SCIENCEFORECAST
A digital science library

Research Article
Published: 09 Sep, 2020

SF Journal of Pulmonology and Respiration Care

Lung Aging Related to Oxidative Stress and Mitochondrial Biogenesis in COPD Patients

Amani S[1], Ines S[1], Bernard G[3] and Sonia R[1,2]*

[1]Laboratory of Physiology, University of Sousse, Sousse, Tunisia

[2]Heart Failure (LR12SP09) Research Laboratory, Farhat HACHED Hospital, Sousse, Tunisia

[3]EA 3072 Physiology Institute, Strasbourg University, France

Abstract

Aims: To identify the physiological and pathological (COPD) non-respiratory lung aging by determining gene expression changes in the airways of genes encoding antioxidants and of mitochondrial biogenesis' markers.

Methods: Three groups were recruited: group I (7 healthy-young-controls, mean age=36.8 years), group II (10 healthy-elderly, mean age=68.7 years) and group III (10 elderly-COPD, mean age=68.7 years). Spirometric measurements and sputum analysis were performed. RNA, isolated from induced sputum, was obtained for gene expression analysis.

Results: Among the studied candidate selected genes: Peroxiredoxin-1 (PRDX1) transcripts levels were significantly decreased in elderly COPD patients, as compared to young healthy subjects and this decline was correlated with advancing age. Diminished Glutathione-Peroxidase-1 (GPX1) in elderly COPD patients correlated significantly with lung functions and cigarette smoke. The decrease of SOD1 expression was significantly correlated with advancing age. Furthermore, mRNA levels of Peroxisome-Proliferator-Activated-Receptor-Gamma-Coactivator-1-beta (PGC1β) and Sirtuin-1 (Sirt1) were significantly increased in elderly COPD patients, compared to young healthy subjects. PGC1β correlated negatively with pulmonary parameters and positively with cigarette smoke.

Conclusions: The up-regulation of genes encoding proteins involving mitochondrial biogenesis might protect airways from an oxidative stress marked by the deterioration of the antioxidant system. These changes were related to cigarette smoke, correlated to lung function and might explain the accelerated lung aging in COPD patients.

Keywords: Aging; COPD; Mitochondrial biogenesis; Oxidative stress; Cigarette smoke

Abbreviations

ANOVA: Analysis of Variance; BMI: Body-Mass-Index; BAX: BCL2-Associated-X-protein; CAT: Calatase; COPD: Chronic-Obstructive-Pulmonary-Disease; COX1: Mitochondrially-encoded-cytochrome-c-oxidase-I; DTT: Dithiothreitol; FEV1: First-second-Forced-Expiratory-Volume; FVC: Forced-Vital-Capacity; LLN: Lower-Limit-of-Normal; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase; GPX1: Glutathione-Peroxidase-1; GSH: Glutathione; LYZ: Lysozyme; PGC1β: Peroxisome-proliferator-activated-receptor-Gamma Coactivator-1-beta; PRDX1: Peroxiredoxin-1; qPCR: Quantitative-real-time PCR; PY: Pack-Years; PostBD: Post Bronchodilator test; R: Correlation-coefficient; r^2: Determination-coefficient; RNA: Ribonucleic-Acid; RPLP0: Ribosomal-Protein-Large P0; RT: Reverse-Transcriptase; SIRT1: Sirtuin-1; SOD1: Superoxide-Disumutase-1; TNF: Tumor-Necrosis-Factor

Introduction

The aging of the population throughout the developed world has been associated with increases in morbidity and mortality attributable to lung diseases [1] such as Chronic-Obstructive-Pulmonary-Disease (COPD), which is considered as a major public health problem [2,3]. COPD shows remarkable age-associated features, such as, increased oxidative stress and altered mitochondria function. With increasing age, the lung undergoes progressive involution with altered pulmonary functions [4] (respiratory or non-respiratory functions: oxidative stress and mitochondrial biogenesis). It is proved that there are a lot of similarities between the lungs of aged people and COPD patients [5].

OPEN ACCESS

*Correspondence:
Amani Sayhi, Faculty of Medicine of Sousse, Laboratory of Physiology, University of Sousse, Sousse, Tunisia.
Tel: +216 21517194
Fax: +21673224899
E-mail: amanisayhi2007@gmail.com

Received Date: 18 Jul 2020
Accepted Date: 04 Sep 2020
Published Date: 09 Sep 2020

Citation: Amani S, Ines S, Bernard G, Sonia R. Lung Aging Related to Oxidative Stress and Mitochondrial Biogenesis in COPD Patients. SF J Pulmonol Resp Care. 2020; 1(2): 1006.

The lung with a large surface area is continuously exposed to external oxidizing environment and is therefore highly vulnerable to exogenous source of Reactive-Oxygen-Species (ROS) [6]: ROS inhaled by cigarette smoke, formed during normal oxygen metabolism [7] and released from leukocyte involved in COPD inflammation [8]. To effectively cope with these various sources of oxidants, various layers of antioxidant defense present in all lung cell types provide adequate protection against their injurious effects and allow for appropriate ROS-mediated biological signaling [8]. Many lung diseases are often associated with altered expression of various antioxidant systems such as Peroxiredoxin and gluthathione-peroxidase as an adaptive response, and with genetic variations in certain antioxidant enzymes [6].

Mitochondria, the main source of ROS within the cells have been proposed to play a central role in aging process [7]. The main marker of mitochondrial biogenesis could be the expression changes of genes involved in pathway leading from PGC1-family of regulated co-activators (PGC1α, PGC1β) [9,10]. Sirtuin-1 regulates also mitochondrial biogenesis [11] but little is known about the regulation of SIRT1 and PGC1β gene expression at transcriptional level [11] in airways.

Many studies employing sample from the peripheral lung [12] or plasma [13] revealed these related age or disease alterations. However, at the transcriptomic level, there have been (at the best of the author knowledge) no studies that used induced sputum to determine molecular changes with age and COPD in airways. Sputum is one of the most non-invasively accessible body fluids and contains exfoliated airway epithelial cells [14]. It represents an important tool for understanding the molecular mechanisms in patients with pulmonary disorders [15].

The present study sought to improve the understanding of the molecular mechanisms that regulate antioxidant responses and mitochondria biogenesis in the airways with advancing age and COPD by investigating the expression of genes in induced sputum that might be involved in these responses.

The aims of the present study were: 1/to identify the physiological and pathological (COPD) non-respiratory lung aging by determining the transcription levels (messenger ribonucleic-acid (mRNA)) of genes encoding antioxidants and of mitochondrial biogenesis' markers 2/to elucidate the relationship between the molecular markers and lung function parameters in older population (healthy and COPD subjects).

Material and Methods

Study design

Study design consisted of a convenience sample of subjects living in the region of Sousse, Tunisia. This is a cross-sectional experimental case control study. It was conducted at three sites by using a sample of induced sputum.

Subjects

Three groups were recruited: Group I: volunteer healthy young adults aged 25-54 years and Group II: volunteer healthy subjects aged ≥60 years. Groups I and II subjects' were recruited among parents of Hospital workers and/or of medical school students and among elderly people residing in a retirement home. The non-inclusion criteria applied for group I and II are detailed in the Supplementary Material.

Group III: ten known COPD patients aged ≥60 years addressed by the local hospital pulmonary department for lung function testing were included. COPD diagnosis was retained when the post-bronchodilator (postBD) first-second-forced-expiratory-volume/forced-vital-capacity (FEV1/FVC) ratio was lower than 0.70 [16,17]. Duration of the COPD patients and medical treatments were recorded.

Medical questionnaire

A detailed questionnaire was administered about smoking history, symptoms, medical history, treatment, occupation and family history. The data were collected according to a pre-established form specifying the epidemiological, clinical, spirometric, therapeutic and evolutionary data of the COPD patients. The ATS-DLD-1978 [18] questionnaire was used to assess the different characteristics of the subjects.

Spirometry measurement

Spirometry was carried out according to the recent guidelines [19] using a spirometer (ZAN 100, Messgerate GmbH, Germany).

The following parameters were measured before (Pre)/PostBD inhalation of 400 mg Salbutamol *via* a large volume spacer [20]: FVC (L), FEV1 (L); FEV1/FVC ratio (absolute value). Spirometric data results were expressed as percentage of local spirometric norms [21]. FEV1 and/or FVC<LLN, were considered abnormal [22]. Obstructive-Ventilatory-Defect (OVD) was defined by FEV1/FVC ratio<Lower-Limit-of-Normal (LLN) [22].

A FEV1/FVC<0.70 PostBD, in an evocative clinical context, confirmed the diagnosis of COPD [16,17]. GOLD 2017 recommendations have been referred to classify the severity of the COPD patients [23].

Sputum induction, analysis and processing

Sputum was induced and processed by the method described by Pizzichini et al., [24] and Paggiaro et al., [25]. Saline nebulization was performed with an ultrasonic nebulizer (GIMA, ROFESSIONAL Medical Products, CE) at a flow rate of 0.2 to 0.7 ml/min and a median aerodynamic particles' diameter of 5 μm.

Cytological analysis was performed after running a total cell count and verifying cell viability with trypan blue and using Malassez cells [26]. The cells are then cytocentrifuged and the cytological formula is studied on non-scaly living cells after staining with May-Grünwald Giemsa (MGG) [26].

Gene expression: RNA preparation and analysis

Total RNA was isolated from the cell pellets of sputum (containing heteregenous cells) using acid-phenol-chloroform precipitation according to Chomczynski and Sacchi (1987) [27] with TRI-Reagent (Invitrogen). RNA was converted to cDNA with SuperScript II reverse transcriptase (Invitrogen, Life Technologies) and hexamers primers according to the supplier's protocol. Quantitative-RT-PCR was performed using the QuantiTectTM SYBR Green PCR kit (Roche) according to the supplier's protocol. Relative abundance of transcript levels was calculated after normalization to Ribosomal-Protein-Large-0 (RPLP0). The data were expressed as relative fold expression compared to group I.

Several genes have been studied: 1-Genes encoding antioxidant enzymes: Glutathione-Peroxidase-1(GPX1), Peroxiredoxin-1 (PRDX1), Catalase (CAT), Superoxide-Disumutase-1 (SOD1); and

Table 1: Anthropometric, Spirometric and quantity of used cigarettes of the three groups.

	Group I Healthy-young Control-group (n=7)	Group II Healthy-elderly-group (n=10)	Group III Elderly-COPD group (n=10)	ANOVA
Sex: Male/Female	4/3	5/5	10/0	*ab
Age (yr)	36.76±10.59	68.67±6.29	68.72±7.06	
BMI (kg/m^2)	28.7±6.2	29.0±4.8	23.2±4.4	*c
Cigarettes use (PY)	0	0	48±19	*bc
PreBDFEV1/FVC (absolute value)	0.91±0.03	0.82±0.12	0.52±0.09	-
PreBD FVC (L)	4±1.02	2.84±0.98	1.97±0.63	-
PreBD FEV1 (L/s)	3.64±0.92	2.3±0.67	1.01 ±.,32	-
PostBD FEV$_1$/FVC (absolute value)	0.91±0.03	0.83±0.05	0.53±0.12	*bc
PostBD FVC (L)	4.08±0.7	2.73±0.7	2.18±0.33	-
PostBD (L/s) (%predicted)	3.72±0.7	2.27±0.75	1,16±0.36	*bc
FEV$_1$	117±19	97±14	43±14	

Data are expressed as mean±SD. n: Number of subjects; FEV1: First-second-forced-Expiratory-Volume; FVC: Forced-Vital-Capacity; ANOVA: Analysis of Variance; PostBD: Post Bronchodilatator test; PreBD: before Bronchodilatator test.
*p< 0.05 (Nonparametric ANOVA Kruskal-Wallis test)
*p< 0.05 (Nonparametric ANOVA Kruskal-Wallis test)
[a]p<0.05 (Dunn's test). Group I vs. Group II
bp<0.05 (Dunn's test). Group I vs. Group III
[c]p<0.05 (Dunn's test). Group II vs. Group III

2-Genes involved in mitochondrial biogenesis: such as sirtuin-1 (SIRT1) which is involved in the regulation of many metabolic processes of aging, Peroxisome-proliferator-activated-receptor-Gamma Coactivator-1-beta (PGC1β) that controls mitochondrial oxidative metabolism, Cyclo-oxygénase1 (COX1), BCL2-associated-X-protein (Bax), Glycéraldéhyde-3-Phosphate Déshydrogénase (GAPDH), and Tumor Necrosis Factor (TNF).

To accomplish the genetic study, we employed Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). Total RNA was isolated from the cell pellets of sputum (containing heteregenous cells) using acid-phenol-chloroform precipitation according to Chomczynski and Sacchi (1987) [27] with TRI-Reagent (Invitrogen). RNA was converted to cDNA with SuperScript II reverse transcriptase (Invitrogen, Life Technologies) and hexamers primers according to the supplier's protocol. Quantitative-RT-PCR was performed using the QuantiTectTM SYBR Green PCR kit (Roche) according to the supplier's protocol. Relative abundance of transcript levels was calculated after normalization to Ribosomal-Protein-Large-0 (RPLP0). The data were expressed as relative fold expression compared to group 1.

Statistical analysis

Variables were checked for normality and expressed as mean±SD or median (interquartile range). Data were analyzed using a statistical package for social sciences (Statistica 6 for windows) and Graphpad prism 5 software. Statistical analyses were performed using Kruskal-Wallis nonparametric ANOVA followed by Dunn's post tests for multiple comparisons. Pearson product-moment correlation coefficient (r) was used to evaluate the associations between relative fold expression gene, independent variables such as spirometric data (PostFEV1 (%), PostBDFEV1/FVC (absolute value)), age and smoking history (Pack-Years, PY). The oxidative stress markers and pertinent clinical characteristics were compared in controls and patients. Oxidant-antioxidants were correlated in the three groups. Correlations were analyzed between the oxidative stress markers against pulmonary function. In addition, the association of oxidative status with predicted FEV1% was also examined. Ap value<0.05 was considered statistically significant.

Results

Subject's clinical and spirometry data

The initial enrollment of the recruited subjects was 50. After applying the non-inclusion criteria the sample size was reduced to 27 (Figure 1).

Table 1 exposes the characteristics of the subjects included in the three groups. 1) Groups II and III were age- matched and significantly older than group I, 2) Compared to group I, group II was BMI-, FEV1- and FEV1/FVC ratio- matched and 3) Compared to groups I and II, group III had significantly lower spirometric data and higher number of PY.

Regarding lifestyle customs and clinical data, ten of our patients had a concept of current active or weaned smoking with a PY average of 48.28±19.31 (24-90 PY).

None of the patients from Groups I and II had obvious respiratory symptoms when Group III patients presented the triad: Cough, morning expectoration and dyspnea.

All Group III patients had severe to very severe proximal OVD. After the bronchodilation test and according to the GOLD 2017 recommendations, eight of our patients had severe to very severe COPD. In the induced sputum of COPD patients (Group III) a clear predominance of neutrophils is noted (Table 2).

Effect of aging on gene expression

Among studied correlation between transcripts levels and

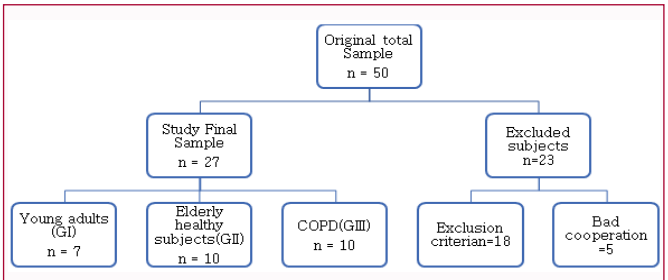

Figure 1: Sampling diagram and distribution of groups.

Table 2: Induced sputum cell count in Group III patients (COPD).

Count/Cell type	Mean±SD
Total (×10^5 cells/ml)	10,28±4,20
Neutrophils (%)	71,19±11,14*
Macrophages (%)	14,51±11,23
Lymphocytes (%)	8,44±4,46
Bronchial epithelial Cells (%)	3,50±2,48
Eosinophils (%)	0,30±0,39

*p<0.05 (Wilcoxon matched pairs test): neutrophils *vs*. other cells.

Table 3: Correlation coefficient between relative fold expression of with age in group I and II.

Relative fold expression	Age (years)
Genes encoded antioxidants enzymes	
CAT	-0.38(0.14)
GPX1	-0.07(0.006)
PRDX1	-0.52(0.27*)
SOD	-0.58 0.34*)
Genes encoded mitochondrial biogenesis	
COX1	-0.12(0.01)
PGC1β	0.22(0.05)
SIRT1	0.37(0.14)

For abbreviations, see abbreviations list. r(r^2)
*p<0.05 Pearson correlation test.

age in two groups healthy-young and elderly, only the decrease of PRDX1 and SOD1 transcripts levels was significantly correlated with advancing age (r=-0.52 and r=-0.58 respectively) (Figure 2, Table 3).

So, according to our study, lung aging affects as shown in Figure 2, the antioxidant system, with a significant decrease in the expression of genes that encode PRDX1 and SOD1.

The comparison of the different studied genes as well as antioxidant enzymes as those of mitochondrial biogenesis between

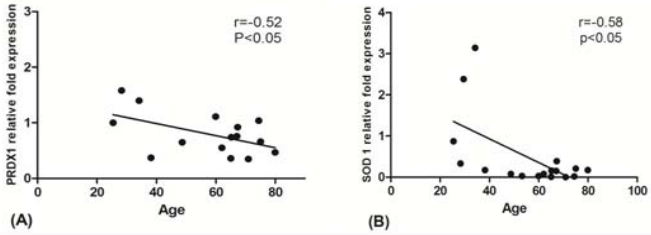

Figure 2: Correlation between relative fold expression of PRDX1 (A) and SOD1 (B) with age in groups I and II. Relationship was analyzed by Spearman's correlation test. A correlation coefficient (r) and p value are indicated. RDX1: Peroxiredoxin-1, SOD: Superoxide-Disumutase.

the 3 groups of the study showed that only PRDX1 and GPX1 were significantly decreased in elderly patients with COPD, compared to healthy young subjects and healthy elderly subjects (Table 4).

Furthermore, the levels of PGC1β and SIRT1 transcripts were significantly increased in elderly patients with COPD compared to healthy young subjects. This is in favor of accelerated aging of both the antioxidant system and mitochondrial biogenesis in COPD.

The impact of COPD on gene expression

Analysis of the expression of the genes associated with the antioxidant enzymes showed that the levels of the Group III PRDX1 and GPX1 (antioxidant enzyme) transcripts were significantly lower than those of Groups I and II (Figure 3).

Compared to Group I, the transcript levels (mRNA) of PGC1β and SIRT1 (elements of mitochondrial biogenesis) of Group III were significantly higher (Figure 4).

Table 5 analyzed correlations of gene expression data with spirometric, age, and smoking data. The mRNA expression for PRDX1 and GPX1 was significantly and positively correlated with FEV1/FVC PostBD ratio. In addition, a positive correlation between PRDX1 transcript levels and PostBD(%) FEV1 (r=0.60) was noted.

Table 4: Relative fold expression of studied candidate genes.

	Group I Healthy-young Control-group (n=7)	Group II Healthy elderly-group (n=10)	Group III elderly COPD patients-group (n=10)
Genes that are significantly down regulated with age and pathology			
PRDX 1	1.000±0.225	0.696±0.085	0.362±0.064**
GPX1	1.000±0.240	0.948±0.128	0.473±0.132***
Genes that are significantly up regulated with age and pathology			
PGC1β	1.000±0.551	5.984±2.236	16.450±2.057**
SIRT1	1.248±0.607	12.720±3.736	21.420±6.775**
Genes where no significant change is observed with age and pathology			
CAT	1.003±0.488	0.509±0.165	0.184±0.050
SOD1	1.000±0.473	0.122±0.038	0.123±0.065
BAX	1.001±0.231	0.942±0.125	1.338±0.123
GAPDH	1.000±0.311	1.256±0.183	0.612±0.118
TNF	0.998±0.283	0.921±0.271	0.448±0.132
LYZ	1.000±0.315	0.934±0.168	0.405±0.212

Data are mean±SEM. Relative abundance of transcript levels was calculated after normalization to Ribosomal Protein Large 0 (RPLP0). The data are expressed as relative fold expression compared to young healthy control group (Group I). PRDX1: Peroxiredoxin-1; GPX1: Glutathione-Peroxidase-1; PGC1β: Peroxisome-proliferator-activated-receptor-Gamma Coactivator-1-beta; SIRT1: Sirtuin-1; CAT: Calatase; SOD: Superoxide-Disumutase; BAX: BCL2-Associated-X-protein; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase; TNF: Tumor Necrosis Factor; LYZ: Lysozyme; COX1: mitochondrially-encoded-cytochrome-c-oxidase-I
p*<0.05: (Kruskal-Wallis test) Group I *vs*. Group II;
p**<0.05: (Kruskal-Wallis test) Group I *vs*. Group III;
p***<0.05: (Kruskal-Wallis test) Group II *vs*. Group III.

Table 5: Correlation coefficient between relative fold expression and lung function data (POSTBDFEV$_1$andPOSTBD FEV$_1$/FVC) in the study sample.

Relative fold expression	Lung function data		Smoking history
	FEV$_1$ (%)	FEV$_1$/FVC ratio (absolute value)	Smoking (Pack-years)
Genes encoded antioxidants enzymes			
CAT	0.31(0.10)	0.39(0.15)	-0.25(0.06)
GPX1	0.39(0.15)	0.45(0.20*)	-0.43(0.18*)
PRDX1	0.60(0.36*)	0.52(0.27*)	-0.47(0.22*)
SOD1	0.32(0.10)	0.34(0.12)	-0.25(0.06)
Genes encoded mitochondrial biogenesis			
COX1	0.12(0.01)	0.28(0.08)	-0.25(0.06)
PGC1β	-0.69(0.48*)	-0.70(0.50*)	0.70(0.50*)
SIRT1	-0.32(0.10)	-0.19(0.04)	0.29(0.08)

%: percentage of predicted value; r: correlation-coefficient; r^2: determination-coefficient
*p**<0.05 Pearson correlation test.
FEV1: First-second-forced-Expiratory-Volume; FVC: Forced-Vital-Capacity; CAT: Calatase; GPX1: Glutathione-Peroxidase-1; PRDX1: Peroxiredoxin-1; SOD: Superoxide-Disumutase; COX1: mitochondrially-encoded-cytochrome-c-oxidase-I; PGC1β: Peroxisome-proliferator-activated-receptor-Gamma Coactivator-1-beta; SIRT1: Sirtuin-1

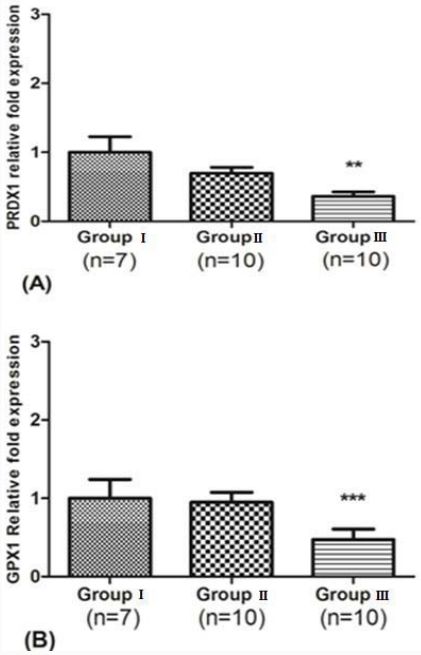

Figure 3: Fold expression of PRDX1 (A) and GPX1 (B) in induced sputum samples from three groups. Group I: healthy young control (n=7); Group II: healthy elderly (n=10) and Group III: elderly COPD patients (n=10). Relative abundance of transcript levels was calculated after normalization to Ribosomal protein large 0 (RPLP0). The data are expressed as relative fold expression compared to group I. Bar graphs show means±SEM. GPX1: glutathione-peroxidase-1, PRDX1: peroxiredoxin-1. *p<0.05: GroupeI (GI) *vs*. GroupeII (GII), **p<0.05: GI *vs*. Groupe III (GIII), ***p<0.05: GII *vs*. GIII (Kruskal-Wallis test).

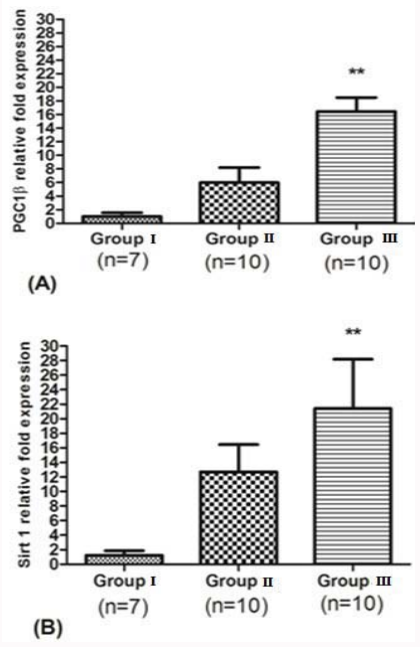

Figure 4: Relative fold expression of PGC1β (A) and Sirt1 (B) in induced sputum samples from three groups. Group I: healthy young control (n=7); Group II: healthy elderly (n=10) and Group III: elderly COPD patients (n=10). Relative abundance of transcript levels was calculated after normalization to Ribosomal protein large 0 (RPLP0). The data are expressed as relative fold expression compared to group I. Bar graphs show means±SEM. PGC1β: Peroxisome-proliferator-activated-receptor-gamma-coactivator-1-beta, SIRT1: Sirtuin-1*p<0.05: Group I (GI) *vs*. Group II (GII), **p<0.05: GI *vs*. Group III (GIII), ***p<0.05: GII *vs*. GIII (Kruskal-Wallis test).

These mRNA levels were negatively correlated with the degree of smoking. In contrast, levels of transcripts for PGC1β were negatively correlated with postBD FEV1(%) and FEV1/FVC PostBD.

Discussion

The main results of this study are: age and pulmonary status modulate expression of genes involved in antioxidant balance and mitochondrial biogenesis. There was an effect of age and markedly of pathology in gene expression related to antioxidant enzymes and mitochondrial biogenesis.

This is the first study, to our knowledge, investigating the (transcriptional) genetic relationship between non-respiratory lung aging and COPD, by studying the expression of panel of genes related to antioxidant enzymes and mitochondrial biogenesis in human cells removed from the respiratory tract (induced sputum). COPD preferentially affects subjects over 65 years old [28]. The average age of the patients in our study was comparable to those in the literature. Indeed, the average age of COPD patients included in the studies interested in oxidative stress varies from 59 to 79 years [29,30]. The sex ratio was variable according to the series. However,

male dominance is notable in most studies [31,32]. At the cytological level, Cell counting at the sputum-induced samples of COPD patients showed a marked predominance of neutrophils compared to other cell types. These data are consistent with the literature results [33].

Effect of age and pathology on sputum antioxidants enzymes related gene expression

The present study showed a significant decrease as a function of age in the transcription levels of the two enzymes of the antioxidant system PRDX1 and SOD1. These levels of antioxidant transcripts were significantly lower in older patients with COPD compared to healthy young and elderly subjects. Aging is associated with marked alterations in gene expression [34,35] which is at least attributable to epigenetic alterations [36]. These changes can be induced by environmental factors, including diet, air pollution exposure and cigarette smoke which have been associated with the development of lung disease [1].

To the best of the authors' knowledge, the present study is the first one that represents relationship between aging and COPD at the transcriptomic level in airways by the investigation of expression of some gene related to antioxidants enzymes and mitochondria biogenesis.

Oxidative stress and imbalances in the host defense mechanisms are believed to play a key role in aging [7] and are considered to be a key mechanism in the pathogenic process in COPD [37]. Previous studies of serum antioxidant concentration and lung outcomes had shown that lower levels of antioxidant defenses are associated with decreased lung function [38] and Tomaki N et al., reported also a decreased expression of antioxidant enzymes such as catalase, glutathione S-transferase in COPD lung tissues compared with non COPD-tissues [12].

Peroxiredoxins are a class of thiol peroxidases that degrade hydroperoxide to water [39,40] and considered as antioxidant enzyme [38]. While a single study of Pierrou et al., reported an up-regulation of PRDX1 with disease in epithelial-cell expression in COPD *vs*. non-diseased and in smokers [41], the present study noted a down-regulation in sputum elderly-COPD-patients, as compared to young-healthy subjects.

Other antioxidant enzyme, GPX1 which was considered for a role in the antioxidant activity of glutathione (GSH) and in GSH recycling, the mRNA expressions in the present study were diminished in elderly-COPD patients as compared to elderly-healthy. Geraphty et al., have demonstrated that the expression of GPX1 is reduced in the lungs of COPD patients [42,43] and they have demonstrated that overexpression of GPX1 prevents the expansion of airspace induced by cigarette smoke in mice [42]. The lungs of mice deficient in GPX1 and exposed to cigarette smoke are more susceptible to inflammation and emphysema [44]. Most human studies have shown that GPX1 increased in COPD and was up-regulated with smoking [38,45]. It's important to note that these conflicting results were due to different methods used for quantitative analysis of gene expression profiles. Pierrou et al., [41] used DNA microarray technologies whereas the present study used the RT-PCR which has more advantages: more robust with small changes in expression [46] and higher degree of analytical precision with a single amplification for each gene and not a large number of transcripts that can be quantified in a single experiment [47].

The decrease of mRNA-expression of PRDX1 and GPX1 correlated with the degree of airway limitation and smoking. Another important result was the decreased transcripts levels of PRDX1 and SOD1 with advancing age in healthy-subjects. In the literature, genes encoding the peroxiredoxins have recently emerged as a new class of gerontogenes that link aging to genome instability and cancer and could be target therapeutically in the treatment of age-related cancers [48]. Their deficiency results in accelerated aging in yeast, worms, flies and rodents [49-52]. These results suggest that impaired protective activity of antioxidants enzymes with age and markedly with smoking (COPD) might lead to an accelerated lung aging.

Effect of age and pathology on sputum mitochondrial biogenesis related gene expression

The metabolic Nicotinamide Adenine Dinucleotide (NAD1)-dependent protein deacetylases have emerged as important regulators of chronic inflammatory diseases, cancer, and aging [53]. These proteins, which belong to class III histone/protein deacetylases (HDACs), are referred to as sirtuins. SIRT1 is a gene that encodes a member of the sirtuin family of proteins. SIRT1 is essential for maintaining silent chromatin *via* the deacetylation of histones. Activation or over-expression of SIRT1 has been shown to increase the lifespan of fly, yeast, worm (up to 70%), and mouse [54-59]. Recently, it has been shown that SIRT1 plays an important role in a wide variety of processes, including apoptosis, senescence, mitochondria biogenesis and aging [53]. In the present study, mRNA levels of Sirt1 were significantly increased in elderly-COPD-patients, compared to young-healthy-subjects. Recent findings showed an up-regulation of Sirt1 under oxidative conditions with a H_2O_2 treatment [11]. This could explain an over-expression of SIRT1 in elderly-COPD in response to an oxidative stress in the airways. Also, this over-expression could protect airways from oxidative stress as reported by Alcendor et al., [60]. Yao et al., demonstrated that SIRT1 itself protects against oxidative stress by positively regulating antioxidant genes such as CAT and SOD. It is considered a new anti-aging protein involved in the regulation of cellular senescence and premature aging [61].

Other genes were involved in the mitochondrial biogenesis. PGC1β, mRNA levels were significantly increased in elderly-COPD-patients compared to young healthy-subjects. These results were associated with cigarette smoke. Little information is reported about the expression of PGC1β in airways in the literature. Meirhaeghe et al., have shown that an over-expression of PGC1β is associated with an increase in the number of mitochondria, and is correlated with increased oxygen consumption [62]. The present study extended the reports of the literature and demonstrated that sputum PGC1β gene expression in airways increased with age and manifestly with cigarette smoke (COPD).

Strengths and Limitations

This study has some limitations that require further investigation. RNA isolated from sputum plugs contains heterogeneous populations of cells with an increased proportion of neutrophils in COPD-group. However, changes in gene expression do not occur without changes in intracellular signal transduction [63]. Gene expression biomarkers predicting age related lung disease would be a useful and feasible sample source to guide treatment strategies. Further studies are needed to understand the relation between gene expression and their related protein levels, underlying mechanisms and related cell type. Despite these limitations, the present study has added significantly to the field of transcriptional data on the expression of some sputum gene

related to antioxidants enzymes and mitochondria biogenesis with age and COPD, combining the use of a non-invasive sample source and powerful technology which will lead to further identification of age related lung disease molecular biomarkers.

Conclusion

In conclusion, pulmonary aging affects both the respiratory and non-respiratory lungs' functions. A protective role of sputum gene against oxidative stress is likely. The up-regulation of genes encoding proteins involved in mitochondrial biogenesis might protect airways from an oxidative stress marked by the deterioration of the antioxidant system. These changes were related to cigarette smoke, and might explain the accelerated lung aging in case of COPD.

Acknowledgment

The authors thank Drs Mohamed Banni, Helmi Ben Saad, Anne-Laure Charles, Gilles Laverny and Daniel Metzger for valuable comments and technical assistance.

Ethical Approval

The study was carried out in compliance with the 'Ethical principles for medical research involving Human subjects' of the Helsinki Declaration (available from: http://www.wma.net/en/30publications/30ethicsmanual/pdf/ethics_manual_arabic.pdf, accessed August 29th 2016). Approval for the study was obtained from the Hospital ethics committee (Farhat-HACHED-Hospital-ethics committee, approval number 2801/2013). Written informed consent was obtained from all subjects.

References

1. Thannickal VJ, Murthy M, Balch WE, Chandel NS, Meiners S, Eickelberg O, et al. Blue journal conference. Aging and susceptibility to lung disease. Am J Respir Crit Care Med. 2015; 191: 261-269.
2. Celli BR, MacNee W, Agusti A, Anzueto A, Berg B, Buist AS, et al. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. Eur Respir J. 2004; 23: 932-946.
3. Daldoul H, Denguezli M, Jithoo A, Gnatiuc L, Buist S, Burney P, et al. Prevalence of COPD and tobacco smoking in Tunisia-Results from the BOLD Study. Int J Environ Res Public Health. 2013; 10: 7257-7271.
4. Mercado N, Ito K, Barnes PJ. Accelerated ageing of the lung in COPD: new concepts. Thorax. 2015; 70: 482-489.
5. Faner R, Rojas M, MacNee W, Agusti A. Abnormal lung aging in chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2012; 186: 306-313.
6. van der Vliet A. Comparative Biology of the Normal Lung. Elsevier. 2015.
7. Harman D. Free radical theory of aging: an update: increasing the functional life span. Ann NY Acad Sci. 2006; 1067: 10-21.
8. Langen R, Korn S, Wouters E. ROS in the local and systemic pathogenesis of COPD. Free Radical Biology and Medicine. 2003; 35: 226-235.
9. Izquierdo JM, Ricart J, Ostronoff LK, Egea G, Cuezva JM. Changing patterns of transcriptional and post-transcriptional control of β-F1-ATPase gene expression during mitochondrial biogenesis in liver. J Biol Chem. 1995; 270: 10342-10350.
10. Perez GI, Trbovich AM, Gosden RG, Tilly JL. Reproductive biology: mitochondria and the death of oocytes. Nature. 2000; 403: 500-501.
11. Hwang JW, Yao H, Caito S, Sundar IK, Rahman I. Redox regulation of SIRT1 in inflammation and cellular senescence. Free Radic Biol Med. 2013; 61: 95-110.
12. Tomaki M, Sugiura H, Koarai A, Komaki Y, Akita T, Matsumoto T, et al. Decreased expression of antioxidant enzymes and increased expression of chemokines in COPD lung. Pulm Pharmacol Ther. 2007; 20: 596-605.
13. Chehab O, Ouertani M, Souiden Y, Chaieb K, Mahdouani K. Plasma antioxidants and human aging: a study on healthy elderly Tunisian population. Mol Biotechnol. 2008; 40: 27-37.
14. Thunnissen F. Sputum examination for early detection of lung cancer. J Clin Pathol. 2003; 56: 805-810.
15. Nicholas B, Djukanović R. Induced sputum: a window to lung pathology. Biochem Soc Trans. 2009; 37: 868-872.
16. Ben HS, Ben LA, Ben SM, Ghannouchi I, Ben ME, Bougmiza I, et al. The diagnosis of COPD is recommendation dependent. La Tunisie medicale. 2014; 92: 474-481.
17. Vestbo J, Hurd SS, Agustí AG, Jones PW, Vogelmeier C, Anzueto A, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med. 2013; 187: 347-365.
18. Ferris B. Epidemiology Standardization Project (American Thoracic Society). Am Rev Respir Dis. 1978; 118: 7-53.
19. Pellegrino R, Viegi G, Brusasco V, Crapo R, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. Eur Respir J. 2005; 26: 948-968.
20. Saad HB, Préfaut C, Tabka Z, Zbidi A, Hayot M. The forgotten message from gold: FVC is a primary clinical outcome measure of bronchodilator reversibility in COPD. Pulm Pharmacol Ther. 2008; 21: 767-773.
21. Saad HB, El Attar MN, Mabrouk KH, Abdelaziz AB, Abdelghani A, Bousarssar M, et al. The recent multi-ethnic global lung initiative 2012 (GLI2012) reference values don't reflect contemporary adult's North African spirometry. Respir Med. 2013; 107: 2000-2008.
22. Ben HS, Ben RAS, Rouatbi S, Ben SM, Garrouche A, Hadj AM, et al. Which definition to use when defining reversibility of airway obstruction? Rev Mal Resp. 2007; 24: 1107-1115.
23. Chronic obstructive pulmonary disease (COPD). 2017.
24. Pizzichini E, Pizzichini M, Efthimiadis A, Evans S, Morris MM, Squillace D, et al. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. Am J Respir Crit Care Med. 1996; 154: 308-317.
25. Paggiaro P, Chanez P, Holz O, Ind P, Djukanović R, Maestrelli P, et al. Sputum induction. Eur Resp J Suppl. 2002; 37: 3s-8s.
26. Boniface S, Lorec A, Badier M, Vervloet D, Magnan A. Induced sputum in allergology: techniques and indications. Rev Mal Respir. 2002; 19: 381-383.
27. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical Biochemistry. 1987; 162: 156-159.
28. MacNee W. Accelerated lung aging: a novel pathogenic mechanism of chronic obstructive pulmonary disease (COPD). Biochem Soc Trans. 2009; 37: 819-823.
29. Vibhuti A, Arif E, Deepak D, Singh B, Pasha MQ. Correlation of oxidative status with BMI and lung function in COPD. Clin Biochem. 2007; 40: 958-963.
30. Tang W, Bentley AR, Kritchevsky SB, Harris TB, Newman AB, Bauer DC, et al. Genetic variation in antioxidant enzymes, cigarette smoking, and longitudinal change in lung function. Free Radic Biol Med. 2013; 63: 304-312.
31. Wiegman CH, Michaeloudes C, Haji G, Narang P, Clarke CJ, Russell KE, et al. Oxidative stress-induced mitochondrial dysfunction drives inflammation and airway smooth muscle remodeling in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol. 2015; 136: 769-780.

32. Numakura T, Sugiura H, Akaike T, Ida T, Fujii S, Koarai A, et al. Production of reactive persulfide species in chronic obstructive pulmonary disease. Thorax. 2017; 72: 1074-1083.

33. Barbaro MF, Carpagnano G, Spanevello A, Cagnazzo M, Barnes P. Inflammation, oxidative stress and systemic effects in mild chronic obstructive pulmonary disease. Int J Immunopathol Pharmacol. 2007; 20: 753-763.

34. Bahar R, Hartmann CH, Rodriguez KA, Denny AD, Busuttil RA, Dollé ME, et al. Increased cell-to-cell variation in gene expression in ageing mouse heart. Nature. 2006; 441: 1011-1014.

35. Brunet A, Berger SL. Epigenetics of aging and aging-related disease. J Gerontol Biomed Sci Med Sci. 2014; 69: S17-S20.

36. Rando TA, Chang HY. Aging, rejuvenation, and epigenetic reprogramming: resetting the aging clock. Cell. 2012; 148: 46-57.

37. MacNee W, Tuder RM. New paradigms in the pathogenesis of chronic obstructive pulmonary disease I. Proc Am Thorac Soc. 2009; 6: 527-531.

38. Bentley AR, Emrani P, Cassano PA. Genetic variation and gene expression in antioxidant related enzymes and risk of COPD: a systematic review. Thorax. 2008; 63: 956-961.

39. Rhee SG, Woo HA. Multiple functions of peroxiredoxins: peroxidases, sensors and regulators of the intracellular messenger H2O2, and protein chaperones. Antiox Redox Signal. 2011; 15: 781-794.

40. Wood ZA, Schröder E, Harris JR, Poole LB. Structure, mechanism and regulation of peroxiredoxins. Trends Biochem Sci. 2003; 28: 32-40.

41. Pierrou S, Broberg P, O'donnell RA, Pawłowski K, Virtala R, Lindqvist E, et al. Expression of genes involved in oxidative stress responses in airway epithelial cells of smokers with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2007; 175: 577-586.

42. Geraghty P, Hardigan AA, Wallace AM, Mirochnitchenko O, Thankachen J, Arellanos L, et al. The Glutathione Peroxidase 1–Protein Tyrosine Phosphatase 1B–Protein Phosphatase 2A Axis. A Key Determinant of Airway Inflammation and Alveolar Destruction. Am J Respir Cell Mol Biol. 2013; 49: 721-30.

43. Geraghty P, Baumlin N, Salathe MA, Foronjy RF, D'Armiento JM. Glutathione Peroxidase-1 suppresses the unfolded protein response upon cigarette smoke exposure. Mediators Inflamm. 2016; 2016: 9461289.

44. Duong C, Seow HJ, Bozinovski S, Crack PJ, Anderson GP, Vlahos R. Glutathione peroxidase-1 protects against cigarette smoke-induced lung inflammation in mice. Am J Physiol Lung Cell Mol Physiol. 2010; 299: L425-L433.

45. Comhair SA, Thomassen MJ, Erzurum SC. Differential induction of extracellular glutathione peroxidase and nitric oxide synthase 2 in airways of healthy individuals exposed to 100% O_2 or cigarette smoke. Am J Respir Cell Mol Biol. 2000; 23: 350-354.

46. Allanach K, Mengel M, Einecke G, Sis B, Hidalgo L, Mueller T, et al. Comparing microarray versus RT-PCR assessment of renal allograft biopsies: similar performance despite different dynamic ranges. Am J Transplant. 2008; 8: 1006-1015.

47. Sinicropi D, Cronin M, Liu ML. Gene expression profiling utilizing microarray technology and RT-PCR. BioMEMS and Biomedical Nanotechnology. 2006.

48. Nyström T, Yang J, Molin M. Peroxiredoxins, gerontogenes linking aging to genome instability and cancer. Genes Dev. 2012; 26: 2001-2008.

49. Lee K-S, Iijima-Ando K, Iijima K, Lee WJ, Lee JH, Yu K, et al. JNK/FOXO-mediated neuronal expression of fly homologue of peroxiredoxin II reduces oxidative stress and extends life span. J Biol Chem. 2009; 284: 29454-29461.

50. Neumann CA, Krause DS, Carman CV, Das S, Dubey DP, Abraham JL, et al. Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. Nature. 2003; 424: 561-565.

51. Oláhová M, Taylor SR, Khazaipoul S, Wang J, Morgan BA, Matsumoto K, et al. A redox-sensitive peroxiredoxin that is important for longevity has tissue-and stress-specific roles in stress resistance. Proc Natl Acad Sci USA. 2008; 105: 19839-19844.

52. Timmermann B, Jarolim S, Rußmayer H, Kerick M, Michel S, Krüger A, et al. A new dominant peroxiredoxin allele identified by whole-genome re-sequencing of random mutagenized yeast causes oxidant-resistance and premature aging. Aging. 2010; 2: 475-486.

53. Michan S, Sinclair D. Sirtuins in mammals: insights into their biological function. Biochem J. 2007; 404: 1-13.

54. Anderson RM, Bitterman KJ, Wood JG, Medvedik O, Sinclair DA. Nicotinamide and PNC1 govern lifespan extension by calorie restriction in Saccharomyces cerevisiae. Nature. 2003; 423: 181-185.

55. Bitterman KJ, Anderson RM, Cohen HY, Latorre-Esteves M, Sinclair DA. Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast sir2 and human SIRT1. J Biol Chem. 2002; 277: 45099-45107.

56. Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, et al. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature. 2003; 425: 191-196.

57. Lin SJ, Defossez PA, Guarente L. Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae. Science. 2000; 289: 2126-2128.

58. Trapp J, Jung M. The role of NAD+ dependent histone deacetylases (sirtuins) in ageing. Curr Drug Targets. 2006; 7: 1553-1560.

59. Tissenbaum HA, Guarente L. Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. Nature. 2001; 410: 227-230.

60. Alcendor RR, Gao S, Zhai P, Zablocki D, Holle E, Yu X, et al. Sirt1 regulates aging and resistance to oxidative stress in the heart. Circulation research. 2007; 100: 1512-1521.

61. Yao H, Rahman I. Perspectives on translational and therapeutic aspects of SIRT1 in inflammaging and senescence. Biochem Pharmacol. 2012; 84: 1332-1339.

62. Meirhaeghe A, Crowley V, Lenaghan C, Lelliott C, Green K, Stewart A, et al. Characterization of the human, mouse and rat PGC1beta (peroxisome-proliferator-activated receptor-gamma co-activator 1beta) gene in vitro and in vivo. Biochem J. 2003; 373: 155-165.

63. George BJ, Reif DM, Gallagher JE, Williams-DeVane CR, Heidenfelder BL, Hudgens EE, et al. Data-driven asthma endotypes defined from blood biomarker and gene expression data. PLoS One. 2015; 10: e0117445.