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 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. ‘Telomereopathy’ 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.

Keywords: interstitial lung disease, idiopathic pulmonary fibrosis, telomerase expression, telomerase, telomere gene

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. ‘Telomereopathy’ is associated with genetic mutations. The most common phenotype related to telomerase mutation is pulmonary fibrosis.

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].

Conclusions

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 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 MgCl2, 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°C at 50 min.

**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 TACCTCACCACAC-3′, (h-TERC) forward primer: 5′-GCCGTGCCGCTTTCACCGTTCA TT-3′, and (h-TERT) reverse primer 5′-CACTG TCTTCCGCAGTTTCAC-3′, (h-TERC) reverse primer 5′-GACTCGCTTCCGTTCCTTTTCCTG-3′. β-Actin sequences were as follows: forward primer: 5′-ACTGTCGCTGCCGCACTTACAT-3′ and reverse primer: 5′-GCAAATAGTCATAGTCC 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°C 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°C, 10 min at 95°C and 40 cycles of denaturation for 15 s, and annealing/extension at 60°C 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 \( C_\text{t} \) method was used to calculate the relative expression of studied gene mRNA. Normalization of all values were done to \( \beta \)-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 \( \chi^2 \)-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) |
| \( \text{PO}_2 \) | 61.80±13.91     |
| \( \text{PCO}_2 \) | 41.65±10.95     |
| \( \text{SO}_2 \) | 89.59±5.23      |
| Spirometry (mean±SD) |
| FVC\( \% \)     | 49.63±17.95     |
| FEV\( \text{I} \)/FVC\( \% \) | 48.07±17.29   |
| 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       | 5 (10.87)       |
| Sarcoidosis     | 0.49±0.37       |
| h-TERT (mean±SD) | 0.66±0.31      |

FEF, forced expiratory flow; FEV\( \text{I} \), 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 pressure; PO\( \text{CO}_2 \), partial pressure for carbon dioxide; PO\( \text{O}_2 \), partial pressure for oxygen; SO\( \text{O}_2 \), 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).

### 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                   | 0.13b        |
| 25–75%                          | 35.25 (60)        | 30.75 (50) | 27 (62)  | 32 (40)              |
| Sex [%]                         |                   |          |          |                      |
| Females                         | 11.00 (61.11)     | 15.00 (93.75) | 5.00 (71.43) | 4.00 (80.00) | 0.17a        |
| Males                           | 7.00 (38.89)      | 1.00 (6.25)  | 2.00 (28.57) | 1.00 (20.00) |
| Smoking status [%]              |                   |          |          |                      |
| Yes                             | 4.00 (22.22)      | 0.00 (0.00)  | 1.00 (14.29) | 0.00 (0.00)  | 0.17a        |
| No                              | 14.00 (77.78)     | 16.00 (100.00) | 6.00 (85.71) | 5.00 (100.00) |
| Bird contact [%]                |                   |          |          |                      |
| Yes                             | 4.00 (22.22)      | 7.00 (43.75)  | 1.00 (14.29) | 2.00 (40.00)  | 0.39a        |
| No                              | 14.00 (77.78)     | 9.00 (56.25)  | 6.00 (85.71) | 3.00 (60.00)  |
| History of DM [%]               |                   |          |          |                      |
| Yes                             | 7.00 (38.89)      | 5.00 (31.25)  | 0.00 (0.00)  | 1.00 (20.00)  | 0.42a        |
| No                              | 11.00 (61.11)     | 11.00 (68.75) | 7.00 (100.00) | 4.00 (80.00)  |
| Autoimmunity (yes) [%]          | 2.00 (25.00)      | 0.00 (0.00)  | 6.00 (75.00) | 0.00 (0.00)  | <0.001a      |
| ABG analysis                    |                   |          |          |                      |
| PO2 (median)                    | 54.00 (50–68.5)   | 58.50 (51.25–72) | 65 (50–70)  | 69 (60–81)   | 0.42c        |
| PCO2 (median)                   | 40.50 (34–43.75)  | 43 (39.5–55.5) | 40 (30–43)  | 40 (27–49)   | 0.34c        |
| SO2 (median)                    | 88.5 (84–92.25)   | 90 (87.25–94.25) | 90 (89–96) | 93 (89–95.5) | 0.30b        |
| Spirometry                      |                   |          |          |                      |
| FVC% (median)                   | 40 (27.25–57)     | 63 (40.75–72.75) | 58 (24–69)  | 63 (40–68)   | 0.06c        |
| FEV1% (median)                  | 37 (29.5–57.25)   | 53.50 (35–68)  | 64 (28–71)  | 55 (36.5–74.5) | 0.33c        |
| Ratio [median (25–75%)]         | 80.5 (69–95.75)   | 82.5 (66–93.5) | 97 (97–114) | 84 (75.5–108) | 0.06b        |
| FEF25–75% (median)              | 45 (43–52)        | 44 (39–50)   | 56 (55–57)  | 45 (39–59.5) | 0.02c        |
| Right heart status [%]          |                   |          |          |                      |
| Dilatation                      | 11.00 (61.11)     | 4.00 (25.00)  | 3.00 (42.86) | 3.00 (60.00)  | 0.18a        |
| High PASP                       | 11.00 (61.11)     | 5.00 (31.25)  | 3.00 (42.86) | 3.00 (60.00)  | 0.33a        |
| Predominant HRCT Pattern [%]    |                   |          |          |                      |
| Ground glass                    | 0.00 (0.00)       | 10.00 (62.50) | 6.00 (85.71) | 0.00 (0.00)  | <0.001a      |
| 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) | 0.17c        |
| 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) | 0.88c        |

ABG, arterial blood gases; DM, diabetes mellitus; FEF, forced expiratory flow; FEV1, forced expiratory volume in first second; FVC, forced vital capacity; HP, hypersensitivity pneumonitis; HRCT, high-resolution computed tomography; h-TERT, 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. *Pearson χ2. **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.

### 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 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  |
| PaO₂                       | -0.32  | 0.03  | -0.15  | 0.32  |
| PCO₂                       | 0.01   | 0.96  | 0.20   | 0.18  |
| SO₂                        | -0.21  | 0.17  | -0.15  | 0.31  |
| FVC%                       | -0.17  | 0.25  | -0.21  | 0.17  |
| FEV₁%                      | -0.11  | 0.47  | -0.20  | 0.19  |
| FVC/FEV₁%                  | 0.12   | 0.41  | -0.06  | 0.70  |
| FEF₂₅₋₇⁵%                  | -0.06  | 0.71  | 0.14   | 0.37  |

**Notes:**
- FEF, forced expiratory flow; FEV₁, 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.
- *Pearson correlation, the sign before (r) represent the direction of correlation, whereas P represents the significant of correlation, *P* < 0.05, significant.

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

| Variables                  | h-TERT | h-TERT |       | h-TERT | 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 | 0.72 | 0.28 | 0.25–0.96 | 0.86 |
| 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 | 0.68 | 0.28 | 0.20–0.97 | 0.60 |
| 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 | 0.29 | 0.20–0.63 | 0.90 |
| 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 | 0.29 | 0.23–0.71 | 0.90 |
| 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 | 0.32 |
| 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 | 0.29 | 0.22–0.54 | 0.32 |
| 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 | 0.29 | 0.12–0.57 | 0.07 |
| 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 | 0.27–0.95 | 0.32 |
| Reticulonodular (n=17)     | 0.84   | 0.38–0.91 | 0.27 | 0.27 | 0.18–0.52 | 0.32 |

**Notes:**
- DM, diabetes mellitus; HRCT, high-resolution computed tomography; h-TERC, telomerase reverse transcriptase RNA component; 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. Gardera 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].

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

| Variables       | h-TERC |          |         | 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|        |          |         |        |          |         |
| 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 pressure; PHTN, pulmonary hypertension.
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.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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