Role of telomerase expression in interstitial lung diseases

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Background Telomeres are hexameric nucleotide sequences. The biological role of telomeres is to prevent shortening of DNA to preserve integrity of the genome. Length of telomeres is determined by age, sex, and environmental exposures. Telomeres are vulnerable to injury by oxidative stress. Telomere length is sustained by telomerase, a ribonucleoprotein telomerase reverse transcriptase (TERT). Telomerase may help cell growth and secure against cell death. 'Telomeropathy' is associated with genetic mutations. The most common phenotype related to telomerase mutation is pulmonary fibrosis.

Objective To investigate the associations of both TERT and telomerase RNA component C with disease progression in patients with interstitial lung diseases (ILDs), which include idiopathic pulmonary fibrosis (IPF), and to compare results between patients with ILD and control.

Patients and methods A total of 46 patients with different types of ILDs were enrolled as well as 15 healthy persons as control. Whole blood sample was obtained from both patients and healthy control for detection of expression of telomerase gene by quantitative real-time PCR.

Results There was a significant negative correlation between telomerase reverse transcriptase (h-TERT) and partial pressure of oxygen (r=–23, P=0.03). Both h-TERT and telomerase reverse transcriptaseRNA component (h-TERC) were relatively more expressed in patients with IPF with pulmonary hypertension, whereas there was a significant elevation of h-TERT relative expression in patients with IPF with honeycombing high-resolution computed tomography pattern in comparison with those with reticulonodular pattern, with median of 0.85 versus 0.29, respectively.

Conclusion Hypoxia may affect DNA damage in the telomere region. Expression of telomerase may take part in pulmonary fibrosis. Exposure to hypoxia or growth factors can stimulate the expression of telomerase on cells of vascular smooth muscle.

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Introduction

Eukaryotic chromosomes contain telomeres which have hexameric nucleotide $(TTAGGG)_n$ repeats on its distant ends [1]. The biological role of telomeres is to prevent shortening of DNA, to provide security to the chromosomes from unsuitable fusions of DNA, and also to prevent breaks of DNA to preserve integrity and stabilization of the genome [2]. Upon cell division, length of telomere (TL) reduces by pairs of 50–200 bases, which conjointly associates with cell aging, eventually ends in an important TL point, which promotes fusions of chromosomal and/or cell death [3].

TLs is determined not only by genetic but also by several other factors including sex, age, and environmental exposures [4]. Susceptibility of telomeres to damage increases by oxidative stress because of their high content of guanine residues [5].

Telomerase reverse transcriptase (TERT) attaches the DNA repeats of TTAGGG to ends of telomere, thereby sustaining the TL through replication of genome. To do this function, TERT needs the template of RNA molecule telomerase RNA component C (TERC) and telomerase-associated proteins [6].

TERT is responsible for telomerase activity (TA), because of this fact, TA expression is hardly controlled, being greatly expressed only in tissues that regularly or continuously renew, such as in the germ cells, hematopoietic system cells, the epidermis, and tumors. In contrast to TERT, expression of TERC is broadly in all cells of any type, but cannot make TA [7].

Biological functions of telomerase are separate from its enzymatic activity in maintenance of telomere. Telomerase may help cell growth and preserve against cell death by using pathways not related to its telomere elongation function [8,9].

'Pulmonary fibrosis' includes a wide range of diseases of lung, including most importantly the idiopathic interstitial pneumonias (IIPs) [10], with idiopathic pulmonary fibrosis (IPF) being the most common and severe clinic-pathologic entity of IIPs [11].

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Implication of the role of maintenance of TL in interstitial lung disease (ILD), especially in IIP, has been found [12,13].

Short telomere syndrome, or 'telomeropathy' is associated with genetic mutations in TERT, TERC, regulator of telomere elongation helicase 1, and poly (A)-specific ribonuclease, which can be distinguished by abnormalities of many systems including pulmonary fibrosis, bone marrow dysfunction, cirrhosis of liver, and greying at early age. Overall, pulmonary fibrosis is the most common phenotype associated with telomerase mutation [14]. Regardless of the diagnosis, rapid disease progression and poor survival are related to this genetic mutation, which suggest that disease development, disease progression, and fibrosis propagation are related to telomere dysfunction [4].

TL may be partly responsible for a significant overlap between the clinical, radiographic, and histopathologic features of IPF and chronic hypersensitivity pneumonitis owing to association of short age-adjusted TL with radiographic and histopathologic 'IPF-like features' in the form of honeycombing, temporal heterogeneity, and fibroblastic foci [15].

Aim

This study tried to investigate the associations of both TERT and TERC with disease progression in patients with ILDs, including IPF, in a trial extending these considerations to human chronic fibrotic lung disease.

Patients and methods

This prospective study was conducted in chest department in collaboration with Molecular Biology and Biochemistry Department, Faculty of Medicine, Cairo and Fayoum Universities. This study included 46 patients already diagnosed with different types of ILDs (the study group) and 15 healthy control participants. All patients were subjected to full history taking including drug history, smoking and history of exposure, detailed clinical examination, arterial blood gases analysis, spirometry, high-resolution computed tomography (HRCT) of the chest to determine the pattern of lung parenchymal affection, and echocardiography to evaluate the right-side heart chamber size and to screen for associated pulmonary hypertension (PH). Moreover, serological tests were done to screen for associated autoimmune features. Whole blood sample was obtained from both patients already diagnosed to have ILD [clinical data, radiologic imaging, and pathologic findings (if lung biopsy is needed) are combined to reach the diagnosis] and healthy control participants for determination of telomerase gene expression by quantitative real-time PCR. The Human Research Ethics Committee, Faculty of Medicine, Fayoum University, has approved the study.

Detection of telomerase gene expression by quantitative real-time PCR

Extraction of total RNA

Removal of total RNA was done from whole blood using SV Total RNA Isolation System (Promega, Madison, Wisconsin, USA), according to the manufacturer's instructions. Ultraviolet spectrophotometer was used to measure the concentrations of RNA and purity.

Synthesis of complementary DNA

SuperScript III First-Strand Synthesis System (Invitrogen, Massachusetts, USA) was used for union of the cDNA to 1 μg RNA according to the manufacturer's protocol (#K1621; Fermentas, Waltham, Massachusetts, USA). In summary, 1 μg of total RNA was mixed with 50 μmol/l oligo (dT) 20, 50 ng/μl random primers, and 10 mmol/l dNTP mix in RNase-free water to a 10-μl total volume. This mixture is kept at 56°C for 5 min and then put on ice for 3 min. Mixing of the reverse transcriptase master was performed, which contained buffer of 2 μl of 10× RT, 4 μl of 25 mmol/l $MgCl_2$, 2 μl of 0.1 mol/l DTT, and 1 μl of SuperScript. Addition of III RT (200 U/μl) to the mixture and placing in an incubator at 25°C for 10 min were performed followed by 50 min at 50°C.

Real-time quantitative PCR

Applied Biosystem with software version 3.1 (StepOne; USA) was used for real-time PCR amplification and analysis. The reaction contained SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA) and gene-specific primer pairs, which were primer sequences of both T and C as follow: (h-TERT) forward primer: 5′-TGACACC TCACCTCACCCAC-3′, (h-TERC) forward primer: 5′-GCCTGCCGCCTTCCACCGTTCA TT-3′, and (h-TERT) reverse primer 5′-CACTG TCTTCCGCAAGTTCAC-3′, (h-TERC) reverse primer 5′-GACTCGCTCCGTTCCTCTTCCTG-3′. β-Actin sequences were as follows: forward primer: 5′-CTGTCTGGCGGCACCACCAT-3′ and reverse primer: 5′-GCAACTAAGTCATAGTCC GC-3′. They were planned with Gene Runner Software (Hasting Software Inc., Hasting, New York, USA) from RNA sequences from the bank of gene. A calculated annealing temperature of 60° was found in all primer sets. In a reaction of volume of 25 μl containing of 2X SYBR Green PCR Master Mix

(Applied Biosystems), 900 nmol/l of each primer and 2 µl of cDNA; quantitative RT-PCR was used.

Conditions for amplification were as follows: 2 min at 50°, 10 min at 95° and 40 cycles of denaturation for 15 s, and annealing/extension at 60° for 10 min. Real-time assay data using the v1·7 sequence detection software from PE Biosystems (Foster City, California, USA) were calculated. The comparative C_t method was used to calculate the relative expression of studied gene mRNA. Normalization of all values were done to β-actin, as it was used as the housekeeping gene control and detected as fold change over background levels found in the diseased groups.

Statistical analyses

Collection, tabulation, and analyses of the statistical data were done using SPSS 22.0 for Windows (SPSS Inc., Chicago, Illinois, USA). All tests were two sided. A *P* value less than 0.05 was considered significant. Continuous variables were expressed as mean, SD, median and 25–75 percentile, and the categorical variables were expressed as a number (percentage). Checking for normality of continuous variables was done using Shapiro–Wilk test. Two sample *t*-test was used to compare between two normally distributed data, whereas Mann–Whitney test was used for two groups with nonnormally distributed data. Analysis of variance test was used to compare between more than two groups of normally distributed data, whereas Kruskall–Wallis *H*-test was applied to compare between more than two groups of non-normally distributed data. Categorical variables percent were compared using Pearson's χ^2-test or Fisher's exact test when appropriate. Pearson's correlation was calculated to assess the correlations between various study parameters, where positive sign indicates positive correlation and negative sign indicates negative correlation.

Results

This prospective study was conducted in the chest department in collaboration with Molecular Biology and Biochemistry Department, Faculty of Medicine, Cairo, and Fayoum Universities. The study included 46 patients with different types of ILDs (the study group) and 15 healthy control participants. The mean±SD age of ILD cases was 44.20±13.40 years, whereas that of control group was 44.30±12.50 years. Regarding sex distribution and smoking status, ILD cases included 11 (23.91%) males and 35 (76.09%) females, and there were five (10.87%) smokers, whereas the control group included seven (46.67%) males and eight (53.33%) females, and there were three (20%) smokers. Both cases and control were matched regarding age, sex, and smoking habits (*P* values of 0.98, 0.09, and 0.38, respectively).

h-TERT and h-TERC markers were relative expressed in both groups (ILD cases and healthy control participants), with mean±SD of 0.49±0.37 and 0.66 ±0.31, respectively, in ILD group, and 1.01±0.01 in control ($P<0.001$). The most important characteristics of ILD group are shown in Table 1.

Table 1 Characteristics of interstitial lung disease group

Variables	ILD group (*n*=46)
Age (mean±SD)	44.24±13.39
Sex [*n* (%)]	
Females	35 (76.09)
Males	11 (23.91)
Smoking status [*n* (%)]	
Yes	5 (10.87)
No	41 (89.13)
Bird contact [*n* (%)]	
Yes	14 (30.43)
No	32 (69.57)
History of diabetes [*n* (%)]	
Yes	13 (28.26)
No	33 (71.74)
Positive autoimmune profile [*n* (%)]	
Yes	8 (17.39)
No	38 (82.61)
Arterial blood gases analysis (mean±SD)	
PO_2	61.80±13.91
PCO_2	41.65±10.95
SO_2	89.59±5.23
Spirometery (mean±SD)	
FVC%	49.63±17.95
FEV_1%	48.07±17.29
FEV_1/FVC%	85.01±16.12
$FEF_{25-75\%}$	48.33±9.27
Right-sided heart status [*n* (%)]	
Dilated chambers	21 (45.65)
High PASP	22 (47.8)
PASP	61.29 (18.60)
Predominant HRCT pattern [*n* (%)]	
Ground-glass opacity	16 (34.78)
Honeycombing	13 (28.26)
Reticulonodular pattern	17 (36.96)
Final diagnosis [*n* (%)]	
Hypersensitivity pneumonitis	16 (34.78)
Idiopathic pulmonary fibrosis	18 (39.13)
Nonspecific interstitial	7 (15.22)
Pneumonia	–
Sarcoidosis	5 (10.87)
h-TERT (mean±SD)	0.49±0.37
h-TERC (mean±SD)	0.66±0.31

FEF, forced expiratory flow; FEV_1, forced expiratory volume in first second; FVC, forced vital capacity; HRCT, high-resolution computed tomography; h-TERC, telomerase reverse transcriptase RNA component; ILD, interstitial lung disease; PASP, pulmonary artery systolic preesure; PCO2, partial pressure for carbon dioxide; PO2, partial pressure for oxygen; SO2, oxygen saturation.

The comparison between the different histopathological subtypes of ILD is presented in Table 2, which revealed a statistically significant difference between the different ILD histopathological subtypes regarding positivity of autoimmune profile, forced expiratory flow 25–75%, and the predominant HRCT pattern.

Regarding the correlation between both markers h-TERT and h-TERC and characteristics of patients with ILD, there was a significant negative correlation between h-TERT and partial pressure of oxygen (r=−23, P=0.03), as shown in Table 3.

Regarding effect of different characteristics of patients with IPF on h-TERT and h-TERC expression, both h-TERT and h-TERC were relative more expressed in female (n=11) than male (n=7), without significant difference, with median of 0.51 versus 0.28 and 0.74 versus 0.72, respectively (Table 4). Moreover, both h-TERT and h-TERC were relative more expressed in patients with IPF with history of PH (n=11) than patients without PH (n=7), without significant difference, with median of 0.51 versus 0.28 and 0.85 versus 0.72, respectively, as shown in Table 5, whereas there was significant elevation of h-TERT relative expression in patients with IPF with honeycombing HRCT pattern (n=11) in comparison with those with reticulonodular pattern (n=7), with median of 0.85 versus 0.29, respectively (P=0.04; Fig. 1). AQ1

Table 2 Comparison between different histopathological subtypes of interstitial lung disease study group

Variables	ILD groups (n=46)				P value
	IPF (n=18)	HP (n=16)	NSIP (n=7)	Sarcoidosis (n=5)	
Age (median)	55.5	42.5	35	38	0.13[b]
25–75%	35.25 (60)	30.75 (50)	27 (62)	32 (40)	
Sex [n (%)]					
Females	11.00 (61.11)	15.00 (93.75)	5.00 (71.43)	4.00 (80.00)	0.17[a]
Males	7.00 (38.89)	1.00 (6.25)	2.00 (28.57)	1.00 (20.00)	
Smoking status [n (%)]					
Yes	4.00 (22.22)	0.00 (0.00)	1.00 (14.29)	0.00 (0.00)	0.17[a]
No	14.00 (77.78)	16.00 (100.00)	6.00 (85.71)	5.00 (100.00)	
Bird contact [n (%)]					
Yes	4.00 (22.22)	7.00 (43.75)	1.00 (14.29)	2.00 (40.00)	0.39[a]
No	14.00 (77.78)	9.00 (56.25)	6.00 (85.71)	3.00 (60.00)	
History of DM [n (%)]					
Yes	7.00 (38.89)	5.00 (31.25)	0.00 (0.00)	1.00 (20.00)	0.42[a]
No	11.00 (61.11)	11.00 (68.75)	7.00 (100.00)	4.00 (80.00)	
Autoimmunity (yes) [n (%)]	2.00 (25.00)	0.00 (0.00)	6.00 (75.00)	0.00 (00.00)	<0.001[a]
ABG analysis					
PO_2 (median)	54.00 (50–68.5)	58.50 (51.25–72)	65 (50–70)	69 (60–81)	0.42[c]
PCO_2 (median)	40.50 (34–43.75)	43 (39.5–55.5)	40 (30–43)	40 (27–49)	0.34[c]
SO_2 (median)	88.5 (84–92.25)	90 (87.25–94.25)	90 (89–96)	93 (89–95.5)	0.30[b]
Spirometry					
FVC% (median)	40 (27.25–57)	63 (40.75–72.75)	58 (24–69)	63 (40–68)	0.06[c]
FEV_1% (median)	37 (29.5–57.25)	53.50 (35–68)	64 (28–71)	55 (36.5–74.5)	0.33[c]
Ratio [median (25–75%)]	80.5 (69–95.75)	82.5 (66–93.5)	97 (97–114)	84 (75.5–108)	0.06[b]
$FEF_{25–75\%}$ (median)	45 (43–52)	44 (39–50)	56 (55–57)	45 (39–59.5)	0.02[c]
Right heart status [n (%)]					
Dilatation	11.00 (61.11)	4.00 (25.00)	3.00 (42.86)	3.00 (60.00)	0.18[a]
High PASP	11.00 (61.11)	5.00 (31.25)	3.00 (42.86)	3.00 (60.00)	0.33[a]
Predominant HRCT					
Pattern [n (%)]					
Ground glass	0.00 (0.00)	10.00 (62.50)	6.00 (85.71)	0.00 (0.00)	<0.001[a]
Honeycombing	11.00 (61.11)	0.00 (0.00)	0.00 (0.00)	2.00 (40.00)	
Reticulonodular	7.00 (38.89)	6.00 (37.50)	1.00 (14.29)	3.00 (60.00)	
h-TERT (median)	0.4 (0.27–0.93)	0.36 (0.24–0.81)	0.22 (0.12–0.59)	0.26 (0.2–0.39)	0.17[c]
h-TERC (median)	0.73 (0.37–0.87)	0.69 (0.33–0.89)	0.81 (0.34–0.97)	0.37 (0.34–0.85)	0.88[c]

ABG, arterial blood gases; DM, diabetes mellitus; FEF, forced expiratory flow; FEV_1, forced expiratory volume in first second; FVC, forced vital capacity; HP, hypersensitivity pneumonitis; HRCT, high-resolution computed tomography; h-TERC, telomerase reverse transcriptase RNA component; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; NSIP, nonspecific interstitial pneumonia; PCO2, partial pressure for carbon dioxide; PO2, partial pressure for oxygen; SO2, oxygen saturation. P<0.05, significant. [a]Pearson χ^2. [b]One-way analysis of variance test. [c]Kruskal–Wallis test.

The best cutoff point level of h-TERT and h-TERC was 0.99; at or below the determent cutoff point of h-TERT and h-TERC, the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of ILD diagnosis were 91.3, 100, 100, 79, and 93.4%, and the area under the curve was 92% for both. Regarding value of h-TERT and h-TERC in IPF diagnosis, the best cutoff point of h-TERT and h-TERC was 0.99 and 0.97, respectively; the sensitivity and specificity of h-TERT and h-TERC at this cutoff point were 94 and 100%, respectively, the area under the curve was 94% for both. At or below the determent cutoff point of h-TERT and h-TERC, the positive predictive value, negative predictive value, and accuracy of IPF diagnosis were 100, 94, and 97%, respectively.

Table 3 Correlation between h-TERT and h-TERC and the characteristics of patients with interstitial lung disease

Variables	h-TERT		h-TERC	
	r	*P*	*R*	*P*
Age	0.12	0.43	–0.08	0.59
PaO_2	–0.32	0.03	–0.15	0.32
PCO_2	0.01	0.96	0.20	0.18
SO_2	–0.21	0.17	–0.15	0.31
FVC%	–0.17	0.25	–0.21	0.17
FEV_1%	–0.11	0.47	–0.20	0.19
FVC/FEV_1%	0.12	0.41	–0.06	0.70
$FEF_{25–75\%}$	–0.06	0.71	0.14	0.37

FEF, forced expiratory flow; FEV_1, forced expiratory volume in first second; FVC, forced vital capacity; h-TERC, telomerase reverse transcriptase RNA component; PCO2, partial pressure for carbon dioxide; PO2, partial pressure for oxygen; SO2, oxygen saturation. $P<0.05$, significant. *Pearson correlation, the sign before (*r*) represent the direction of correlation, whereas *P* represents the significant of correlation, $P<0.05$, significant.

Discussion

Over the past decades, a countless of number of human diseases including Werner syndrome, dyskeratosis congenita, Bloom syndrome, ataxia–telangiectasia, Fanconi anemia and Nijmegen breakage syndrome are showing aging of cells, which are regulated by telomerases [16].

Throughout the previous 5 years, the scale of diseases influenced by length disequilibrium of telomere has been greatly increased. Among these, the lung disease, IPF, is the important frequent manifestation of telomere-mediated disease [14].

Table 4 Effect of different characteristics of patients with interstitial lung disease on h-TERT and h-TERC expression

Variables	h-TERTC			h-TERT		
	Median	25–75%	*P*	Median	25–75%	*P*
Sex						
Female (*n*=35)	0.52	0.34–0.89	0.76	0.29	0.22–0.59	0.86
Male (*n*=11)	0.76	0.39–0.85		0.28	0.25–0.96	
Smoking status						
No (*n*=41)	0.74	0.35–0.89	1.00	0.29	0.23–0.72	0.81
Yes (*n*=5)	0.72	0.56–0.81		0.28	0.20–0.97	
Bird contact						
No (*n*=32)	0.73	0.34–0.87	0.55	0.29	0.24–0.91	0.60
Yes (*n*=14)	0.68	0.37–0.94		0.29	0.20–0.63	
DM						
No (*n*=33)	0.74	0.36–0.89	0.70	0.29	0.23–0.90	0.90
Yes (*n*=13)	0.52	0.34–0.86		0.29	0.23–0.71	
Right heart						
Dilated (*n*=21)	0.85	0.37–0.90	0.30	0.28	0.22–0.55	0.30
Normal (*n*=25)	0.72	0.34–0.86		0.43	0.24–0.92	
PHTN status						
No (*n*=24)	0.73	0.34–0.87	0.36	0.40	0.23–0.92	0.32
PASP (*n*=22)	0.69	0.37–0.90		0.29	0.22–0.54	
Autoimmune						
No (*n*=38)	0.74	0.35–0.89	0.44	0.29	0.26–0.90	0.07
Yes (*n*=8)	0.57	0.35–0.82		0.22	0.12–0.57	
Predominant HRCT						
Pattern						
Ground glass (*n*=16)	0.60	0.33–0.89	0.38	0.36	0.22–0.59	0.23
Honeycombing (*n*=13)	0.51	0.36–0.86		0.53	0.27–0.95	
Reticulonodular (*n*=17)	0.84	0.38–0.91		0.27	0.18–0.52	

DM, diabetes mellitus; HRCT, high-resolution computed tomography; h-TERC, telomerase reverse transcriptase RNA component; PHTN, pulmonary hypertension.

Table 5 Effect of different characteristics of patients with idiopathic pulmonary fibrosis on h-TERT and h-TERC expressions

Variables	h-TERTC			h-TERT		
	Median	25–75%	*P*	Median	25–75%	*P*
Sex						
Female (*n*=11)	0.74	0.37–0.91	0.61	0.51	0.23–0.92	0.58
Male (*n*=7)	0.72	0.37–0.85		0.28	0.27–0.98	
Smoking status						
No (*n*=14)	0.63	0.37–0.90	0.87	0.40	0.25–0.92	0.52
Yes (*n*=4)	0.74	0.72–0.83		0.62	0.27–0.98	
Bird contact						
No (*n*=14)	0.73	0.37–0.86	0.52	0.40	0.25–0.94	0.59
Yes (*n*=4)	0.71	0.41–0.94		0.57	0.29–0.91	
DM						
No (*n*=11)	0.74	0.37–0.89	0.34	0.29	0.23–0.93	0.68
Yes (*n*=7)	0.51	0.35–0.87		0.51	0.28–0.93	
Right heart						
Dilated (*n*=11)	0.72	0.37–0.89	0.82	0.28	0.28–0.93	0.87
Normal (*n*=7)	0.85	0.37–0.76		0.51	0.23–0.98	
PHTN status						
No (*n*=7)	0.72	0.37–0.76	0.82	0.28	0.23–0.98	0.89
PASP (*n*=11)	0.85	0.37–0.89		0.51	0.28–0.93	
Autoimmune						
No (*n*=16)	0.74	0.38–0.89	0.43	0.52	0.28–0.93	0.17
Yes (*n*=2)	0.55	0.36–0.74		0.22	0.23–0.96	
Predominant HRCT						
Pattern						
Honeycombing (*n*=11)	0.72	0.36–0.86	0.12	0.85	0.28–0.96	0.04
Reticulonodular (*n*=7)	0.74	0.39–0.93		0.29	0.23–0.51	

DM, diabetes mellitus; HRCT, high-resolution computed tomography; h-TERC, telomerase reverse transcriptase RNA component; PASP, pulmonary artery systolic preesure; PHTN, pulmonary hypertension.

TA is widely demonstrated in cells of cancer and unnoticed in somatic cells of adult, but it is temporarily induced in many tissues subjected to repair, injury, and fibrosis. Hypoxia, bleomycin, and silica-induced lung injury and fibrosis in rodents are marked by the promotion of telomerase in cells of epithelium and fibroblasts [17,18].

This study tried to investigate the associations of both TERT and TERC with disease progression in patients with ILDs, involving IPF, and tried to compare results between patients with ILD and control.

In this study, both cases and control were matched regarding age and sex, with *P* value of 0.98 and 0.09, respectively; therefore, there was no significant difference between them regarding age and sex.

The mean±SD age of ILD cases was 44.20±13.40 years, whereas that of control group was 44.30 ±12.50 years.

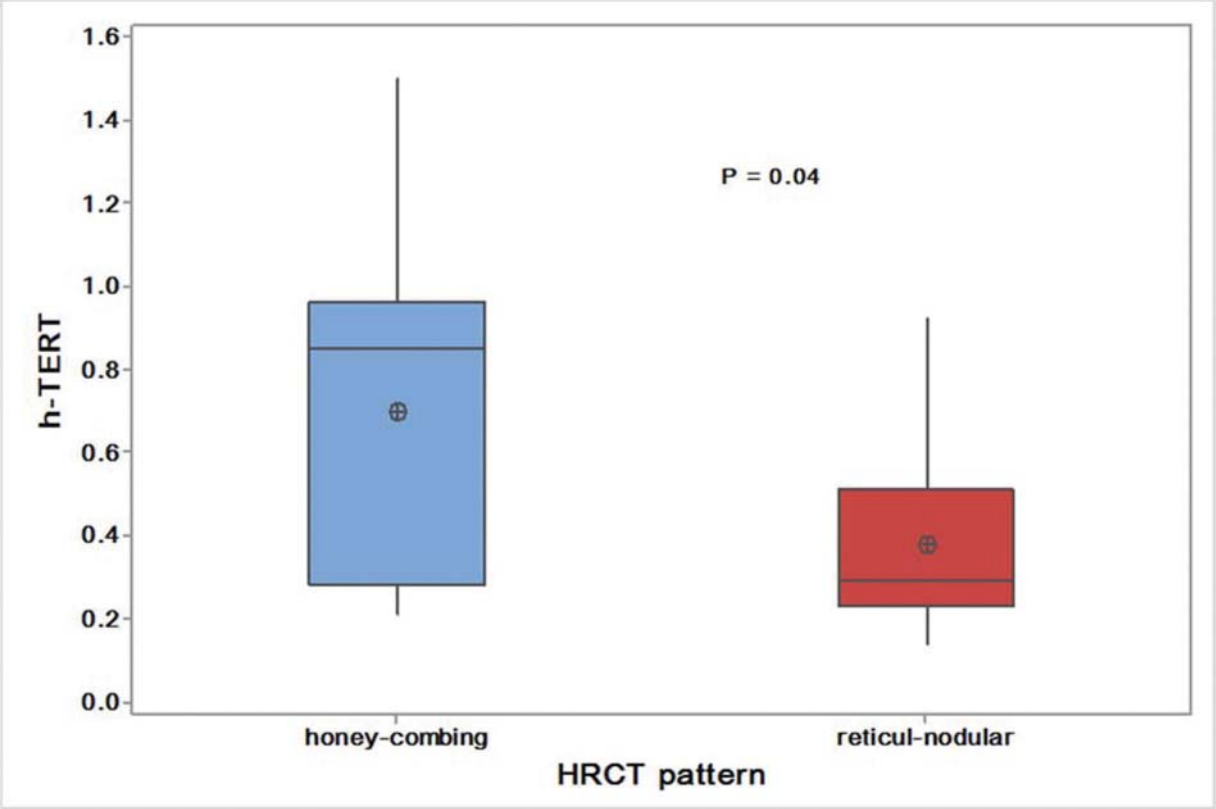
There was no correlation of significance between both markers h-TERT and h-TERC and the age (*P*=0.43 and 0.59, respectively), sex (*P*=0.86 and 0.76, respectively) of patients with ILD. However, the result of this study found in patients with IPF, h-TERT was relative more expressed in female (*n*=11) than male (*n*=7), without significant difference, with median of 0.51 versus 0.28.

In contrast, the study by Savale *et al*. [19] revealed that TL was shorter in control males than control females.

In general, age shortens telomeres, and their length may be affected by stressors, such as diet, physical activity, chronic inflammation, and occupational and environmental exposure and thus forming an association between TL and aging and associated diseases [20,21].

Different hypotheses found a relation between sex and TL. Shiel *et al*. [22] found that TL is related inversely to chronological age in humans. Gardnera *et al*. [23] found that there might be a relation between sex and TL, with females with longer telomeres than males, and that this connection might become evident with increasing age. Other hypotheses proposed that steroid of sex hormones may be a good regulator of physiology in expression of h-TERT [24].

Figure 1



h-TERT expression in IPF patients as regarding its HRCT pattern.

Despite that oxidative stress is triggered by smoking [25], which may influence telomere shortening [24], this study found no significant correlation between h-TERT and h-TERC expression and smoking history in patients with ILD.

In contrast, previous studies by Morla *et al.* [26], Valdes *et al.* [27], and Chan *et al.* [28] revealed cigarette smoking effect on telomere shortening.

Morla *et al.* [26] found a dose–response connection between cumulative exposure to tobacco smoking in life and TL.

A study by Schulz *et al.* [29] found that the relation between TL and lung function indices in a random sample was essentially owing to smokers, which revealed that lung function mainly shows aging owing to extrinsic factors rather than intrinsic aging in the absence of substantial pollution of air.

Similarly, this study found no significant correlation between both markers h-TERT and h-TERC and extrinsic factors (as smoking and bird exposure) on pulmonary function in patients with ILD.

Moreover, the study done by Dai *et al.* [30] found that data of PFT including forced vital capacity, forced expiratory volume in 1 s, and diffusion capacity for carbon monoxide of lung revealed no differences of significance between the two groups with patients with IPF (group with TERT/TERC gene mutations and second group without TERT/TERC gene mutations).

There are two options regarding the connection between induction of hypoxia and TA. Damage of DNA in the telomere region can expand its effect by hypoxia, which would lead to expression of hypoxia-inducible factor-1-produced telomerase to save the destroyed ends of chromosome; or an antiapoptotic response may be generated by the hypoxic induction of telomerase [31].

Hypoxia-inducible factor-1α is the vital activating transcription factor to cause transcription of TERT, which sequentially can control the transcription of TERT and increase its expression and TA [32,33]. Similarly our study found that there was a negative significant correlation between h-TERT and partial pressure of oxygen (r=–23, P=0.03). In contrast, other study revealed that

oxidative stress may increase the shortening of telomeres of circulating leukocytes in patients with obstructive sleep apnea [34].

This study showed there was a significant elevation of h-TERT relative expression in patients with IPF with honeycombing HRCT pattern (n=11) in comparison with those with reticulonodular pattern (n=7), with median of 0.85 versus 0.29, respectively (P=0.04).

Similar to our study, Nozaki *et al.* [17] found that expression of telomerase may take part in pulmonary fibrosis, which revealed that the affected tissue of lung and isolated fibroblasts of lung from rats with bleomycin-induced pulmonary fibrosis is induced by TA.

In contrast Newton *et al.* [4] found that between different telomere-related genetic mutation patients, there is a poor genotype–ILD phenotype relation. The site of the lung fibrosis, the pattern of lung damage (honeycombing either microcystic vs. macrocystic), the extracellular matrix nature deposition in the lung, and the extent of re-modelling are controlled by genetic and environmental factors. For example, there is proof of fibrosis around central airway and air trapping in patients with chronic hypersensitivity pneumonitis; in these cases, more cycles of cell division and more telomere shortening of airway epithelia around the small airways of lung after inhalation of fibro-genic environmental agent exist [4].

This study found that both h-TERT and h-TERC were relatively more expressed in patients with IPF with history of PH (n=11) than patients without PH (n=7) without significant, with median of 0.51 versus 0.28 and 0.85 versus 0.72, respectively.

PH is a crucial complication of IPF [35]. The principal targets that lead to growth of pulmonary vascular cells excessively, hypertrophic remodeling of the arterial wall, and PH are pulmonary artery smooth muscle cells [36]. Expression of telomerase on vascular smooth muscle cells can be done on provocation by growth factors or subjected to hypoxia [37], supporting a part for telomerase in dysregulated growth of vascular cells [38]. In contrast, a study by Mouraret *et al.* [39] found that patients with idiopathic pulmonary artery hypertension (iPAH) have excessive expression of TERT in lung and in mice with experimental PH, indicating that serotonin-transporter overexpression and not hypoxia was the essential factor responsible for telomerase expression.

Limitations

This study has many limitations. First, the sample size may not have been large enough to test the associations between telomerase expression in each of the ILD subsets individually with patients characteristics and extra-pulmonary manifestations of telomere-mediated disease. Second, TL was not measured in lung tissue biopsy.

Recommendation

Further researches will be required to detect the possible application of predicting biomarker of gene mutations and TL for therapy and outcomes in patients with ILD, especially with IPF.

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Conflicts of interest

There are no conflicts of interest.

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Author Queries???

AQ1: Please check we have ignore your correction because after correction table 4 not cited in the text.