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RBC membrane damage and decreased band 3 phospho-tyrosine phosphatase activity are markers of COPD progression

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1. ABSTRACT

Injury to red blood cell (RBC) membrane by oxidative stress is of clinical importance in chronic obstructive pulmonary disease (COPD) which leads to oxidative stress (OE) during disease progression. Here, we studied the impact of this stress on injury to RBC membrane. Blood samples from both healthy volunteers (HV, n = 11) and controlled COPD patients (n=43) were divided according to their GOLD disease stage (I=7, II=21, III=10, IV=5). Plasma levels of paraoxonase (PON) activity, protein carbonyls (PC), conjugate dienes, lipohydroperoxides (LPH) and malondialdehyde (MDA) were determined and the PTPase, and the oxidative parameters were measured in RBC ghosts. Plasma from patients with COPD showed an increased oxidation of lipids and proteins, that correlated with the disease progression. PON activity decreased from GOLD stages II to IV and correlated with an increase in LPH ($p < 0.0001$, $r = -0.8115$). There was evidence of an increase in the oxidative biomarkers in RBCs, while the PTPase activity was diminished in stage III and IV of COPD. In conclusion, OE-induced injury associated with COPD is associated with an oxidative damage to the RBC membrane, with a concomitant decrease in the PTPase activity and altered function of anionic exchanger (AE_1).

2. INTRODUCTION

COPD is a major cause of chronic morbidity and mortality throughout the world. Because further increases in the prevalence and mortality of the disease are predicted for the coming decades, a unified, international effort is required to reverse these trends. The **G**lobal Initiative for Chronic **O**bstructive **L**ung **D**isease (1) outlines a simple classification system that divides the severity of this disease into four stages: GOLD I-Mild; GOLD II-Moderate; GOLD III-Severe; and GOLD IV-Very severe. Oxidative stress (OE) has been implicated in the pathogenesis and progression of COPD. Reactive oxygen species (ROS) from inhaled cigarette smoke or those endogenously formed by inflammatory cells constitute an increased intrapulmonary oxidant burden. Data from *in vitro* experiments and *in vivo* studies indicate that two general effects of oxidative stress are relevant to COPD. Oxidative stress causes structural changes to essential components of the lung, leading to irreversible damage to both the parenchyma and the airway wall (2). Moreover, the serious imbalance between ROS production and antioxidant defenses leads to activation of transcription factors (such as nuclear factor-NF-KB), inactivation of anti-proteases, increased sequestration of neutrophils in the pulmonary microvasculature, and oxidative tissue and cellular injury (2). ROS are generated principally from leukocytes in the blood/air spaces or inhaled in the form of environmental oxidant pollutants, including cigarette smoke (3), particulate matter (4), and wood smoke (5).

Biomarkers of ROS in plasma can potentially be used to evaluate both the injury and degradation of circulating bio-molecules and endothelial cell membranes. Additionally, biomarkers can help to determine the extent of oxidative injury, identify the source of the oxidant, and characterize the chemical nature of the damage to biomolecules (lipids, proteins, carbohydrates etc.), including modifications and breakage. The amount of biomarkers in plasma depends on the number of susceptible substrates for ROS, including circulating proteins, hormones (6-7), and unsaturated fatty acids of lipoproteins. Inducing lipoperoxidative and chemical modifications, especially in phospholipids and proteins. Cells show a wide range of responses upon exposure to ROS, which range from increased proliferation, prevention of cell division, necrosis, apoptosis and cell death. In some cells, such as erythrocytes, senescence or lysis are possible outcomes of oxidative injury due to the absence of nuclei and mitochondria. Red blood cells (RBC) show a moderate OE response, which is characterized by a decrease in ion exchange functional capacity. Such affected exchanges include O_2 and CO_2 uptake from the lungs or peripheral tissues, respectively, and CO_2 extrusion as HCO_3- through the band 3 anion exchanger (8). The band 3 anion exchanger forms a scaffold for assembly of a protein complex that can transmit signals from the exterior and modulate the transport and mechanical properties of the erythrocyte (9).

Pathological alterations in the lungs may lead to deficient hemoglobin oxygenation and ultimately pulmonary hypoxemia, which limits intramuscular oxidative metabolism and decreases aerobic adenosine triphosphate (ATP) production. Additionally, the OE present in COPD patients induces structure and function modifications. Thus, it should be considered that pulmonary dysfunction has detrimental effects on RBCs (10). The RBC has been proposed as a biosensor of COPD progression due to the ability to detect oxidative modifications in these cells during the GOLD II stage and after experimental exposure to ROS and reactive nitrogen species (RNS). Typically, these RBC modifications are studied by scanning electron microscopy and flow-cytometric analysis. Furthermore, pilot studies (11) have shown alterations in the RBC ultrastructure, including relevant changes in the spectrin cytoskeleton and glycophorin expression. N-acetylcysteine (NAC) treatment was able to significantly counteract these changes caused by oxidative stress. These altered RBCs have a reduced transport capacity and a reduced peripheral release of O_2 (11).

In this study, we assessed the oxidative damage associated with various disease stages of COPD. We measured plasma biomarkers of oxidative injury caused by ROS generation, which is known to affect circulating or endothelial biomolecules (12) and the antioxidant paraoxonase (PON) activity associated with HDL lipoproteins (13). We also quantified the oxidative injury to the RBC membrane and the band 3 anion exchanger AE1 phospho-regulation in the various GOLD stages by measuring the activity of phospho-tyrosine phosphatase PTPase.

Our results demonstrate a progressive increase in the oxidative injury biomarkers in both plasma and RBCs from COPD patients that correlates with the different stages of GOLD progression. In addition, there was a decrease in plasmatic PON and RBC PTPase activities, which affected the band 3 anion exchanger (AE1) activity and consequently decreased the HCO_3^- extrusion, leading to reduced elimination of CO_2 from lungs. We conclude that the OE present in the COPD patients was increased and correlated with the progression of the disease. Furthermore, the OE showed a correlation with the intensity of oxidative injury. This oxidative injury adversely affected the erythrocyte membrane function by altering its ability to uptake O_2 and eliminate CO_2.

3. MATERIALS AND METHODS

3.1. Patients

Forty-three consecutive patients with a diagnosis of COPD and 11 healthy controls were enrolled in this study. COPD patients were ex-smokers and belonged to a cohort of subjects who were evaluated during a clinically stable period, which was free of exacerbations during the previous 6 weeks. These subjects had a history of tobacco smoking of at least 10 packs/year. COPD diagnosis was established according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines (14). Subjects who met the criteria for COPD but had an alternative respiratory disorder (i.e. bronchiectasis or asthma) were excluded. The spouses of COPD patients who had no history of chronic illness such as diabetes, rheumatoid arthritis, or lung disease were invited to participate as a control. Controls with a history of tobacco smoking were excluded. The protocol of this study (B10-08) was approved by the Ethics Committee at the National Institute of Respiratory Diseases. All subjects were informed, and their written consents were obtained.

3.2. Treatment strategy

Subjects underwent treatment for post-bronchodilator spirometry following the procedures recommended by the ATS and ERS (15). We used Mexican standard reference equations for predicted values (16). For spirometry we used a dry rolling-seal volume spirometer (Sensormedics, Yorbalinda, CA). Subjects also underwent testing for their blood count and lipid profile.

3.3. Biochemical analysis

Blood samples (5 mL) from both healthy volunteers and COPD patients were obtained by venipuncture. These were centrifuged, and the plasma and erythrocyte ghosts were obtained and used for biomarker analysis. Open erythrocyte membranes were prepared by hypotonic lysis of RBCs as originally described by Steck. (17) The entire process was carried out at 4ºC and the assays were performed on the same day. To evaluate

Table 1. Demographics and lung function characteristics

Variable	COPD Group Mean (SD) n=43	Control Group Mean (SD) n=11	p-value
Demographics			
Gender M (%)	33 (72)	2(17)	0.001*
Age (years)	68.4 (9.7)	62.2 (9.3)	0.05
Height (cm)	160.0 (0.08)	153 (0.09)	0.02
Weight (kg)	67.5 (11.4)	65 (9.6)	0.51
BMI (kg/m2)	26.5 (4.7)	27.6 (3.3)	0.46
Cigarette Smoke Exposure (pack-years)	46 (16)		
Post-bronchodilator Pulmonary Function			
FEV1 (liters)	1.49 (0.63)	1.89 (0.60)	<0.0001
FEV1 % predicted	58.43 (23.62)	106 (21.88)	<0.0001
FVC (liters)	2.85 (0.99)	2.95 (0.72)	<0.0001
FVC % predicted	86.6 (24.9)	104.7 (21.07)	0.001
FEV1/FVC	0.51 (0.13)	0.79 (0.04)	<0.0001

SD=standard deviation; BMI=body mass index; FEV_1=first forced expiratory volume; FVC=forced vital capacity. *Chi square test

Table 2. Hematological biometry values

	HV	GOLD I	GOLD II	GOLD III	GOLD IV	SS
Leukocytes (4-10 x 10^3 mm^3)	6.74(2.97)	6.41(1.94)	6.55(0.98)	6.76(1.29)	7.9(1.16)	NS
Neutrophils (1-4 x 10^3 mm^3)	3.78(2.39)	3.6(1.14)	3.84(1.37)	3.45(0.62)	4.82(0.96)	NS
BI (0-0.75 mg/dL)	0.399(0.173)	0.371(0.121)	0.435(0.24)	0.503(0.121)	0.418(0.27)	NS
Glucose (55-115 mg/dL)	97.18(11.26)	95.75(6.42)	94.45(6.61)	102.7(11.93)	97.86(7.80)	NS
Hemoglobin (11.5-17 g/dL)	14.45(0.93)	14.90(0.99)	15.84(1.09)	15.49(2.02)	15.6(3.10)	NS
Erythrocytes (4.5-5.2 X 10^6 mm^3)	4.78(0.56)	4.93(0.46)	5.07(0.52)	4.97(0.62)	4.98(0.85)	NS

The data are the MEAN (SD), SS= Statistical significance; BI= bilirubin indirect.

ipoperoxidation, 1-methyl-2-phenylindole (Sigma-Aldrich, MO) was used as a standard. Aliquots of plasma were used to measure malondialdehyde (MDA) at 586 nm, and the values obtained were expressed as nmoles of MDA per mg of dry weight (18). To evaluate lipid peroxides, we used the assay conditions described by El-Saadani *et al* (19). The test solution was mixed with a color reagent of a commercially available kit for the enzymatic determination of cholesterol (CHO-iodide; ROCHE). This assay quantifies lipid peroxides by testing their ability to convert iodide to iodine, which can be measured photometrically at 365 nm. Calibration curves were obtained using peroxides such as t-butylhydroperoxide. The concentration of lipid peroxides was calculated by using the molar absorptivity of I_3, measured at 365 nm (ϵ= 2.46 ± 0.25 x $10^4 \cdot M^{-1} \cdot cm^{-1}$). The conjugated dienes were obtained after extraction once with methanol-chloroform and quantified using a molar extinction coefficient of 28 x $10^3 \cdot M^{-1} \cdot cm^{-1}$ and photometric analysis at 284 nm (20).

Protein damage was evaluated by the carbonyl group content of the erythrocyte membranes and was determined by treatment with 2,4-dinitrophenylhydrazine (DNPH), which reacts with the protein carbonyl derivates to form stable hydrazones that have an absorption spectrum at 370 nm (21). A molar extinction coefficient of 21 x 10^3 M^{-1} cm^{-1} was used to quantify carbonyl content. Paraoxonase activity was evaluated by the hydrolysis of diethyl p-nitrophenyl phosphate (paraoxon) and the absorbance was measured (22) at 412 nm. PTP activity was determined by using p-nitrophenyl phosphate (p-NPP) as substrate according to previously published procedures (23). The release of p-nitrophenol from p-NPP was measured spectroscopically at 410 nm.

3.4. Statistics

Data are expressed as the mean ± standard deviation. One-way ANOVA and Bonferroni's Multiple Comparison Test, linear regression, and Pearson's correlation were used for statistical analysis. Differences were considered significant when the p-value was < 0.05. Data analyses were performed using the Statistical Package for Social Sciences (version 10.0 for Windows; SPSS Inc., Chicago, Ill).

4. RESULTS

4.1. Respiratory probes

There were significant differences between the control and patient groups with respect to post-bronchodilator FEV_1 % predicted, forced vital capacity (FVC) % predicted, and the FEV_1/FVC ratio (Table 1). There were no significant differences between the two groups with respect to age, body mass index (BMI), lipid profile, and C-reactive protein (PCR). The mean cumulative tobacco consumption for COPD patients was 45 ± 26 pack-years. The only difference between COPD patients and the control group was that the patients had statistically higher hematocrit values in the blood count. According to the GOLD classification, 7 patients (11.5%) were in stage I, 21 (34.4%) were in stage II, 10 (21.3%) were in stage III, and 5 (8.2%) were in stage IV. The only difference between GOLD stage groups was in the spirometry parameters, as was calculated by using ANOVA analysis (Table 1).

4.2. Clinical Parameters

Within the lipid profile (Table 2), only the total triglyceride concentration was significantly different when compared to the HV for all of the GOLD stages. Specifically, there is a decrease in the triglyceride concentration from GOLD stage I to IV (191.58 to 212.14 mg/dL). Table 3 shows the hematic biometry values.

4.3. Biochemical parameters

Figure 1 shows that the plasma concentrations of LHP and malondialdehyde (MDA) were elevated in the patient group compared to the healthy volunteers (HV).

Table 3. Lipid profile

Lipids	HV	GOLD I	GOLD II	GOLD III	GOLD IV	SS
TAG (50-200 mg/dL)	155.4(62.03)	176.12(95.66)	154.26(74.08)	136.77(51.85)	72(35.54)	*I vs IV
CT (140-200 mg/dL)	208.54(26.95)	191.58(28.14)	214.83(45.97)	206.11(59.68)	212.14(28.74)	NS
Phospholipids (80-250 mg/dL)	226.661(2.39)	213.57(23.52)	243.71(46.14)	217.05(46.14)	212.6(25.17)	NS
HDL (35-65 mg/dL)	53.88(14.64)	46.42(17.07)	53.63(17.82)	58(15.24)	60.36(7.34)	NS
LDL (0-150 mg/dL)	131.82(26.18)	113.62(20.79)	134.52(40.42)	127.1(47.53)	126.4(23)	NS
CRP (0-80 mg/dL)	0.483(0.44)	0.587(0.674)	0.466(0.392)	0.487(0.335)	0.634(0.4069)	NS
APO-A (107-214 mg/dL)	154.45(26.27)	149.27(35.83)	148.10(28.14)	151.73(34.5)	150.2(22.94)	NS
APO-B (51-171 mg/dL)	104.50(34.89)	107.67(21.95)	111.6(34.172)	101.47(28.78)	97.52(21.44)	NS
Non-HDL-C (<160 mg/dL)	154.67(31.61)	159.98(28.13)	161.20(46.48)	133.3(68.63)	151.78(31.06)	NS

SS= Statistical significance; TAG= triacylglycerols; CT= cholesterol total; CRP= C-reactive protein. The data are the MEAN(SD) * There is a significant difference with a $p < 0.001$ between GOLD I and GOLD IV stages

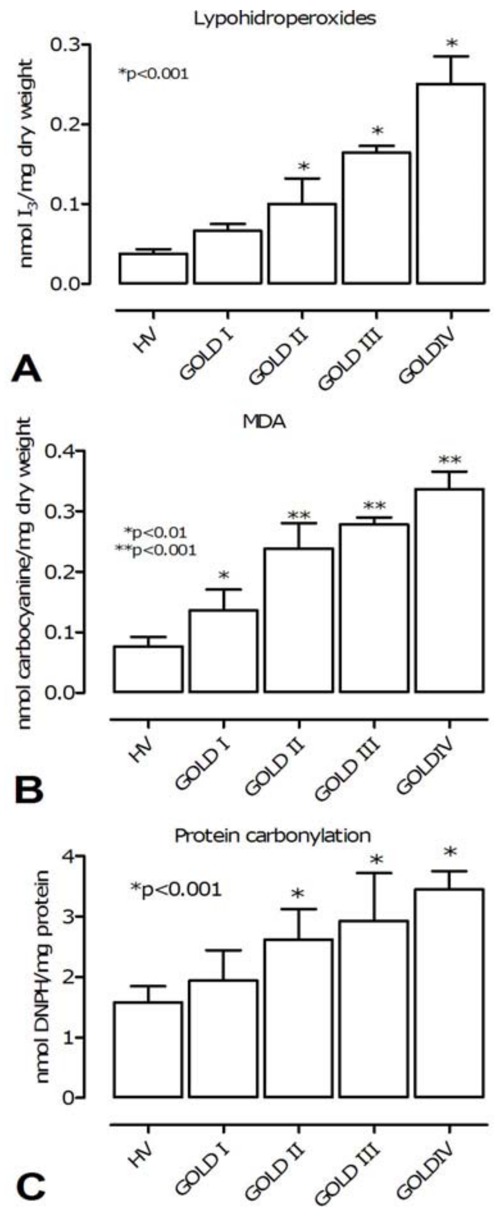

Figure 1. (A) The Lipohydroperoxide (LHP), (B) Malondialdehyde (MDA), and (C) carbonylation concentration measured from patient plasma during COPD progression expressed as GOLD stages (I to IV) compared to healthy volunteers (HV).

The COPD patients showed a 2.5-fold increase in LHP (Figure 1-A) compared to the values of the stage II GOLD (0.100 ± 0.031 nmol of I_3/mg dw, $p < 0.001$) and reached a concentration of 0.251 nmoles of I_3/mg dw at stage IV. The MDA concentration increased with subsequent GOLD stages ($P < 0.001$) (Fig 1-B) compared to the control (0.07 ± 0.01) and the MDA concentrations were 0.13 ± 0.03, 0.23 ± 0.04, 0.27 ± 0.01 and 0.33 ± 0.02 nmoles of carbocyanine/mg dw for GOLD I to IV, respectively. Figure 1-C shows the amount of protein carbonylation in the control group (1.58 ± 0.26 nmoles) compared to the values of the different GOLD stages, which show significant differences ($p < 0.001$) after stage II (2.60 ± 0.53 nmoles). In fact, the GOLD IV values of GOLD IV are 2.25-fold greater than the control values. Figure 2 shows the paraoxonase activity (Figure 2-A) and its correlation with the amount of its substrate (LPH) formed during the lipoperoxidative process. A significant decrease in the enzymatic paraoxonase activity (detected by the catalytic hydrolysis of the LHP formed during lipoperoxidation) was evident after stage II ($p < 0.001$), in which there was a 31% decrease in activity (5.49 ± 1.58) compared to the health volunteer (8.31 ± 0.87 nmoles/mg Apo A/min). In GOLD IV the hydrolytic efficiency of PON was decreased 5-fold (Figure 2-A) compared to the HV activity. This difference is not due to HDL or apo A concentration differences between COPD patients and the control group (Table II); thus the decrease in PON activity may be attributed to oxidative injury of PON that negatively correlates with carbonylation of plasmatic proteins (Fig 1-C). The inverse correlation between PON activity and LHP concentration is highly significant ($p < 0.001$) and had a negative correlation coefficient of – 0.811 (Figure 2-B). Additionally the protein carbonylation (Fig 2-C) had a significant negative correlation coefficient ($r = 0.589$) when compared to PON activity throughout the GOLD stages ($p < 0.001$).

The RBC membranes showed oxidative injury, which increased from GOLD I to IV when compared to the healthy volunteers. In Figure 3, three steps of the lipoperoxidation process are shown. Although the MDA values were not significant (Figure 3-C), conjugate diene and LHP formation were significant after GOLD II (Figure 3-A and 3-B) and increased in significance ($P < 0.0001$) in GOLD III and IV. The diene formation was almost 3-fold higher in GOLD IV (9.65) compared with HV RBCs (3.33), while the LHP increase in GOLD IV patients showed a 2.5-fold increase (0.077) compared to the control

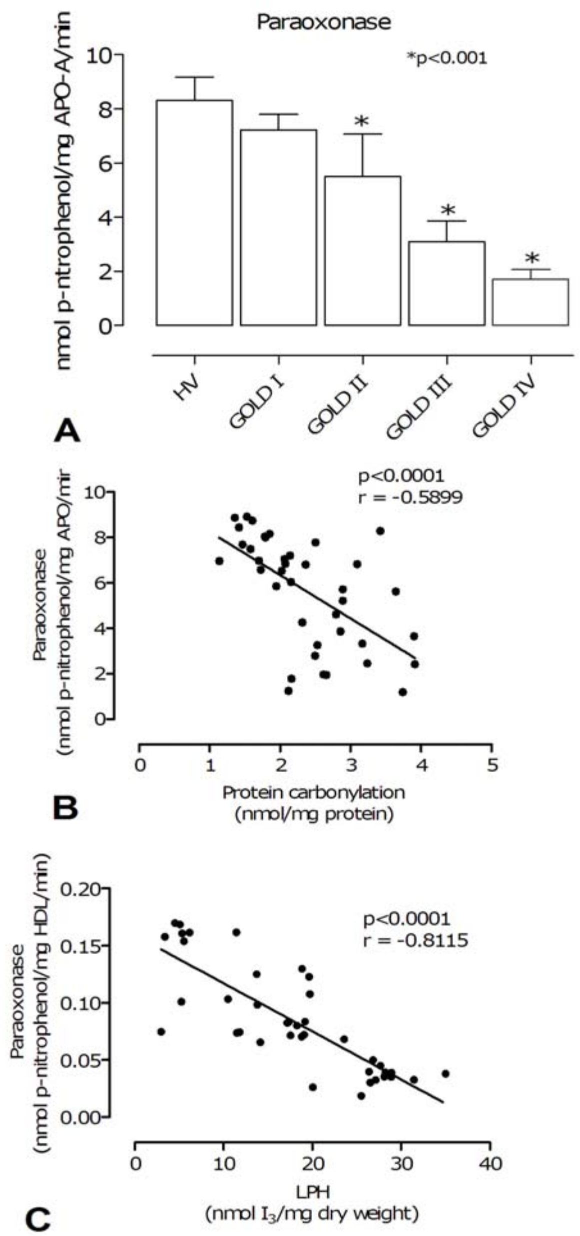

Figure 2. (A) The plasma paraoxonase activity of COPD patients during each GOLD stage compared to healthy volunteers (HV), and its correlation with (B) protein carbonylation and (C) lipohidroperoxide concentrations (LHP).

group (0.032). The membrane protein carbonylation was increased in all of the GOLD stages (Figure 4-A), while the PTPase activity associated with the membrane band 3 was significantly decreased in the erythrocytes from GOLD III and IV patients. There is no significant correlation between the protein oxidative injury (carbonylation) and phospho-tyrosine phosphatase activity.

5. DISCUSSION

Chronic obstructive disease affects various lung structures and function, leading to limitation of air flow (1). In addition to these pulmonary abnormalities, COPD is associated with significant detrimental effects in organs distant from the lungs. These so-called systemic effects of COPD (24) include weight loss, nutritional abnormalities, skeletal muscle dysfunction, an increased risk of cardiovascular disease, and several neurological and skeletal effects. The mechanisms underlying these systemic effects are unclear, but likely interrelated and multifactorial, and may include inactivity, systemic inflammation, tissue hypoxia, and oxidative stress among others. In a previous study, it was observed that plasma from COPD patients showed a progressive increase in protein carbonylation, which correlated with both the GOLD stage and the protein C concentration (12). From the results obtained here, several parmeters of plasma oxidative lipoperoxidation were elevated during progression of COPD. Additionally, the antioxidant catalytic efficiency of paraoxonase (arylesterase activity) contained in Apo A_1 of the HDL (13), which measures both its activity and relation with lipid hydroperoxides (LHP) concentration, was also elevated with COPD progression (Table 2, Figure 2).

In an attempt to understand the source and consequences of OE damage to the erythrocyte membrane during COPD progression, we correlated oxidative stress with the measurement of several plasma biomarkers (Fig 1). Our data show that the selected biomarkers significantly increased from GOLD stages I to IV, while the patients showed only a slight loss in the body mass index weight, which inversely decreased with the GOLD stage (Table 1). Furthermore, there was a decreased total triglycerides concentration (Table 2), which correlated with the BMI (25). The characteristics of chronic and progressive oxidative stress were similar to our previous report (12) (Figure 1). Although the RBCs obtained from patients were similar to those of the HV group (Table 3), the oxidative injury to the erythrocyte membranes was higher in the patient population. This was likely due to a decrease of PTPase activity as a consequence of the oxidative stress, and was related to band 3 anion exchanger dysfunction, which altered HCO_3^- extrusion.

Band 3, also termed the anion exchanger (AE_1), constitutes the most abundant polypeptide in the RBC membrane and comprises 25% of the total membrane protein. The 55-kD membrane-spanning domain can be cleaved into 2 independent domains, transverses the bilayer 12 times, and serves to catalyze the exchange of anions (mainly Cl^- for HCO_3^-) across the membrane during gas transport in the blood (11). The membrane-spanning domain may also mediate removal of senescent RBCs (10) and carry a number of common blood group antigens. The structure and function of the short (33 residue) C-terminal cytoplasmic tail (cdb3) of band 3 mediates the transfer of nitric oxide from bound deoxyhemoglobin to the cell exterior through the intermediary cysteine groups of cdb3 (26). The cdb3 domain functions primarily as an anchoring site for other membrane associated proteins, including glyceraldehydes-3-phosphate dehydrogenase (GAPDH), phosphofructokinase, aldolase, hemoglobin, hemichromes, and the protein tyrosine kinase (PTK). These band 3 interactions in turn modulate cell flexibility, cell shape,

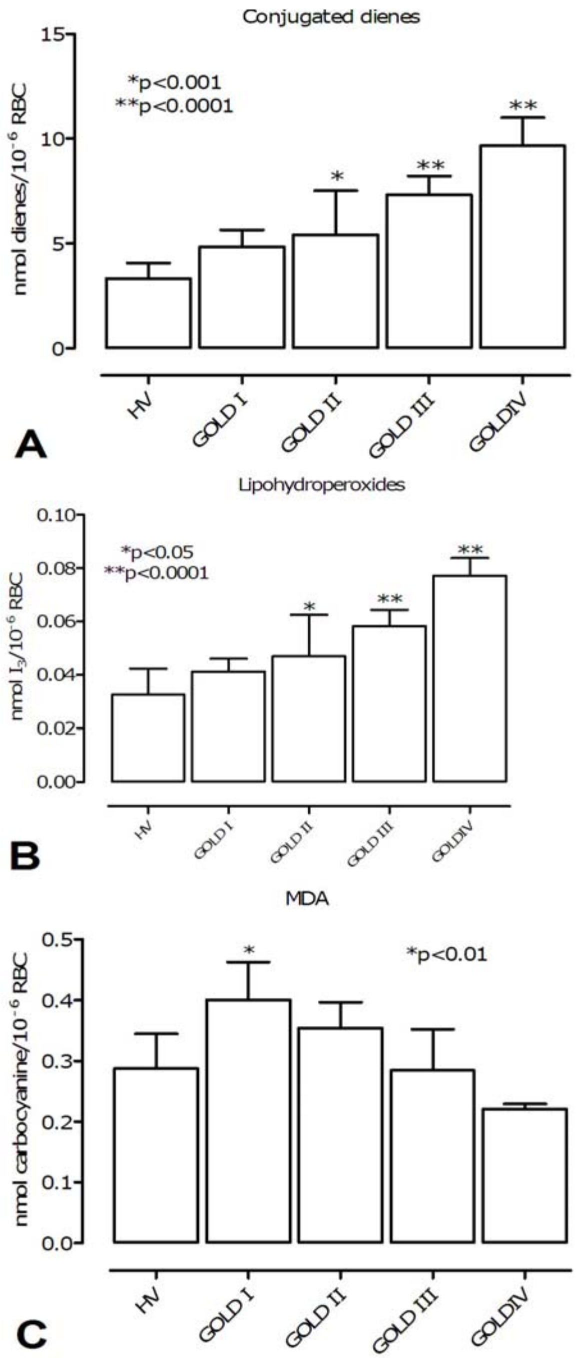

Figure 3. (A) The conjugated diene, (B) Lipohidroperoxide (LPH), and (C) malondialdehyde (MDA) concentration measured in RBC ghosts during various GOLD stages of COPD progression.

glucose regulation, ion transport, and cell lifespan. Tyrosine phosphorylation at the NH_2 terminus of band 3 (26) and deoxygenation of RBCs reversibly releases glycolytic enzymes (GEs) (such as lactic dehydrogenase, LDH, and pyruvate kinase, PK) (27) to the cytoplasm. This subsequently causes an increase in glucose metabolism, NADPH and H^+ generation, and binding of deoxyhemoglobin to the NH_2 terminus of band 3. This, in turn, leads to a decrease in anion exchanger AE_1 function. Our observation that PTPase (Figure 4) in RBCs from COPD patients showed a significant decrease (43%) in activity at stages III and IV of the disease suggests that patient RBCs had a decreased ability to emit HCO_3^- caused be a glycolysis and pentose shunt increase. Band 3 tyrosine phosphorylation can be induced by the inhibition of phospho-tyrosine phosphatase (PTP) with vanadate, thiol oxidation, peroxynitrite (10-100 μM (28)), activation of PTK by hypertonic NaCl or an increase in intra-cellular Ca^{2+} (29) and peroxynitrite (200-1000 μM (28)). The evident increase in plasma oxidative stress injury biomarkers (Figure 1) and their relationship to COPD progression (Table 1) suggests that RBCs under this oxidative *milieu* also had oxidative injury to their membranes (Figure 3). These damaged RBCs demonstrated a metabolic shift with an increase in glycolysis, which induced a burst of activity for the hexose monophosphate shunt (27). Increased glycolysis also led to an increase in glucose-6 phosphate availability to generate sufficient NADPH and H^+ to increase the antioxidant capacity and prevent cell lysis.

The Apo-A and HDL concentrations were not statistically different between the patient and control groups (Table 1). However, the PON activity localized to Apo-A was different between the groups and showed a significant decrease in the patients (Figure 2). The exact mechanism by which PON exerts its protective effect is not well established, although it has been proposed that this antioxidant effect can be associated with the peroxidase-like activity of PON (13). The hydrolysis of preformed lipid peroxides is an essential step in the lipoperoxidation process that concludes with the breaking of unsaturated free acids to produce several aldehydes (30). The progressive decrease in PON activity found in the COPD patients correlated with both an increase in plasma carbonyls (which shows the injury to plasma proteins) (Figure 2-B), and an increase in LHP (Figure 2-C). The mechanism of PON inactivation could be caused by adduct formation and modification of several proteins, including Apo-B-100 of the LDLs and insulin (13). As we report here, PON activity decreased under oxidative conditions and allowed the progression of lipoperoxidation in plasma, cells, and tissues. PON activity decreased during GOLD stages II to IV and in RBC membrane injury, as was observed by an increase in the generation of conjugate dienes and hydroperoxides (Figure 3, A and B). However, the membrane MDA did not show any differences, which was perhaps due to the adduct formation with membrane proteins such as the PTPase of band 3. Erythrocytes typically have efficient antioxidant mechanisms because their physiological function depends on membrane integrity. The inhibition of PTPase found in stages III and IV might be related to patient deterioration due to the disease progression. This may result in poor oxygenation of tissues, leading to secondary COPD effects (31) and deficient anion exchange caused by membrane oxidation. The RBCs in these patients may alter the band 3 functions to survive the increased glucose utilization and decreased anion exchange.

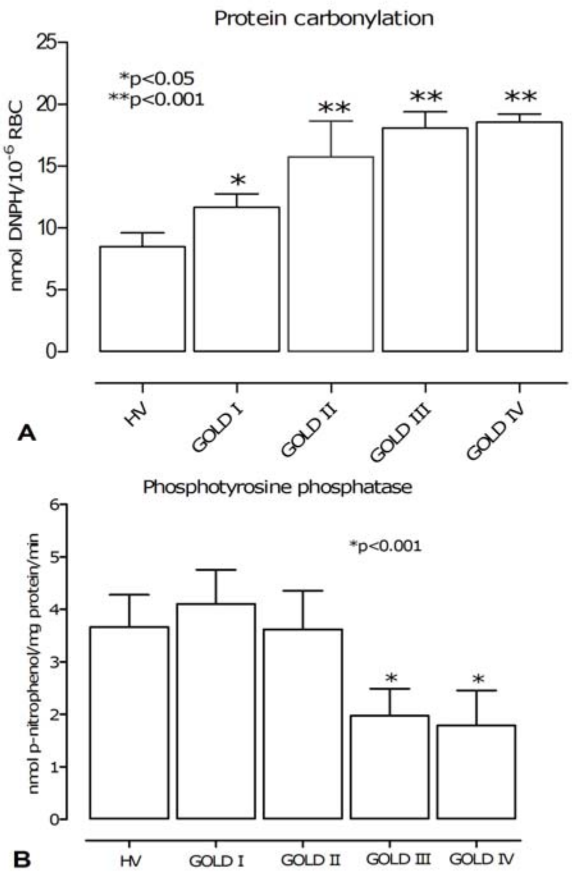

Figure 4. The phosphotyrosine phosphatase activity and carbonylation of erythrocyte membranes throughout the various COPD stages. (** $p < 0.001$ when compared to the HV group)

In summary, we have demonstrated that reactive aldehydes, including MDA are generated as a by-product of ROS activity during the progressive oxidative stress in patients with COPD. These products are capable of forming adducts with the RBC membrane proteins, and subsequently, they decrease MDA in membranes. However, the previous steps in lipoperoxidation that conjugate dienes and LPH were progressively increased in different stages of GOLD and showed the following characteristics: (1) a decrease in phosphor-tyrosine phosphatase activity; (2) a significant inverse correlation caused by a decrease in plasma PON activity and an increase in both LPH concentration and protein carbonylation; (3) no evidence for a correlation between decreased PTPase activity and carbonylation of the RBC membrane (Figure 4); and (4) an increased tendency of oxidative injury caused by OE to correlate with the progression of GOLD disease stages. Finally, we considered whether the erythrocyte membrane oxidative injury induced a decrease in PTPase activity and could decrease the band 3 anion-exchanger AE_1 activity and interfere with bicarbonate extrusion and thus CO_2 elimination. However, PTPase inhibition increased glycolysis in RBCs [32] and the availability of glucose-6 phosphate for the reduction of $NADP^+$ in the catalysis for glucose-6 phosphate dehydrogenase [33]. This, in turn, produced NADPH and H^+, which prevents lysis of RBCs in COPD patients.

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