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Original Research Article

Genotyping and receiver operating characteristic-based evaluation of Paraoxonase-1 in stable chronic obstructive pulmonary disease: a case-control study from Central India

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ABSTRACT

Background: Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide, particularly in low- and middle-income countries like India. Oxidative stress is a key contributor to COPD pathogenesis, and the Paraoxonase 1 (PON1) enzyme plays a crucial antioxidant role. This case-control study from Central India investigated the association of PON1 gene polymorphisms-Q192R and L55M-with PON1 enzyme activity and lipid peroxidation marker malondialdehyde (MDA) in stable COPD patients.
Methods: Sixty COPD patients and 60 age and sex-matched healthy controls were enrolled. PON1 activity was measured spectrophotometrically, and genotyping was performed using PCR-RFLP. Receiver operating characteristic (ROC) analysis assessed the diagnostic performance of PON1 activity.
Results: COPD patients had significantly lower PON1 activity than controls ($p<0.001$). The RR genotype and R allele of the Q192R polymorphism were more frequent in COPD patients and associated with reduced enzyme activity (OR 2.7; $p=0.02$). L55M polymorphism showed no significant intergroup difference. An optimal PON1 activity cutoff of 118.2 U/l (Youden's index) yielded 80% sensitivity and 86.7% specificity in distinguishing COPD from controls. Findings suggest the R allele and RR genotype as potential genetic risk markers for COPD, with decreased PON1 activity indicating impaired antioxidant defence.
Conclusions: This is the first report of PON1 polymorphism data in COPD patients from India, offering novel insights into gene-environment interactions and genetic susceptibility. Larger studies are needed to confirm these results and assess the role of PON1 genotyping in risk stratification and disease management.

Keywords: Praoxonase-1, Diagnostic accuracy, Stable COPD, Q192R, Oxidative stress

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory lung disease characterized by persistent airflow limitation and is currently a leading cause of morbidity and mortality worldwide. According to the global burden of disease study, COPD accounted for approximately 3.23 million deaths globally in 2019, making it the third leading cause of death.[1] The prevalence and disease burden of COPD are particularly high in low- and middle-income countries, including India, where risk

factors such as tobacco use, indoor air pollution, and occupational exposures are widespread.[2]

Oxidative stress plays a pivotal role in the pathogenesis of COPD, contributing to airway inflammation, alveolar destruction, and systemic effects. Increased production of reactive oxygen species (ROS) and impaired antioxidant defenses lead to lipid peroxidation and cellular damage in the lungs.[3,4] Among the various antioxidants, the enzyme PON1, primarily synthesized in the liver and associated with high-density lipoprotein (HDL), has emerged as a key modulator of oxidative stress and inflammation.[5] PON1 hydrolyzes lipid peroxides and prevents the oxidation of low-density lipoproteins (LDL), thereby exerting a protective effect against oxidative injury in pulmonary tissues.[6]

The PON1 gene, located on chromosome 7q21.3, exhibits significant genetic polymorphism, which can influence enzyme activity and disease susceptibility. Two common single nucleotide polymorphisms (SNPs), Q192R (rs662) and L55M (rs854560), have been widely studied. The Q192R polymorphism results in a glutamine-to-arginine substitution at position 192, while L55M results in a leucine-to-methionine substitution at position 55. These variants modulate enzyme stability and substrate specificity, potentially altering the antioxidant capacity of PON1.[7] Recent studies have indicated that individuals carrying the R allele at codon 192 or the M allele at codon 55 may exhibit reduced PON1 activity and increased susceptibility to oxidative stress-related diseases, including cardiovascular conditions and respiratory disorders such as asthma and COPD.[8,9] However, the distribution of these polymorphisms and their functional significance in COPD patients remains under-investigated in Indian population, which is genetically diverse and underrepresented in genomic studies.

This study aims to evaluate the frequency of PON1 Q192R and L55M polymorphisms and their association with PON1 enzyme activity and malondialdehyde (MDA), a key lipid peroxidation marker, in stable COPD patients from Central India. To the best of our knowledge, this is the first report assessing PON1 genotypes in Indian COPD patients, offering potential insight into genetic susceptibility and personalized risk profiling in this population.

METHODS

Setting

The study was carried out at Bhopal Memorial Hospital and Research Centre, Bhopal, Madhya Pradesh, during January 2017 to December 2019.

Study design and participants

A case-control study was conducted at a tertiary care hospital in Central India, comprising 120 subjects-60 patients diagnosed with COPD and 60 age- and sex-matched healthy controls. The study protocol was approved by the institutional ethics committee, and written informed consent was obtained from all participants in accordance with the declaration of Helsinki.

Inclusion and exclusion criteria

COPD diagnosis was confirmed based on global initiative for chronic obstructive lung disease (GOLD) guidelines using pulmonary function tests (PFTs) performed according to American thoracic society criteria. Patients with coexisting pulmonary, renal, cardiovascular, oncological diseases, active infections, inflammation, or neurological dysfunctions influencing oxidative status were excluded. Controls were free of any pulmonary, renal, cardiovascular, or oncological diseases and had no infections, inflammatory conditions, or neurological dysfunctions. Controls were recruited from the same population base as the COPD cases.

Demographic and clinical data collection

Data were collected from medical records (for patients) and structured questionnaires (for control subjects). Demographic variables included age, sex, and ethnicity. Physical parameters comprised height and weight. Clinical variables such as PFT results, disease duration, and risk factors affecting oxidative status (e.g., smoking and alcohol consumption) were recorded.

Sample collection

A 5 mL venous blood sample was collected from each participant by a trained phlebotomist under sterile conditions using EDTA vacutainers.

Biochemical and molecular analysis

PON1 enzyme activity assay

PON1 enzyme activity was quantified spectrophotometrically using phenyl acetate as the substrate, based on the modified method of Jyothi et al. The reaction was monitored at 270 nm at 37°C to determine arylesterase activity.

DNA extraction and genotyping

Genomic DNA was extracted from the peripheral leukocytes using the standard phenol-chloroform extraction method.

Genotyping of PON1 polymorphisms

Two polymorphisms of the PON1 gene (Q192R and L55M) were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique:

Q192R polymorphism: amplified with primers:

Forward: 5′-TAT TGT TGC TGT GGG ACC TGA G-3′.

Reverse: 5′-CAC GCT AAA CCC AAA TAC ATC TC-3′. PCR conditions involved an initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 1 min, 60°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 10 min. The 99 bp product was digested with 8 U of BspI at 55°C overnight, and fragments were separated on 3% agarose gel stained with ethidium bromide.

L55M polymorphism: Amplified with primers:

Forward: 5′-GAA GAG TGA TGT ATA GCC CCA G-3′.

Reverse: 5′-TTT AAT CCA GAG CTA ATG AAA GCC-3′. PCR and cycling conditions were identical to those for Q192R. The 170 bp PCR product was digested with NlaIII enzyme at 37°C overnight in the presence of BSA and resolved on a 3% agarose gel.

All genotype interpretations were made independently by two blinded researchers to ensure accuracy. Allele and genotype frequencies were calculated by direct counting, and Hardy-Weinberg equilibrium was assessed using the chi-square test.

Statistical analysis

Data analysis was conducted using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). The Mann-Whitney U test was applied to compare continuous variables between groups. Genotype and allele frequencies were evaluated using the chi-square test. A $p<0.05$ was considered statistically significant.

RESULTS

Demographic and clinical characteristics

A total of 120 participants were enrolled in this case-control study, including 60 patients with COPD and 60 age-and sex-matched healthy controls. There were no statistically significant differences in age and sex distribution between groups ($p>0.05$), confirming effective matching. The COPD group had significantly reduced pulmonary function (mean FEV1 and FEV1/FVC ratio) compared to controls ($p<0.001$). The smoking and alcohol consumption rates were significantly higher among COPD patients, serving as major risk factors (Table 1).

PON1 enzyme activity

PON1 enzyme activity measured by arylesterase activity was significantly decreased in the COPD group compared to the control group ($p<0.001$), suggesting compromised antioxidant defence mechanisms in COPD patients (Table 2 and Figure 1).

Table 1: Demographic and clinical characteristics of the COPD patients and controls.

Variables	COPD, (n=60)	Controls, (n=60)	P value
Age (in years)	59.4±8.6	58.8±7.9	0.72
Male (%)	68.3	66.7	0.85
Smoking (%)	61.7	18.3	<0.001
Alcohol (%)	35.0	13.3	<0.01
FEV1 (%)	48.6±13.2	89.7±8.4	<0.001

Table 2: PON1 enzyme activity in COPD and controls.

Groups	PON1 activity (U/L)	P value
COPD	104.3±22.7	<0.001
Control	137.8±24.1	

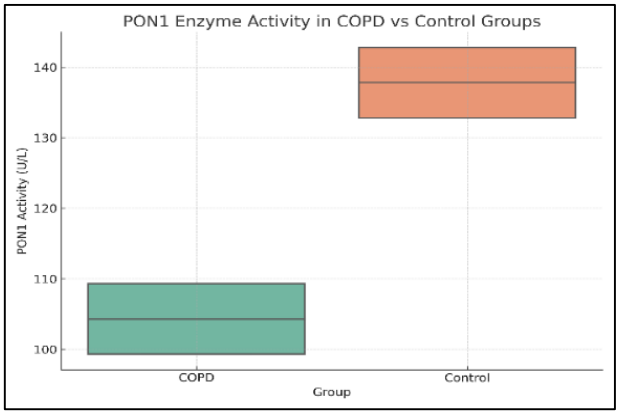

Figure 1: PON1 enzyme activity in COPD vs control groups.

ROC curve analysis

To evaluate the diagnostic performance of PON1 activity in distinguishing COPD patients from healthy controls, ROC curve analysis was performed. The area under the curve (AUC) was 0.88, indicating excellent discriminatory ability. An optimal cutoff value of 118.2 U/L was identified using Youden's index, yielding a sensitivity of 80% and a specificity of 86.7%. These results suggest that PON1 activity may serve as a potential biomarker for COPD risk stratification (Figure 2).

Genotype and allele frequencies of PON1 polymorphisms

PON1 Q192R polymorphism

The frequency of the RR genotype was significantly higher in COPD patients (9%) compared to controls (1.7%), while the QQ genotype was less frequent in COPD (33.9%) than controls (51.7%). The difference in genotype distribution was statistically significant ($p<0.05$). The R allele frequency was also elevated in COPD patients (Table 3).

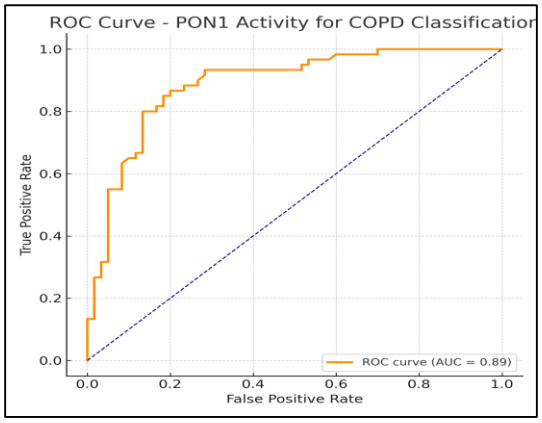

Figure 2: ROC curve showing the diagnostic performance of PON1 activity in distinguishing COPD patients from controls (AUC=0.88).

Table 3: Allele frequency of Q192R genotype in COPD and controls.

Genotypes	COPD (%)	Control (%)	P value
QQ	33.9	51.7	0.045
QR	56.4	46.6	
RR	9.0	1.7	
Q allele	0.62	0.75	0.04
R allele	0.38	0.25	

PON1 L55M polymorphism

No significant differences were observed in L55M genotype frequencies between the COPD and control groups, although the MM genotype was slightly more frequent in COPD patients (Table 4).

Table 4: Allele frequency of L55M genotype in COPD and controls.

Genotypes	COPD (%)	Control (%)	P value
LL	40.0	48.3	0.28
LM	43.3	40.0	
MM	16.7	11.7	
L allele	0.61	0.68	0.31
M allele	0.39	0.32	

Hardy-Weinberg equilibrium

Genotype distributions in both COPD and control groups conformed to Hardy-Weinberg equilibrium for both polymorphisms ($p>0.05$), confirming the population's genetic stability and lack of genotyping errors.

DISCUSSION

In this case-control study conducted on a Central Indian population, we investigated the association of PON1 gene polymorphisms (Q192R and L55M) and enzyme activity with susceptibility to COPD. Our findings reveal a significantly reduced PON1 enzyme activity in COPD patients compared to healthy controls, along with an increased frequency of the R allele and RR genotype of the Q192R polymorphism, supporting the role of oxidative stress and genetic susceptibility in pathogenesis of COPD.

Oxidative stress plays a pivotal role in the progression of COPD, and antioxidant enzymes such as PON1 serve as crucial defense mechanisms. The significantly lower PON1 enzyme activity observed in our COPD cohort aligns with previous studies that have reported compromised antioxidant potential in COPD patients.[10,11] The reduced enzymatic activity may be attributed to both environmental factors, such as cigarette smoke, and genetic predispositions, notably polymorphisms in the PON1 gene.

The Q192R polymorphism, located in the coding region of the PON1 gene, results in a substitution of glutamine (Q) with arginine (R), leading to altered enzymatic activity and substrate affinity. Our data show a significantly higher frequency of the RR genotype and R allele in COPD patients, which is consistent with findings from similar population-based studies in Turkey and Egypt, suggesting that the R allele may be associated with increased oxidative burden and greater disease susceptibility.[12,13]

Interestingly, we did not observe a statistically significant difference in the distribution of L55M genotypes between COPD and control groups. The L55M polymorphism affects PON1 protein levels rather than catalytic efficiency, and its influence may be more subtle or modulated by other environmental or genetic factors. Similar results were reported by Dutta et al in a North Indian population, where the L55M variant showed no significant correlation with COPD risk.[14]

Moreover, we found a significant association between smoking status and COPD, reinforcing the established role of smoking as a primary risk factor. However, our data also suggest that even among smokers, individuals harbouring the R allele may be at elevated risk due to impaired antioxidant defence, indicating a gene-environment interaction.

Our study is strengthened by well-matched controls and rigorous genotyping; however, it is limited by a modest sample size and the lack of serum PON1 protein quantification. Future studies with larger cohorts and functional assays are warranted to elucidate the precise mechanisms by which PON1 polymorphisms influence COPD pathophysiology.

Several studies have evaluated the diagnostic utility of PON1 activity using ROC curve analysis in patients with COPD. Tekin et al demonstrated that PON1 arylesterase activity showed a significantly high AUC, supporting its role in COPD diagnosis and monitoring oxidative stress burden.[15] Similarly, Kamal et al in a meta-analysis, confirmed the consistent discriminatory power of PON1 activity across populations, reinforcing its biomarker potential.[16] In an Indian cohort, Ibrahim et al reported

elevated AUC values for PON1 activity in ROC analysis, suggesting it could serve as a non-invasive predictor of oxidative imbalance in COPD patients.[17] These findings align with our study, where PON1 activity yielded an AUC of 0.88, indicating excellent performance in distinguishing COPD patients from controls. The consistency of these results across diverse populations strengthens the case for considering PON1 activity as a clinically useful biomarker for COPD risk assessment and disease stratification.

The ROC curve analysis supports the utility of PON1 activity as a diagnostic tool for COPD, with an AUC of 0.88 indicating high accuracy. The identified cutoff value of 118.2 U/L demonstrates a favorable balance between sensitivity and specificity. These findings reinforce the role of oxidative stress and impaired antioxidant defense in COPD pathogenesis and highlight the potential of PON1 as a clinically relevant biomarker.

However, the present study has certain limitations, including a relatively small sample size, which may reduce the statistical power and generalizability of the findings. Additionally, the cross-sectional design precludes causal inference, and the absence of serum PON1 protein quantification or longitudinal follow-up limits the ability to assess temporal changes in enzyme activity. Future large-scale, multi-centric, and longitudinal studies are warranted to validate these observations and explore mechanistic pathways in greater detail.

CONCLUSION

In conclusion, our findings support the hypothesis that PON1 Q192R polymorphism, particularly the RR genotype and R allele, may contribute to decreased antioxidant protection and heightened susceptibility to COPD. These genetic variants, in conjunction with environmental exposures such as smoking, may serve as valuable biomarkers for COPD risk stratification and management.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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