Relationship Between Attitude of Optimism and Pessimism with Leukocytes Telomere Length

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Abstract:

Background & Aim: Human vision for the future includes two kinds of optimism and pessimism. Combination of low positive and high negative attitudes about the future increases the risk of diseases and premature death. One way to influence the person's attitude on the phenomena of life is its effect on leukocyte telomere length.

Methods: For this purpose, 50 participates (25 males and 25 females) with a mean age of 41.74 ± 7.48 were selected. R-LOT questionnaire was filled about them and 5 ml of blood was collected and DNA extracted. The terminal repeats sequences of leukocyte telomeres were examine with use of Real time PCR.

Results: The results indicated that optimism was a marginally relationship with leukocyte telomere length(r=0.31, p= 0.07) but in contrast, pessimism has been linked strongly and negatively with telomere length (r= - 0.55, p= 0.001). Also optimism is associated with leukocyte telomere length (correlation coefficient = r= 0.21) but pessimism correlation with telomere length is 0.68. The coefficient of determination, or R^2 in optimism and pessimism, were 0.04 and 0.46 respectively (P= 0.001). Conclusion: Based on these findings, we can conclude that the negative attitude can be considered as one of the factors contributing to the life and shortens the telomere length in human cells.

Keywords: optimism; pessimism; telomere and real time PCR

Introduction

Human lifespan is a complex phenotype that is influenced by various factors such as diet, physical activity, environmental conditions, etc. Although non-genetic factors play an important role in human lifespan, genetics researchers believe that more than 50% of the difference in human lifespan depends on their genetic differences, and they have always been trying to discover the genetic secrets of the aging process. Some of these factors can be prevented, and especially by modifying the lifestyle, some of the factors can be prevented or changed if they occur [1, 2].

Among these factors, we can mention the attitude towards life. Human attitude towards the future includes two types of optimism and pessimism. The combination of low positive and high negative attitudes about the future and its related path (i.e. low optimism and high pessimism) increases the risk of diseases and premature death. This may be by reducing the activity level of the body's immune system or other biological activities [3].

Optimism and pessimism are the basic qualities of personality and affect how people adapt to life events. These two factors affect people's mental expectations when facing problems and their behavior when overcoming problems. In several studies, the relationship between optimism and suffering and unhappiness has been investigated in groups that have faced problems or adversities, including heart surgery experiences (4), adaptation to MS disease [5] and cancer diagnosis [6]. In general, it has been shown that optimistic people experience less suffering than pessimistic people in facing life problems. Maybe optimists do certain things when dealing with problems, which causes them to adapt better

Nowadays, the topic of longevity and old age is discussed in many scientific societies. There are several definitions for old age depending on its criteria. In different parts of the world, based on different cultures, old age is defined in different ways. The World Health Organization has classified different periods of life after puberty based on calendar age as follows: from 35 years to 59 years old middle age, from 60 years old to 74 years old, from 75 years old to 90 years old and from 90 years old to Above is considered very old [7]. The combination of low optimism and high pessimism is more related to negative mood and weak exposure to stress. Expanded negative expectations for the future can increase susceptibility to cognitive stress [8, 9].

There are different methods to study the aging process. Recently, new biomarkers of aging include changes in telomere length as one of the most important ones [10, 11].

The ends of eukaryotic chromosomes are covered by the hexameric sequence (TTAGGG) and related proteins. These sequences have a size of about 10 to 15 kilo base pairs ([12] and are known as telomeres. Telomeres stabilize chromosome ends during replication [13, 14]. The telomere length of peripheral blood leukocytes shows the size of the systemic telomere length in the body. Systemic telomere length is considered as an indicator of biological aging. Systemic telomere length is a matter that is mainly genetic, but several factors are also known as intervening factors: age (telomeres are shorter in older people), father's age at birth (telomeres in people with older fathers are birth is longer), and gender (telomeres are shorter in men). Also, evidence shows that higher levels of oxidative stress and inflammation cause a further increase in the rate of destruction of telomeres [15].

A new and acceptable method that is recently desired by scientists is the use of Real-Time PCR technique, which can determine the number of copies of the desired gene at the end of the telomeres at any moment [16].

In this regard, in this article, an attempt was made to investigate the relationship between the telomere length of leukocyte chromosomes and the positive or negative attitude of the studied subjects towards life.

Materials and methods:
People in the study:

Fifty people (25 women and 25 men) aged between 40 and 44 years with medium economic and social level were selected from the northwest region of the country by random sampling method. These people were the companions of people who referred to two medical diagnosis laboratories in two economically different areas of Tabriz. The main reason for this choice was, first of all, the ease of blood donation and the reason for choosing two laboratories in two different urban areas was to neutralize the effect of possible bias in the referring people in terms of economic and cultural level. After completing the demographic questionnaire, the consent form and the R-LOT test including 10 standard questions, 5 ml of peripheral blood was collected in completely standard and sterile conditions using the Japan Trimo vacuum system and poured into special vials with EDTA.

Methods:
1. Revised-Life Orientation Test (R-LOT):

Scheier and colleagues compiled a summary self-report Life Orientation Test (LOT) to assess optimism and later revised it [17]. This test measures individual differences in optimism-pessimism. The type of optimism evaluated by LOT is a personality trait that is characterized by favorable personal expectations in the future. Many researchers have shown that optimism and pessimism of nature have a good relationship with a wide range of related constructs such as self-esteem, hopelessness and psychological distress, and this shows the convergent validity of this questionnaire. Respondents are asked to rate their level of agreement for each of the questions based on a 5-point Likert scale ranging from 0 (strongly disagree) to 4 (strongly agree) [17].

2. Real-Time Polymerase Chain Reaction (Real Time-PCR) technique:

One of the important types of PCR is the Real-Time Polymerase Chain Reaction (Real Time-PCR) technique. In general, Real Time PCR is a technique for continuously observing the progress of the PCR reaction over time. Also, with this method, the amounts of PCR products (DNA, cDNA or RNA) can be measured. Using a special extraction kit, RNA from peripheral blood and according to the protocol included in the kit and using Oligo-dT primer and Reverse Transcriptase enzyme, total RNA was converted into cDNA. The concentration and optical density of the purified DNA were determined at 260 and 280 nm wavelengths with a ND-1000 Nanodrop spectrophotometer (BioRad, USA). DNA samples with a wavelength ratio of 260/280 nm between 1.6 and 1.8 were used to perform Real-time PCR.

To perform quantitative PCR and calculate the copy of the Human Telomerase Reverse Transcriptase (hTERT) gene, the following path was taken:

From the obtained cDNAs, using specially designed primers and probes, Real time PCR was performed with the Taq/Man method according to Table 1.

Primer type	Sequence
Forward primer of the target gene	5′- CGG TTT (GTTTGG)5 GTT-3′
Target reverse gene primer	5′- GGC TTG (CCTTAC)5 CCT-3′
Forward primer reference gene	5′-CCCATTCTATCATCAACGGGTACAA-3′
Reference reverse gene primer	5′-AGGTAGAAGGCCACATCACC-3′

Table 1: Primers used in Q-PCR reaction

This PCR was performed for two hTERT genes as the target gene and acidic ribosomal phosphoprotein P0 on chromosome 12 (as internal control and reference gene) and Δct was calculated and the expression level of hTERT gene was reported quantitatively. This amount of gene expression is compared with the variables participating in the project and the final report is presented in the results section.

ABI 7300 Sequence Detection System Biosystems Real-Time PCR device was used for this research. The time program of the device was carried out in three stages: the first stage, which leads to the denaturation of DNA molecules and the activation of the polymerase enzyme, is $95^{\circ C}$ for 10 minutes, the second stage is 95°C for 15 seconds and $54^{\circ C}$ for 2 minutes for 18 consecutive cycles and the final stage for drawing the dissociation curve or melting curve was performed at $95^{\circ C}$ for 15 seconds, $60^{\circ C}$ for 30 seconds and $95^{\circ C}$ for 15 seconds.

The reactions were performed in triplicate in a final volume of 25 microliters. The mixture of each reaction includes: 12.5 microliters (SYBR-Green PCR Master Mix (Applied Biosystems Warrington, UK), 1 microliter of forward and reverse primers specific to each gene, 5 microliters of template DNA, and the rest of the mixed distilled water to reach the final volume of 25 microliters.

In this research, the green fluorescent dye SYBR Green I (as a specific fluorescent probe) was used, which can be located between the small grooves of the double-stranded DNA molecule and produce fluorescent light. The amount of fluorescent light production is directly proportional to the amount of PCR product production.

In quantitative data relative measurement studies, changes in the target gene compared to the reference gene are investigated and compared. Therefore, it is necessary and necessary to calculate the efficiency and effectiveness of the multiplication reaction for both genes. Therefore, after the completion of the reaction, successive dilutions of standard DNA as a template, a standard curve is drawn based on the logarithm of DNA concentration (horizontal axis) and the threshold cycle or Ct (vertical axis) for each gene. The t/s ratio can also be used to express telomere length, where t is the number of repeated copies of the telomere and s is the number of single gene copies. But stating the number of base pairs at the end of the telomere makes it easier to understand the problem. This scale has also been used in this research.

Results:

In order to investigate the relationship between optimism and pessimism with leukocyte telomere length, Pearson's correlation coefficient was used. The results showed that optimism was closely related to telomere length (r=0.31 and p=0.7), but on the other hand, pessimism was strongly related to telomere length and its relationship was negative and inverse. (r=-0.55 and p=0.001)

Correlation and regression were also used in relation to examining the severity of pessimistic and optimistic attitudes with telomere length. The data of this test is included in table 2.

Telomere length	R	R^2	β	p
Optimism	0.21	0.04	0.27	0.39
Pessimism	0.68	0.46	0.68	0.001**

** $P=0.001$

Table 2: Regression analysis between optimism and pessimism with telomere length

The contents of the mentioned table show that optimism is related to leukocyte telomere length with a correlation coefficient of 0.21, but the correlation rate of pessimism with telomere length is 0.68. While the coefficient of determination or R^2 is 0.04 in optimism and 0.46 in pessimism, which is highly significant in this last case (P=0.001).
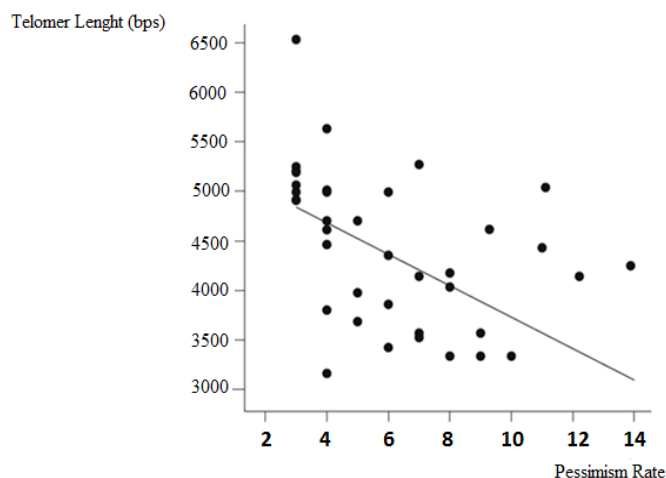

Figure 1: Correlation between pessimism and chromosomal telomere length in base pairs

As shown in Figure 1, the amount of pessimism has an inverse and significant relationship with telomere length.

Conclusion:

Regarding the study of the relationship between optimism and telomere length, because the level of p = 07 was obtained, therefore

these two components are closely related (r = 0.31 and p = 0.7), but on the other hand, the mood of pessimism It is strongly related to telomere length and its relationship is negative and inverse (r=0.55 and p=0.001). That is, the higher the level of negative attitude in a person, the shorter the telomere length (Figure 1). In line with the findings of the present study, the findings of Epel *et al.*'s research are in line with our research; They state that high levels of pessimism are associated with telomere length. This group of scientists also investigated chronic stress with telomere length and telomerase activity and showed that the amount of time people are exposed to life pressures is inversely related to telomere length [18, 19].

In 2009, O'Donovan et al. investigated the level of optimism and pessimism with the level of IL-6 and determined that pessimism in people is directly related to the increase of IL-6 level. They did not find a relationship between optimism and telomere length, but there was a strong relationship between pessimism and the length of telomeric repeats [20]. The work of these scientists is also in line with the results of the present research.

Regarding the relationship between pessimistic and optimistic attitudes and telomere length, it was found that the greater the pessimism, the lower the telomere length and its terminal repeat. In the research done by Ludlow et al., it shows that in pessimistic people, the length of the cell cycle of leukocyte cells decreases by 30 to 35 cycles for each year of life, and this means that telomeres and the telomerase enzyme are capable of terminal replication. It gradually loses the chromosome and the higher the level of pessimism, the lower the number of DNA replication [20-24].

Recently, in another studies by Puterman *et al.* in 2015[25] and Tomiyama *et al.* in 2012, the relationship between life pressures and stress and cortisol secretion as well as telomere length has been investigated, which is in line with the results of the present study [26]. This type of studies, especially in recent years, all indicate the importance of the subject and attention to the type of life attitudes with telomere activity and the role of telomerase.

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Conflict of interest:

The authors declare that there is no conflict of interest in conducting this research.

Contribution of the authors:

In this research, the works related to the design of the topic and molecular genetic experiments were done by Seyed Mahmoud Tabatabaei (participation rate 40%), Vahid Pour Saeid helped in the interpretation of the results (participation rate 20%), Naser Herfe doost in obtaining the questionnaires and participated (20% participation rate) and Masoumeh Hafezi in participation of article and statistic analyzing (20% participation rate).

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