Telomere length assessment in blood leukocytes of patients with sarcoidosis

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ABSTRACT. Background: Accelerated aging and telomere shortening have been studied in many chronic diseases such as interstitial pulmonary fibrosis and chronic obstructive pulmonary disease. Different studies have shown that patients with these diseases have shorter telomere lengths than controls; this can be a marker of the progression and outcome of the disease. So far, a few studies have been evaluated the telomere length in sarcoidosis. In this study we determine the telomere length in patients with sarcoidosis and compare it with control subjects. **Objective:** Our aim is to compare telomere length in patients with sarcoidosis and normal population. Methods: We select 58 patients with sarcoidosis who were visited in the sarcoidosis clinic of Masih Daneshvari Hospital. 58 sex and age-matched (with±2 years) healthy control subjects were selected. Telomere length was measured by quantitative real time PCR as described by Cawthon on peripheral blood sample. The telomere repeat copy number (T) to single-gene copy number(S) ratio was calculated using the comparative Ct method. Results: The mean and standard deviation of telomere length in the patient and control group was 0.65 ± 0.05 and 0.72 ± 0.07 respectively. There was a statistically significant difference between the two groups. (P = 0.031). Conclusion: Sarcoidosis is an inflammatory disease that can involve many organs. Like other chronic diseases, aging phenomenon occurs in that; which led to decrease cellular and tissue telomere length. This article demonstrates shorter telomere length in Iranian sarcoidosis patients compared to normal population.

INTRODUCTION

Sarcoidosis is a multiorgans chronic granulomatous inflammatory disease with unknown etiology (1, 2). Variable incidence has been reported in different studies ranging from 0.73 per 100,000 to 71 per 100,000 in Japanese and American population, respectively (3, 4). Recently it has been declared that sarcoidosis incidence in Iranian population is almost 1-2 cases per 100,000 people (1). However, its etiology and pathogenesis is not completely understood.

Recently more attention has been paid to the association between the phenomenon of aging and cellular oxidative stress and the occurrence of chronic pulmonary diseases such as chronic obstructive pulmonary disease (COPD) and interstitial pulmonary fibrosis (IPF). The process of "aging" or "senescence" is defined as a condition associated with the

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progressive reduction of homeostasis in the body, which begins after the fertility phase has been completed, leading to an increased number of diseases and also disorders of DNA repair (5-7).

Human telomeres consist of thousands of hexameric nucleotides, TTAGGGs, and related proteins, including the Shelterin complex, or the Telosome (8). They play a key role in establishing the integrity of the chromosomes and their stability (9, 10). In many cells with high proliferation rate, the length of the telomere is dynamic. The telomere length in human somatic cells decreases from 20 to 200 bp in each cell division. So this process gradually causes chromosomal shortening in any cell division. In long term this phenomenon causes telomere attrition which leads to apoptosis or irreversible ending of the cell cycle (11). Telomeres, as a replication index, determine the number of cell divisions throughout the life; eventually transmit senescence as a signal. Many causes such as the imbalance of oxidant / antioxidant factors lead to telomeres attrition, accelerated aging and shorter life span (12-14). The rate of telomeres length shortening is similar in different tissues. Therefore, measuring telomere length in cells such as peripheral blood leukocytes or oral mucosal cells that are readily accessible can reflect telomere length in other tissues in patients with chronic diseases and healthy individuals (11, 15).

In many chronic diseases such as IPF and COPD, accelerated aging and telomere shortening have been studied. Different studies have shown that these individuals have shorter telomere lengths than controls; which can be a marker of the progression and outcome of the disease (16-19).

There are some studies that have evaluated the telomere length in sarcoidosis (20, 21). In our best knowledge, this study is the first one in Iran, which aimed to determine the telomere length in patients with sarcoidosis and compare it with control subjects.

Methods

Fifty-eight sarcoidosis patients who were visited in the sarcoidosis clinic in Masih Daneshvari Hospital were enrolled in the study. Their diagnosis was confirmed with clinical, radiological and pathological data. The patients with any other chronic lung disease, active cardiovascular disease, malignancy,

infection or other chronic inflammatory diseases like inflammatory bowel

disease, were excluded. A group of 58 sex and age-matched (±2 years) healthy control subjects, were selected. This study was approved by the Ethical Committee of the National Research Institute of Tuberculosis and Lung Disease (NRITLD) and written consent was obtained from all the participants.

The blood samples [Angiotensin converting enzyme (ACE), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP)] are obtained at the first visit in the clinic. The blood sample for PCR was also drawn.

Blood samples were drawn from all patients and controls (5ml) and transferred into EDTA-containing tubes. All samples were centrifuged at 1000×g for 10 minutes at 4°C and stored at -70°C for DNA extraction. Genomic DNA was extracted from buffy coats with Qiagen (QIAamp DNA Blood Mini Kit, Germany) and quantified by spectrophotometer (Hitachi 1800, Japan). Telomere length was measured by quantitative Real Time-PCR as described by Cawthon (22) .All samples were run in triplicate, using the SYBR green method and 35 ng of DNA. The sequences of the telomere primers for tel-1 and tel-2 and 36B4 as single copy gene for normalized technique were as follows:

Tel-1F 5'-CGGTTTGTTTGGGTTTGGGTT TGGGTTTGGGTTTGGGTT-3'
Tel-2R 5'-GGCTTGCCTTACCCTTACCCT-TACCCTTACCCTTACCCT-3'
Single-1F 36B4F, 5'-CAGCA AGTGGGAA-GGTGTAATCC-3
Single-2R 36B4R, 5'-CCCATTCTATCAT-CAACGGGTACAA-3'

The telomere repeat copy number (T) to single gene copy number(S) ratio was calculated using the comparative Ct method. Positive controls (extracted from a normal healthy person) and negative controls (DDW+ master mix) were added for every PCR run. PCR was performed by a BioRad RT-PCR system (BioRad, USA).

Descriptive statistics in terms of mean, Standard deviations and percentages were used to describe characteristics of the studied patients. Comparison of categorical variables was conducted by Chi-Square test or Fisher's exact test accordingly. After

assessment of normality distribution of variables by Shapiro test, Student t-test and ANOVA test were used if data had normal distribution while Mann–Whitney and Kruskal–Wallis test were used in skewed data. A P-value less than 0.05 was considered a significant test. SPSS version 22 was used for all statistical analysis.

Results

In patients with sarcoidosis, there were 43% (n=25) male and 57% (n=33) female. Their mean age was 48 ± 9 years old. The mean and standard deviation of telomere length in the patient and control group was 0.65 ± 0.05 and 0.72 ± 0.07 respectively. There was a statistically significant difference between the two groups. (P = 0.031)(Figure1)

According to Scadding staging 23 patients (39.7%) were in stage one, 31 patients (53.4%) in stage two and 4 patients (6.9%) in stage 3 pulmonary involvement. No significant correlation between telomere length and stage of pulmonary involvement was seen based on ANOVA test. (P-value=0.488) (Figure 2)

Extrapulmonary organs (skin, eyes, liver and kidneys) were also involved in 27 patients (46.6%). No significant difference was seen with more organ involvements (Table 1).

The T/S ratio (indicative of telomere length) was 0.70 ± 0.05 in women and 0.68 ± 0.04 in men (P-value = 0.174). In both sexes, telomere length was studied in correlation of age separately with Spearman's rho. There was no statistically significant
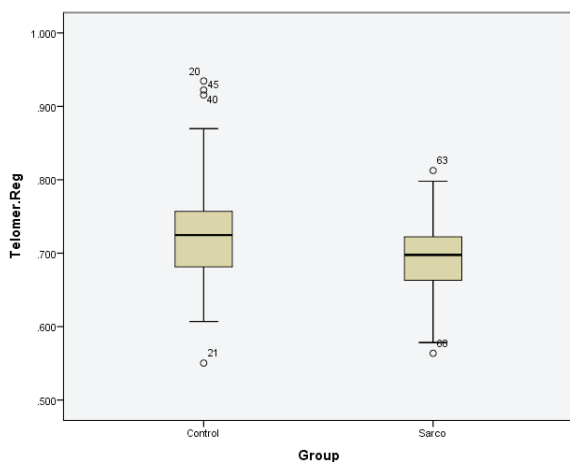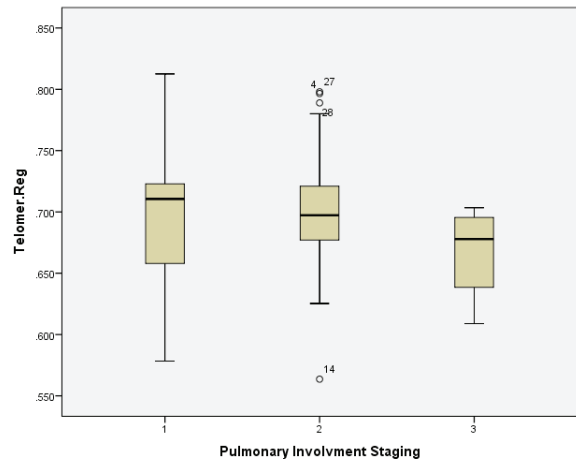

Figure 2. Comparison of telomere length among different stage of pulmonary involvement.

Table 1. Comparison of telomere length in patients with pulmonary involvement and in patients with pulmonary and extrapulmonary involvements.

Involvement	N	Mean	Standard Deviation	p-value
Pulmonary	31(53.4%)	0.69341	0.051745	
Pulmonary + extra pulmonary	27(46.6%)	0.69922	0.054435	0.678

Figure 1. Comparison of telomere length in patients with sarcoidosis and controls.

Table 2. The relationship between telomere length and age in sarcoidosis subjects in each sex.

		Telomere	
Male	Age	Correlation Coefficient	0.249
		p-value	0.231
		Telomere	
Female	Age	Correlation Coefficient	-0.273
		p-value	0.124

correlation between age variation in subjects with sarcoidosis with different sex and telomere length. However, there was an inverse relationship between age and telomere length in female group, but it was not statistically significant (Table2).

There was no significant correlation between serum ACE level and telomere length in patients with sarcoidosis (P value = 0.84). According to the analysis performed by the spearman correlation coefficient, with increasing serum CRP, the telomere length decreased (P-value = 0.003) (Table 3).

No significant correlation between serum levels of ESR and telomere length in patients with sarcoidosis was seen (P-value = 0.86).

Discussion

Attrition of telomere length with aging and oxidative stress can be seen in many inflammatory situations and chronic disease. Our study demonstrated shortening of telomere length in Iranian sarcoidosis patients.

Telomere length is one of the cellular aging markers. With prolonged cell lifespan, the telomeres are shortened to reach the Hay-flick limit and the cell division is stopped, and thus the cell phenomenon may senescence and / or apoptosis (23,24). Cawthon has shown that telomere shortening will cause an increase in mortality rate specially in age-related diseases (25). Thus, length of telomere can be considered as a biomarker of cell destruction, oxidative stress and aging (13).

Interstitial lung disease (ILD) is a heterogeneous group of diseases which primarily involves the interstitium. Studies have shown shortening the telomere length in ILDs, especially in idiopathic pulmonary fibroses IPF (26). In 2015 Sadr and his colleagues showed that COPD patients have a shorter telomere (T/S ratio) in comparison to control group (19).

Table 3. The relationship between telomere length and CRP level in subject with sarcoidosis.

	CRP	N	Mean	Standard deviation	p-value
T/S regression	No	43	0.70804	0.050707	0.003
	Yes	15	0.66192	0.043266	

(0.61±0.08 in comparison to 0.69±0.09/P-value <0.001)

This is the first study to evaluate the telomere length in Iranian patients with sarcoidosis. This study showed that the telomere length is shorter in patients with sarcoidosis compared to the control group. Results of this study are compatible with Madea and Guan findings (20, 27). None of our patients were smokers; so oxidative stress was not an important or effective parameter in the telomere length analysis. Extrapulmonary organs (skin, eye, liver and kidney) were also involved in 27 patients (46.6%). It was expected that with increased organ involvement, the inflammation and stress levels in the body would increase; and therefore telomeres would have a greater reduction than single organ involvement. However the comparison of telomere length between these two groups didn't show any remarkable difference. Although Thompson showed that the length of telomere in patients with ocular sarcoidosis is decreased in comparison to control group, the researches done in field of telomere and Sarcoidosis didn't study this comparison comprehensively (28).

Average length of telomere was 0.7±0.05 and 0.68±0.04 in women and men with sarcoidosis (P-value=0.174) respectively. Also no meaningful correlation was found between aging in both sexes with sarcoidosis and length of telomere. Although a reverse correlation was seen between aging and length of telomere in women, but it was not statistically significant. In Madea et al study, it has been shown that aging associated change in telomere length in both control and Sarcoidosis groups, and in both men and women, is the same. The only difference was higher decreasing rate of telomere length in men (27). Guan et al, reached to the same results in 2007 (20).

ACE is secreted by epithelioid cells in granuloma. Studies show that there is no difference between ACE level, sex and age. Most of the studies showed that patients with higher ACE level have more severe disease and more inflammation (29). Also there is a reverse correlation between Sarcoidosis chronicity and ACE level (30-33). In our study, there was no significant correlation between ACE level and telomere length; which was in agreement with Guan et al study.

Telomere length is decreased with increasing oxidative stress and inflammation. Wrong et al, have shown that with each ng/ml increase in CRP,

telomere would be shortened by 2.6x10-2 bp (34). In 2015 a negative correlation between telomere length of leukocyte in blood and CRP level was shown in women with polycystic ovary syndrome (35). Here we report that with increase in CRP, telomere length will be decreased (P-value=0.03). Although there was no significant correlation between telomere length and ESR level (P-value=0.86).

Conclusion

Sarcoidosis is an inflammatory disease that can involve many organs. Like other chronic diseases, aging phenomenon occurs in sarcoidosis; which lead to decrease cellular and tissue telomere length. This study demonstrated that patients with Sarcoidosis have statistically significant shorter Telomere length compared to healthy individuals, in an Iranian population.

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