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Shorter telomeres in non-smoking patients with airflow limitation

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ABSTRACT

Background: Cross-sectional and longitudinal studies describe shorter telomeres in patients with chronic obstructive pulmonary disease (COPD) compared to matched non-COPD controls, but the relationship is confounded by tobacco consumption. We hypothesized that telomere shortening would be similar between non-smoking and smoking individuals with airflow limitation and shorter than non-obstructed controls.

Methods: Telomere length (T/S) was measured by qPCR in blood leukocytes of 80 non-smoking patients and 80 age-matched smokers with airflow limitation. Forty non-smoker healthy individuals served as controls. Anthropometrics, lung function, previous and current comorbidities were recorded in all individuals. Relationship between telomere length and clinical and functional variables were explored in the three groups.

Results: Telomeres length was similar in non-smokers and smoker individuals with airflow limitation (T/S = 0.61 ± 0.19 vs. 0.60 ± 0.23, p > 0.05) respectively. Telomere length was significantly shorter in both groups compared to healthy controls (T/S 0.79 ± 0.40; p = 0.01) independent from age and sex. No significant association was found between the telomere length and clinical or lung function parameters.

Conclusions: Telomere shortening is associated with airflow limitation independent of smoking status. Weather premature ageing or biologically determined shorter telomeres are responsible for this finding remain to be determined.

1. Introduction

Chronic obstructive pulmonary disease (COPD), a major cause of morbidity and mortality throughout the world, is thought to result from the interaction of environmental agents such as tobacco smoking or exposure to biomass fuel and inherited genetic factors [1]. COPD is characterized by the presence of airflow limitation that frequently co-exists with other age-related co-morbidities such as osteoporosis, cardiovascular disease, lung cancer, depression and diabetes [2,3].

Tobacco smoking has been considered the most important risk factor for developing COPD. However, close to 20% of patients with poorly reversible airflow limitation, similar to COPD have never smoked. Other factors such as chronic asthma, outdoor air pollution, biomass and coal smoke, environmental smoke exposure (ETS), occupational exposure, diet, tuberculosis sequelae, repeated lower respiratory-tract in early childhood, intrauterine growth retardation, poor nourishment, and poor socioeconomic status, have been reported to be related to airflow obstruction similar to COPD in non-smokers [1,4,5].

Furthermore, it has been suggested that COPD is a disease related to accelerated ageing [6–8]. Telomere length is a biomarker of biological age [9–11], and its length has been related to mortality and poor outcomes in COPD patients [12]. Telomeres consist of stretches of repetitive hexanucleotides (5′-TTAGGG-3′) that get progressively shorter as cells divide, as a consequence of the known end-replication process. When telomeres reach a critical length, their ability to protect the DNA decreases, resulting in cell cycle arrest and eventually leading to cellular senescence or cell death by apoptosis [11,13].

Shorter telomeres have been described in circulating leukocytes of patients with COPD when compared to smokers without COPD [14–16].

Abbreviations: COPD, Chronic Obstructive Pulmonary Disease; T/S, Relative Telomere length ratio; qPCR, Quantitative Real Time Polymerase Chain Reaction; FEV_1, Forced Expiratory Volume in one second; FVC, Forced Vital Capacity; BMI, Body Mass Index; GOLD, Global Initiative for Chronic Obstructive Lung Disease; PaO_2, Partial Oxygen Tension; K_{CO}, Diffusion Capacity of Carbon Monoxide; IC/TLC, Inspiratory to Total Lung Capacity Ratio; 6MWD, 6-min walk distance; SD, Standard deviation

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However, no studies regarding telomere shortening in non-smoking patients with airflow limitation similar to COPD have been conducted before.

We hypothesized that telomere shortening is a feature of individuals with airflow limitation independent of smoking. To test this hypothesis, we explored the interaction between telomere attrition and clinical and lung function parameters, as well as outcomes in a very well characterized cohort of non-tobacco smoking individuals with airflow limitation similar to COPD. Smoking individuals with COPD and non-smoking individuals without airflow limitation served as controls.

2. Methods

2.1. Study individuals

Patients: 370 individuals suspected of COPD were included in this study which were patients of the two main hospitals in the Canary Islands: the Hospital Universitario de Gran Canaria, Doctor Negrín and Hospital Universitario N/S de Candelaria, Tenerife, Spain. From these individuals, 80 were non-tobacco users with post-bronchodilator airflow limitation that were age-matched with a control group of 80 tobacco-smokers with COPD (smoking history of > 15 pack-year) (Fig. 1). Inclusion criteria: age > 40 years, and post-bronchodilator FEV_1/FVC ratio < 0.70 measured 20 min after administration of 400 mg of albuterol. All patients were clinically stable (no exacerbation for at least 6 weeks) at the time of evaluation. Spirometry lung volumes, pulmonary function test, and exercise capacity were measured according to ATS-ERS guidelines [17–19] and severity was graded by the Global Initiative for Obstructive Lung Disease (GOLD) [20]. Dyspnoea was evaluated by mMRC scale [21] and the BODE Index was calculated as previously described [22]. Co-morbidities were quantified using the Charlson index [23]. Exclusion criteria included uncontrolled co-morbidities such as malignancy at baseline. Asthma was defined by physician diagnosis. Controls: based on the availability of healthy controls with blood sample and > 44 years old from general population (CDC project) [24], only forty non-smoker healthy individuals were included in a second analysis to contrast them with age-matched patients within the two airflow obstruction groups (Fig. 1). All participants were informed of study purposes and provided written informed consent. The study was approved by the ethical committee board at both hospitals.

2.2. Telomere length measurement

DNA from venous blood was obtained at baseline from leucocytes using the QIAamp DNA Mini Kit (GE Healthcare). Telomere length was measured using a qPCR based protocol as already published [16,25,26]. The primer sequences and cycling conditions for the measurement of telomere length were the same used by others [26] and albumin was used as a reference gene. qPCR reactions were performed in 20 µL reactions for each individual: 10 µL SYBRGreen PCR Master Mix (BioRad), 0.9 µM of telomere primers and 0.6 µM of albumin primers. All the reactions were performed on triplicates on the iQ Cycler Real-Time PCR Instrument (BioRad).

Telomere length was calculated as a ratio of telomere (T) to albumin (S) as previously described [26]. The telomere length standardized to the reference single copy gene (T/S) was calculated using the "ΔΔCp with efficiency correction" calculation method [27].

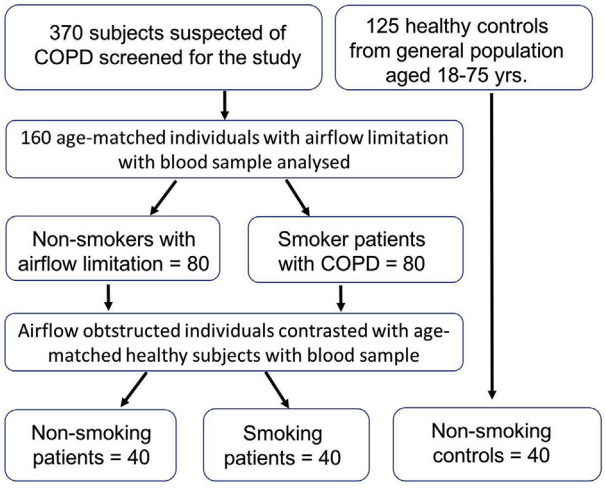

Fig. 1. Consort diagram representing the assessment of patients and healthy control individuals considered to analysis in the present study.

2.3. Statistical analysis

For the cross-sectional analysis, non-tobacco smoking patients with airflow limitation were categorized in three groups by telomere length ratio (T/S) tertiles: shorter, medium or longer telomeres.

A t-Student, ANOVA, Chi^2, Fisher Exact, Kruskal-Wallis test were used to test differences in means and proportions of clinical and lung function characteristics between non-smokers and smoker patients with COPD. The association between baseline telomere length (T/S) with clinical and/or pulmonary function variables was explored using Pearson's correlation coefficients. A multiple logistic regression was performed to test the association of telomere length with COPD adjusting for age, sex and pack years. SPSS 21.0 IBM Co software was used for all statistical analyses and two-tailed p-values < 0.05 were considered significant.

3. Results

Table 1 shows the clinical and lung function data of the patients

Table 1
Baseline characteristics of non-smoking patients with airflow obstruction versus smokers with COPD included in the study.

Variable	Non-smokers with airflow obstruction (N = 80)	Smokers with COPD (N = 80)	p-value
Sex (male %)	45	57	0.261
Age (years)[a]	64 ± 9	64 ± 9	1.000
BMI (Kg/m^2)[a]	29 ± 5	28 ± 6	0.089
Smoking habit (pack-yrs)[a,c]	0	63 ± 30	–
Active smoking (%)	0	55	–
T/S ratio[a]	0.61 ± 0.19	0.60 ± 0.23	0.768
FEV_1 (L)[a]	1.69 ± 0.78	1.46 ± 0.62	**0.045**
FEV_1 (% pred)[a]	66 ± 19	60 ± 23	**0.064**
FVC (% pred)[a]	93 ± 21	90 ± 25	0.337
FEV_1/FVC (%)[a]	58 ± 8	53 ± 12	**0.004**
IC/TLC (%)	39 ± 10	34 ± 9	**0.009**
6MWD (m)[a]	518 ± 108	470 ± 85	**0.004**
mMRC dyspnoea[b]	1 (0–2)	1 (0–2)	0.275
Exacerbations[b]	1 (0–2)	0 (0–1)	0.381
Charlson index[b]	0 (0–1)	0 (0–1)	0.150
Inhaled b2-agonist (%)	73	80	0.354
Inhaled anticholinergic (%)	83	45	**0.001**
Inhaled corticosteroid (%)	54	79	**0.003**

BMI: body mass index; T/S ratio: relative telomere length; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; % pred: percent predicted; IC/TLC: inspiratory to total lung capacity ratio; 6MWD: six-min walk distance test.

[a] Data are presented as mean ± SD.

[b] Data are presented as median (25^{th}-75^{th}pc).

[c] Number of packs of cigarettes smoked per day x number of years smoking.