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The Role of Nf-Kappa and HDCA and Implication of Oxidative Stress in Airway Inflammation of Chronic Obstructive Pulmonary Disease

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Abstract: Oxidative stress has been implicated in the pathogenesis of Chronic Obstructive Pulmonary Disease (COPD), due to its effect on proinflammatory gene transcription. Oxidants/antioxidants imbalance is responsible for disease development. The study was designed to assess oxidative stress and inflammation in COPD patients. The level of TNF-α and IL-8 in sputum and BAL were compared. NF-κB and HDAC were measured in BAL leucocytes. Oxidative stress was evaluated by assessment of MDA levels in BAL and antioxidant markers: TAS, GSH, GSH-Px activity, vitamin E and vitamin C in BAL and uric acid in serum. Design and Methods. Sixty patients with COPD were recruited from Medical Biochemistry Department, in collaboration with the Chest hospital, Ain Shams University and compared to 20 control (smokers and nonsmokers) subjects. The patients were divided to three groups (GOLD stage II, III and IV) according to the severity of disease. Results. Concentrations of TNF-α and IL-8 were significantly elevated in both sputum and BAL compared to controls. MDA level increased while antioxidants (TAS, GSH, GSH-Px, vitamin E, vitamin C and uric acid) decreased in patients compared to the controls. Oxidants stress also alters the activity of HDAC and NF-κB. There was a significant negative correlation between MDA and all antioxidant markers ($p<0.000$), also HDAC and NF-κB ($p<0.000$). Conclusion. There is evidence of increased oxidative stress in the airways of patients with COPD. Understanding the mechanisms of TNF-α inhibition, NF-κB regulation, histone acetylation/deacetylation balance may support the development of novel therapies that limit lung inflammation and injury.

Keywords: COPD, Oxidative Stress, Inflammation, NF-κappa, HDAC

Introduction

Chronic Obstructive Pulmonary Disease (COPD) causes a significant health burden worldwide, in terms of morbidity and mortality. The principal abnormalities in airways are the presence of a persistent inflammatory response as well as a structural remodeling that thickens the airway wall and destruction of alveoli. The epithelial cells are damaged by the inhalation of noxious particles; there is an activation of innate and adaptive immune cells. These cells are responsible for the release of proteases, cytokines/chemokines and mediators, which lead to inflammation and remodeling (Moermans *et al*., 2011).

The involvement of oxidative stress in the pathogenesis of COPD appears to be crucial for the manifestation of the inflammatory response of the lung. The increase of oxidative stress in patients with COPD results from the action of exogenous and endogenous oxidants produced during the inflammatory process. One of the main targets of oxidative stress is the polyunsaturated fatty acids of cell membranes and the oxidizing species, leading to lipid peroxidation where Malondialdehyde (MDA) is recognized as an indicator of increased lipid peroxidation, therefore, an indicator of oxidative damage (Cristóvãoa *et al*., 2013).

NF-κB plays a central role in inflammation and stress response by controlling gene network

Science Publications

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expression. The members of the NF-κB/Rel family of transcription factors are known to activate expression of many prionflammatory genes involved in lung inflammation. The expression of inflammatory mediators can be regulated by the activation of redox-sensitive transcription factor, NF-κB, stimulated in response to Reactive Oxygen Species (ROS). In addition to ROS, cellular redox status, particularly thiol status, can be directly involved in activation of NF-κB, signal transduction and gene expression involved in cellular pathophysiologic activities. The activation of NF-κB is redox-sensitive and regulated by the changes in the oxidant/antioxidant balance (Rajendrasozhan *et al*., 2008).

Direct acetylation and deacetylation of NF-κB play an important role in the regulation of its functions. Acetylations have different effects on NF-κB transcriptional activity and NF-κB-dependent gene expression. The crucial part of NF-κB acetylation and deacetylation in the regulation of NF-κB-mediated gene expression raises the idea to modulate inflammatory responses by controlling NF-κB acetylation level with HDAC inhibitors (Ghizzoni *et al*., 2011).

The aim of the present study is to evaluate the different biological pathways, such as inflammation, injury and repair; relate the effect of oxidative stress on activation of transcription factor nuclear factor kappa B (NF-κB) and Histone Deacetylase (HDAC) nuclear enzyme that change the chromatin structure by regulating expression of inflammatory genes; investigate the effect of oxidant/antioxidant imbalance and inflammatory markers on the COPD stages; and give recommendation on better lifestyles that help to decrease the symptoms of the disease.

Subjects and Methods

Study Design and Subject Characteristics

The study was conducted on eighty subjects, 20 healthy volunteers of matched age and sex were recruited for participation as control subjects and 60 patients affected with COPD. All individuals were subjected to full clinical examination. They were classified according to clinical examination into the following groups:

- Control group: 20 Nonsmokers, ex-smokers and smokers control subjects, with no history of lung disease and their age 58±9 years
- GOLD stage II: 20 Moderate COPD patients clinically stable for 6 weeks, age 60±9 years
- GOLD stage III: 20 COPD Patients, age 62±5 years, who will have severe exacerbations, requiring hospital admission during exacerbations
- GOLD stage IV: 20 Patients, age 60±9 years, who will have very severe exacerbations and respiratory failure requiring mechanical ventilation and ICU admission

Specimen Collections and Preparation

Ten mL blood specimens were collected; sera were separated and kept at-80°C. All subjects were undergoing fiber optic bronchoscopy, under local anesthesia. Topical lignocaine was applied to the nasopharynx, vocal cords and major airways. Sputum specimens were aspirated as proximal large airway secretion from the trachea and main bronchi. Distal small airway specimens in the form of Broncho Alveolar Lavage (BAL) from a segment of the middle lobe or lingual, by using up to 240 mL warmed saline in 30 mL aliquots, were aspirated immediately using a low suction rate. Sputum was weighed and an equal volume of 0.1% dithiotreitol was added. The sample was put in ice for 15 min, diluted with an equal volume of Phosphate Buffered Saline (PBS) and then filtered through 48 mm nylon gauze. The supernatant was centrifuged at 3000 rpm for 10 min at 4°C. The cell pellet was discarded and aliquots of supernatant were stored at-80°C. BAL fluid was filtered and centrifuged at 1000 rpm for 10 min at 4°C. The supernatant was decanted and centrifuged at 3000 rpm for 10 min at 4°C. The aliquot was kept at-80°C for further analysis. The extraction of proteins from cells and nuclei was performed according to Cayman Chemicals Nuclear Extraction Kit (Catalog no. 10009277) and nuclear fraction was stored in micro centrifuge tube at-80°C.

Specimens taken were subjected to the following examinations:

- Airway inflammation markers: Tumor Necrosis Factor Alpha (TNF-α) as described by Bio source TNF-α EASIA Kit (Catalog no. KAP1751), interleukin-8 (IL-8) in sputum and BAL as Orgenium Laboratories Interleukin-8 ELISA Kits (Catalog no. 1L08001) and Histone Deacetylase Activity (HDAC) in BAL as determined by Bio vision Colorimetric Kit (Catalog no. K331-100)
- Antioxidative markers: Total Antioxidant Status (TAS) according to Cayman Chemical Kits (Catalog no.70900), reduced glutathione (GSH) levels as described by Bio vision Colorimetric Assay Kit (Catalog no. K264-100), Glutathione Peroxidase (GSH-Px) by Cayman Chemical Kits (Catalogno. 703102), vitamin E by method of Hashim and Schultringer (1996) and vitamin C according to the method of Kyaw (1978), which were estimated in BAL fluid and uric acid was estimated in serum as the method described by Vassault *et al*. (1986)
- Malondialdehyde (MDA) levels as described by Esterbauer *et al*. (1982)

- Nuclear translocation of the oxidant sensitive, proinflammatory transcription factors nuclear factor-kB (NF-kB) was evaluated according to the method of Bio source Immunoassay Kit (Catalog no. KH00371) in the nuclear extract from mixed leucocytes obtained from BAL fluid

Biochemical Parameters Assay

The determination of total protein was performed according to Bradford (1976). Cytokines (TNF-α and IL-8) were measured by ELISA technique; the antibodies and standards were purchased from Bio source and Orgenium laboratories, respectively. Total antioxidant status and glutathione peroxidase activity were assessed using Cayman Chemical Kits and GSH concentration was determined using Bio vision Glutathione Colorimetric Assay Kit, while uric acid was measured in sera according to the method of Vassault *et al*. (1986); vitamin E and vitamin C were measured according to the methods of (Hashim and Schultringer, 1996; Kyaw, 1978) respectively.

The lipid peroxidation was assessed by determination of MDA according to the method of Esterbauer *et al*. (1982), NF-κBp 65 was measured by ELISA technique using Bio source Immunoassay Kit and HDAC activity was assayed by a fast and convenient colorimetric method provided by Bio vision Colorimetric Kit.

The results obtained were statistically Analyzed using a One-way Analysis of Variance (ANOVA) for comparison between groups according to technique described by Daniel and Cross, (2012; Bailey, 1995) and the data were expressed as mean ± Standard Deviation (SD). The studied parameters for inflammation and oxidative stress were expressed as median (IQR) for continuous variables and Chi-square test for categorical analyses. A p value <0.05 was considered as statistically significant.

Results

Cytokines Inflammatory Markers

The BAL and sputum cytokine levels were shown in "Table 1"; sputum cytokines values for both TNF-α and IL-8 were higher than the value of BAL samples with ratios of 1.5 and 1.2, respectively "Fig. 1". Patients "cutoff" value for sputum TNF-α and IL-8 were 139.9 and 10.8 pg mL^{-1} protein and their sensitivity percentages were 93.3 and 91.7%, respectively. Cytokines levels were significantly higher in BAL and sputum for all COPD patients, TNF-α ($p \leq 0.001$ and $p \leq 0.05$, respectively) and IL-8 ($p \leq 0.001$) for both parameters as compared to those of controls. Moreover there was a significant positive correlation between TNF-α and IL-8 levels where r = 0.950, 0.971 for BAL and r = 0.967, 0.969 for sputum, respectively.

Oxidative Stress Markers

The mean levels of TAS, reduced glutathione, glutathione peroxidase, vitamin E, vitamin C in BAL and serum uric acid were significantly decreased ($p \leq 0.001$), while the mean levels of BAL-MDA were significantly increased in COPD cases when compared to controls and are statistically highly significant ($p \leq 0.001$). "Table 2" showed a comparative analysis of oxidative stress markers level between controls and COPD patients that indicate the increase in oxidative stress and decrease in antioxidant levels in COPD cases when compared to controls.

"Table 1" Data demonstrate a significant difference between the value of studied markers of tumor necrosis factor and interleukin-8 in the large airway (sputum samples) for both control group and different patient groups. BAL results for TNF-α show non-significant relation between healthy control group and COPD patient GOLD stage II; however, values for GOLD stages III and IV are highly statistically significant with control group and other patient groups. IL-8 results for BAL also show highly significant difference between healthy control group and COPD patients of all different stages.

GSH has high concentration in epithelial lining fluid (10-100 times more than in the plasma) and is representing one of the primary lung defenses against cigarette smoke. A strong positive and highly significant correlation ($p \leq 0.001$) between GSH and antioxidant was studied (r = 0.670, r = 0.948, r = 0.964, r = 0.942 and r = 0.959) for BAL TAS, GPx, serum uric acid, BAL vitamin E and vitamin C, respectively as shown in "Fig. 2A". While a highly significant negative correlation was observed with MDA (r = -0.934) the existing imbalance between oxidant/antioxidant was shown by the negative correlation between MDA and different antioxidant markers studied (r = -0.612, -0.856, -0.925, -0.961 and -0.961) for TAS, GPx, uric acid, vitamin E and vitamin C, respectively as shown in "Fig. 2B". All inflammatory markers show statistical significant difference as disease progresses from GOLD stage II to GOLD stage IV. However, there was not any significant difference in clinicopathological factor for gender and smoking status.

Transcription Factors NF-κB and HDAC

"Table 3" illustrates the values of NF-κB and HDCA in nuclear extract of BAL cell. The mean concentration of NF-κB was significantly increased in COPD patients for all GOLD stages (stage II, P = 0.001; stage III and IV, p<0.000) when compared to controls. The percentage of patients with NF-κB concentration more than the cutoff value (0.29 pg mL^{-1} protein) was 90.0% "Fig. 3". The

level of HDAC was significantly decreased in GOLD stages III and IV ($p<0.000$); there was no significant relation observed in GOLD stage II ($P = 0.052$) between COPD patients group and controls subjects. The percentage of patients with HDAC concentration more than the cutoff value (14.3 μM mg^{-1} protein) was 90.0% "Fig. 4". However, a highly significant correlation was found in relation to disease severity ($p<0.001$) for GOLD stage II versus III, stage II versus IV and stage III versus IV for both parameters. As NF-κB expression is regulated by HDCA concentration, a strong negative correlation ($r = -0.929$; $p<0.001$) was found between HDAC and NF-κB "Fig. 5".

Data illustrated in "Table 3" showed a significant difference in the level of studied parameters (NF-κappa and HDAC) between patients in different groups and controls, except for the level of HDAC which was non-significantbetween GOLD group II and controls.

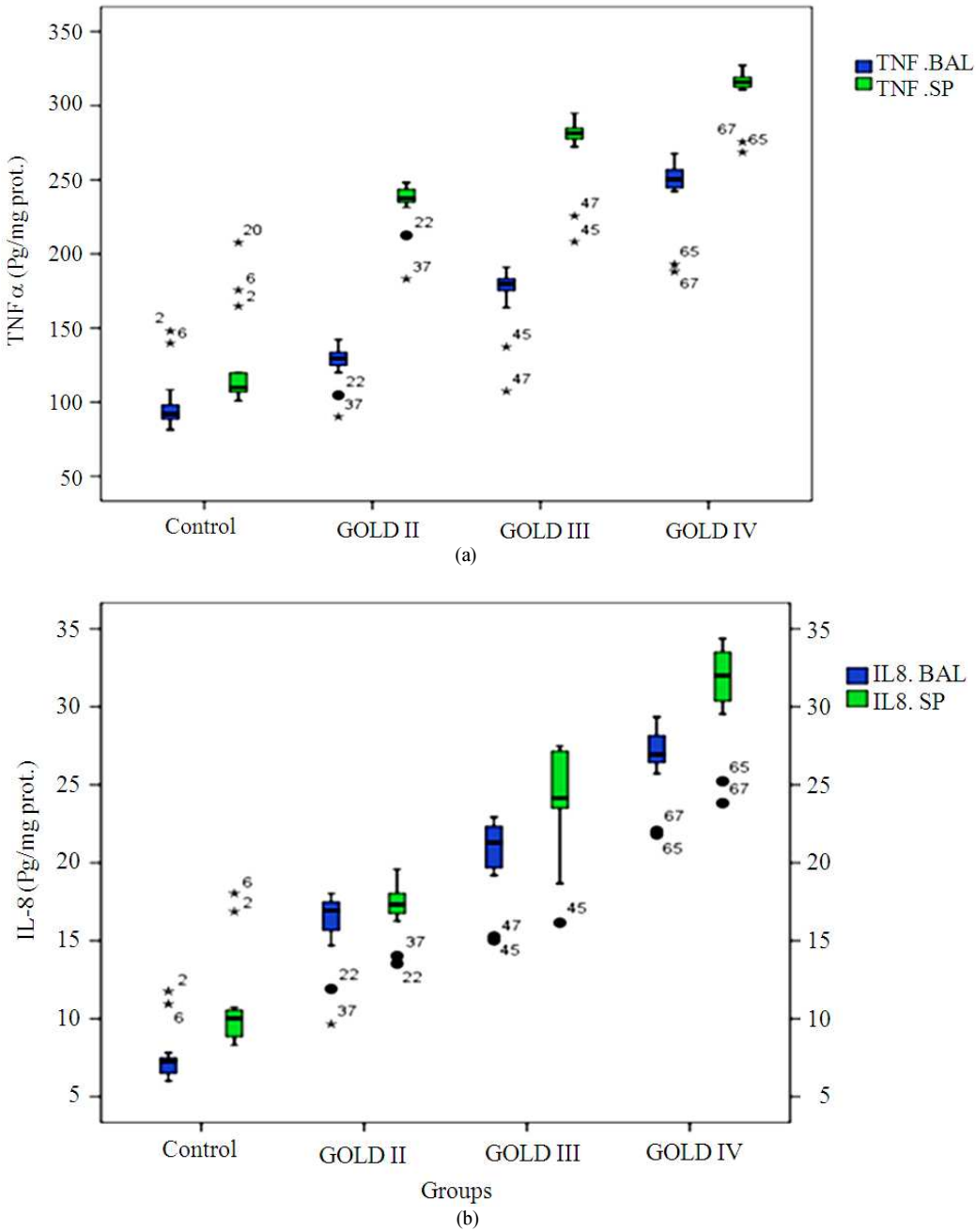

Fig. 1. Statistical significance of tumor necrosis factor-α (TNF-α) and interleukein-8 (IL-8) of COPD patients groups and healthy controls compared to samples of BAL and sputum

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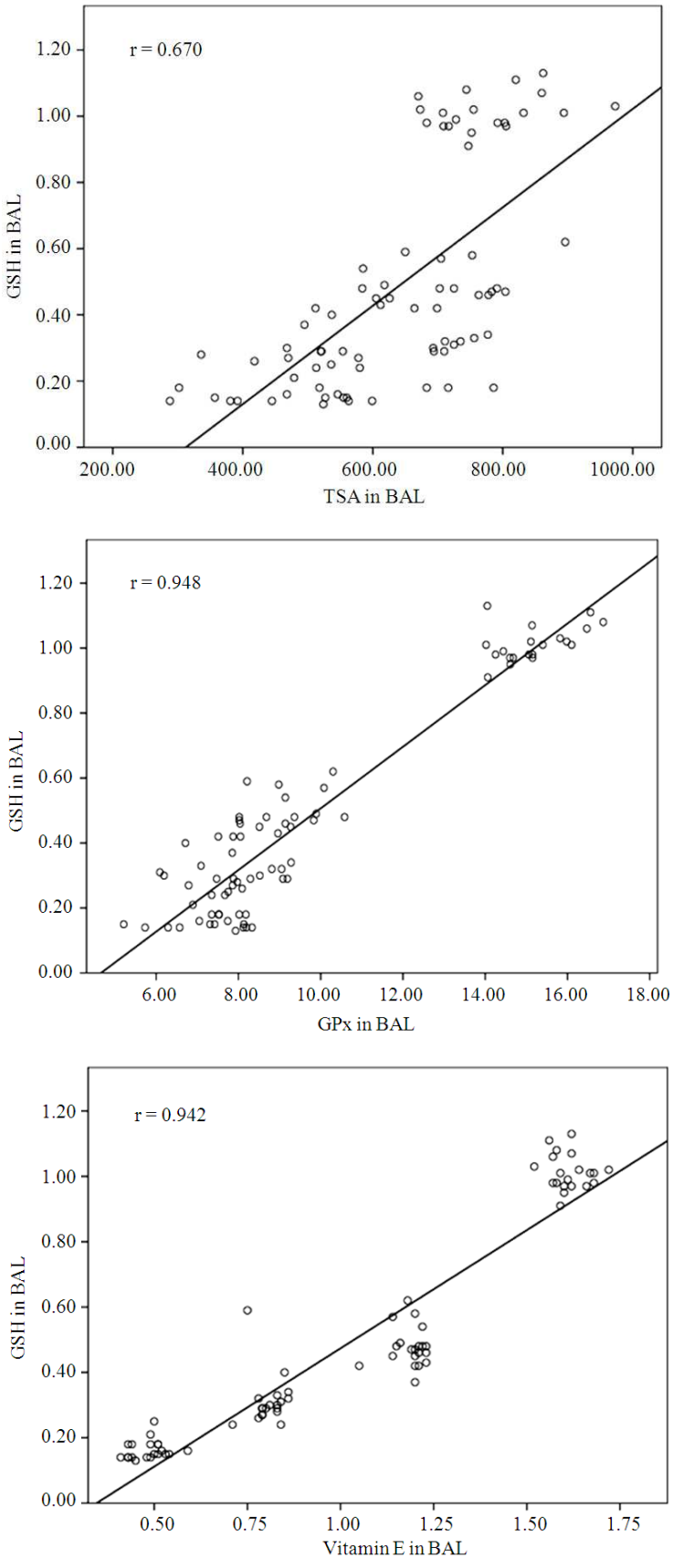

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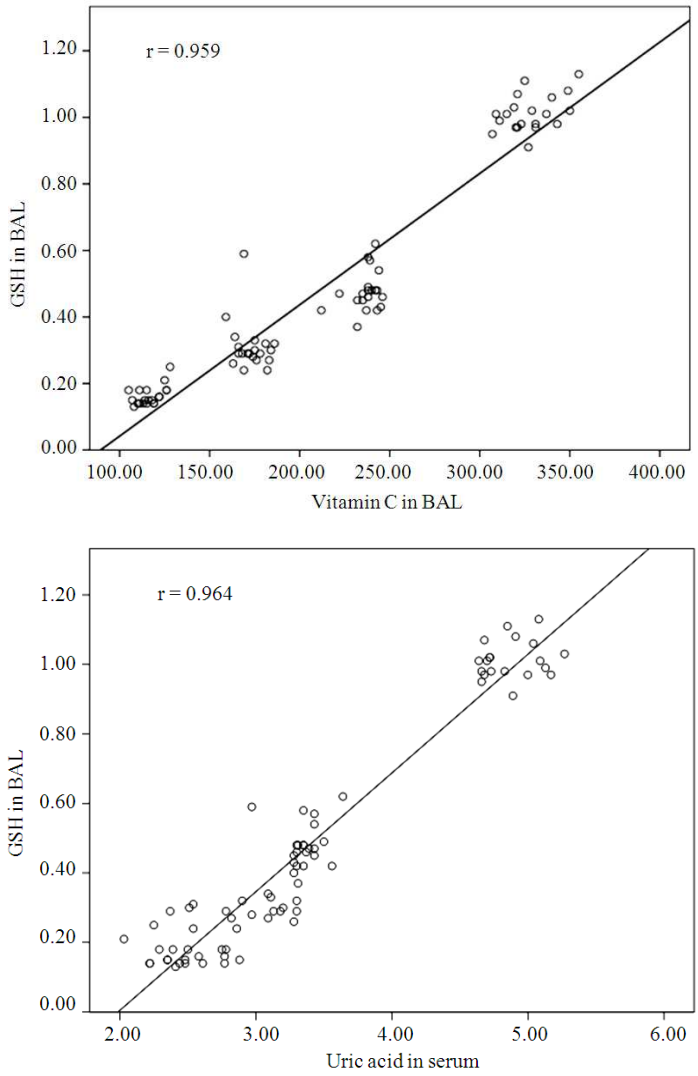
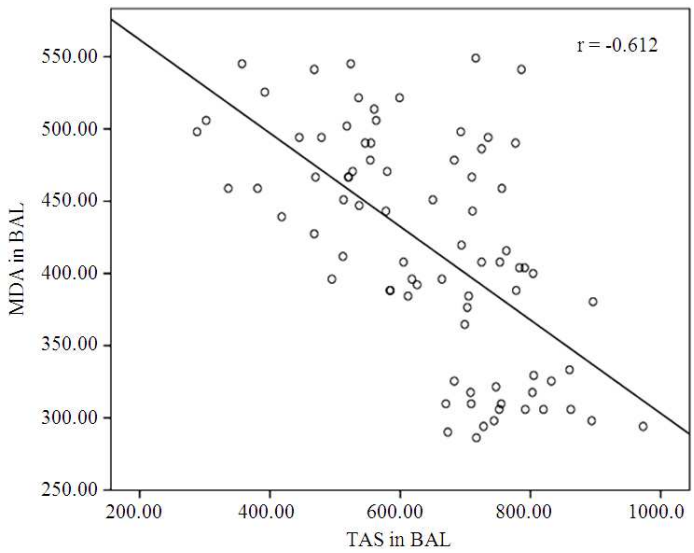
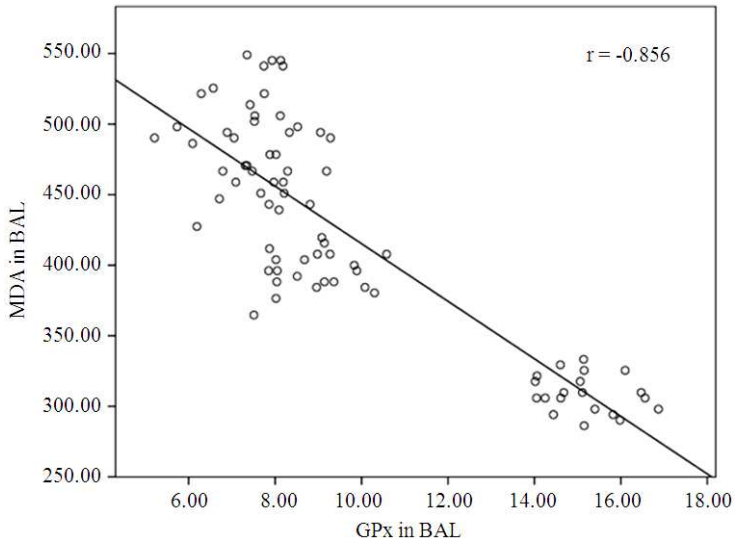
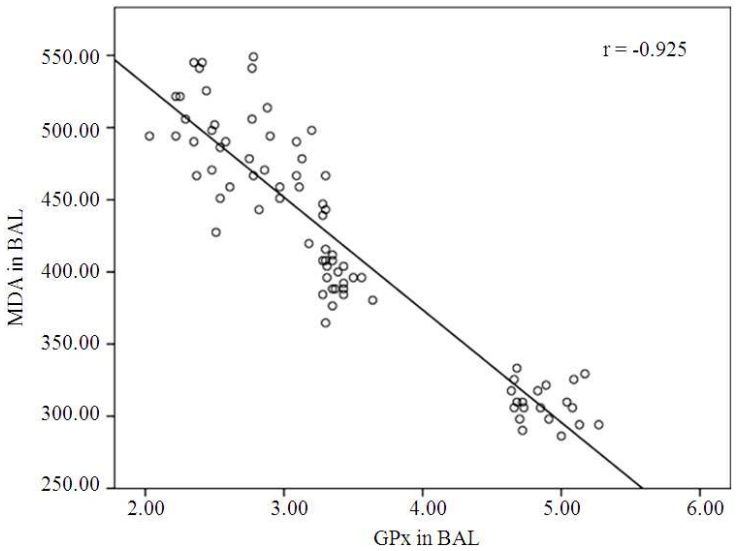


Fig. 2A. Correlations of GSH with antioxidant markers studied




r = -0.856
MDA in BAL
550.00
500.00
450.00
400.00
350.00
300.00
250.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
GPx in BAL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MDA in BAL
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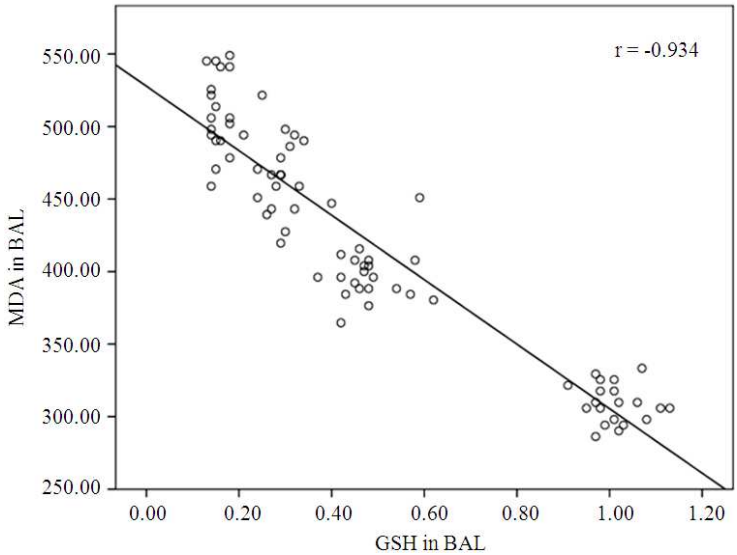
500.00
450.00
400.00
350.00
300.00
250.00
2.00
3.00
4.00
5.00
6.00
GPx in BAL


r = -0.934
MDA in BAL
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500.00
450.00
400.00
350.00
300.00
250.00
0.00
0.20
0.40
0.60
0.80
1.00
1.20
GSH in BAL

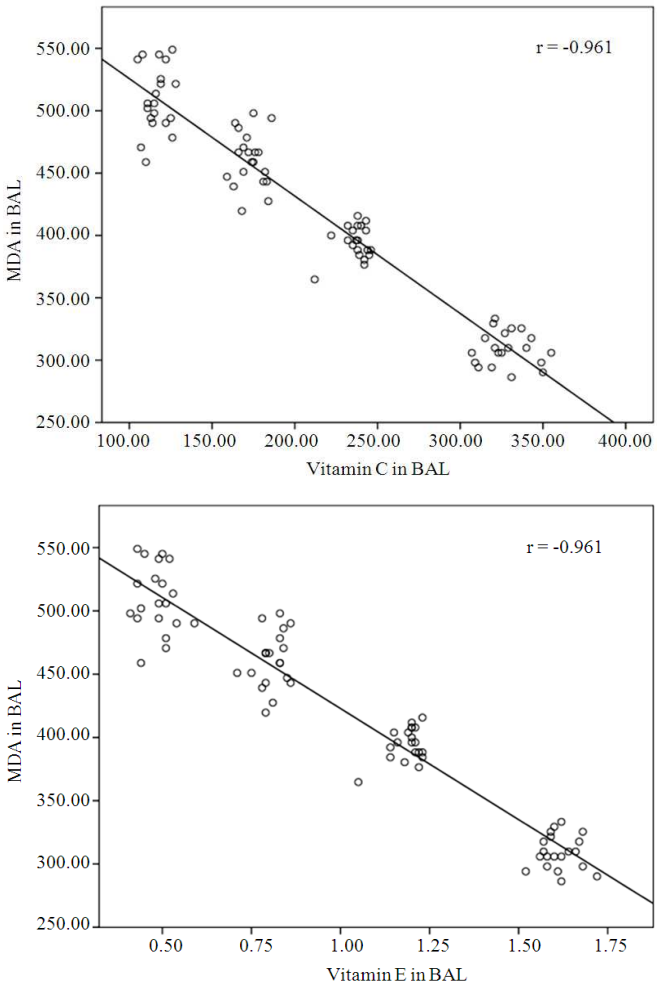
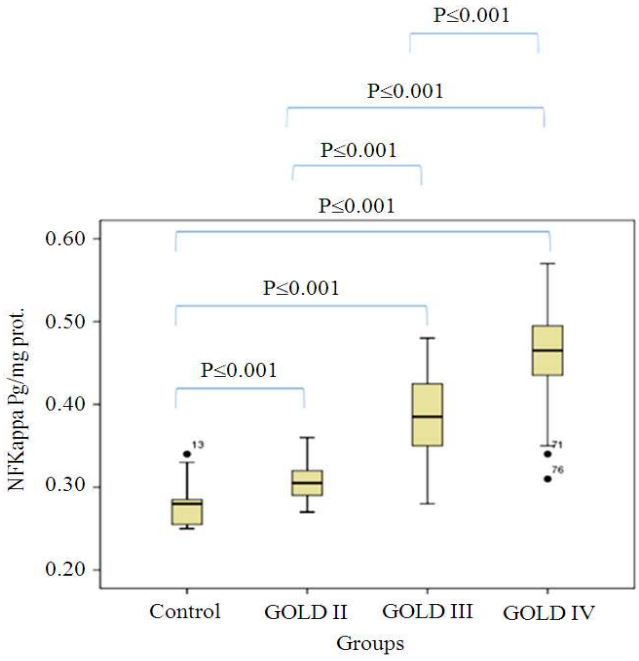


Fig. 2B. Correlations of MDA with antioxidant markers studied



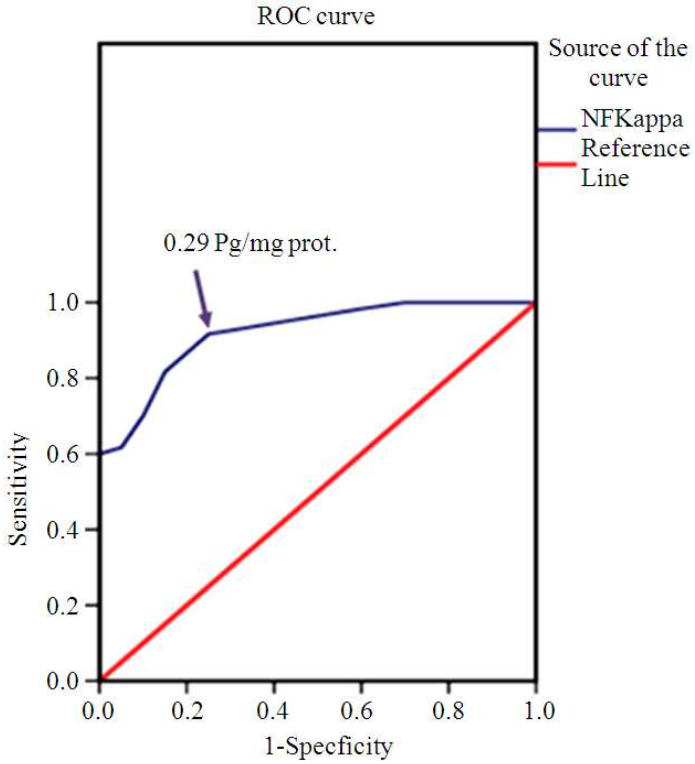
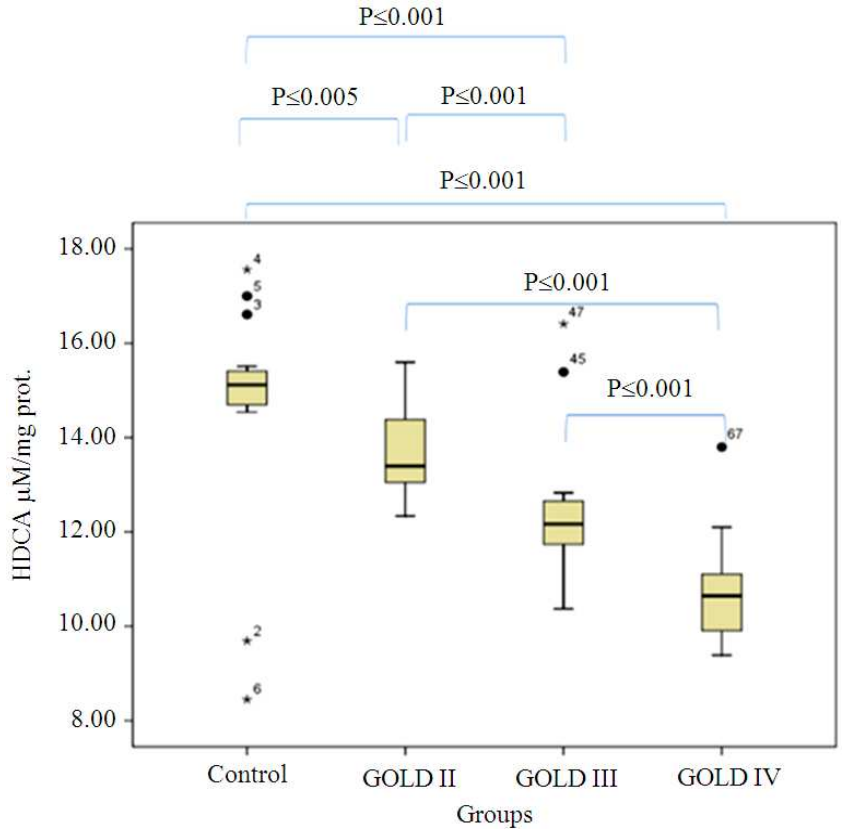


Fig. 3. Graphical presentation of NF-κappa from nuclear extract of BAL cell for COPD patient groups compared to control by whisker box plot and ROC curve



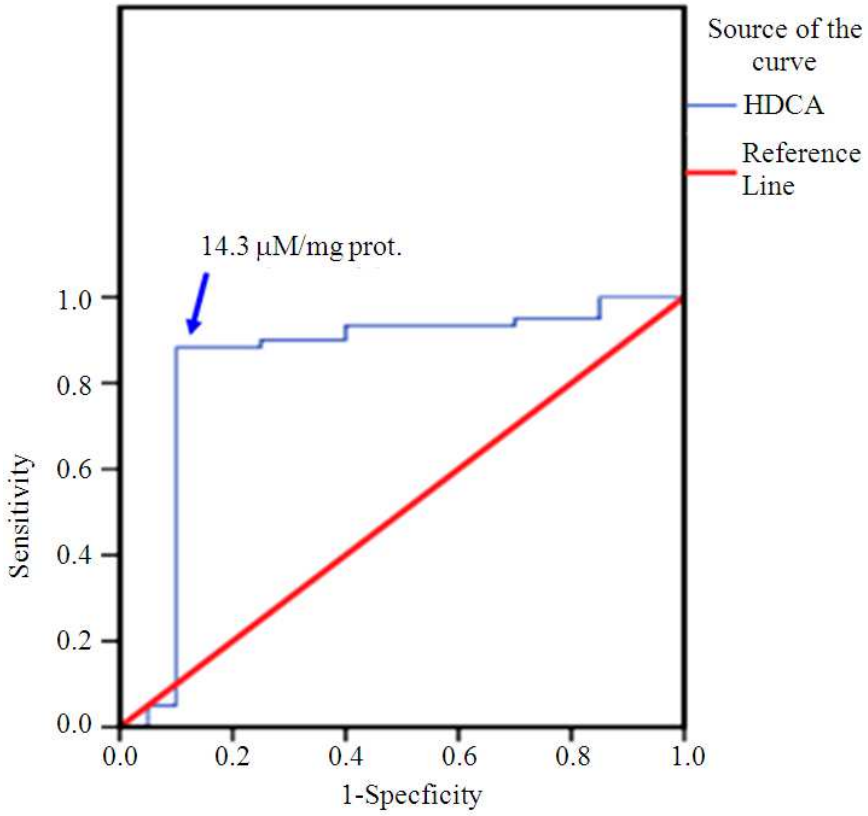


Fig. 4. Graphical presentation of HDAC from nuclear extract of BAL cell for COPD patient groups compared to control by whisker box plot and ROC curve

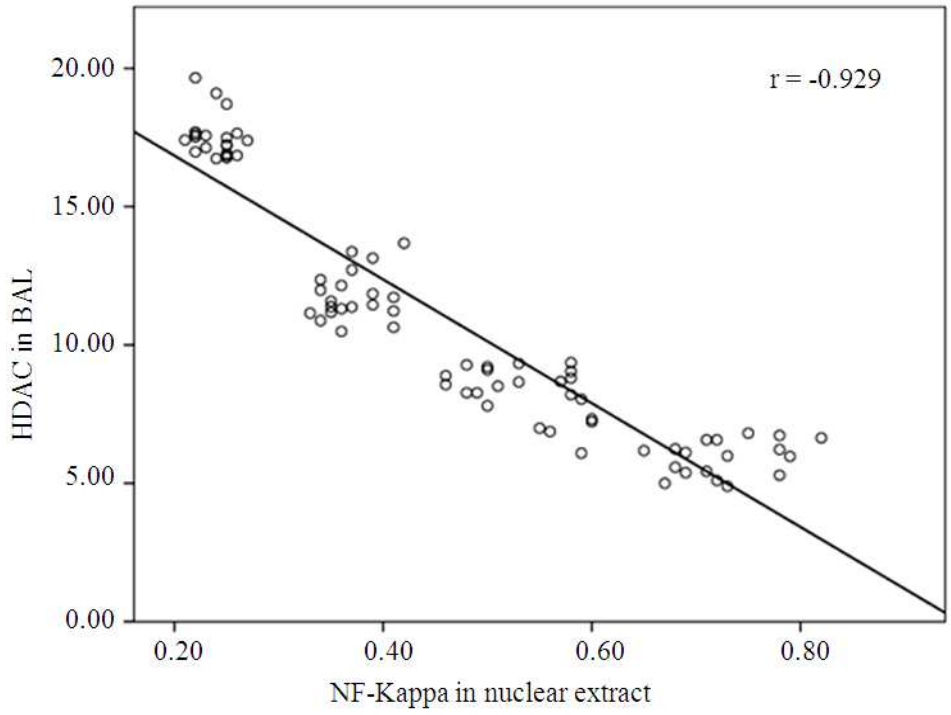


Fig. 5. Correlations of HDCA and NF-κB in nuclear extract of BAL cells

Table 1. Statistical significance of tumor necrosis factor-α (TNF-α) and interleukein-8 (IL-8) in BAL and sputum of COPD patient groups compared to healthy controls

Groups	TNF-α (Pg/mg prot.)		IL-8 (Pg/mg prot.)	
	BAL	Sputum	BAL	Sputum
Healthy control				
Range	(88.6-158.2)	(120.9-227.7)	(8.0-13.3)	(8.3-16.4)
Mean ± SD	107.910±17.206	141.305±28.491	9.354±1.453	10.033±1.883
COPD GOLD Stages:				
Stage II: Range	(80.2-132.2)	(83.1-214.5)	(6.7-15.0)	(8.5-16.1)
Mean ± SD	116.942±11.898	161.410±28.584	12.192±2.291	11.909±1.548
P versus control	0.061	0.032*	0.000***	0.001***
Stage III: Range	(82.4-187.9)	(140.0-274.4)	(8.0-17.6)	(7.2-18.4)
Mean ± SD	149.168±26.979	212.972±39.153	13.834±2.416	14.135±2.867
P versus control	0.000***	0.000***	0.000***	0.000***
P versus stage II	0.000***	0.000***	0.033*	0.004**
Stage IV: Range	(138.1-255.8)	(152.0-285.2)	(11.0-19.4)	(14.2-26.4)
Mean ± SD	199.405±27.597	241.702±35.250	16.723±2.283	22.054±3.368
P versus control	0.000***	0.000***	0.000***	0.000***
P versus stage II	0.000***	0.000***	0.000***	0.000***
P versus stage III	0.000***	0.020*	0.000***	0.000***
Total: Range	80.2-255.8	83.1-285.2	6.7-19.4	8.5-26.4
Mean ± SD	155.17±41.184	205.361±47.740	14.251±2.969	16.183±5.313
P versus control	0.000***	0.000***	0.000***	0.000***

Table 2. A significant difference in the level of antioxidant markers (TSA, GSH-Px, GSH, uric acid, vitamin E and vitamin C) and oxidative Marker (MDA) of COPD patients compared to controls. Statistical significance of oxidative stress of COPD patient groups compared to control

	Healthy control		COPD		
Groups	Range	Mean ± SD	Range	Mean ± SD	P value
TAS (mM/mg Prot.)	590-973	767.45±93.041	288-778	533.90±104.305	0
GPx (nM/mg Prot.)	8.9-15.9	13.653±1.678	7.2-15.4	9.995±1.699	0
GSH (µg/mg Prot.)	0.37-0.78	0.669±0.105	0.33-0.83	0.513±0.122	0
Uric acid (mg/dl)	2.70-4.77	4.284±0.523	2.93-4.46	3.593±0.305	0
Vitamin E (µg/mL)	1.14-1.68	1.566±0.135	0.41-1.37	0.851 ±0.295	0
Vitamin C (µg/mL)	192-305	269.75±25.600	182-280	217.87±24.588	0
MDA (Mg/dl.)	336-481	366.274±29.927	350-454	396.831±25.184	0

Table 3. Statistical significance of nuclear factor kappa (NF-κappa) and Histone Deacetylase Activity (HDAC) in nuclear extract of BAL cell for COPD patient groups compared to control

Groups	NF-κappa (Pg/mg prot.)	HDAC (µM/mg prot.)
Healthy control		
Range	(0.25-0.34)	(8.5-17.6)
Mean ± SD	0.279±0.026	14.750±2.108
GOLD stage II		
Range	(0.27-0.36)	(12.3-15.6)
Mean ± SD	0.307±0.024	13.704±0.991
P versus control	0.001***	0.052
GOLD stage III		
Range	(0.28-0.48)	(10.4-16.4)
Mean ± SD	0.388±0.056	12.366±1.393
P versus control	0.000***	0.000***
GOLD stage IV		
Range	(0.31-0.57)	(9.4-13.8)
Mean ± SD	0.458±0.066	10.726±1.024
P versus control	0.000***	0.000***

*** Highly significant at p value ≤0.001

Discussion

The most important nonmalignant lung disease, caused by cigarette smokers, is Chronic Obstructive lung Disease (COPD), a globally escalating problem nowadays. COPD is characterized by irreversible airflow limitations and is associated with an abnormal inflammatory response of the lung to noxious particles and gases (Emin *et al*., 2010).

Lung is representing a unique tissue for oxidant stress among most organs because it is directly exposed to higher oxygen tensions. In the resting state, the balance between antioxidants and oxidants is sufficient to prevent the disruption of normal physiologic functions; however, either increases in oxidants or decreases in antioxidants can disrupt this balance. The state of imbalance is collectively referred to as oxidative stress and is associated with diverse airway pathologies (Büyükbaş *et al*., 2008).

Inflammatory and oxidative stress markers assessed in current study are found to be significantly indicative of COPD for GOLD stages III and VI; however, in some parameters there was no significant difference between controls and stable COPD (GOLD stage II, mild stage of disease).

The main concentration of proinflammatory cytokine TNF-α and chemokine IL-8 in BAL fluid and sputum were significantly higher in patients with COPD in comparison to controls. The levels of IL-8 is positivelycorrelated to the concentration of TNF-α in both BAL fluid and sputum. Moreover, both markers levels were increased in sputum when compared to BAL samples. The results obtained were in agreement with that of Kristan *et al*. (2012), who reported significant elevated levels of IL-8 and TNF-α in patients with COPD in comparison to normal smokers and nonsmokers and that reported by Hong *et al*. (2010) showed significantly higher release of IL-8 induced by TNF-α in COPD patients as compared to smokers and normal controls, as TNF-α is an important inflammatory mediator produced by several kinds of cells, playing a role in activating the neutrophils and stimulating the release of IL-8. Its over-expression can lead to the chronicity of inflammatory response and the aggravation of lung injury (Xie *et al*., 2010). The concentration of IL-8 in sputum was closely associated with the degree of airflow obstruction and is suggested as a biomarker to evaluate the severity of airway inflammation. The secretion of IL-8 was suggested to be regulated transcriptionally by several transcription factors, among which NF-κB is predominant (Hong *et al*., 2010).

Increased epithelial permeability produced by cigarette smoke is likely to be mediated through depletion in total antioxidants capacity. The oxidant/antioxidant balance is essential for normal lung function (Raut, 2012).

In the present study a significant decreased levels of antioxidant markers TAS, GPx, GSH, vitamin E and vitamin C in BAL and uric acid in sera were observed in patients' groups in comparison to controls. This is in accordance with Raut (2012) who documented lower mean plasma levels of total antioxidant capacity in patients with chronic bronchitis than controls and (Vibhuti *et al*., 2007) who reported decreased antioxidants level (GSH $p = 0.005$ and GSH-Px $p = 0.035$, respectively) in patients with COPD in comparison to controls. Their explanation was that there is a complex endogenous defense system designed to protect tissues from ROS induced cell injury. Special enzymes such as glutathione peroxidase, reduced glutathione and vitamins form a network of functionally overlapping defense mechanisms. In critically ill patients, there are reduced stores of antioxidant, free electron scavengers and cofactors in plasma or intracellular concentrations and decreased activities of enzymatic systems involved in the detoxifications of ROS. Also, the antioxidant effect can be either enzymatic (glutathione and Glutathione Peroxidase (GPx)) or nonenzymatic (vitamin C, vitamin E and uric acid) (Büyükbaş *et al*., 2008). Glutathione (GSH) is a major low-molecular weight antioxidant thiol; its concentration in the Epithelial Lining Fluid (ELF) is 10 to 100 times more than in the plasma. GSH levels can influence both inflammation and oxidative stress, which are the two major contributing factors to COPD. The lung's ability to produce a GSH adaptive response to environmental oxidants may be a key factor in minimizing inflammation and oxidative damage to the lung (Gould *et al*., 2010).

The present study supports that TAS and GSH evaluation were the best parameters studied to prove the antioxidant burden on the patient's lung. The present data also explored the highly significant difference between antioxidant and different disease stages.

Malondialdehyde (MDA) has been widely studied as a product of polyunsaturated fatty acid peroxidation. High MDA levels have been observed in several biological fluids from patients with COPD (Bartoli *et al*., 2011). In this study, when compared to control, COPD patients had significantly increased levels of MDA for GOLD stage III ($P = 0.001$) and stage IV ($p<0.000$); no significant relation was observed in GOLD stage II ($P = 0.074$). The cutoff value showed nonsignificant relation between MDA and clinicopathological factors in COPD regarding gender ($P = 0.768$) and smoking status ($P = 0.929$), while there is significant relation with degree of disease ($P = 0.004$). This is in parallel with the study of Bartoli *et al*. (2011), who stated that within the COPD group, subjects with severe COPD

showed higher levels of MDA than patients with moderate COPD.

NF-κB controls a wide spectrum of biological effects ranging from immune and stress-induced responses to cell fate decisions such as proliferation, differentiation, tumorigenesis, apoptosis and tissue remodeling. Thus NF-κB is viewed as a master regulator of inflammatory responses because it plays an essential role in the evolution as well as the resolution phase of inflammation. Inappropriate activation of NF-κB is associated with many inflammatory disease states. In the context of lung inflammatory diseases, NF-κBis implicated in the pathogenesis of chronic obstructive pulmonary disease. Studies indicate that proinflammatory transcriptional programs that are activated by NF-κB to shape the inflammatory response vary depending on the stimulus and the cell type involved. Understanding how information from various inputs is relayed to NF-κB in pulmonary vascular cells is of fundamental importance in controlling specific proinflammatory transcriptional programs associated with inflammatory disease in the lung (Rahman and Fazal, 2011).

Posttranslational modifications of proteins, such as acetylation, are important regulatory events in eukaryotic cells. Reversible acetylations of histones and nonhistone proteins regulate gene expression and protein activity. Acetylation levels of proteins are regulated by a dynamic equilibrium between acetylation by histone acetyltransferases and deacetylation by histone deacetylases. Focusing on the crucial regulatory roles of protein acetylation in NF-κB-mediated inflammation and the potential applications of small-molecule inhibitors of acetylation can help in treatment of inflammatory diseases (Massimo *et al*., 2011). HDAC2 activities in lung from COPD patients are reduced: This reduction is correlated with the increase in disease severity, in gene expression of IL-8 and in acetylation of histone associated with the NF-κB binding site on the IL-8 promoter. In patients with very severe COPD (GOLD stage 4) the expression of HDAC2 was less than 5% of that seen in normal lung (Jun-Ping *et al*., 2013).

NF-kB is oxidant sensitive transcription factor that has been implicated in the regulation of numerous proinflammatory mediators pertinent to lung inflammation. The present study assessed the nuclear translocation of NF-kB and HDAC in nuclear extract of BAL cell. There was a significant increase in the levels of NF-κB observed in the COPD patients ($p<0.000$) while a high significant decrease in the levels of HDAC ($p<0.000$) compared to controls. NF-κB and HDAC showed nonsignificant relation for the clinicopathological factors in COPD regarding gender and smoking status, while there is significant relation with degree of disease ($p\leq0.01$). These findings prove that NF-κB and HDAC are good indicatives for disease progression.

Conclusion

COPD is not considered effectively treatable and many people will have to deal with it for the rest of their lives. Cytokines are increased in the COPD as a consequence of systemic inflammation that is worth the disease severity. The study speculates that changing lifestyle, smoking cessation and taking healthy levels of diet rich in fruits and vegetables will help the body fight inflammation. Also, suppression of the inflammatory response is a logical approach to the treatment of COPD by interfering with inflammatory mediators that regulate migration and activation of inflammatory cells, inhibitors of proinflammatory pathways, or activators of anti-inflammatory pathways like TNF-α, chemokine and NF-κB inhibitors.

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Ethics

This article is original and contains unpublished materials. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

Kyaw, A. 1978. A simple colorimetric method for ascorbic acid determination in blood plasma. Clin. Chim. Acta, 86: 153-157.
DOI: 10.1016/0009-8981(78)90128-6

Bartoli, M.L., F. Novelli, F. Costa, L. Malagrinò and P.L. Paggiaro *et al*., 2011. Malondialdehyde in exhaled breath condensate as a marker of oxidative stress in different pulmonary diseases. Mediators Inflamm., 2011: 7-7. DOI: 10.1155/2011/891752

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254. DOI: 10.1016/0003-2697(76)90527-3

Büyükbaş, S., K. Uzun, E. Demirkapı and K. Başaralı, 2008. Oxidative stress and antioxidant status in bronchoalveolar lavage fluid, plasma and erythrocyte of critically mixed ill with respiratory failure. Eur. J. General Med., 5: 140-146.

Cristóvãoa, C., L. Cristóvão, F. Nogueira and M. Bicho, 2013. Evaluation of the oxidant and antioxidant balance in the pathogenesis of chronic obstructive pulmonary disease. Rev. Port. Pneumol., 19: 70-75. DOI: 10.1016/j.rppneu.2012.09.002

Emin, S., K. Yordanova, D. Dimov, V. Ilieva and T. Vlaykova *et al*., 2010. Total antioxidant capacity of plasma determined as ferrous reducing ability of plasma in patients with COPD. Trakia J. Sci., 8: 205-213.

Esterbauer, H., K.H. Cheeseman, M.U. Dianzani, G. Poli and T.F. Slater, 1982. Separation and characterization of the aldehydic products of lipid peroxidation stimulated by ADP-Fe2+ in rat liver microsomes. Biochem. J., 208: 129-140.

Gould, S.N., E. Min, S. Gauthier, H.W. Chu and B.J. Day *et al*., 2010. Aging adversely affects the cigarette smoke-induced glutathione adaptive response in the lung. Am. J. Respir. Crit. Care Med., 182: 1114-1122. DOI: 10.1164/rccm.201003-0442OC

Ghizzoni, M., H.J. Haisma, H. Maarsingh and F.J. Dekker, 2011. Histone acetyltransferases are crucial regulators in NF-κB mediated inflammation. Drug Discovery Today, 16: 504-511. DOI: 10.1016/j.drudis.2011.03.009

Hashim, S.A. and Schultringer, 1996. Tocopherols determination (macro-and microquantities) in plasma. Am. J. Chen. Nutr., 19: 137-137.

Hong, C., D. Wang and X. Wang, 2010. Role of airway epithelium-origin chemokines and their receptors in COPD. J. Epithelial Biol. Pharmacol., 3: 26-33.

Jun-Ping, W.U., W.U. Qi, S.U.N. Xin and S.U.N. Hong-fen, 2013. Perspective corticosteroid resistance in chronic obstructive pulmonary disease: New uses of theophylline. Chin. Med. J., 126: 965-965.

Kristan, S.S., M.M. Marc, I. Kern, M. Flezar and P. Korosec *et al*., 2012. Airway angiogenesis in stable and exacerbated chronic obstructive pulmonary disease. Scandinavian J. Immunol., 75: 109-114. DOI: 10.1111/j.1365-3083.2011.02623.x

Massimo, G., H.J. Haisma, H. Maarsingh and F.J. Dekker, 2011. Histone acetyltransferases are crucial regulators in NF-κB mediated inflammation: Drug Discov. Today, 16: 504-511. DOI: 10.1016/j.drudis.2011.03.009

Moermans, C., V. Heinen, M. Nguyen, M. Henket and R. Louis *et al*., 2011. Local and systemic cellular inflammation and cytokine release in chronic obstructive pulmonary disease. Cytokine, 56: 298-304. DOI: 10.1016/j.cyto.2011.07.010

Rahman, A. and F. Fazal, 2011. Blocking NF-κB: An inflammatory issue. Proc. Am. Thorac. Society, 8: 497-503. DOI: 10.1513/pats.201101-009MW

Rajendrasozhan, S., S.R. Yang, I. Edirisinghe, H. Yao and I. Rahman *et al*., 2008. Deacetylases and NF-κB in Redox Regulation of Cigarette Smoke-Induced Lung Inflammation: Epigenetics in Pathogenesis of COPD. Antioxid Redox Signal, 10: 799-811. DOI: 10.1089/ars.2007.1938

Raut, M.A., 2012. Study of glutathione metabolism oxidative stress and the effect of vitamin E supplementation in COPD. Int. J. Biomed. Res., 3: 322-322. DOI: 10.7439/ijbr.v3i7.178

Vassault, A., D. Grafmeyer, J. de Graeve, R. Cohen and J. Bienvenu *et al*., 1986. Protocole de validation de techniques. Ann. Bio. Clin., 44: 686-686.

Vibhuti, A., E. Arif, D. Deepak, B. Singh and M.A. Qadar Pasha *et al*., 2007. Correlation of oxidative status with BMI and lung function in COPD. Clin. Biochem., 40: 958-963. DOI: 10.1016/j.clinbiochem.2007.04.020

Xie, J., X.Y. Yang, J.D. Shi, X.Q. Deng and W. Long, 2010. A new inflammation marker of chronic obstructive pulmonary disease-adiponectin. World J. Emerg. Med., 1: 190-195. PMID: 25214966.

Bailey, N.T., 1995. Statistical Methods in Biology. 1st Edn, Cambridge University Press, Cambridge, New York, ISBN-10: 052146983X, pp: 255.

Daniel, W.W. and C.L. Cross, 2012. Biostatistics: A Foundation for Analysis in the Health Sciences. 1st Edn., Wiley Global Education, Hoboken, ISBN-10: 111847578X, pp: 960.