measurements are usually concordant in the literature, some conflicting data regarding the two PON1 activity measurements due to the different substrate activity polymorphisms was also reported.\textsuperscript{19,22} Consequently, we think that, this contradictory finding between the two PON-1 activities in the present study, may be related to polymorphism influence and differences in the rate of PON1 hydrolysis. Another issue is that in clinical studies there is lack of a cut of value for serum PON1 activity in different clinical situations.\textsuperscript{6,13,15} In our study we found that a serum PON1 activity lower than 182 U/L has a 68.4% sensitivity and 97.1% specificity for indicating the presence of PH. To our knowledge this finding is the first described in literature.

Contrast to few previous studies,\textsuperscript{23,24} in the present study we didn’t observe any difference between the PH patients and healthy controls in the means of other oxidative status (TAC, TOS and OSI). There are literature data indicating that the currently approved agents used in the treatment of PH\textsuperscript{4,25,26} such as endothelin receptor antagonists, phosphodiesterase-5 inhibitors, and soluble guanylate cyclase stimulators, attenuate the oxidant level.\textsuperscript{27-29} Additionally, any treatment such as statin, vitamins C and E which has anti-oxidative properties may have some beneficial effects on decreasing the oxidant level.\textsuperscript{30,32} In the present study, participants were treated with various single or multiple drug regimens including calcium channel blockers, endothelin receptor antagonists, PDE-5 inhibitors and synthetic analogues of prostacyclin. Consequently, heterogeneity of treatment options and small sample size may be related to this contradictory result.

BNP and uric acid are the most known biochemical markers with prognostic value in patients with PH.\textsuperscript{32,33} In the current study, although serum NT-proBNP and uric acid values were not a predictor of death in PH patients in binary regression analysis, we found that serum NT-proBNP and uric acid concentrations were significantly higher in PH patients who died during the follow-up period. This finding may be linked to small size of our study. When considered to literature data and our study findings, BNP and uric acid seems to be the useful biochemical parameters for the prognosis and follow up of the PH patients.

Parallel to previous data,\textsuperscript{1,34} in our study also observed that the mean 6MWT of patients who couldn’t survive was significantly lower than the surviving PH patients.

The main limitation is that we studied the serum PON-1, ARLS activities and oxidative status only once. Repeated measurements would be helpful in identifying any clinical worsening of patients. The small number of patients is the second limitation. The third limitation is that this was a single center study and the patients and the control subjects were reflecting a local area which may affect the parameters.

In a conclusion, serum PON-1 activity of PH patients is lower than healthy population and a serum PON-1 activity below 182 U/L may indicate the presence of PH with a 68.4% sensitivity and 97.1% specificity. PON-1 did not achieved being a prognostic marker for PH. Besides that, to our knowledge, the roles of oxidative-antioxidative biomarkers in PH patients has been relatively little studied\textsuperscript{4}, and further investigations assessing oxidative-stress markers in patients with pulmonary hypertension are needed.

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**Conflicts of interest:** No conflict of interest was declared.

**Authors’s contribution:**

**Data gathering and idea owner of this study:** Muhsin Kalyoncuoglu, Murat Baskurt

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Table 1. The clinical characteristics of the study patients.

|                          | Pulmonary Hypertension | Control Group | p   |
|--------------------------|------------------------|---------------|-----|
| Age, years, mean±SD      | 46.5±12.6              | 45.7±5.9      | 0.71|
| Sex, female, n(%)        | 21(55.3%)              | 23(65.7%)     | 0.36|
| Body mass index, kg/m²   | 25.1±2.9               | 26.9±3.8      | 0.15|
| Diabetes Mellitus, n(%)  | 0                      | 0             | 1.00|
| Hypertension, n(%)       | 0                      | 0             | 1.00|
| Smoking, n(%)            | 2(5.3%)                | 2(5.7%)       | 0.66|
| Hyperlipidemia, n(%)     | 0                      | 0             | 1.00|
| Systolic PAP, mmHg, mean±SD | 95.3±25.7            | NA            | NA  |
| Mean PAP, mmHg, mean±SD  | 68.7±20.4              | NA            | NA  |
| NT-proBNP, pg/ml, mean±SD | 2033±132.4           | NA            | NA  |
| Uric acid, mg/dl, mean±SD | 6.5±1.9               | 6.0±0.95      | 0.14|
| 6MWT, meters, mean±SD    | 321.3±98.8             | NA            | NA  |
| FC NYHA I/II/III/IV, n(%)| 0(0)/16(42.1)/20(5.3) | 35(100)/0/0/0 | 0.001|

FC NYHA: functional capacity New York Heart Association; N: number; NT-proBNP: N-terminal pro B-type natriuretic peptide; PAP: pulmonary arterial pressure; SD: standart deviation; 6MWT: 6 minutes walking test; NA: not available

Table 2. Comparison of the biochemical parameters between the two groups.

|                          | Pulmonary Hypertension | Control Group | p   |
|--------------------------|------------------------|---------------|-----|
| PON-1 activity, U/L, median | 175.5±90.9             | 384.2±204.2   | 0.01|
| TAC, µmolTrolox equivalent/L, mean±SD | 0.64±0.4          | 0.69±0.1      | 0.49|
| TOS, µmol H2O2 equivalent/L, mean±SD | 14.8±6.0          | 16.1±3.7      | 0.26|
| OSI, Units, mean±SD      | 2.6±1.5                | 2.5±1.0       | 0.84|
| ARLS, U/L, median         | 2.80                   | 2.2           | 0.20|

ARLS: arylesterase; OSI: oxidative stres index; PON: paraoxonase-1; SD: standart deviation; TAC: total antioxidative capacity; TOS: total oxidant status; U/L: Units/Liter.

Table 3. Comparison of clinical characteristics and biochemical parameters between survivors and nonsurvivors in PH group.

|                          | PH non-survivors | PH survivors | p   |
|--------------------------|------------------|--------------|-----|
| Age, years, mean±SD      | 50.7±15.8        | 45.4±11.7    | 0.29|
| Body mass index, kg/m², mean±SD | 26.4±3.3         | 24.8±2.7     | 0.15|
| sPAP, mmHg, mean±SD      | 89.3±18.6        | 97.9±26.7    | 0.40|
| mPAP, mmHg, mean±SD      | 59.8±20.4        | 69.8±19.8    | 0.29|
| dPAP, mmHg, mean±SD      | 44.5±21.5        | 50.4±18.6    | 0.52|
| PON1, U/L, median         | 181              | 161          | 0.73|
| ARLS, U/L, median         | 2.1              | 3.8          | 0.49|
| TAC, µmolTrolox equivalent/L, mean±SD | 15.47±3.4       | 15.0±6.0     | 0.52|
| TOS, µmol H2O2 equivalent/L, mean±SD | 14.1±6.3       | 15.0±6.2     | 0.52|
| OSI, U, mean±SD          | 8.3±4.1          | 8.6±5.2      | 0.11|
| NT-proBNP, pg/ml, median  | 4284             | 1312         | 0.04|
| Uric acid, mg/dl, mean±SD | 8.6±2.7          | 6.0±1.7      | 0.002|
| 6 MWT, meters, mean±SD    | 246.8±94.9       | 341.2±91.3   | 0.01|

ARLS: arylesterase; dPAP: diastolic pulmonary arterial pressure; PH: pulmonary hypertension; mPAP: mean pulmonary arterial hypertension; NT-proBNP: N terminal pro B-type natriuretic peptide; OSI: oxidative stress index; PON1: paraoxonase-1; SD: standart deviation; sPAP: systolic pulmonary arterial pressure; TAC: total antioxidative capacity; TOS: total oxidant status; 6MWT: 6 minutes walking test.
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