Oxidative stress, telomere length, and frailty in an old age population

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Abstract

Background and objectives: A global aging population requires focus on the risk factors for unhealthy aging, preventive medicine, and chronic disease management. The identification of adverse health outcomes in older adults has been addressed by the characterization of frailty as a biological syndrome. On the other hand, oxidative stress and telomere length have been suggested as biomarkers of aging. Here we evaluated the association of oxidative stress, telomere length, and frailty in an old age population.

Research design and methods: This was a cross-sectional study based on 2015 data from 202 members from the Cohort of Obesity, Sarcopenia and Frailty of Older Mexican Adults (n=202; gender F/M ratio: 133/69; mean age: 69.89 ± 7.39 years). Reactive oxygen species (ROS) were measured by dichlorofluorescin diacetate, and lipid peroxidation by malondialdehyde. Telomere length was determined using qPCR with SYBR Green Master Mix.

Results: We found no effect of oxidative stress on telomere length or frailty, but association between telomere length and frailty. Oxidative stress, measured only as ROS and lipid peroxidation, seems to reach a homeostatic level in our older population, which has no effect on telomere length or frailty status. On the other hand, telomere length is associated with frailty, an accurate identifier of health outcome.

Discussion and implications: Hence, it would seem that telomere length could eventually be used as a marker to discriminate between healthy and unhealthy aging, but oxidative stress is not suited as a biomarker but perhaps just as part of the progression of unhealthy aging.

Highlights

- Oxidative stress has no effect on telomere length or frailty status in older adults
- Telomere length is associated to frailty status in older adults
- Biomarkers to help define frailty are needed for a better assessment of healthy ageing

Background and Objectives

A global aging population has motivated researchers to focus on risk factors, preventive medicine and chronic diseases in order to prepare for the coming health issues that this phenomenon will cause. Economy and society itself will suffer from the change in population age distribution around the globe. Between the year 2015 and 2050 the proportion of the world's population with more than 60 years of age will increase from 900 millions to 2000 million, an increase from 12 to 22% ("WHO | 10 facts on ageing and health," 2017). In 2015, Mexico had one older adult (60 years of age and older) for every 10 young adults (15 years old), a figure that is expected to double by 2050. At present, Mexico City is the entity with the highest index of ageing in Mexico with 61.1 older adults for every 100 individuals (González, 2016).

The concept of aging suggests the emergence and accumulation of chronic conditions such as heart disease, diabetes mellitus (DM), cancer, and cerebrovascular diseases, which are also the main causes of death at this stage of life. Hence, the identification of adverse health outcomes in older adults has been addressed by the characterization of frailty as a biological syndrome. Frailty has been described as the loss of physiologic reserve and resistance to stressors that involves multiple physical, mental, and emotional deficits (Buckinx et al., 2015a). As frailty advances, vulnerability to dependence increases, and the risk of adverse events such as functional decline, falls, hospitalization, institutionalization, and mortality become more plausible (Buckinx et al., 2015b; Fried et al., 2001).

Hence the search for biomarkers that can help define frailty at a biological level could be used to improve diagnosis of frailty, to identify pre-frail patients at an earlier stage, and to evaluate the biological outcome when frailty is modified by factors such as healthy eating or physical exercise. On the matter, biomarkers such as MCP1 or β2-microglobulin have been recently associated to frailty in humans (Annweiler et al., 2011; Yousefzadeh et al., 2018). Other markers such as telomere length which has been associated with lifespan (Boonekamp, Simons, Hemerik, & Verhulst, 2013), have had a controversial role on frailty description. Although no correlation was found for Chinese (Yu, Tang, Leung, & Woo, 2015) and German (Breitling et al., 2016) populations, we recently observed shorter telomeres associated with frailty in a Mexican population (Ortiz-Ramírez et al., 2018).

Telomeres are protective and regulatory structures of the genome, their shortening is associated with chronic degenerative diseases such as diabetes (Zee, Castonguay, Barton, Germer, & Martin, 2010), obesity, and hypertension (Nordfjäll et al., 2008), and has been associated to lifespan (Boonekamp et al., 2013). Lifespan depends on many internal and external factors such

as aging, pollution, quality of life, and education, among others. Consequently, telomere shortening and frailty would seem to be intimately related.

Most of the associations with telomere length mentioned above involve the production of oxidative stress that can affect DNA in a direct form. Oxidative stress has been associated with chronic damage in several conditions including DM and hypertension (Pan, Zhang, Chang, Li, & Sui, 2008; Pawluk, Pawluk, Robaczewska, Kędziora-Kornatowska, & Kędziora, 2017; Safar, O'Rourke, & Frohlich, 2014). At a molecular level, oxidative stress is capable of altering DNA and has been linked to telomere length reduction (Ahmed & Lingner, 2018). In order to determine the possible association between ROS, telomere length and frailty, we evaluated the effect of oxidative stress on telomere length as a first approach to explain telomere length shortening in frail patients. Then, we assessed both telomere length and oxidative stress, as frailty biomarkers.

Research Design and Methods

Participants

This is a cross-sectional study based on the baseline 2015 data from the "Cohort of Obesity, Sarcopenia and Frailty of Older Mexican Adults" (COSFOMA) a study involving 1,252 adults ≥60 years of age affiliated to the Instituto Mexicano del Seguro Social (IMSS), residents of Mexico City. A total of 202 individuals from the 1,252 involved in COSFOMA, agreed to participate in the study. In the COSFOMA study, participants were chosen based on their residence through a simple random selection form the list of older adults affiliated to IMSS from 48 Clinics of Mexico City. Written letters delivered to their home addresses were used as invitations to inform them of the nature of the study and to provide them with an appointment at

their clinic. When the older adult did not attend the appointment, a phone invitation was made and in some cases, a home visit [17]. Blood samples were obtained by venipuncture of the median cubital vein using the vacutainer system. Samples collected from April to September 2015 were used for this study.

Ethics Statement

This research protocol was conducted with the approval of the National Committee of Scientific Research as well as by the Ethics Committee for Health Research of the Instituto Mexicano del Seguro Social (R-2012-785-067). All participants gave written informed consent. This study was performed according to the World Medical Association Declaration of Helsinki.

Evaluation of reactive oxygen species (ROS)

To evaluate the formation of ROS by fluorometry, 5 µl of serum and 85 µl of PBS 1X buffer was added to a 10 µl solution of dichlorofluorescein diacetate (DCDHF) (CAS Number 4091-99-0, Sigma-Aldrich), incubated in darkness for 30 minutes at 37ºC for the oxidation of the DCFH to the fluorescent compound 2-7-dichlorofluorescin (DCF) by the presence of peroxide hydrogen to occur. The samples were then read at 498 nm excitation and 522 nm emission (Cytation 5 Cell Imaging Multi-Mode Reader), previously calibrated with a standard curve.

Determination of lipid peroxidation

To evaluate lipid peroxidation we measured malondialdehyde (MDA) as a reaction of thiobarbituric acid (TBA) (CAS Number 504-17-6, Sigma-Aldrich) 50 µl of serum with 25 µl of PBS 1X were added to 50 µl of TBA. The sample was placed in a boiling bath at 94°C for 20 minutes, then centrifuged at 10.500 rpm for 15 min, the supernatant was read at 532 nm, with a spectrophotometer (EPOCH), which was previously calibrated with a standard curve.

Sample processing and telomere length assessment

Genomic DNA was extracted from the peripheral leukocytes by the salting out procedure. Purified DNA samples were aliquoted in a concentration of 10ng/µl and stored at -70 °C until use. For telomere length assessment, we followed the qPCR method published by O´Callaghan and Fenech. The number of copies of telomere repeats was determined by the standard curve of Tel STD, while the standard curve of 36B4 STD was used as a housekeeping gene.

After the qPCR reaction on a StepOnePlus Real-Time PCR System (Applied Biosystem), the Ct values of each sample were extrapolated in their corresponding curves by a linear regression test to determine the telomere length. The Maxima SYBR Green/ROX qPCR Master Mix 2X (Thermo Scientific, California, USA) was used. The cycling conditions for both genes were: 10 minutes at 95°C, followed by 40 cycles of 95°C for 15 seconds, 60°C for 1 minute, followed by a melting curve.

Frailty

Operationalization of the frailty phenotype was performed using the five criteria proposed by Fried (Fried et al., 2001): weight loss, exhaustion, low physical activity (washburn), slowness and weakness. The criteria for low physical activity, slowness and weakness were adapted for the study population (Sánchez-Garcí-a et al., 2017). Participants were classified as non-frail (score 0), pre-frail (score 1–2), and frail (score 3–5).

Statistical analysis

In general, the data analysis and visualizations were carried out with free (R software ("R: The R Project for Statistical Computing," n.d.)) and commercial (SPSS and GraphPad) software. In particular, we used ggplot2 (Wickham, 2009) and ggsignif (Ahlmann-Eltze, n.d.) R packages. In

order to explore the distribution of DCFH, MDA and telomere length between frailty categories, we generated boxplots and calculated their p-value by Mann-Whitney-Wilcoxon test. The relationship between the target variables was explored by scatter plot and linear regression. Quantitative variables are presented as arithmetic mean and standard deviation (mean ± SD), and evaluated with one-way analysis of variance (ANOVA). The distribution of telomere and oxidative stress among elderly groups was evaluated calculating p-value obtained by Kruskal-Wallis non-parametric test. The association between these variables with frailty was analyzed by multiple linear regression analysis with p-values obtained by Student's t test adjusted by age, BMI, education level (without studies, 1-6 years, and ≥ 7 years), and sex.

To establish the magnitude of the association between oxidative stress and telomere length (independent variables) in frailty we grouped the non-frail and pre-frail participants into the Non-frail group. The comparison between Non-frail and Frail groups (dependent variables) was then performed by logistic regression analysis. The odds ratio (ORs) with 95% confidence intervals (CI 95%) was obtained for precision, adjusted by age, BMI, education level (without studies, 1-6 years, and ≥ 7 years), and sex..

Results and Implications

We recruited 202 old adults (≥60 years of age) who agreed to participate from a random selection of the Cohort of Obesity, Sarcopenia and Frailty of Older Mexican Adults (COSFOMA). The general characteristics as well as reactive oxygen species (ROS) measured by DCFH, lipid peroxidation measured by MDA, and telomere length measurements of the elder participants, 133 females (65.8%) and 69 males (34.2%), ranging between 60 and 95 years (mean age: 68.9 ± 7.4) are shown in Table 1. According to the frailty categories, 14.4% of participants

were classified as non-frail and 85.6% had functional decline, corresponding to 60.4% pre-frail and 25.2% frail.

Table 1. General characteristics of Mexican older population ranging between 60-95 years

Old age population	Participants	Frequency *n* (%)
Age (mean ± SD years)	69.89 ± 7.39	202 (100)
BMI (mean ± SD kg/m^2)	27.71 ± 6.59	
Reactive oxygen species (DCFH)	50.27 ± 24.58	
Lipid peroxidation (MDA)	33.53 ± 13.18	
Telomere length (mean ± kb)	5.31 ± 1.72	
Sex	Female	133 (65.8)
	Male	69 (34.2)
Nutritional status	Underweight	8 (3.96)
	Normal weight	63 (31.19)
	Overweight	78 (38.61)
	Obesity	53 (26.24)
Education level	Without studies	16 (7.9)
	1-6 years	78 (38.6)
	≥ 7 years	108 (53.5)
Frailty status	Non-frail	29 (14.4)
	Pre-frail	122 (60.4)
	Frail	51 (25.2)

The aim of this study was to determine a possible association between oxidative stress (DCFH), lipid peroxidation (MDA), telomere length, and frailty status. First, as oxidative stress has been shown to alter telomere length, we evaluated the relationship between DCFH and MDA on telomere length (Figure 1). There was no global effect of DCFH (r = -0.031, p= 0.66) or MDA (r= 0.095, p= 0.18) in telomere length, even when the analysis was carried out for each frailty category (Figure 1). In addition, as the effect of obesity or adiposity on telomere length has been

reported previously (Lee, Martin, Firpo, & Demerath, 2011; Mundstock et al., 2015), and although a negative association of BMI with telomere length is reduced among older people (Lee et al., 2011), we explored the possible correlation between telomere length and nutritional status (underweight, normal weight, overweight, and obesity), but no significant association was found for neither all participants: underweight (r= 0.640, p= 0.088), normal weight (r= 0.089, p= 0.49), overweight (r= 0.110, p= 0.33), and obesity (r= 0.027, p= 0.85) nor when divided by frailty categories (data not shown).

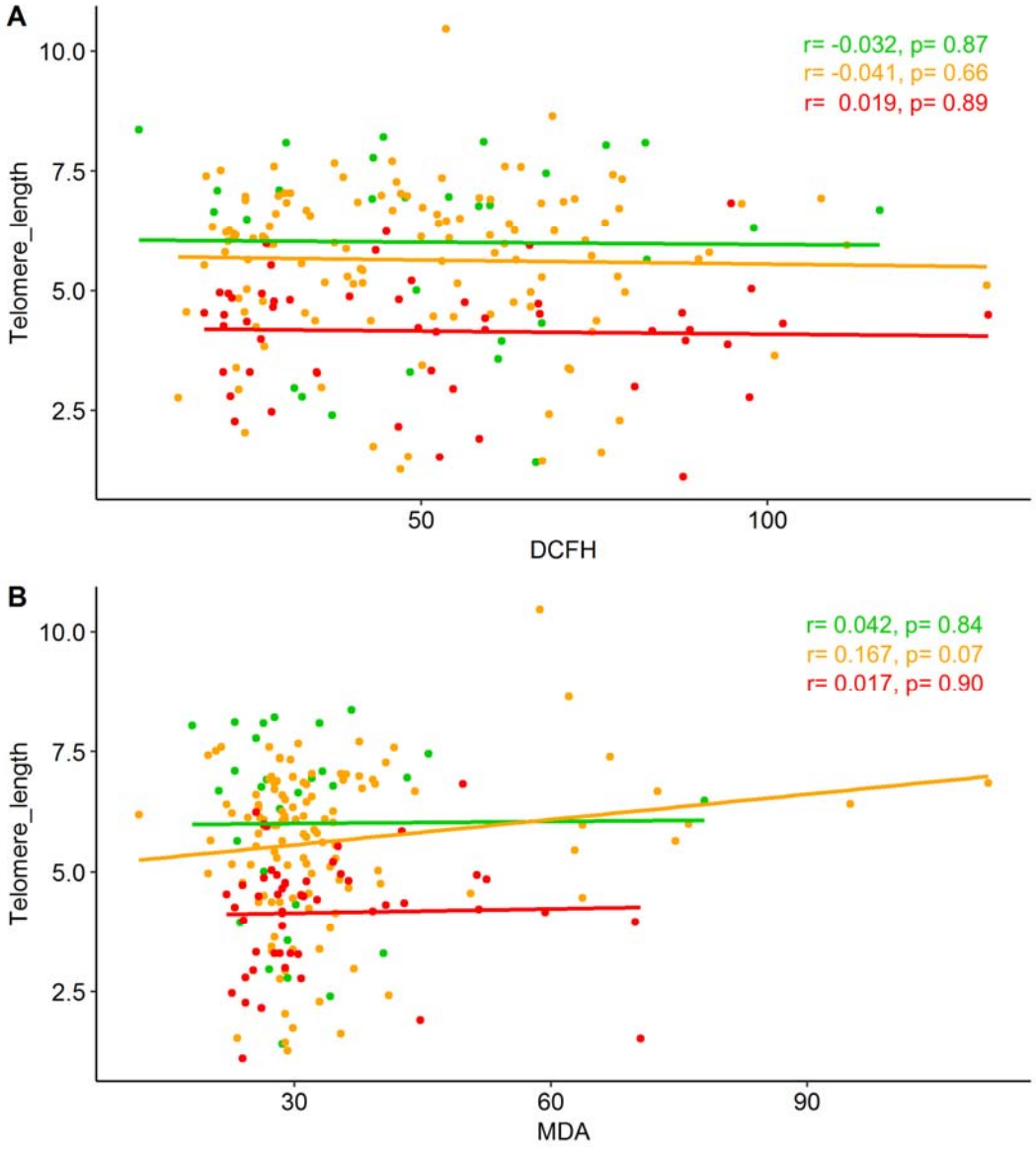

Figure 1. Relationship between DCFH or MDA and telomere length. The figure shows the correlation analysis for (A) DCFH and (B) MDA, with telomere length. The analysis was carried on the overall older population. Linear regression for non-frail (green), pre-frail (orange), and frail (red) participants with Pearson correlation coefficients (r) and p-values adjusted for age, BMI, educational level, and sex are shown.

Then, we assessed the distribution between oxidative stress, lipid peroxidation, and telomere length according to frailty groups (Table 2 and Figure 2). Across frailty categories, we did not find significant differences between the distributions values of ROS (DCFH, p= 0.76) or lipid peroxidation (MDA, p= 0.37), but we did find a significant difference on the value distribution with telomere length (p< 0.001). This difference was explained by lower telomere length in the frail group compared against the non-frail (p< 0.001) and pre-frail (p< 0.001) groups, while differences between non-frail vs. pre-frail did not reached significance (p= 0.065), obtained by Mann-Whitney-Wilcoxon test with Bonferroni correction.

Table 2. Association between telomere length and oxidative stress with frailty status

Elderly groups	Non-frail	Pre-frail	Frail		
	n= 29	n= 122	n= 51		
	mean ± SD	mean ± SD	mean ± SD	β	p-value
Lipid peroxidation	31.18 ± 11.0	34.1 ± 14.27	33.5 ± 11.53	0.017	0.807
Reactive oxygen species	52.55 ± 24.32	49.11 ± 23.12	51.75 ± 28.2	-0.035	0.606
Telomere length	6.00 ± 2.02	5.63 ± 1.6	4.15 ± 1.20	-0.114	< **0.001**

Multiple linear regression analysis with frailty group as dependent variable, and telomere length (kb), lipid peroxidation (MDA) as well as oxidative stress (DCFH) were used as independent variables. SD, standard deviation; ROS, reactive oxygen species. The p-values were obtained by Student's t test adjusted by age, BMI, educational level, and sex. Bold text indicates a statistically significant difference (p-value cut-off <0.05).

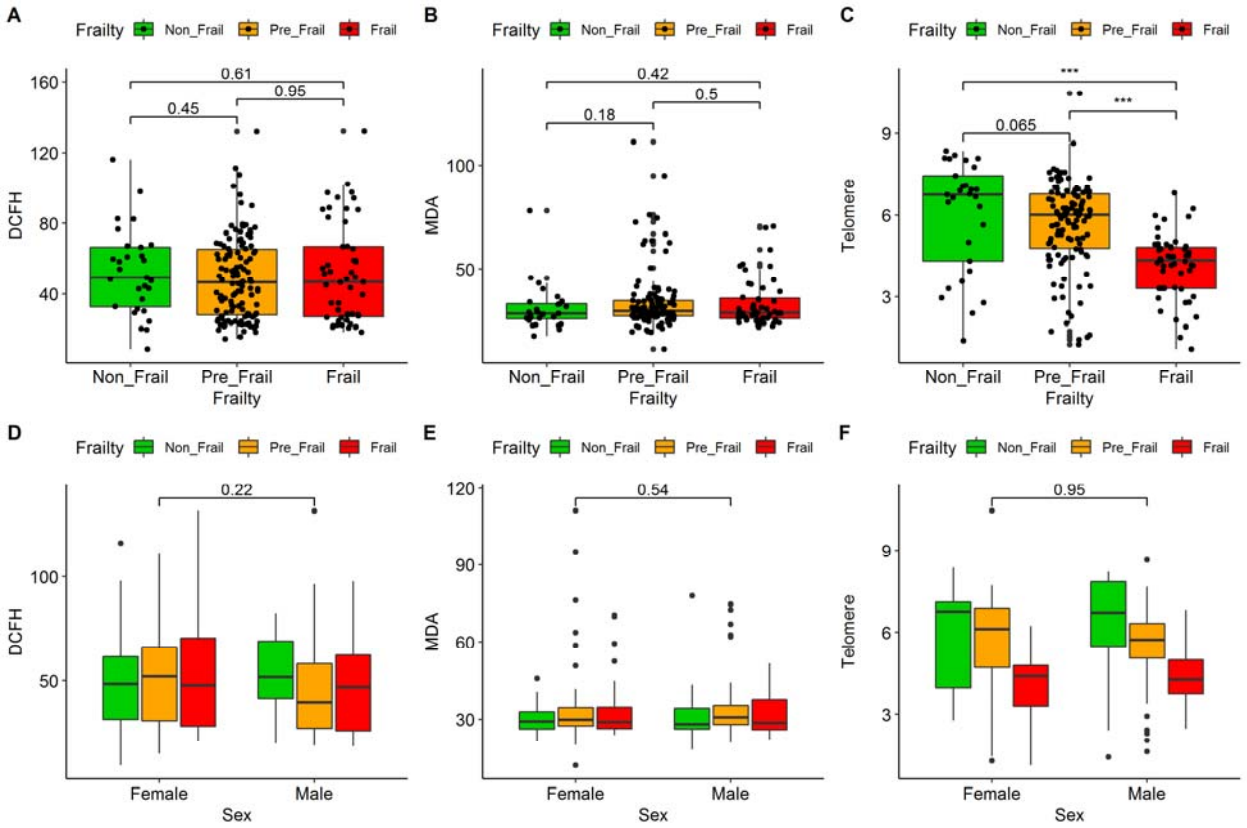

Figure 2. Oxidative stress, lipid peroxidation and telomere length among old adults groups. This figure shows the distribution of DCFH (p= 0.76), MDA (p= 0.37), and telomere length (p< 0.001), among elder groups. The p-value was obtained by Kruskal-Wallis nonparametric test. The A, B, and C panels show the differences with significant lines within frail categories while panels D, E, and F show the differences with significant lines between females and males by Mann-Whitney-Wilcoxon test. Bonferroni correction < 0.016 was calculated to assign significant differences; p-values <0.001 ("***"). The p-value was obtained by Kruskal-Wallis nonparametric test.

Contrary to our hypothesis, we observed no correlation between DCFH or MDA with telomere length in the elder frail groups. Significant differences between the frailty groups and telomere length were confirmed (p<0.01) with a Mann-Whitney-Wilcoxon test (Figure 2 and Table 2).

Finally, Figure 3 and Table 3 show the magnitude and precision of the association of oxidative stress and telomere length on a frail elder population. Risk of frailty increases 1.007 per each µmol of MDA (CI 95% [0.980-1.035] p=0.59), and 1.002 per each pmol of DCFH (CI 95% [0.988-1.016] p=0.75). On the other hand, every kb of telomere (a longer telomere) protects 0.55 times from frailty (CI 95% [0.448-0.697] p<0.001).

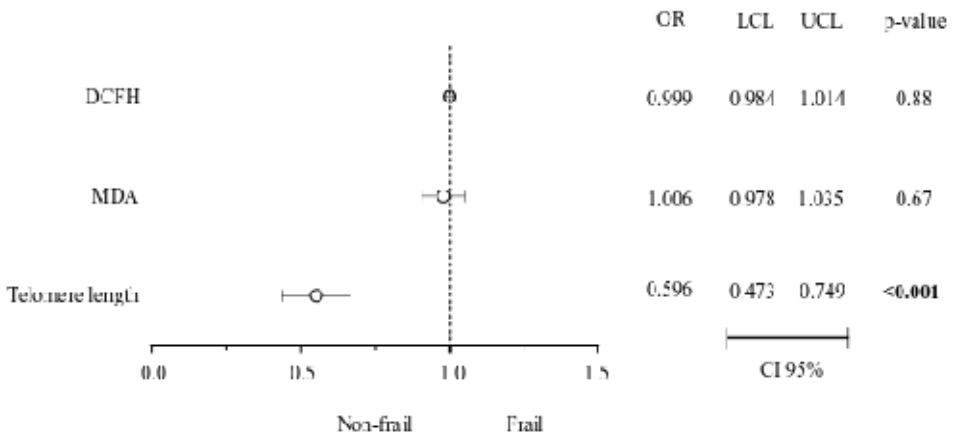

Figure 3. Association of oxidative stress and telomere length on a frail elder population.

A multiple linear regression analysis with telomere length as the dependent variable and frailty, ROS and lipid peroxidation as independent variables showed a non-significant association (p=0.095) of lipid peroxidation suggesting that higher levels of lipid peroxidation are associated to shorter telomeres. When applying to frailty, we found that pre-frail participants have a 1.035kb shortage in telomere length compared to non-frail and frail participants, which increase that range to 2.07kb (Table 3).

Table 3. Association between frailty and oxidative stress with telomere length

Telomere length	Unstandardized β	95% CI		Standardized β	p-value
		LCL	UCL		
Lipid peroxidation	0.014	-0.003	0.031	0.11	0.095
Reactive oxygen species	-0.002	-0.011	0.007	-0.029	0.658
Frailty groups					
No frail	Reference			Reference	
Pre-frail	-1.035	-1.393	-0.677	-0.374	< **0.001**
Frail	-2.07	-2.786	-1.354	-0.748	< **0.001**

Multiple linear regression analysis with telomere length as dependent variable, and frailty, lipid peroxidation (MDA) as well as oxidative stress (DCFH) as independent variables. SD, standard deviation; ß, standardized regression coefficient; MDA, malondialdehyde; DCFH, dichlorodihydrofluorescein diacetate. The p-values were obtained by Studet's t test. Bold text indicates a statistically significant difference (p-value cut-off <0.05)

Discussion and implications

Due to a global demographic transition, successful aging (avoidance of disease/disability, high physical and cognitive function, and sustained engagement with social activities) has become a priority (Rowe & Kahn, 1997). On that matter, frailty has shown to be a good predictor of adverse events in elders, and biomarkers such as telomere length and oxidative stress have shown to be intimately related to lifespan. Short telomeres have been associated to cardiovascular disease (D'Mello et al., 2015), and diminished longevity (Boonekamp et al., 2013); while oxidative stress has been reported to increase in a wide variety of chronic diseases such as diabetes mellitus and hypertension (Radak et al., 2017).

Here, the three variables were evaluated in an aged population of Mexico City in order to define their association and correlation. A first analysis on the distribution between oxidative stress, lipid peroxidation, and telomere length according to frailty groups showed no significant differences between ROS or lipid peroxidation, but a significantly different distribution with telomere length. A second approach of the effect of oxidative stress on telomere length, showed no significant correlation between levels of either reactive oxygen species or lipid peroxidation but a mild association (non significant p=0.095) of increased lipid peroxidation and shorter telomere length. Lipid peroxidation is a reflection of increased oxidative stress, so this mild association could be interpreted as an effect of oxidative stress on telomere length. A significant difference could be reached perhaps with a larger population study.

Also, another approach regarding oxidative stress is the quantification of 8-hydroxy-2'-deoxyguanosine (8OHdG), a specific biomarker of oxidative stress in DNA(Te Koppele et al., 1996). An increase in 8OHdG provides a molecular explanation for how ROS interferes with telomere extension by affecting the telomerase (Forsyth, Evans, Shay, & Wright, 2003). At the time, we were unable to do this measurement, which would be more relevant to telomere length. On the matter, even though oxidative stress has been found to increase in chronic disease and in response to several environmental factors, it has also been reported that a chronic disease such as diabetes mellitus produces an increase in oxidative stress when firstly acquired but diminishes as the body reaches homeostasis within disease (Pérez & Núñez, 2007). It has been reported that oxidative stress is increased when comparing aged to young adults (Muñoz Montero, 2017), but not an association of this increment to other age related diseases. Perhaps, an increased oxidative stress, as part of a general lost of homeostasis related to ageing, does not have a real influence in life span, as do telomere length and frailty.

Here, frailty was found to be associated to telomere length, even for pre-frail patients. This specific variable has become of importance for elders since despite the philosophical debate about the definition of frailty; it has shown to have a predictive value. For one, we have found a direct biological outcome (telomere length), associated to a shorter life span. Research on this direction may help determine frailty in a more quantitative manner with a biological background. Although this procedure is currently not widely available, its future implementation should be considered to diagnose frailty risk.

Interestingly, telomere length has been found to have modifiable factors such as smoking and obesity, which are associated to shorter telomeres while exercise and a healthy diet preserve telomere length (Rowe & Kahn, 1997; Shammas, 2011). On that matter, a recent study showed

that frailty can be modified by exercise and nutrition, reversing the frailty state to a non-frail (Michel, Cruz-Jentoft, & Cederholm, 2015). Hence, telomere length determination for frailty diagnosis could help apply prompt intervention on the above mentioned factors in order to reverse this condition. If frailty is assessed on time, more costly medical outcomes could be prevented and quality of life improved.

Conclusions

In conclusion, we found no effect of oxidative stress on telomere length or frailty for our population. Our main limitation was the number of participants of the study to whom all the measurements were made. Nonetheless, we reaffirmed an effect of the frailty phenotype on telomere length and found a low association between lipid peroxidation and shorter telomere length. Weather oxidative stress is the main cause for telomere length shortening due to frailty remains to be proven.

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Conflict of interest

None

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