

## **EVALUATION OF THE ROLE OF ANTIOXIDANT ENZYME PARAOXONASE 1 IN COPD**

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### **INTRODUCTION**

Chronic obstructive pulmonary disease (COPD) is characterized by progressive, irreversible airflow limitation associated with airway inflammation (Pauwels *et al.*, 2001). It is a leading cause of chronic morbidity and mortality worldwide. The importance of oxidative stress in both lungs and systemic circulation is well established in the pathogenesis of COPD (Chavez *et al.*, 2007). In healthy subjects there is a balance between oxidants and antioxidants, keeping the extracellular environment in a reduced state. But there is limited information on the respiratory epithelial antioxidant defenses in smokers, and less in COPD.

Cigarette smoking, air pollution, an increase of free radicals in respiratory epithelial cells by inflammation and infections are the leading causes of oxidative stress in COPD (Chen *et al.*, 2004; Pryor and Stone, 1993). Free radicals cause an imbalance between oxidants and antioxidants. An imbalance in favor of oxidants may cause oxidative damage to the air space epithelial cells. Lipid peroxidation occurs at the cell membrane by the effect of free radicals (Pryor and Stone, 1993).

Paraoxonase1 (PON1) in the lung may have a protective role from oxidative stress. PON1 is an antioxidant enzyme which is localized in Clara cells, endothelial cells and type 1 cells of the alveolar epithelium in the lung and may have a role in protection from oxidative stress. It is able to hydrolyze paraoxon, which is a potent inhibitor of cholinesterases. It is entirely complexed to HDL and has an antioxidant role in the protection of LDL from oxidative stress in blood. Clara cell is one of the oxidant resistant airway cells in all species and secretions of Clara cells have a role in protection from oxidative stress. Clara cells are replaced by mucous cells in smokers and there is a reduction in Clara cells in COPD patients and in smokers. Smoking which causes vital damage at the airspace epithelium may also cause reduction in the levels of PON1 (Chavez *et al.*, 2007). In the recent years, several studies have showed that extracts of cigarette smoke inhibited the activity of PON1 (Aharoni *et al.*, 2004; Moren *et al.*, 2008). The paraoxonases (PON1, PON2, PON3) are basically lactonases with one of the broadest known substrate specificities (Chow, 1993; Eckerson *et al.*, 1993). All three PONs metabolise 5-hydroxy cicosate traecomic acid 1,5 lactone and 4-hydroxy docosahexaenoic acid which are derived from arachidonic acid. PON3 exclusively hydrolyses lovastatin and spironolactone whereas organophosphates are exclusively hydrolysed by PON1 which has additional esterase activity.

PON1 protein is synthesized mostly in liver and is released by a docking process, i.e. HDL particles transiently associate with the cell membrane and remove PON1 from the membrane (Isik *et al.*, 2005). Unlike PON2 and PON3, it has efficient esterase activity towards many organophosphates (OPs) including paraoxynase (Isik *et al.*, 2005). The PON1 gene is located on the long arm of chromosome 7 at q21-q22 regions (Draganov *et al.*, 2005).

Components of inflammatory cascade such as inflammatory cells, cytokines, oxidative stress biomarkers, and inflammatory indicators like C-reactive protein (CRP) are the potential biomarkers that can be used in the diagnosis and prognosis of COPD. Chronic inflammation in the pulmonary tissue is also associated with systemic effects. CRP is often used as a clinical marker of acute systemic inflammation. Since low-grade inflammation is evident in COPD, high sensitive CRP (hs-CRP) levels are raised in these patients (Yende *et al.*, 2006). C-Reactive Protein (CRP) is an acute phase protein synthesized predominantly by the hepatocytes in response to tissue damage or inflammation.

In this study, we investigated the possible relationship between serum PON levels in the COPD patients, smokers without COPD and the non-smoker healthy subjects and its correlation with hs CRP.

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### **MATERIALS AND METHODS**

#### **Methods**

The study was conducted at Viswanathan Chest hospital attached to Vallabhbhai Patel Chest Institute (VPCI), University of Delhi over a period of 1 year. Written informed consent was obtained from each patient prior to admission to the study.

#### **Study Subjects**

A total of 60 patients with the diagnosis of chronic obstructive pulmonary disease (COPD) were recruited. They consisted of 15 patients each of mild, moderate, severe and very severe COPD as per GOLD Guidelines.

30 subjects were taken as control; they consisted of 15 normal subjects and 15 healthy smokers. These patients and controls were subjected to a detailed clinical examination that includes, general physical examination, systemic examination of the respiratory and cardiovascular systems.

#### **High Sensitivity C-reactive Protein (hs-CRP)**

Hs-CRP was measured in patients' serum and control serum by the ELISA method. Normal expected values: 0.068 – 8.2 mg / lit.

#### **Paraoxonase 1 (PON1)**

PON 1 was measured using spectrophotometry method and values were expressed in kU/L.

SPSS software version 16 was used for statistics involved. P value of  $\leq 0.05$  was considered significant.

### **RESULTS AND DISCUSSION**

#### **Results**

##### **Profile of Patients and Controls in the Study**

The mean age of Controls was  $24.93 \pm 2.79$  yrs, Healthy smokers was  $38.67 \pm 9.82$  yrs, Mild COPD was  $52.47 \pm 13.35$  yrs, Moderate COPD was  $53.27 \pm 18.04$  yrs, severe COPD was  $58.93 \pm 7.38$  yrs & very severe COPD was  $55.73 \pm 7.83$  yrs (Table 1).

##### **Paraoxonase 1 (PON 1)**

The level of PON1 was seen to decrease with the increasing severity of COPD (Table 2). In mild COPD it was  $146.18 \pm 61.20$  KU/L, in moderate COPD it was  $90.30 \pm 29.99$  KU/L, in severe COPD it was  $84.45 \pm 34.89$  KU/L and very severe COPD it was  $143.19 \pm 35.76$  KU/L. Levels were lower in healthy smokers ( $143.26 \pm 55.64$  KU/L) as compared to controls ( $192.55 \pm 54.71$  KU/L). The difference of means was significant on ANOVA. The difference of means between control and moderate COPD was  $102.24 \pm 17.15$  KU/L ( $p < 0.01$ ), between control and severe COPD was  $108.09 \pm 17.15$  KU/L ( $p < 0.01$ ), between healthy smoker & moderate COPD was  $52.96 \pm 17.15$  KU/L ( $p = 0.041$ ), between healthy smoker and severe COPD was  $58.80 \pm 17.15$  KU/L ( $p = 0.014$ ), between mild and moderate COPD was KU/L  $55.88 \pm 17.15$  ( $p = 0.024$ ), between mild and severe COPD was  $61.72 \pm 17.15$  KU/L ( $p = 0.008$ ), between moderate & very severe COPD was  $52.89 \pm 17.15$  KU/L ( $p = 0.041$ ) and between severe and very severe COPD was  $58.74 \pm 17.15$  KU/L ( $p = 0.014$ ).

##### **High Sensitive C - reactive protein (hsCRP)**

The levels of hsCRP were seen to increase with the increasing severity of COPD (Table 2). In mild COPD it was  $0.94 \pm 0.68$  mg/L, in moderate COPD it was  $2.54 \pm 0.61$  mg/L, in severe COPD it was  $4.87 \pm 2.22$  mg/L and very severe COPD it was  $6.74 \pm 5.36$  mg/L. The mean levels of hsCRP in healthy smokers ( $0.93 \pm 0.71$  mg/L) were higher than control ( $0.65 \pm 0.43$  mg/L). The difference of mean was found to be significant on ANOVA. The difference of means between control & severe COPD was  $4.23 \pm 0.88$  mg/L ( $p < 0.01$ ), between control & very severe COPD was  $6.09 \pm 0.88$  mg/L ( $p < 0.01$ ), between healthy smoker & severe COPD was  $3.94 \pm 0.88$  mg/L ( $p < 0.01$ ), between healthy smoker & very severe COPD was  $5.81 \pm 0.88$  mg/L ( $p < 0.01$ ), between mild & severe COPD was  $3.93 \pm 0.88$  mg/L ( $p < 0.01$ ), between mild & very severe COPD was  $5.79 \pm 0.88$  mg/L ( $p < 0.01$ ) and between moderate & very severe COPD was  $4.20 \pm 0.88$  mg/L ( $p < 0.01$ ).

##### **Pulmonary Function Tests**

The mean values of spirometry parameters of controls and patients are depicted in Table 3.

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### Paraoxonase1 (PON1)

PON1 correlated positively with pulmonary function parameters. FEV<sub>1</sub> Pre (r = 0.386, p < 0.01), FEV<sub>1</sub> Post (r = 0.373, p < 0.01) (Figure 1), FVC Pre (r = 0.339, p < 0.01), FVC Post (r = 0.339, p < 0.01) (Figure 2), FEF<sub>25-75</sub> pre (r = 0.410, p < 0.01) and FEF<sub>25-75</sub> Post (r = 0.372, p < 0.01) (Figure 3).

### HS-CRP

hsCRP correlated negatively with PON1 (r = - 0.273, p < 0.01) (Figure 4) and Pulmonary function parameters FEV<sub>1</sub> Pre (r = - 0.600, p < 0.01), FEV<sub>1</sub> Post (r = -0.602, p < 0.01), FVC Pre (r = - 0.521, p < 0.01), FVC Post (r = - 0.509, p < 0.01), FEF<sub>25-75</sub> pre (r = - 0.494, p < 0.01) and FEF<sub>25-75</sub> Post (r = - 0.489, p < 0.01).

## Discussion

### Systemic Inflammatory Markers in COPD

#### High Sensitivity C-Reactive Protein (hsCRP) in COPD

C-Reactive protein (CRP) was first discovered by Tilet and Francis (1930) in the plasma of pneumonia patients, and was named because of its ability to bind and precipitate the C-polysaccharide of pneumococcus (Gillman *et al.*, 2000). CRP is synthesized in the liver and is normally present in serum or plasma at levels less than 0.3 mg/dl. Its physiological roles are multiple but with several functions similar to those immunoglobulins, CRP appears to function in host defense (Baumann *et al.*, 1994).

CRP is one of the acute-phase proteins, which rise during general, nonspecific response to a wide variety of diseases. This includes infections by gram-positive and gram-negative organisms, rheumatoid arthritis, malignancies, Guillain –Barre syndrome and multiple sclerosis, tuberculosis and many other necrotic and inflammatory (Yudkin *et al.*, 1999)

Aronson *et al.*, (2006) demonstrated an inverse linear relationship exists between CRP concentrations and measures of pulmonary function in subjects without pulmonary disease and in never-smokers. There was a strong inverse association between CRP levels and quartiles of FEV<sub>1</sub> (Aronson *et al.*, 2006). In our study to the levels of hsCRP was seen to increase with the increasing severity of COPD.

Dahl *et al.*, (2007) found that CRP is a strong and independent predictor of future COPD outcomes. Serum CRP was measured at baseline, and COPD admissions and deaths were recorded as outcomes. The number of COPD hospitalizations and COPD death were increased in individuals with baseline CRP > 3 mg/L versus < or = 3 mg/L (Dahl *et al.*, 2007).

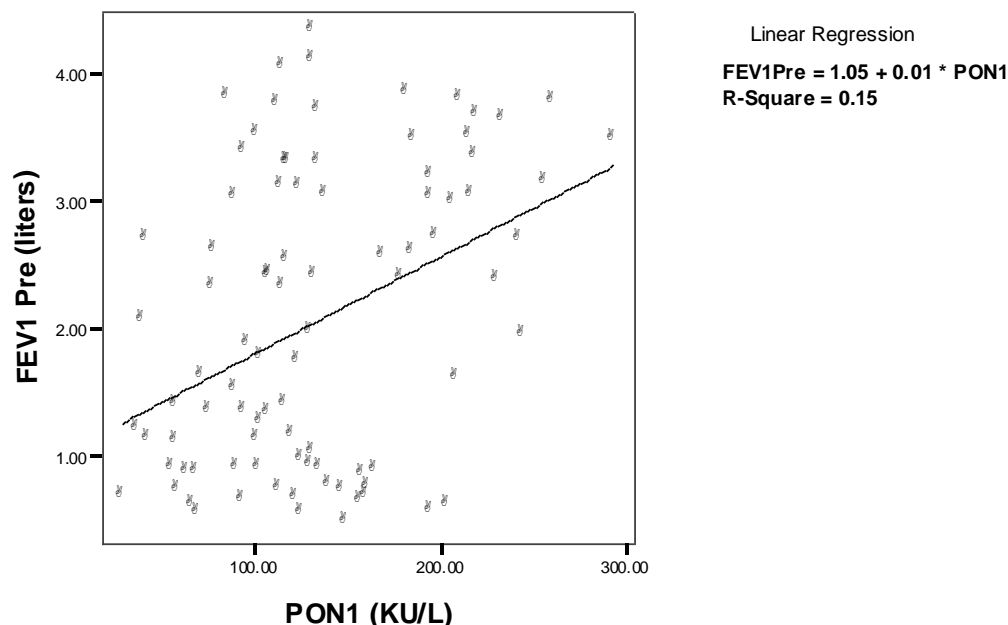
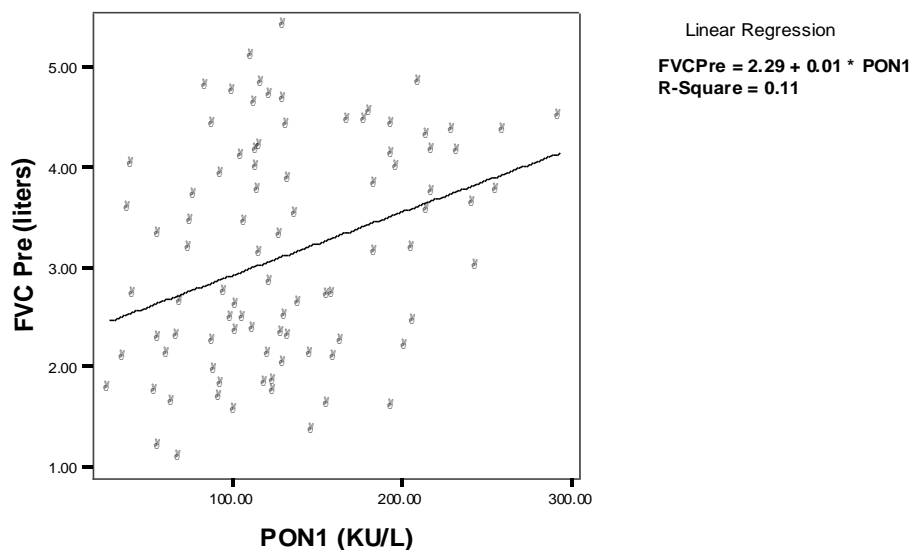
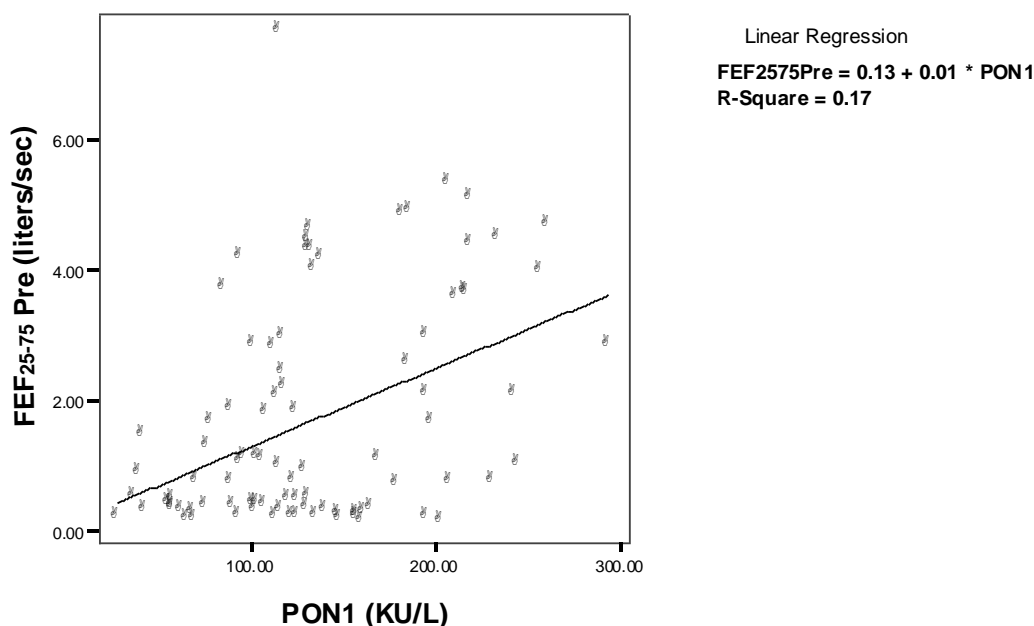


Figure 1: Correlation of PON 1 with FEV1

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**Figure 2: Correlation of PON 1 with FVC**



**Figure 3: Correlation of PON1 with FEF<sub>25-75</sub>**

Man *et al.*, (2006) concluded CRP measurements provide incremental prognostic information beyond that achieved by traditional markers of prognosis in patients with mild to moderate COPD, and may enable more accurate detection of patients at a high risk of mortality (Man *et al.*, 2006).

These findings are in agreement with our results. Hs-CRP was found to be positively correlating with the severity of COPD indicating a more severe disease and intense inflammation in the very severe group.

### **Paraoxonase 1 (PON 1) in COPD**

Paraoxonase1 (PON1) is a calcium-dependent esterase closely associated with HDL-containing apoA-I that has been reported to confer antioxidant properties on HDL by decreasing the accumulation of lipid peroxidation products. PON1 activity is under genetic and environmental regulation and appears to vary widely among individuals and populations.

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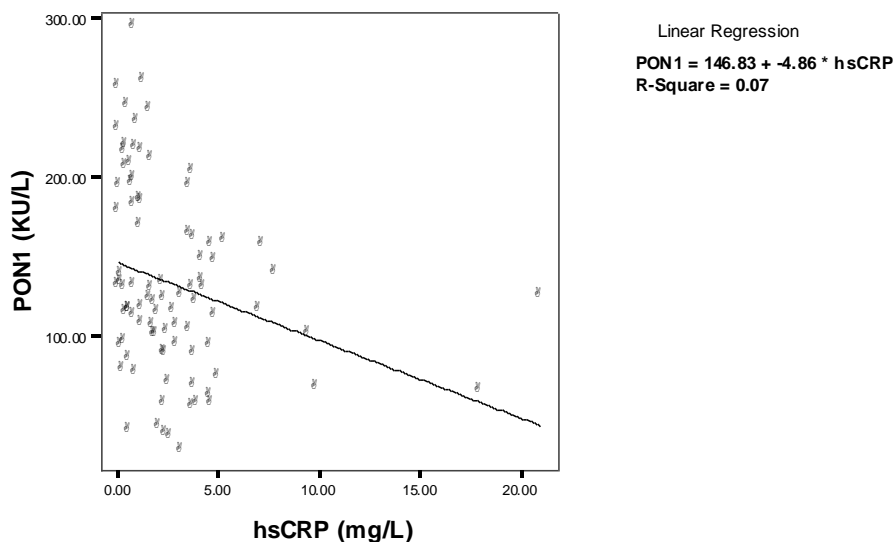


Figure 4: Correlation of hs-CRP with PON 1

Table 1: Demographic Profile of Different Groups

	Age (yrs)	Ht (cm)	Wt (Kg)	BMI (kg/m <sup>2</sup> )
Control	24.93 ± 2.79	163.80 ± 7.63	61.80 ± 11.05	22.92 ± 3.28
HS	38.67 ± 9.83	171.47 ± 6.83	68.47 ± 14.08	23.24 ± 4.32
Mild	52.47 ± 13.35	165.07 ± 6.36	62.47 ± 9.33	23.15 ± 3.20
Moderate	53.27 ± 18.04	158.73 ± 10.45	58.87 ± 12.13	23.44 ± 3.93
Severe	58.93 ± 7.38	160.20 ± 7.78	59.40 ± 15.14	22.76 ± 5.06
V. Severe	55.73 ± 7.83	165.60 ± 6.29	53.27 ± 10.15	19.44 ± 3.71

HS: healthy smokers

V. severe: very severe COPD

Table 2: Levels of systemic inflammatory markers in different stages of COPD

	hsCRP (mg/L)	PON1 (KU/L)
Control	0.65 ± 0.43	192.55 ± 54.71
HS	0.93 ± 0.71	143.26 ± 55.54
Mild	0.94 ± 0.68	146.18 ± 61.20
Moderate	2.53 ± 0.61	90.30 ± 29.99
Severe	4.87 ± 2.22	84.45 ± 34.89
V. Severe	6.74 ± 5.36	143.19 ± 35.76

Table 3: Mean Values of spirometry parameters in different stages of COPD

	FEV <sub>1</sub> Pre	FEV <sub>1</sub> post	FVC Pre	FVC Post	FEF <sub>25-75</sub> Pre	FEF <sub>25-75</sub> Post
Control	3.30 ± 0.53	3.38 ± 0.51	3.83 ± 0.72	3.88 ± 0.70	4.18 ± 0.89	4.43 ± 0.88
HS	3.39 ± 0.52	3.46 ± 0.52	4.26 ± 0.47	4.34 ± 0.51	3.44 ± 1.51	3.56 ± 1.69
Mild	2.49 ± 0.50	2.65 ± 0.58	3.84 ± 0.75	3.89 ± 0.76	1.39 ± 0.52	1.68 ± 0.71
Moderate	1.57 ± 0.57	1.70 ± 0.62	2.67 ± 0.82	2.80 ± 0.82	0.74 ± 0.40	0.86 ± 0.554
Severe	0.97 ± 0.25	1.023 ± 0.26	2.17 ± 0.75	2.35 ± 0.80	0.35 ± 0.10	0.36 ± 0.13
V. Severe	0.67 ± 0.11	0.72 ± 0.12	2.04 ± 0.43	2.18 ± 0.43	0.23 ± 0.06	0.24 ± 0.60



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Birgül *et al.*, (2005) concluded paraoxanase activity was significantly lower in COPD patients as compared to control group (Birgul *et al.*, 2005). In our study we also noticed that the level of PON1 decreased with the increasing severity of COPD.

Mackness *et al.*, (1998) showed that higher levels of CRP seem to be generally associated with low levels of PON1 activity, providing a mechanistic link between inflammation and the development of atherosclerosis (Mackness *et al.*, 1998).

In present study PON1 correlated positively with pulmonary function parameters while hsCRP correlated negatively with and pulmonary function parameters.

### **Conclusion**

These results suggest serum PON-1 levels together with estimation of hs CRP may be important in assessing severity in COPD patients. PON 1 and hs-CRP may be explored as markers of COPD deterioration and hospitalizations.

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