Original Article

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TANAFFOS

Telomere Shortening in Blood Leukocytes of Patients with Chronic Obstructive Pulmonary Disease

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Introduction: Chronic obstructive pulmonary disease (COPD) is characterized by airflow limitation that is not completely reversible by administration of inhaled bronchodilators. Many studies propose that telomere length shortening might have occurred in COPD patients. We aimed to determine the telomere length in COPD patients and compare the results of non-smoking and smoking control subjects.

Materials and Methods: In our case-control study, 84 clinically stable COPD patients were recruited on admission to Masih Daneshvari Hospital. Eighty-five healthy controls were also selected including 45 non-smokers and 40 smokers admitted for diseases other than COPD. Spirometry was done for all subjects. Telomere length was measured by quantitative real time PCR as described by Cawthon. The telomere repeat copy number (T) to single-gene copy number(S) ratio was calculated using the comparative C_t method.

Results: The mean ±SD of age was 64.33±10.04 years in patients and 65.06 ±10.02 years in controls (P=0.693). The mean ±SD of FEV_1 was 1.62±0.75 L in patients, 2.84±0.54 L in smoker controls and 2.83±0.56 L in non-smoker controls; significant differences were detected in this regard between cases and controls (P<0.001). T/S ratio was significantly lower in COPD patients (0.61±0.08) than in the control subjects (0.69±0.09) (P<0.001). However, telomere length was shorter in the patients than in controls in each age group (P<0.001). Additionally, there were no statistically significant differences in telomere length between the smoker and non-smoker control subjects. Regarding the correlation between BMI and telomere length, there were no significant differences among the patients and control groups.

Conclusion: In conclusion, we found that telomere length in COPD patients was shorter than that in smoker and non-smoker controls, irrespective of age, sex, spirometric variables, BMI and history of cigarette smoking.

Key words: Chronic obstructive pulmonary disease, Aging, Telomere, Telomere length

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is the most important cause of morbidity and mortality in the world imposing a growing burden on developing countries (1,2). Tobacco use is a known major risk factor of diseases and can alter the structure of tissues and organs especially the respiratory tract (3). According to the World Health Organization (WHO) health topic on the global burden of disease, COPD is estimated to be the third cause of death in 2020 (1).

COPD is characterized by airflow limitation that is not completely reversible by administration of inhaled bronchodilators (4).

Decreased forced expiratory volume in 1 second (FEV_1) and reduced FEV_1/FVC ratio less than 70% are the main spirometric findings of COPD (5-7).

COPD can significantly affect the lungs but it can have other systemic manifestations as well such as inflammatory response, cancer and cardiovascular diseases that may occur as the disease progresses (8). Cardiovascular events and cancer are the two most common causes of mortality particularly in end-stage patients (9,10).

Atherosclerosis, anxiety, depression, osteoporosis and low BMI as age-related conditions are the most common systemic manifestations of COPD (9,11). Such similarities suggest that COPD can relate to advanced age and its prevalence increases with age (5,12). COPD is rare before the age of 40 years but its prevalence increases to 30% after the age 70 (12). Evidence suggests a correlation between COPD and advanced age. Reactive oxygen species and other causes of oxidative stress such as cigarette smoking and noxious gases can promote the process of premature aging (12-14) when the length of telomere reaches to a condition which is called Hay- flick limit(15,16).

Telomeres are DNA sequences with high amounts of G-rich nucleotides located at the end of chromosomes, which shorten with age in all somatic cells after birth (17,18). The end replication problem can lead to telomere shortening with each cellular division in cell cycle (14). Since telomere length determines the number of cell divisions and proliferation rate of somatic cells, it has been considered as a replicometer or biomarker of aging. They protect the end of chromosomes from end to end fusion and genomic instability (19-21).

Impaired balance between the protease and antiprotease and the impaired balance between oxidative stress and antioxidant defense can accelerate the process of telomere shortening.

Furthermore, telomere attrition in circulating blood leukocytes has been suggested as a biomarker of systemic inflammation and oxidative stress and also as a predictor of biological aging (5,10).

Many studies propose that telomere length shortening might have occurred in COPD patients (8,18,12,22).

Although smoking as a trigger of oxidative stress may affect telomere shortening, there are many investigations showing that telomere length decreases in COPD patients irrespective of smoking (10).

In the current study, we aimed to determine the telomere length in COPD patients and compare the results of non-smoking and smoking control subjects.

MATERIALS AND METHODS

Study population

Our case-control study was performed on 84 clinically stable COPD patients confirmed by a pulmonologist according to ATS/ERS criteria recruited on admission to Masih Daneshvari Hospital, Tehran, Iran.

The inclusion criteria consisted of at least 10/pack/year history of tobacco consumption and the ratio of EFV1/FVC less than 70%. The exclusion criteria were presence of cardiovascular diseases, malignancies or other chronic inflammatory diseases.

Eighty-five healthy controls were selected including 45 non-smokers and 40 smokers who were admitted for diseases other than COPD. Their FEV_1 was greater than 80% and FEV_1/FVC ratio was more than 70%. We tried to select sex-matched and age-matched control subjects with±2 years of age.

This study was approved by the Ethical Committee of Tehran University of Medical Sciences and the National Research Institute of Tuberculosis and Lung Disease (NRITLD).

All patients and controls underwent spirometry and their FEV_1, FVC, FEV_1/FVC and BMI were recoded.

Laboratory investigations:

Blood samples were drawn from all patients and controls (5ml) and transferred into EDTA-containing tubes.

All samples were centrifuged at 1000×g for 10 minutes at 4°C and stored at -70°C for DNA extraction.

Genomic DNA was extracted from buffy coats with Qiagen (QIAamp DNA Blood Mini Kit, Germany) and quantified by spectrophotometer (Hitachi 1800, Japan).

Telomere length was measured by quantitative Real Time-PCR as described by Cawthon (23)

All samples were run in triplicate, using the SYBR green method and 35 ng of DNA. The sequences of the telomere primers for tel-1 and tel-2 and 36B4 as single copy gene for normalized technique were as follows:

Tel-1F *5'-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3'*

Tel-2R *5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3'*

Single-1F *36B4F, 5'-CAGCA AGTGGGAAGGTGTAATCC-3*

Single-2R *36B4R, 5'-CCCATTCTATCATCAACGGGTACAA-3'*

The telomere repeat copy number (T) to single-gene copy number(S) ratio was calculated using the comparative C_t method.

Positive controls (extracted from a normal healthy person) and negative controls (DDW+ master mix) were added for every PCR run.

PCR was performed by a BioRad RT-PCR system (BioRad, USA).

Statistical analysis

The data were compared between COPD patients and control subjects including smoker and non-smoker controls. Differences between patients and controls were tested using the Student's t test.

Quantitative variables were recorded as mean± SD and their correlation was analyzed by Pearson's correlation or by non-parametric Kendalls' correlation tests.

P values less than 0.05 were considered significant. Data were analyzed using SPSS for windows (version 22.00, IBM).

RESULTS

The characteristics of understudy subjects are shown in Table 1.Patients and controls did not have statistically significant differences regarding age or sex because of the type of selection as age- and sex-matched.

The mean ±SD of age was 64.33±10.04 years in patients and 65.06 ±10.02 years in controls (P=0.693)(Table 1).

Table 1. Age and sex characteristics of understudy subjects

Variables	Case N=84	Control N=85	Total	P-value
Age(Yrs.)	64.33±10.04	65.06±10.04	64.70±10.02	0.693
Sex				
Male	68 (81.0%)	69 (81.2%)	137 (81.1%)	0.970
Female	16 (19.0%)	16 (18.8%)	32 (18.9%)	

The mean ±SD of FEV_1 was 1.62±0.75 L in patients, 2.84±0.54 L in smoker controls, and 2.83±0.56 L in non-smoker controls, which were significantly different between cases and controls (P<0.001) (Table 2).

In comparison between smoker and non-smoker control subjects, no significant difference was shown in FEV_1 (P=0.968).

Table 2. Comparison between case and control subjects regarding spirometric variables

Variables	Case N=84	Control Smoker N=40	Control Non-smoker N=45	P-value
FEV_1 (L)	1.62±0.75	2.84±0.54	2.83±0.56	<0.001
FEV_1/FVC (%)	62.11±7.63	79.2±4.78	79.93±4.48	<0.001

The mean ±SD of FEV_1/FVC was 62.11±7.63% in patients, 79.93±4.48% in smoker controls and 79.20±4.78 percent in non- smoker controls; these values were significantly different between cases and controls (P<0.001) (Table 2).

In comparison between smoker and non-smoker control subjects, no significant difference was noted in FEV_1/FVC (P=0.499).

T/S ratio was significantly lower in COPD patients (0.61±0.08) than in control subjects (0.69±0.09) (P<0.001) (Figures 1 and 2).

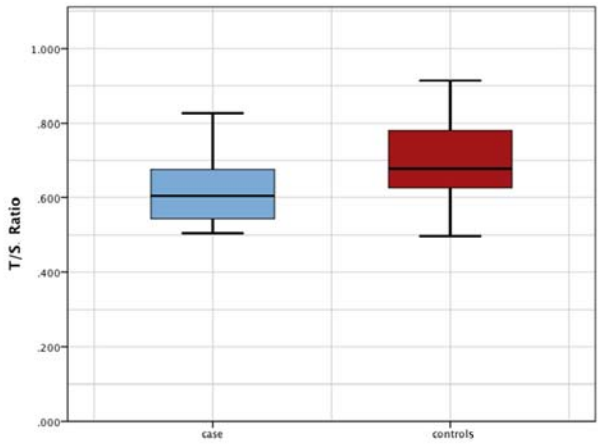

Figure 1. Comparision between COPD patients and control subjects regarding telomere length

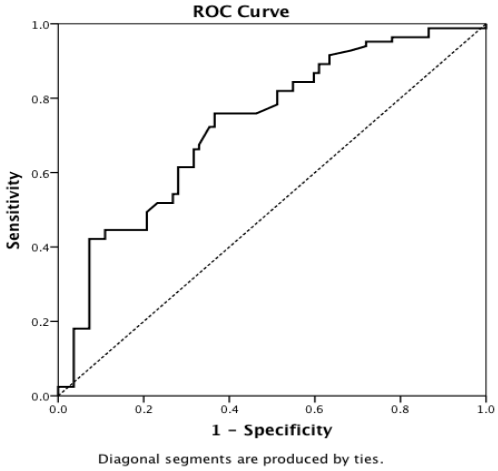

Figure 2. ROC curve distinguishing control subjects from patients by telomere length.

However, telomere length was shorter in the patients than in the controls in each age group (P<0.001).

Additionally, there were no statistically significant differences in telomere length between the smoker and non-smoker control subjects.

The mean±SD values of telomere length in patients and controls based on sex are listed in Table 3. There were no statistically significant differences in telomere length and sex between patients and control subjects.

After stratification of patients into three age groups of <50 years, 50-60 years and > 60 years, the differences between patients and controls in telomere length remained (Figure 3).

Regarding the correlation between BMI and telomere length, there were no significant differences among the three understudy groups (Table 4).

Table 3. Comparison of patients and control subjects in terms of sex and telomere length

Group		Sex	N	Mean	Std. deviation	P-value
Case	T/S ratio	Male	68	.61448	.080547	0.841
		Female	16	.60975	.098851	
Control smoker	T/S ratio	Male	35	.66082	.087778	0.645
		Female	5	.66560	.123488	
Control non-smoker	T/S ratio	Male	34	.65991	.088084	0.944
		Female	11	.66209	.094236	

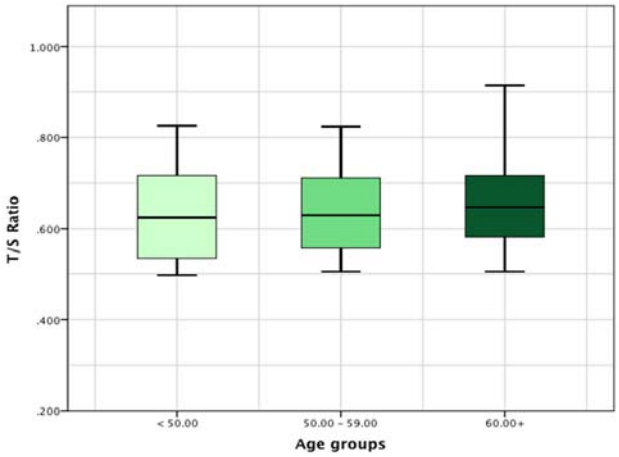

Figure 3. Comparision among the three age groups regarding telomere length

Table 4. Correlation between BMI and telomere length among patients and control subjects

Group				BMI
Case	Kendall's tau_b	T/S Ratio	Correlation coefficient	-0.038
			Sig. (2-tailed)	0.614
			N	84
Control smoker	Kendall's tau_b	T/S Ratio	Correlation coefficient	-0.109
			Sig. (2-tailed)	0.339
			N	40
Control non smoker	Pearson's correlation	T/S Ratio	Correlation coefficient	-0.042
			Sig. (2-tailed)	0.783
			N	45

DISCUSSION

The main finding of the present study was that the telomere length in COPD patients was shorter than that in smoker and non-smoker controls, irrespective of age, sex, spirometric data, BMI and smoking. Our results were in accord with those of Savale et al (10).

Telomere length decreased in both male and female COPD patients compared with the control subjects.

Savale et al, (10) in their study showed that telomere length was shorter in males than females only in control subjects. We found no sex-related statistically significant difference in telomere length between patients and controls.

We found no relationship between telomere length and smoking in patients or controls.

Morla et al. (24), Valdes et al. (25), and Chan et al. (26) showed the effect of cigarette smoking on telomere shortening.

Morla and colleagues (24) in their study, done by fluorescence in situ hybridization (FISH) reported a dose-dependent relationship between telomere length and exposure to smoking. They could not show the influence of smoking on telomere length based on presence of COPD.

In contrast with their results, we showed that telomere length was affected by COPD irrespective of cigarette smoking. Our data are in accordance with those of other previous studies (10,12,14).

Although cigarette smoking, among the oxidative stress agents, can play an important role in cellular senescence and possibly telomere shortening, our findings similar to those of other studies (10,12) confirm that the role of COPD in telomere shortening is more prominent than that of smoking.

Tsuji et al. found that the telomere length in alveolar type II cells of patients with emphysema was shorter than in control subjects (27).Muller et al. reported that telomere length did not differ in cultured parenchymal fibroblasts of patients with emphysema(28).

Adler et al, in their experimental investigation found that a short telomere can act as a predisposing factor, which increases the susceptibility to cigarette smoking-induced emphysema in mice (29).

In our study, similar to a previous study (10), no correlation was found between telomere shortening and change in spirometric variables such as FEV_1, FVC and FEV_1/FVC.

We found no relationship between BMI and telomere length in our current investigation similar to Savale's study. In contrast, Valdes et al, (25) and Cui et al. (30) found a correlation between telomere shortening and obesity in women.

Finally, our findings regarding decreased telomere length in COPD patients can support the involvement of accelerated aging in pathogenesis of this disease, and measurement of telomere length can be considered as a diagnostic biomarker for COPD as well as premature aging.

Similarities between COPD and aging suggest that COPD can relate to advanced age, and its prevalence increases with age (5,12,22,31).

In conclusion, we found that telomere length in COPD patients was shorter than in smoker and non-smoker controls, irrespective of age, sex, spirometric variables, BMI and history of cigarette smoking.

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