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Original Article

Paraoxonase Activity an Independent Contributor in SARS-CoV-2 Infection

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ABSTRACT

Background The aim of the present study was estimation of serum paraoxonase (PON1) activity in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Material and Methods In this cross sectional study we estimated serum paraoxonase activity in 73 patients with SARS-CoV-2 infection and 73 healthy controls.

Results The results showed that PON1 activity was significantly decreased in patients with SARS-CoV-2 (1.30±0.55 kU/L) than in healthy controls (1.913±0.48 kU/L, $p<0.05$). In addition we found that the level ALT/AST, bilirubin, creatinine and urea tests were significantly increased in patients with SARS-CoV-2 than normal subjects ($p<0.05$). Multivariate logistic regression reveals PON1 activity is independently associated with SARS-CoV-2 infection.

Conclusions SARS-CoV-2 may decrease the PON1 activity in patients which needs more clarification.

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Keywords: *Paraoxonase, severe acute respiratory syndrome coronavirus 2, high density lipoprotein.*

Introduction

Paraoxonase (PON) is an aryldialkylphosphatase (EC 3.1.8.1) located on the long arm of chromosome 7 (7q21-22). The PON gene family has three members (*PON1, PON2* and *PON3*); they share structural properties and enzymatic activities.[1] PON1 is shown to reside over high density lipoprotein (HDL) tightly bound to Apo A1 having organophosphate hydrolase, lactonase, arylesterase activities and also has both antioxidant and antiatherogenic functions.[2-5] It also shows various polymorphisms.[6,7] It was observed that patients with low levels of HDLs showed an increased risk of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and worse outcome.[8-10] Therefore, it was assumed that PON1 activity may be associated with SARS-CoV-2 infection.

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Coronaviruses are a large family of viruses that causes clinical conditions ranging from common colds to severe lung conditions, such as severe acute respiratory syndrome (SARS) caused by SARS-CoV and Middle East respiratory syndrome (MERS) caused by MERS-CoV. SARS-CoV-2 is a novel strain of coronavirus, and has been identified as the causal pathogen of an ongoing worldwide epidemic since 2019.[11]

As antioxidant enzyme, PON1 is inactivated under inflammation-induced oxidative stress and PON1 activity is found to be decreased in endothelial dysfunction, various inflammatory and infectious diseases.[3,4,6] In SARS-CoV-2 infected patients clinical deterioration often occurs 7-10 days after the onset of symptoms, in association with declining viral titres,[12] suggesting that pathology is driven by inflammatory cascade than direct viral injury. As suggested by increase in inflammatory markers in patients with severe SARS-CoV-2.[13,14] With this backdrop the present study was carried to evaluate activity of PON1 in SARS-CoV-2 patients.

Material and Methods

This is a cross sectional case-control study designed to assess the activity of PON1 in SARS-CoV-2 patients admitted to S.R.T.R. Govt. Medical College Ambajogai. The study has been undertaken with due approval from the Institutional Ethics Committee and consent were taken from study participants. Cases and controls were selected randomly. Inclusion criteria for cases were RT PCR-positive subjects admitted in hospital covid wards. Inclusion criteria for controls, they were subjects attending the outpatient department (OPD) for regular medical check-up and are RT PCR-negative. Exclusion criteria for cases (n: 73) and controls (n: 73) were a history of cardiovascular, renal or hepatic disease, diabetes mellitus, hypertension and endocrine disorders. With all aseptic precautions early morning fasting blood samples were collected by venepuncture from cases and control subjects, blood samples were collected in plain tube, centrifuged, serum was separated and stored at -80 ºC until analysis. Serum samples from RT PCR-positive patients were collected within 48 hours of admission.

Paraoxonase Activity Assay

The rate of formation of p-nitrophenol was measured on fully automated clinical chemistry analyzer XL-640 (Erba Mannheim/Transasia) using working reagent containing 25 mmol/L of triethanolamine-HCL buffer of pH 7.4 and 1 mmol/L $CaCl_2$ over 225 sec after 100 sec lag time in total volume of 600 µl using 2 µl of serum. The activity expressed in kU/L based on the molar absorptivity (14,000 M^{-1}/cm^{-1}) of p-nitrophenol at 405 nm. As p-nitrophenol liberated is being measured, its linearity was checked and if the activity was beyond the linearity, then the serum was diluted to linearity concentration of p-nitrophenyl acetate.[7,15] Intra assay CV 1.6% and inter assay CV was 6%.

Serum ALT and AST activity, bilirubin, creatinine, urea, $Na^+$and K^+ levels were estimated in XL-640 autoanalyzer using Erba Mannheim kits.

Statistical Analysis

The continuous variables were tested for normality by Shapiro-Wilk test. Results are presented as mean±standard deviation. Student's unpaired t-test was used for statistical analysis of continuous variables, Chi square test was used for categorical variables, Pearson's correlation was performed for correlation of paraoxonase with other variables under study. Univariate logistic regression was performed to assess contribution of variable under study towards presence of SARS-CoV-2 infection. Variables found significant in univariate logistic regression were modelled through multivariate logistic regression. $p<0.05$ was considered as statistically significant. Statistical analysis was performed using Microsoft excel and Mystat 12 software.

Results

PON1 activity in SARS-CoV-2 infected patients was significantly decreased than in control (*Figure 1*). All other baseline or biochemical parameters (age, sex, bilirubin, AST, ALT, creatinine, urea and electrolytes) shows significant difference ($p<0.05$) between cases and controls while Na^+ and K^+ were not significantly different ($p>0.05$) (*Table 1*). To assess the association of variables under study, univariate logistic regression was

Table 1. Baseline parameters.

Variables	Cases (n: 73) (Mean±SD)	Controls (n: 73) (Mean±SD)	P-value
Age (years)	53.93±18.02	45.17±16.62	0.002*
Sex (M/F)	48/25	42/31	NS
Total bilirubin (mg/dL) (Diazo method)	1.16±1.81	0.72±0.65	0.05
AST (IU/L) (IFCC method)	46.33±32.27	30.43±16.36	<0.001*
ALT (IU/L) (IFCC method)	41.06±29.84	26.3±13.79	<0.001*
Creatinine (mg/dL) (Creatinase enzymatic)	1.04±1.00	0.75±0.25	<0.001*
Urea (mg/dL) (GLDH method)	47.04±37.22	27.72±9.56	<0.001*
Sodium (mmol/L) (ISE method)	142.17±6.46	141.64±4.43	0.560
Potassium (mmol/L) (ISE method)	3.96±0.82	3.87±0.61	0.480

*p<0.05

Table 2. Univariate logistic regression.

Variables	Estimate	Odd's Ratio	95% Confidence interval		P-value
			Lower	Upper	
Age	-0.029	0.971	0.953	0.991	0.003**
Sex	0.349	1.417	0.725	2.770	0.308
NPON	2.277	9.746	4.253	22.331	0.000**
Bilirubin	-0.474	0.622	0.351	1.103	0.023*
AST	-0.030	0.970	0.953	0.988	0.000**
ALT	-0.038	0.963	0.942	0.984	0.000**
Creatinine	-1.528	0.217	0.067	0.705	0.001**
Urea	-0.062	0.940	0.912	0.968	0.000**
Sodium	-0.018	0.983	0.926	1.042	0.558
Potassium	-0.162	0.850	0.541	1.336	0.479

done which shows association of variables like age, paraoxonase, bilirubin, AST, ALT, creatinine towards the SARS-CoV-2 infection which was significant except Sex, Na^+ and K^+ (*Table 2*). Multivariate logistic regression (*Table 3*) showed low PON1 activity (odd's ratio 2.7-18.8) and urea were independently associated with SARS-CoV-2 infection. The inclusion of PON1 with these parameters in the diagnostic algorithm had high sensitivity and specificity (AUC=0.853) (*Figure 2*).

Table 3. Multivariate logistic regression

Variables	Estimate	Odd's Ratio	95% Confidence interval		P-value
			Lower	Upper	
Age	0.016	1.016	0.987	1.046	0.271
NPON	1.980	7.239	2.784	18.827	0.000**
Bilirubin	-0.256	0.775	0.459	1.307	0.33
AST	0.000	1.000	0.973	1.028	0.989
ALT	-0.018	0.982	0.950	1.016	0.302
Creatinine	0.316	1.371	0.247	7.613	0.718
Urea	-0.050	0.952	0.914	0.991	0.017*

Discussion

In present study, it was found that the PON1 activity was significantly decreased in SARS-CoV-2 patients (1.30±0.55 kU/L) compared to healthy individuals (1.913±0.48 kU/L). We also found that serum PON1 activity was decreased in cases, to levels near about half of that of the controls.

Defective high density lipoprotein and endothelial dysfunction leads to increase in oxidative stress which could be the possible mechanism behind low PON1 activity.[16,17] Vascular endothelium activation and damage occur as part of SARS-CoV-2 infection.[18-21] SARS-CoV-2 infected patients are known to often have low HDL levels[22] and recent studies reported that patients with severe SARS-CoV-2 had decreased HDL cholesterol and/or HDL functionality.[23-25] Begue et al.[26] found that the HDL cholesterol concentration of SARS-CoV-2 patients admitted to the Intensive Care Unit was about half that of healthy individuals and that their HDL particles were enriched in various inflammatory proteins and depleted in PON1. All these studies suggest that there is change in HDL molecules and its contents hence it becomes more inflammatory. This could be the reason behind dramatic decrease in PON1 activities.

This study found that liver (bilirubin, AST, ALT) and kidney function (urea, creatinine) values were impaired. While these parameters increased significantly when univariate logistic regression was performed, multivariate logistic regression showed that urea was independently associated with SARS-CoV-2 infection. These findings are in agreement with previous studies stating that liver injury occurs during highly pathogenic human coronavirus infections, moreover abnormalities in laboratory indexes of blood biochemical parameters, may be associated with the severity of multiple organ dysfunction.[14,27-29]

Out of 73 patients 13 patients died from SARS-CoV-2 infection having decreased PON1 activity (1.19±0.46 kU/L) as compared to survivors (1.31±0.56 kU/L) ($p>0.05$, data not shown). Among all variables examined, PON1 and urea were found to be associated with SARS-CoV-2 infection. Variables related to SARS-CoV-2 infection in univariate logistic regression were modelled by multivariate logistic regression, showing that PON1 and urea are independently related to SARS-CoV-2 infection. Low PON1 levels appeared to be associated with increased SARS-CoV-2 infections (Odd's ratio 7 with 95% confidence interval 2.7-18.8).

The PON1 enzyme could be a valuable biomarker for understanding SARS-CoV-2 viral

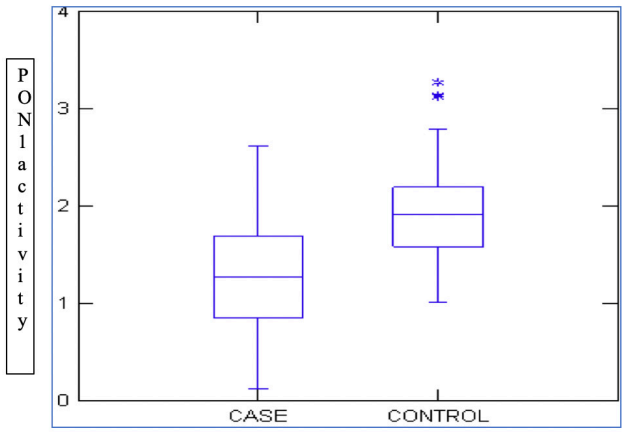

Figure 1. Boxplot for PON1 activity.

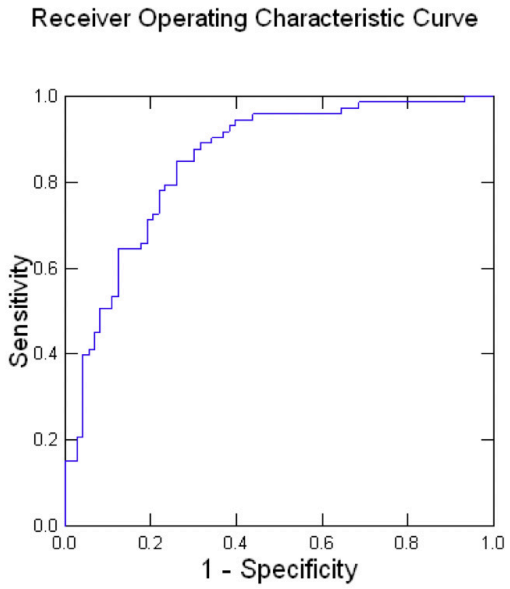

Figure 2. ROC Curve (Area under ROC=0.853).

infection. PON1 estimation requires inexpensive tools such as colourimeters available in remote healthcare facilities in developing and low-income countries. Determination of serum PON1 arylesterase activity is simple and economical, requires p-nitrophenyl acetate as substrate, and can be used as a primary screening test in developing and low-income countries where an ELISA reader is available. The detailed elucidation of inflammatory pathways, identification of inflammation triggers and the role of PON1 could eventually lead to the discovery of a new therapeutic target.

Conclusions

PON1 activity is significantly reduced in SARS-CoV-2 patients. In addition, as in univariate logistic regression, ALT/AST activity, bilirubin, creatinine and urea test levels increased significantly in patients with SARS-CoV-2 ($p<0.05$). Also, multivariate logistic regression reveals that PON1 activity and urea are associated with SARS-CoV-2 infection. In the future, including PON1 activity as a biomarker in SARS-CoV-2 infection may aid diagnostic algorithms with increased sensitivity and specificity. However, extensive multicenter studies may be required to determine its role in SARS-CoV-2 infection.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Ethical Approval

This study has been duly approved by IEC (Institutional Ethical committee).

Authors' Contribution

MRM and PSR researched literature and conceived the study. MRM, MGD & RMZ was involved in protocol development, gaining ethical approval, patient recruitment and data analysis. PSR wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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