Turkish Journal of Thoracic and Cardiovascular Surgery 2021;29(3):377-383
http://dx.doi.org/doi: 10.5606/tgkdc.dergisi.2021.20522

Original Article / Özgün Makale

The role of telomerase in the etiology of primary spontaneous pneumothorax

Primer spontan pnömotoraks etiyolojisinde telomerazın rolü

Mehmet Akif Tezcan[1], İbrahim Ethem Özsoy[1], Fatih Gürler[1], Çiğdem Karakükçü[2]

[1]Department of Thoracic Surgery, University of Health Sciences, Kayseri Health Practice and Research Center, Kayseri, Turkey
[2]Department of Medical Biochemistry, University of Health Sciences, Kayseri Health Practice and Research Center, Kayseri, Turkey

ABSTRACT

Background: This study aims to investigate the role of telomerase activity in the risk of primary spontaneous pneumothorax, which is most frequently encountered in the practice of thoracic surgery.

Methods: A total of 61 patients (56 males, 5 females; median age: 29.4 years; range, 17 to 43 years) who underwent treatment for primary spontaneous pneumothorax and 19 age- and sex-matched healthy controls (10 males, 9 females; median age: 29.1 years; range, 23 to 43 years) were included in this prospective study between January 2018 - August 2018. Telomerase activity was evaluated with enzyme-linked immunosorbent assay. The correlation between telomerase activity and clinical and demographic parameters was examined.

Results: The mean serum telomerase level was 3.4±0.6 ng/mL in the primary spontaneous pneumothorax group and 1.9±0.5 ng/mL in the control group, indicating significantly higher levels in the patient group (p<0.001). There was no significant association between the telomerase levels and presence of blebs and/or bullae on thoracic computed tomography, extent of pneumothorax, laterality (right, left, or bilateral), and pack years of cigarette smoking.

Conclusion: Telomerase levels of patients with primary spontaneous pneumothorax are significantly higher than healthy individuals. Future genetic studies may ultimately clarify a potential relationship between primary spontaneous pneumothorax and short telomere syndrome.

Keywords: Cigarette, spontaneous pneumothorax, telomerase, telomere.

ÖZ

Amaç: Bu çalışmada göğüs cerrahisi pratiğinde sıklıkla karşılaşılan primer spontan pnömotoraks riskinde telomeraz aktivitesinin rolü araştırıldı.

Çalışma planı: Bu prospektif çalışmaya Ocak 2018 - Ağustos 2018 tarihleri arasında primer spontan pnömotoraks nedeniyle tedavi edilen toplam 61 hasta (56 erkek, 5 kadın; medyan yaş: 29.4 yıl; dağılım, 17-43 yıl) ve yaş ve cinsiyet açısından eşleştirilmiş 19 sağlıklı kontrol (10 erkek, 9 kadın; medyan yaş: 29.1 yıl; dağılım, 23-43 yıl) alındı. Telomeraz aktivitesi enzim bağlı immünosorbent testi ile değerlendirildi. Telomeraz aktivitesi ile klinik ve demografik parametreler arasındaki ilişki incelendi.

Bulgular: Ortalama serum telomeraz düzeyleri primer spontan pnömotoraks grubunda 3.4±0.6 ng/mL ve kontrol grubunda 1.9±0.5 ng/mL olup, hasta grubunda düzeyler anlamlı ölçüde daha yüksekti (p<0.001). Telomeraz düzeyleri ile toraks bilgisayarlı tomografide bleb ve/veya bül varlığı, pnömotoraks derecesi, taraf (sağ, sol veya iki taraflı) ve sigara paket yılı arasında anlamlı bir ilişki yoktu.

Sonuç: Primer spontan pnömotorakslı hastaların telomeraz düzeyleri, sağlıklı bireylere kıyasla, anlamlı ölçüde daha yüksektir. İleride yapılacak genetik çalışmalar, primer spontan pnömotoraks ile kısa telomer sendromu arasındaki olası ilişkiyi daha net olarak ortaya koyabilir.

Anahtar sözcükler: Sigara, spontan pnömotoraks, telomeraz, telomer.

Gene stability is critical to the organism's life and health. Complex hemochromatin structures at the end of linear chromosomes contain numerous distinct protein elements.[1] Telomeres are the regions of hemochromatin which contain specialized deoxyribonucleic acid (DNA) repeat sequences that serve to protect the chromosome and to ensure gene stability.[2,3]

Received: July 23, 2020 *Accepted:* November 09, 2020 *Published online:* July 26, 2021

Correspondence: Mehmet Akif Tezcan, MD. SBÜ, Kayseri Sağlık Uygulama ve Araştırma Merkezi, Göğüs Cerrahisi Kliniği, 38080 Kayseri, Türkiye.
Tel: +90 352 - 315 77 00 e-mail: mehmetakiftercan@gmail.com

Cite this article as:
Tezcan MA, Özsoy İE, Gürler F, Karakükçü Ç. The role of telomerase in the etiology of primary spontaneous pneumothorax. Turk Gogus Kalp Dama 2021;29(3):377-383

Human telomeres consist of consecutive TTAGGG repeats. The length of these tandem repetitions is approximately 10,000 to 15,000 base pairs. The end regions of the telomeres are single-chain structures. Telomerases and telomerase activity function to protect the telomeres. Telomerase activity results in telomere elongation. This is critical since, at the end of each duplication event, a small number of base pairs at the end of each chromosome disappear; after numerous duplication events, overt shortening and loss of chromosomal material can be observed. The actions of telomerase address this issue, as chromosomes can be returned to their critical length and structure, thereby, triggering irreversible growth cessation and ageing.[1-4] A pivotal study demonstrated that, in the absence of telomerase, DNA replication resulted in the physical loss of the end portions of all the chromosomes, leading to a defined number of cell divisions, after which cell death was ensued.[5] Short telomeres are associated with an increased risk of cardiovascular disease, cirrhosis of the liver, hypertension, atherosclerosis, and cancer.[6] Although the telomere length has a genetic basis, environmental factors, air pollution, genotoxic stress, and smoking may accelerate the telomeric loss.[6]

Primary spontaneous pneumothorax (PSP) occurs in the absence of clinical or radiological evidence of lung disease or trauma history. Although the diagnosis is in no way rare, the etiology of PSP is still unknown. Independent risk factors for PSP include lung fibrosis, general frailty, young age, and the increased body length-to-width ratio. Another critical etiologic factor is smoking in otherwise healthy men.[5] Given our lack of understanding with respect to disorder, we aimed to investigate the role of telomerase activity in the risk of PSP. To the best of our knowledge, this is the first study to consider telomerases and telomerase activity as having a role in the pathogenesis of this condition.

PATIENTS AND METHODS

This single-center, prospective study was conducted at University of Health Sciences, Kayseri Health Practice and Research Center, Department of Thoracic Surgery between January 2018 and August 2018. A total of 61 patients (56 males, 5 females; median age: 29.4 years; range, 17 to 43 years) who underwent treatment for PSP and 19 age- and sex-matched healthy controls (10 males, 9 females; median age: 29.1 years; range, 23 to 43 years) were included. Only the patients having no chronic diseases (i.e., chronic obstructive pulmonary disease [COPD], asthma, hypertension, coronary artery disease, congestive heart failure, and diabetes mellitus) and undergoing thoracic computed tomography (CT) for screening of PSP risk were included. A written informed consent was obtained from each participant. The study protocol was approved by the Adana City Education and Research Hospital Ethics Committee (Date: 27.03.2018/No:176). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Data including age, sex, number of PSP episodes, presence of chronic disease, telomerase activity, cigarette smoking, extent of pneumothorax, result of CT (bronchoalveolar thickening, localized emphysema, and blebs and/or bullae), treatment modality (observation, tube thoracostomy and video-assisted thoracoscopic surgery [VATS] or thoracotomy), length of hospital stay, chest tube drainage duration, neutrophil-to-leukocyte ratio (NLR), and family history were recorded from all participants. The extent of pneumothorax was calculated in percentages by the method described by Kircher and Swartzel.[7] The patients were further divided into two groups according to the size of pneumothorax: partial (small or moderate pneumothorax, <50%) and total (large pneumothorax, ≥50%).

Telomerase levels were evaluated in serum samples with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Elabscience Biotechnology Co., Ltd, Bethesda, MD, USA) using the sandwich-ELISA principle. The micro-ELISA plate provided in this kit is pre-coated with an antibody specific to human telomerase. The sensitivity of the test per the manufacturer's instructions was 0.1 ng/mL, and the detection range is 0.16 to 10 ng/mL.

Statistical analysis

Statistical analysis was performed using the IBM SPSS version 22.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean ± standard deviation (SD), median (min-max) or number and frequency, where applicable. The Kolmogorov-Smirnov normality test were applied to the data from each of the groups. Parametric tests were used for normally distributed variables, while non-parametric tests were used for non-normally distributed variables. Analysis of variance was performed for to compare more than two variables. The Spearman and Pearson correlation analyses were used for correlation analysis. A p value of <0.05 was considered statistically significant.

RESULTS

Demographic and clinical characteristics of the study population are shown in Table 1. There was

Table 1. Demographic and clinical data of patients

	PSP group (n=61)			Control group (n=19)		
	n	%	Mean	n	%	Mean
Age (year)			29.4			29.1
Sex						
Males	56			10		
Females	5			9		
Laterality						
Left	30	49.2				
Right	31	50.8				
Size of pneumothorax						
Partial	41	67.2				
Total	20	32.8				
Episode						
First	49	80.4				
Second	10	16.4				
Third	2	3.2				
Smoking states						
Smoker	47	77		1	5.3	
Never	14	23		18	94.7	
Length of hospital stay (days)			6.9			
Therapy						
Observation	5	8.2				
Tube drainage	46	75.4				
Videothoracoscopy	5	8.2				
Thoracotomy	5	8.2				

PSP: Primary spontaneous pneumothorax.

no significant difference in the age between the two groups (p=0.8). Telomerase activity was not associated with age or sex (p=0.09 and p=0.24, respectively). There were no associations between age and the side on which pneumothorax developed (p=0.68), the number of episodes (p=0.95), length of hospital stay (p=0.16), duration of chest tube drainage (p=0.27), NLR (p=0.63), and treatment modality (p=0.68).

The mean telomerase levels in blood samples from the study participants were measured as 3.4±0.6 ng/mL in the PSP group and 1.9±0.5 ng/mL in the healthy

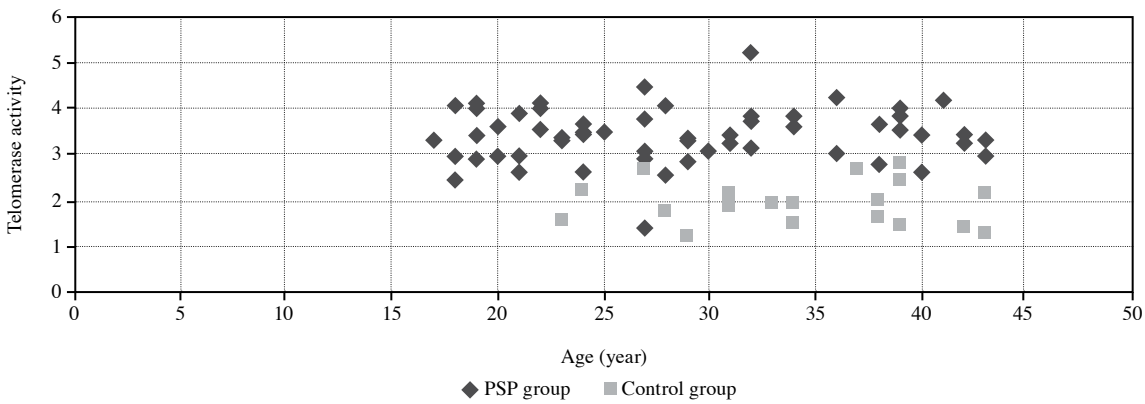

Figure 1. The distribution of telomerase activities of PSP and control groups.
PSP: Primary spontaneous pneumothorax.

Table 2. Correlation of smoking with NLR, telomerase activity, and bullae distribution

PSP group smoker	n=12[1]	n=10[2]	n=25[3]	*p*
PCS	5.94	6.89	6.38	0.34
Telomerase activity	3.47	3.48	3.36	0.25
NLR	2.65	2.92	4.17	0.54
BBE	8	8	8	0.22

NLR: Neutrophil-to-leukocyte ratio; PSP: Primary spontaneous pneumothorax; PCS: Pack years of cigarette smoking; BBE: Bullae/Bleb/Emphysema; [1]: <6 months; [2]: 6-12 months; [3]: >13 months.

controls (Figure 1). Telomerase levels were significantly different between the two groups (p<0.001).

Thirty (49.2%) patients had left-sided pneumothorax, while 31 (50.8%) patients had right-sided pneumothorax. There was no significant relationship between the telomerase levels and the side on which pneumothorax developed (p=0.85). Similarly, 41 (67.2%) of the patients had a partial pneumothorax, whereas 20 (32.8%) were diagnosed with total pneumothorax. Totally, 49 (80.4%) of the patients reported this event as their first episode of pneumothorax. Of the remaining patients, 10 (16.4%) experienced a second episode and two (3.2%) experienced a third episode of pneumothorax. There was no significant relationship between the number of episodes and type of pneumothorax (partial or total) (p=0.84). Telomerase activity was not associated with the number of episodes or partial/ total/ bilateral pneumothorax (p=0.15 and p=0.63, respectively).

In the PSP group, 47 (77%) patients were smokers and 14 (23%) were non-smokers. In the control group, only one participant (5.3%) was a smoker and 18 (94.7%) were non-smokers. The pack years of cigarette smoking, NLR, distribution of bullae in the PSP group and telomerase levels are shown in Table 2. The relationship between the pack years of cigarette smoking and episodes of PSP is shown in Table 3. No significant associations were found between telomerase levels and smoking status, number of cigarettes smoked per day, or number of years of smoking (p=0.4). Similarly, no significant differences were found between the number of episodes of PSP and side of the current pneumothorax, smoking status, number of cigarettes per day, or duration of smoking (p=0.1).

Evaluation of CTs from the patients with PSP revealed that 65.6% (n=40) of the images included bronchoalveolar thickening, localized emphysema, and blebs and/or bullae. These findings were not detected in 34.4% of the scans (n=21). Bullous structures were observed in six of the patients who were non-smokers. There were no significant associations between the levels of telomerase activity and presence or absence of bullous structures (p=0.3).

According to treatment modality, five of the patients (8.2%) improved with oxygen therapy, 46 patients (75.4%) underwent tube thoracostomy, and 10 patients (16.4%) required an additional surgical intervention including VATS (8.2%, n=5) or thoracotomy (8.2%, n=5). There was no significant correlation between the telomerase levels and treatment protocols (observation, tube thoracostomy, and surgical intervention; p=0.78). The median duration of chest tube drainage was 5.5 (range, 3 to 14) days among the patients who underwent tube thoracostomy. The median length of hospital stay was 6.9 (range, 3 to 17) days for all patients. There was no significant relationship between telomerase levels and length of time of drainage and length of hospital stay (p=0.26 and p=0.7, respectively).

Table 3. Correlation between cigarette smoking and PSP episode

PSP group			
Cigarette smoking	1st PSP Episode	2nd PSP Episode	3rd PSP Episode
<6 months	10	2	-
6-12 months	8	1	5
>13 months	19	1	1

PSP: Primary spontaneous pneumothorax.

In the PSP group, the median NLR at the time of admission was 3.6 (range, 0.86 to 31.6). No significant correlation was found between the telomerase activity and NLR (p=0.29). Moreover, there was no significant association between NLR and number of episodes of PSP (p=0.72), laterality (p=0.56), extent of pneumothorax (p=0.76), drainage duration (p=0.35), and length of hospital stay (p=0.26). Neither the patients, nor the healthy controls had any history or underlying evidence of chronic disease (i.e., COPD, asthma, hypertension, coronary artery disease, congestive heart failure, and diabetes mellitus).

DISCUSSION

Several studies focusing on telomerase activity and short telomere syndrome have been conducted in patients with different disorders, including COPD, dyskeratosis congenita, acquired aplastic anemia, pulmonary fibrosis, liver disease, esophageal cancer, head and neck squamous-cell carcinomas, skin and anorectal cancers, acute myeloid leukemia, heart disease, and acute myocardial infarction.[6,8,9] However, to the best of our knowledge, this is the first study to examine telomerase activity in PSP.

Primary spontaneous pneumothorax is diagnosed frequently in a standard clinical setting and reports have shown that there are between 6 and 24 cases per 100,000 individuals annually.[10] A study in Denmark reported that the national incidence of PSP was actually lower than previously reported, at 7.3 per 100,000 individuals. There are also significant differences in the incidence rates between the two sexes; the incidence of PSP among men is approximately six times higher than that among women.[11] Previous reports from single-center studies conducted 45 years ago in the United States and 30 years ago in Sweden have shown that the incidence of PSP is 7.4 to 18 per 100,000 and 1.2 to 6 per 100,000 annually in men and women, respectively.[12,13] More recently, two large epidemiological studies from the United Kingdom and France have indicated a rising trend with respect to this disorder, with a reported combined hospital admission rate for PSP and secondary spontaneous pneumothorax as 16.7 to 22.7 per 100,000.[14,15] The incidence of PSP among men was observed to reach its peak at the ages of 16 to 25 years, similar to that reported in previous studies.[11,12,14,15] In a study conducted in Turkey, PSP occurred most frequently in the second decade of life.[16] The factors underlying this sex difference still remain unknown, but may relate to more active tobacco consumption and specific anthropometric characteristics in men.[17]

Sex has been shown to be associated with the telomere length in females having longer telomeres on average than males.[18] An earlier study reported that recurrence rates were higher in tall men and women,[19] while another study showed a relationship between low body mass index and bilateral and contralateral pneumothoraces.[20] The increased chest length contributes to the increase in subpleural bullae. Pleural pressure decreases by about 0.20 cmH_2O per cm going from cephalad to caudad; as such, negative pressure at the apex increases in individuals who are unusually tall. Consequently, alveoli in the lung apex are subjected to greater pressures. Over time, the pressure differential results in the formation of subpleural blebs and genetic factors may be predisposing factors with respect to bleb formation. Bullae and blebs have been shown to be associated with the pathogenesis of the PSP. Destructive processes, such as those associated with emphysema, promote the formation of bullous structures in the lung parenchyma. Among patients with PSP, emphysema-like changes (ELCs) were identified in 89% of same-side PSPs and 80% among those occurring bilaterally.[21] Alpha-1-antitrypsin deficiency is genetically associated with ELCs; however, this underlying disorder explains only 1 to 2% of severe cases of emphysema.

Both emphysema and idiopathic pulmonary fibrosis (IPF) belong to a group of disorders associated with the shortened telomere lengths, also known as short telomere syndrome. A recent study has revealed that 90% of those with emphysema and IPF have short telomere syndrome.[22] These disorders are associated with a mutation in the gene encoding the catalytic subunit, hTERT.[23] Telomerase mutations and short telomere syndrome may be directly associated with emphysema secondary to smoking. In emphysemas associated with telomere mutations, destruction of the air cavities increases the risk of pneumothorax via its impact at the lung apex. Interestingly, pneumothorax occurs more often than COPD among patients with emphysema associated with telomerase mutations.[21] However, there are no studies that document a cause-and-effect relationship between ELCs and PSP.

In the present study, bullae and blebs were identified during thoracotomy, VATS, or sternotomy. In a series of studies, bullae and blebs were reported at a rate of 48 to 100%.[24] There were no significant relationships between anatomical structure, number and distribution of the bullae and blebs, and rate of recurrence. In our study, recurrence was observed in 16.4% of patients who were treated with oxygen and tube thoracostomy. Although the bullous structures were present in 40 (65.6%) of 61 patients as evaluated

by CT, 21 (34.4%) had no bullous structures. There was also no significant relationship between the telomerase activity and presence of bullous structures. Nonetheless, pathophysiological changes due to diffuse respiratory bronchiolitis found in smokers have a significant impact on the rate of recurrence of PSP. Deterioration of the small airways depends on the number of cigarettes smoked. Defects with respect to lung expansion can result in pneumothorax; the size and extent of the pneumothorax increase proportionally with the defects involved.[25] There was no significant relationship between the telomerase activity and the extent of pneumothorax in our study.

Smoking is associated with a shorter telomere length.[26] The coal-tar pitch extract alters the telomere length and telomerase activity.[27] Inflammatory changes may play a role in promoting the pathogenesis of PSP. The risk of PSP increases nine times in men and 22 times in women among those who smoke and depends directly on the number of cigarettes consumed daily. There is a definitive relationship between the size of the pneumothorax and cigarette smoking, as well as smoking amount (packs per year; $p<0.001$). Smoking has a reversible impact on the pathogenesis of spontaneous pneumothorax, although it takes a substantial amount of time for the effects of smoking to disappear.[28] In this study, the patients who were non-smokers had also a high rate of recurrence. The pathogenesis of pneumothorax in these patients may be different from those associated with smoking. In the future, we would emphasize the fact that non-smoking status is an independent risk factor for recurrence of PSP, once existing lung disease has been excluded. No significant relationships were identified between telomerase levels and pack years of cigarette smoking in the present study.

Inflammatory activity is frequently proposed as a contributor to biological aging in general, and leukocyte telomere shortening, in particular.[29,30] Numerous studies have revealed that the infiltration of inflammatory cells, particularly macrophages, undergoes an increase in the small airways of smokers.[29,30] Endobronchial obstruction due to the accumulation of inflammatory cells between the pulmonary parenchyma and bronchial tree may result in the increased pressure in the alveolar tissue, thereby, promoting the parenchymal rupture. This hypothesis is supported by the increased incidence of pneumothorax in combat pilots and divers subjected to sudden changes in the environmental pressure.[31] A strong association between the PSP with low atmospheric pressure and significant pressure reduction in a study conducted in Turkey was shown previously.[32] Histopathological examination and electron microscopic analysis of the tissues of patients who underwent bullectomy after spontaneous pneumothorax revealed obstruction and stenosis in the distal airways, as well as inflammation in the bronchial wall, and peribronchial fibrosis. In our study, no significant relationship between the NLR and the number of episodes, the side on which pneumothorax developed, type of pneumothorax (partial or total), duration of tube thoracostomy or duration of hospital stay was identified. There were also no significant relationship between the NLR and telomerase levels.

Familial cases of pneumothorax are quite rare, and the mode of inheritance of this disorder has not been elucidated yet. Although mutations in the gene encoding folliculin (FLCN) are rare, this finding may be also implicated in the pathogenesis of PSP. In the lungs, FLCN found in the connective tissue of cells allows the lungs to contract and expand while breathing. It may also play a role in the repair of the damaged lung tissue. However, it is still unclear how FLCN gene mutations lead to the formation of blebs and increase the risk of PSP. One of the proposed theories is that the altered FLCN protein may induce inflammation within the lung tissue, thereby, promoting damage and blebs.[33] In our study, we identified two patients with a family history of PSP.

The main limitations of the present study are its single-center design with a relatively small sample size. Excluding those over the age of 45 years and those with chronic diseases is also another limitation. In addition, genetic mutations with telomerase activity were unable to be analyzed in this study.

In conclusion, our study results showed a significant relationship between primary spontaneous pneumothorax and serum telomerase levels in our patient cohort, although this finding alone did not reveal significant information on the etiology of this disorder. The high levels of telomerase in young adults may be among the factors which can predict the development of primary spontaneous pneumothorax in later life. Therefore, serum telomerase levels can be used as an influencing factor for the treatment planning and prognosis of the disease in the future. However, further large-scale, long-term, prospective studies are needed to draw a firm conclusion.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

REFERENCES

1. Kaminker PG, Kim SH, Desprez PY, Campisi J. A novel form of the telomere-associated protein TIN2 localizes to the nuclear matrix. Cell Cycle 2009;8:931-9.
2. Wood AM, Laster K, Rice EL, Kosak ST. A beginning of the end: New insights into the functional organization of telomeres. Nucleus 2015;6:172-8.
3. Cusanelli E, Chartrand P. Telomeric noncoding RNA: Telomeric repeat-containing RNA in telomere biology. Wiley Interdiscip Rev RNA 2014;5:407-19.
4. Ouellette MM, McDaniel LD, Wright WE, Shay JW, Schultz RA. The establishment of telomerase-immortalized cell lines representing human chromosome instability syndromes. Hum Mol Genet 2000;9:403-11.
5. Olovnikov AM. A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. J Theor Biol 1973;41:181-90.
6. Calado RT, Young NS. Telomere diseases. N Engl J Med 2009;361:2353-65.
7. Kircher LT, Swartzel RL. Spontaneous pneumothorax and its treatment. JAMA 1954;155:24-29.
8. Young NS. Telomere biology and telomere diseases: Implications for practice and research. Hematology Am Soc Hematol Educ Program 2010;2010:30-5.
9. Garcia CK, Wright WE, Shay JW. Human diseases of telomerase dysfunction: Insights into tissue aging. Nucleic Acids Res 2007;35:7406-16.
10. Tschopp JM, Bintcliffe O, Astoul P, Canalis E, Driesen P, Janssen J, et al. ERS task force statement: Diagnosis and treatment of primary spontaneous pneumothorax. Eur Respir J 2015;46:321-35.
11. Olesen WH, Titlestad IL, Andersen PE, Lindahl-Jacobsen R, Licht PB. Incidence of primary spontaneous pneumothorax: A validated, register-based nationwide study. ERJ Open Res 2019;5:00022-2019.
12. Melton LJ 3rd, Hepper NG, Offord KP. Incidence of spontaneous pneumothorax in Olmsted County, Minnesota: 1950 to 1974. Am Rev Respir Dis 1979;120:1379-82.
13. Bense L, Eklund G, Wiman LG. Smoking and the increased risk of contracting spontaneous pneumothorax. Chest 1987;92:1009-12.
14. Gupta D, Hansell A, Nichols T, Duong T, Ayres JG, Strachan D. Epidemiology of pneumothorax in England. Thorax 2000;55:666-71.
15. Bobbio A, Dechartres A, Bouam S, Damotte D, Rabbat A, Régnard JF, et al. Epidemiology of spontaneous pneumothorax: Gender-related differences. Thorax 2015;70:653-8.
16. Çelik B, Nadir A, Şahin E, Kaptanoğlu M, Demir H, Furtun K. Nüks spontan pnömotorakslı olgularda risk faktörleri, klinik ve radyolojik değerlendirme. Turk Gogus Kalp Dama 2008;16:107-12.
17. Lippert HL, Lund O, Blegvad S, Larsen HV. Independent risk factors for cumulative recurrence rate after first spontaneous pneumothorax. Eur Respir J 1991;4:324-31.
18. Gardner M, Bann D, Wiley L, Cooper R, Hardy R, Nitsch D, et al. Gender and telomere length: Systematic review and meta-analysis. Exp Gerontol 2014;51:15-27.
19. Sadikot RT, Greene T, Meadows K, Arnold AG. Recurrence of primary spontaneous pneumothorax. Thorax 1997;52:805-9.
20. Huang TW, Lee SC, Cheng YL, Tzao C, Hsu HH, Chang H, et al. Contralateral recurrence of primary spontaneous pneumothorax. Chest 2007;132:1146-50.
21. Mitlehner W, Friedrich M, Dissmann W. Value of computer tomography in the detection of bullae and blebs in patients with primary spontaneous pneumothorax. Respiration 1992;59:221-7.
22. Armanios M. Telomerase and idiopathic pulmonary fibrosis. Mutat Res 2012;730:52-8.
23. Armanios M, Chen JL, Chang YP, Brodsky RA, Hawkins A, Griffin CA, et al. Haploinsufficiency of telomerase reverse transcriptase leads to anticipation in autosomal dominant dyskeratosis congenita. Proc Natl Acad Sci U S A 2005;102:15960-4.
24. Guo Y, Xie C, Rodriguez RM, Light RW. Factors related to recurrence of spontaneous pneumothorax. Respirology. 2005;10:378-84.
25. Sayar A, Kök A, Citak N, Metin M, Büyükkale S, Gürses A. Size of pneumothorax can be a new indication for surgical treatment in primary spontaneous pneumothorax: A prospective study. Ann Thorac Cardiovasc Surg 2014;20:192-7.
26. Latifovic L, Peacock SD, Massey TE, King WD. The influence of alcohol consumption, cigarette smoking, and physical activity on leukocyte telomere length. Cancer Epidemiol Biomarkers Prev 2016;25:374-80.
27. Feng F, Yang Y, Li Z, Song J, Zhu H, Wang L, et al. Changes in telomere length and telomerase activity in human bronchial epithelial cells induced by coal tar pitch extract, Toxicology Research 2015;4:1535-44.
28. Bense L, Wiman LG. Time relation between sale of cigarettes and the incidence of spontaneous pneumothorax. Eur J Respir Dis 1987;71:362-4.
29. Carrero JJ, Stenvinkel P, Fellström B, Qureshi AR, Lamb K, Heimbürger O, et al. Telomere attrition is associated with inflammation, low fetuin-A levels and high mortality in prevalent haemodialysis patients. J Intern Med 2008;263:302-12.
30. Chung HY, Kim HJ, Kim KW, Choi JS, Yu BP. Molecular inflammation hypothesis of aging based on the anti-aging mechanism of calorie restriction. Microsc Res Tech 2002;59:264-72.
31. Tueller EE, Crise NR, Belton JC, McLaughlin RF Jr. Idiopathic spontaneous pneumothorax. Electron microscopic study. Chest 1977;71:419-21.
32. Akyıl M, Tezel Ç, Evman S, Tokgöz Akyıl F, Vayvada M, Bayram S, et al. Correlation between meteorological changes and primary spontaneous pneumothorax: Myth or fact? Turk Gogus Kalp Dama 2018;26:436-40.
33. Chan-Yeung M, Malo JL. Aetiological agents in occupational asthma. Eur Respir J 1994;7:346-71.