Telomere length in chronic obstructive pulmonary disease

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Background Telomere length (TL) is considered a biomarker of cellular aging. Chronic obstructive pulmonary disease (COPD) is found to be associated with premature aging and the senescence hypothesis is now accepted as a molecular pathway for COPD development.

Purpose The aim of this study was to measure TL in COPD patients and to study its relation to demographic data, spirometric indices, and arterial blood gases parameters.

Participants and methods We measured TL using quantitative PCR in 20 patients with severe to very severe COPD and 11 age-matched and sex-matched nonsmokers.

Results TL was significantly shorter in COPD patients ($P < 0.001$). Among COPD patients, TL was significantly shorter in current smokers than ex-smokers. In COPD patients, TL was correlated positively with SpO_2%, pH ($P < 0.05$), PaO_2 ($P < 0.01$), FVC% ($P < 0.05$) and FEV_1%, and $FEF_{25–75}$% ($P < 0.001$) and not correlated with pack-year. The BODE (Body mass index, airflow Obstruction, Dyspnea, and Exercise capacity) index was correlated negatively with TL ($P < 0.01$); among BODE index parameters, the dyspnea score correlated negatively ($P < 0.05$) with TL. TL was shorter in very severe COPD than severe COPD ($P < 0.001$).

Conclusion Our data support accelerated cellular senescence in COPD represented by shortening of TL; TL was correlated positively with airflow limitation and it may be related to impaired physical activities in COPD, which is a manifestation of the aging process.

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Introduction

Although chronic obstructive pulmonary disease (COPD) is primarily a respiratory disease, systemic complications contribute considerably toward the prognosis. Most of these systemic complications, including weight loss, skeletal muscle dysfunction, osteoporosis, and atherosclerosis, are considered age-related abnormalities [1].

Telomere attrition in circulating white blood cells has been proposed as a marker for cumulative oxidative stress and inflammation and, therefore, as an indicator of the pace of biological aging [2]. Telomeres are DNA sequences and associated proteins that cap and stabilize the ends of linear chromosomes, thereby maintaining genome integrity and stability. Telomere length (TL) is not only related to the basic biology of aging as a trigger of cellular senescence but also reflects the balance between oxidative stress and antioxidant defense mechanisms [3].

The existing hypothesis for the pathogenetic mechanism of COPD is explained in terms of proteases, oxidants, and inflammation, whereas a new hypothesis is explained in terms of apoptosis, proliferation, and senescence. The two hypotheses can be integrated into a single hypothesis by linking senescence and inflammation [4].

Aim of the work

The aim of this work is to measure TL in patients with COPD and to study its relation to demographic data, spirometric indices, and arterial blood gases parameters.

Participants and methods

This case–control study was carried out at Al-Zahraa University Hospital on 31 age-matched men; they were divided into two groups.

(1) *Group I (control group)*: It included 11 healthy lifelong nonsmoker volunteers. None of them had any symptoms suggestive of any disease and their spirometric indices and arterial blood gas values were in the normal range.
(2) *Group II (COPD group)*: It included 20 COPD patients with symptoms of chronic airflow limitation and fulfilled the spirometric criteria set out by the GOLD 2011 guideline. The patients were included only if they had a stable course of disease with regular follow-up during the preceding 1 year and no hospitalization for COPD-related illness during the preceding 6 months. All of them had irreversible/partially reversible obstruction of airflow. COPD patients had a postbronchodilator FEV_1/FVC% of less than 70%. They had an increase in FEV_1 of less than 200 ml or less than

12% of baseline value 20 min after two puffs of inhaled salbutamol (100 μg) administered by a metered-dose inhaler. Most patients were using medications to treat COPD, including β2-agonists (salbutamol or formoterol), theophylline, and inhaled corticosteroids (budesonide); none of them was using oral steroids or long-term oxygen therapy.

Individuals known to have infective or interstitial lung diseases, bronchial asthma, cardiac, hepatic, renal, gastrointestinal, metabolic or endocrine diseases, malignancy, and inflammatory diseases such as diabetes mellitus were excluded from the study.

The study was approved by the ethical committee of Al-Azhar University. An informed written consent was obtained from all participants before their enrollment into the study.

All participants were subjected to the following studies: detailed assessment of history and complete clinical examination were performed, and age, BMI, pack-years of smoking (current smokers and ex-smokers), and mean arterial blood pressure (MAP) were recorded. The BODE (Body mass index, airflow Obstruction, Dyspnea, and Exercise capacity) index score was calculated [5]. Dyspnea was assessed using the modified medical research council dyspnea scale (mMRC scale), whereas exercise was assessed by the 6-min walk test, which was carried out according to the ATS [6].

Spirometry was carried out using spirometry Spirosift 5000 manufactured for Fukuda Denshi USA, INC. 7102-A 180th Avenue Northeast Redmond, WA, 98052 by Nippon Systemhouse CO., LTD; made in Japan. Spirometric indices were calculated using the best of three technically satisfactory trials in accordance with the recommendations of the ATS.

Telomere length measurement

Fasting venous blood samples (5 ml) were drawn into EDTA-containing tubes in the morning (08:00–09:00 a.m.) and were stored at −70°C. DNA was extracted from white blood cells. Leukocyte TL was measured using the quantitative real-time method described by Cawthon [7], which measures the relative TL by determining the ratio of telomere repeat copy number (T) to the single-copy gene (S) number (T/S ratio) in experimental samples relative to a reference sample. The target gene in our study was the telomere and the reference gene was the acidic ribosomal phosphoprotein PO (36B4).

The TL of peripheral blood leukocytes were standardized to the reference single-copy gene (S) to yield a T/S ratio using the comparative C_t method

$(T/S = 2^{-\Delta\Delta C_t})$.

The ΔC_t value for each sample was determined by calculating the difference between the C_t value of the target gene and the C_t value of the endogenous reference gene [7].

Statistical presentation and analysis of the study data were carried out using Statistical Package for the Social Sciences (SPSS) version 17. Parametric data were expressed as mean ± SD and nonparametric data were expressed as number and percentage of the total. An unpaired Student *t*-test was used to compare between two groups in quantitative data according to the computer program SPSS for Windows. The analysis of variance test was used for comparison when there were more than two groups in quantitative data. The linear correlation coefficient was used to detect the correlation between two quantitative variables in one group. *P* value greater than 0.05 was considered nonsignificant, *P* value less than 0.05 was considered significant, and *P* value of 0.01 or less was considered highly significant.

Results

Figures 1–8 indicate that the TL ratio in COPD patients shows a statistically significant positive correlation with FVC%, FEV_1%, $FEF_{25–75}$%, SpO_2%, pH, and PaO_2. Moreover, TL ratio showed a significant negative correlation with the BODE index; among BODE index parameters, it was negatively correlated with the dyspnea score.

TL was not correlated with age, smoking/year, smoking duration, MAP, vital capacity, FEV_1/FVC%, $PaCO_2$, HCO_3, BMI, and six-min walk distance (6MWD).

In addition, TL was significantly shorter in patients with very severe COPD (FEV_1 <30) than those with severe COPD (FEV_1 >30) ($P = 0.00$).

Discussion

There is a growing realization that COPD involves several processes present in aging and cellular senescence [8]. Some have suggested that COPD is a disease of accelerated aging [9].

In the current study, peripheral blood leukocytes were assessed for TL not only because they were readily available, easily obtained, and processed, but also because they reflect the amount of stress on immune cells because of cigarette smoke and/or other environmental stresses [10]. Several studies have shown that TL in peripheral blood mononuclear cells is representative of that of many tissues; the intraindividual correlation between TL in different tissues is high [11].

The real-time PCR approach was chosen for TL measurement as a method based on PCR requires a lesser amount of DNA and can be completed in a short time. In addition, this method targets only the telomeric region and does not include the subtelomeric region as the TL restriction fragments method does [7]. For practical reasons, quantitative PCR has become the favored technique in telomere research over the past few years [12]. We have chosen the 36B4 gene as a single-copy gene for normalization because it has already been validated for gene–dosage studies [7].

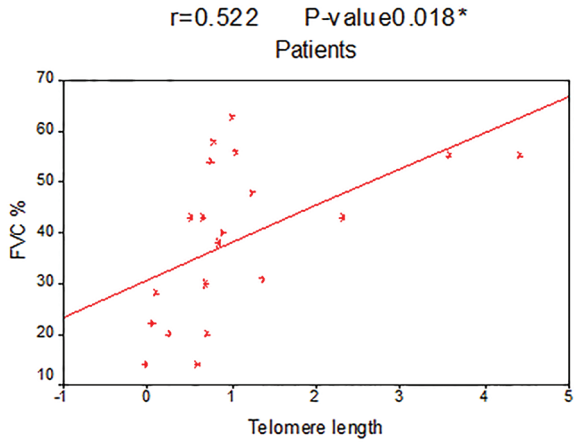
Patients with diseases other than COPD were excluded from the study as many diseases were associated with TL shortening. Also, COPD patients who were receiving systemic steroids were excluded from the study because of the immune modulating effect of steroids [13]. Exposure of human T lymphocytes to cortisol is associated with a significant reduction in telomerase activity [14].

In the current study, the COPD patients and control participants selected were men to avoid TL variation with sex. Many studies confirmed that in adulthood the age-adjusted TL is shorter in men than in women [15–18]. TL in adults may have potential differences because of sex differences and exposures to oxidative stress [19]; also, it may be attributed to potential telomerase upregulation by estrogens [20].

In the current study, there was no significant correlation between TL and BMI in both groups ($P > 0.05$). McGrath *et al.* [18] and Lee *et al.* [9] did not find a significant relationship between TL and BMI.

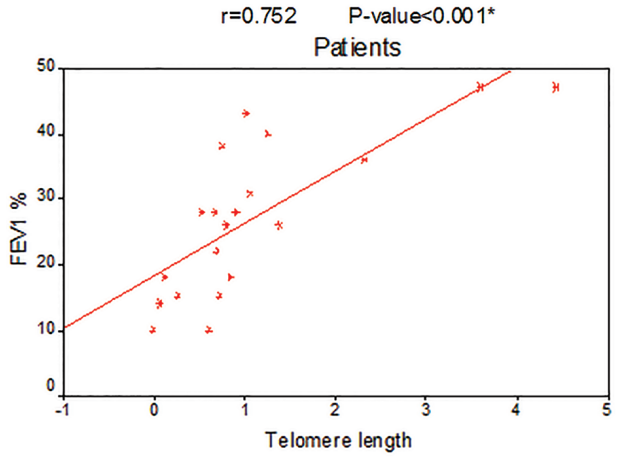
TL showed no significant correlation with MAP. Jeanclos *et al.* [21] reported that TL correlated inversely

Fig. 1



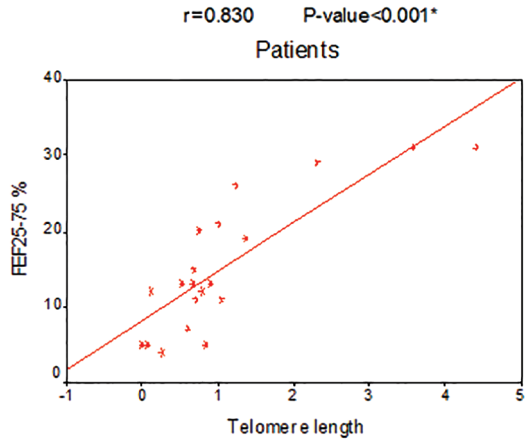
Correlation between telomere length (T/S ratio) and FVC% in the COPD group. COPD, chronic obstructive pulmonary disease; FVC, forced vital capacity.

Fig. 2



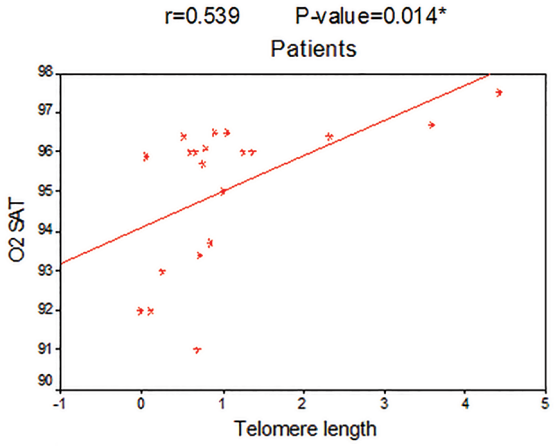
Correlation between telomere length and FEV_1% in the COPD group. COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s.

Fig. 3



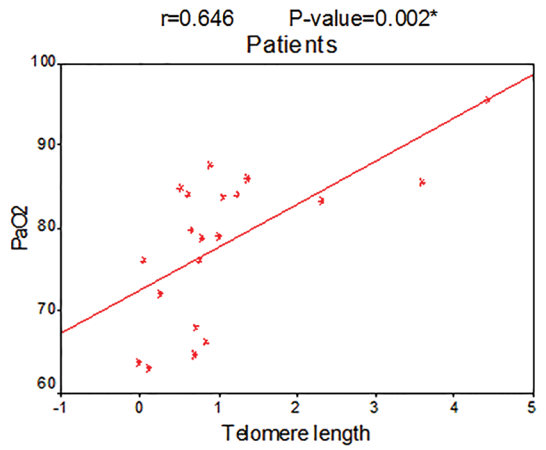
Correlation between telomere length and $FEF_{25–75}$% in the COPD group. COPD, chronic obstructive pulmonary disease; FEF, forced expiratory flow.

Fig. 4



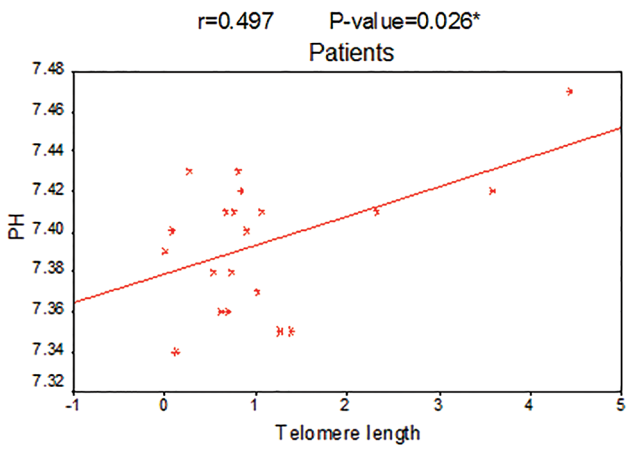
Correlation between telomere length and SpO_2% in the COPD group. COPD, chronic obstructive pulmonary disease.

Fig. 5



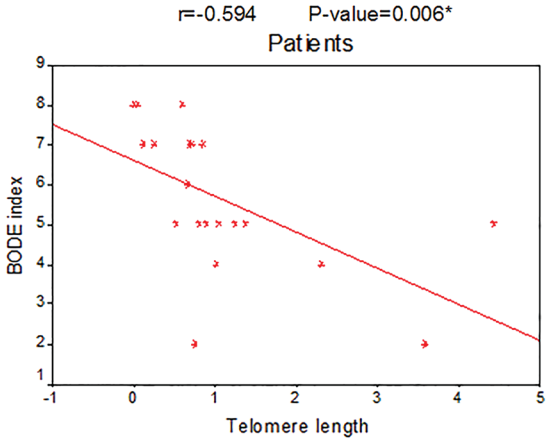
Correlation between telomere length and PaO_2 in the COPD group. COPD, chronic obstructive pulmonary disease.

Fig. 6



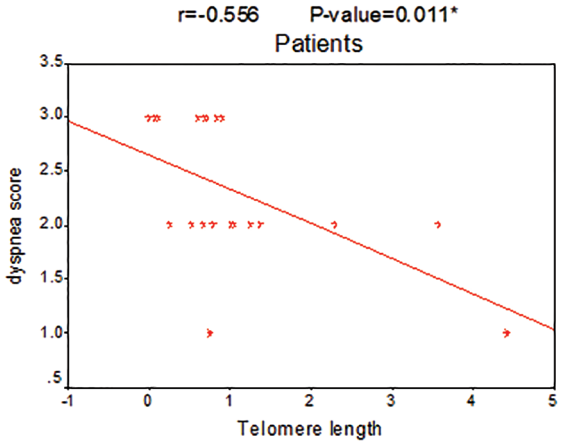
Correlation between telomere length and pH in the COPD group. COPD, chronic obstructive pulmonary disease.

Fig. 7



Correlation between telomere length and the BODE index in the COPD group. BODE, Body mass index, airflow Obstruction, Dyspnea, and Exercise capacity; COPD, chronic obstructive pulmonary disease.

Fig. 8



Correlation between telomere length and dyspnea score in the COPD group. COPD, chronic obstructive pulmonary disease.

with pulse pressure. This difference may be explained by the fact that all participants in our study had normal blood pressure.

TL was significantly shorter in COPD [1.1 ± 1.127 (0.001–4.427)] in comparison with the control group [4.352 ± 2.611 (1.0–8.143)] (Table 1). Thériault *et al.* [22] and Mui *et al.* [23] found that COPD patients had significantly shorter TL compared with healthy controls. Savale *et al.* [2] reported decreased TL in peripheral leukocytes from COPD patients compared with both healthy nonsmoker and smoker participants. Houben *et al.* [24] documented that TL was significantly shorter in peripheral blood lymphocytes of COPD patients than controls. Morlá *et al.* [25] found shorter TL in circulating lymphocytes from smoker COPD compared with healthy controls. Tomita *et al.* [26] found reduced TL of alveolar macrophages (AM) from bronchoalveolar of smokers than nonsmokers, whereas there was no difference between healthy smokers and smokers with COPD and shorter TL in smokers (with and without COPD) than nonsmokers. Tsuji *et al.* [27] reported that TL in alveolar type II cells and endothelial cells was significantly shorter in patients with emphysema than in asymptomatic nonsmokers, but no difference was detected between COPD and non-COPD smokers (Tables 2 and 3).

We studied TL in smoker COPD and healthy controls and found that TL was significantly shorter in smokers (COPD) [1.1 ± 1.127 (0.001–4.427)] than in nonsmoker controls [4.352 ± 2.611 (1.0–8.143)] (Table 1). TL was shorter in current smokers (0.631 ± 0.416) compared with ex-smokers (1.484 ± 1.385); the difference was statistically nonsignificant, but when compared with healthy controls, it showed significant shortening (Table 4). However, TL was not correlated with cigarette smoke exposure (pack-

Table 1 Comparison of all variables between both groups

Indices	Control (n = 11)	COPD (n = 20)	t-test	
			t	P
Demographic data				
Age (years)	50.0 ± 7.0	53.6 ± 6.5	−1.44	0.16
BMI (kg/m^2)	25.5 ± 3.2	26.1 ± 3.9	−0.47	0.63
MAP (mmHg)	92.7 ± 7.1	93.1 ± 6.4	−0.17	0.86
Spirometric indices				
VC%	90.2 ± 4.5	39.0 ± 14.4	11.37	0.00*
FVC%	93.3 ± 4.5	38.7 ± 15.5	11.26	0.00*
FEV_1%	93.6 ± 5.7	27.0 ± 11.8	17.44	0.00*
FEV_1/FVC	84.0 ± 3.4	56.9 ± 9.0	9.47	0.00*
$FEF_{25–75}$%	85.5 ± 6.4	15.1 ± 8.7	23.05	0.00*
ABG parameters				
SpO_2%	96.6 ± 1.2	95.0 ± 1.8	2.43	0.02*
PaO_2 (mmHg)	95.3 ± 2.1	78.0 ± 9.1	6.11	0.00*
$PaCO_2$ (mmHg)	40.5 ± 3.4	41.2 ± 5.2	−0.38	0.70
pH	7.4 ± 0.0	7.3 ± 0.0	1.01	0.31
HCO_3 (mEq/l)	25.0 ± 1.5	24.7 ± 2.8	0.37	0.71
TL	4.35 ± 2.6	1.10 ± 1.12	4.85	0.00*

ABG, arterial blood gas; COPD, chronic obstructive pulmonary disease; FEF, forced expiratory flow; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; MAP, mean arterial blood pressure; SpO_2, oxygen saturation; VC, vital capacity; *Significant difference.

Table 2 Smoking status in the COPD group

Smoking status	Current smokers [n (%)]	Ex-smokers [n (%)]	
COPD	9 (45)	11 (55)	
Pack-year in COPD patients			
	Pack-year		t-test
	Range	Mean ± SD	t	P
Current smoker	37.5–120.0	74.8 ± 30.9	2.49	0.11
Ex-smoker	30.0–80.0	49.4 ± 17.9		

COPD, chronic obstructive pulmonary disease.

Table 3 BODE index in the COPD group

BODE index items	Range	Mean ± SD
BODE index	2.00–8.0	5.6 ± 1.7
BMI (kg/m^2)	20.4–32.3	26.18 ± 3.92
FEV_1%	10.0–47.0	27.00 ± 11.85
6MWD (m)	120.0–350.0	234.00 ± 65.64
Dyspnea score	1.00–3.0	2.3 ± 0.66

BODE, Body mass index; airflow Obstruction, Dyspnea, and Exercise capacity; FEV_1, forced expiratory volume in 1 s; 6MWD; six-min walk distance.

Table 4 Comparison of TL among control participants, current smokers COPD, and ex-smokers COPD

Groups	TL		ANOVA	
	Range	Mean ± SD	F	P
Control	1.0–8.1	4.35 ± 2.61		
COPD current smoker	0.07–1.2	0.63 ± 0.4	12.4	0.001*
COPD ex-smoker	0.001–4.4	1.48 ± 1.3		
	Tukey's test			
Control vs. current smokers	Control vs. ex-smokers	Current smokers vs. ex-smokers		
<0.001	0.002	0.54		

ANOVA, analysis of variance; COPD, chronic obstructive pulmonary disease; TL, telomere length; *Significant difference.

year smoking) (P = 0.87). Although smoking status was not associated with a significant difference in TL, the TL from COPD patients, who all had significant smoking history (on average more than 10 pack-years), was significantly shorter than that of the control participants of the same age. These data suggest that previous cigarette exposure or COPD accelerates telomere attrition, leading to short telomeres. Similar results have been reported by many investigators; Houben *et al.* [24] concluded that shorter TL was not related to smoking exposure in COPD. Mui *et al.* [23] found no association between TL and pack-year, and no difference was found between current and ex-smokers. However, Valdes *et al.* [28] recorded a dose-dependent TL correlation with smoking, and each pack-year smoked was equivalent to an additional five base pair loss of TL. Their results emphasize the proaging effects of cigarette smoking. McGrath *et al.* [18] reported significantly shorter TL in healthy smokers than healthy nonsmokers. They did not observe an association between smoking status (current vs. former; never vs. ever) and TL among control participants. Similarly, Morlá *et al.* [25] have reported previously that TL was reduced in peripheral blood lymphocytes in smokers with and without COPD; they observed a dose–response relationship between cumulative lifetime exposure to tobacco smoking and TL. Lee *et al.* [9] found no significant difference in TL between the quitter group and the smoker groups (sustained quitters vs. intermittent quitters and sustained quitters vs. continuous smokers); however, a significant difference existed when adjusted for age. TL of the control group was significantly longer than all three smoker groups. Tomita *et al.* [26] reported evidence that smoking induces shortening of TL in AMs from both young and old individuals. Their findings suggested that smoking might induce AMs senescence and COPD might be associated with premature aging. Tsuji *et al.* [27] reported no significant differences in TL between patients with emphysema and asymptomatic smokers. Shen *et al.* [29] reported that TL was associated inversely with pack-years of smoking among controls. The significant relationship found between a history of smoking and TL reported in some studies, but not in others, including our study, may be because of differences in smoking history, age, and stages of COPD.

In the present study, no significant correlation was found between TL and years of smoking; however, Shen *et al.* [29] found a significant positive interaction between TL and years of smoking. McGrath *et al.* [18]

reported that age-adjusted TL was five base pair shorter for every pack-year smoked and observed a significant correlation between TL and pack-years of smoking. Hou *et al.* [30] reported that TL tended to decrease with increasing pack-years of cigarette smoking. Several factors may explain this discrepancy, such as different white blood cells that were studied, the fact that our study and that of Morlá *et al.* [25] included only men, and the relatively small population sizes of both studies.

In the present study, TL was shorter in COPD patients than in the healthy controls (Table 1); among COPD patients, it was shorter in those with FEV_1% less than 30% than those with FEV_1% greater than 30%, and it was correlated positively with FVC%, FEV_1%, and $FEF_{25–75}$%. The same results were obtained by Tsuji *et al.* [27] as they reported that TL in type II pneumocytes and endothelial cells was correlated positively with FEV_1%. Schulz *et al.* [31] reported that FEV_1, FVC, but not flow rates, were correlated positively with TL. Mui *et al.* [23] reported a strong, but nonsignificant, correlation between TL and FEV_1% in COPD, whereas a significant positive relationship was found between TL and FEV_1/FVC in COPD, but no association was found between TL and FVC% in COPD. However, Savale *et al.* [2] found no relationship between TL and FEV_1, and FVC, and reported no significant difference in TL between patients with FEV_1 less than or greater than 50%. Lee *et al.* [9] found no significant relationship between TL and FEV_1%. This discrepancy may be explained by the different stages of airflow limitation, which was severe to very severe in our study and moderate to severe in Savale *et al.* [2] and mild to moderate in Lee *et al.* [9].

In the current study, there was a significant positive correlation between TL and SpO_2, pH, and PaO_2, whereas $PaCO_2$ showed an inverse nonsignificant correlation (Figs. 4–6); the same results were obtained by Savale *et al.* [2], who found a strong positive correlation of TL with both PaO_2 and SpO_2 as well as a negative correlation with $PaCO_2$.

In the current study, TL was not correlated with age ($P > 0.05$). The same result was obtained by Lee *et al.* [9], Shen *et al.* [29], and McGrath *et al.* [18]; they found no significant relationship between TL and age. However, many studies observed that TL shortened linearly with age [2,15,24,28,32]. Morlá *et al.* [25] found that TL significantly decreased with age in smokers, but no correlation was found in never-smokers. The exact reason for this discrepancy is unclear; it may be explained by the fact that smoking also reduces TL and attenuates the relationship between chronological age and TL and the narrow age spectrum in our study. It may also be because TL is considered to be a biomarker of biological age rather than chronological age [33].

In the current study, in COPD, there was a significant negative correlation between TL and the BODE index ($r = -0.594$; $P = 0.006$); among the BODE components, the dyspnea score was correlated negatively with TL ($P = 0.011$) (Figs. 7 and 8). Savale *et al.* [2] reported no relationship between TL and the BODE index; among the BODE components, 6MWD was correlated positively with TL. Mui *et al.* [23] found no significant relationship between TL and BMI.

The present study has some limitations that deserve comments. First, the sample size of the present study is relatively small. Second, all patients had severe to very severe COPD; thus, the relationship of TL across the full range of COPD severity is unknown.

Conclusion and recommendations

The results of this study support the link between TL shortening and COPD, which confirms accelerated cellular senescence in COPD.

TL was correlated positively with airflow limitations and it may be related to impaired physical activities in COPD patients. Early smoking cessation for all COPD patients to decrease the rate of cellular senescence is mandatory. Further study of TL involving a large number of patients and different stages of COPD to assess the effect of disease severity is required. A cohort study to detect the rate of telomere attrition in COPD and its relation to morbidity and mortality is required.

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Conflicts of interest

There are no conflicts of interest.

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