# INFLUENCE OF LONG-TERM HEARTFULNESS MEDITATION PRACTICE ON hTERT GENE EXPRESSION, PERCEIVED STRESS AND EMOTIONAL WELLNESS: A CASE-CONTROL STUDY

**Parmita Contractor[1*], Kinjal A. Patel[2], Kruti A. Mehta[3], Prabhudas Patel[4]**

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## Abstract

**Background**: Meditation confers health and longevity, both of which are considerably determined by one's experiences in terms of stress and emotions. Involved in diverse cellular and physiological functions, hTERT gene relates with health and longevity. The influence of a long-term meditation practice "Heartfulness" on hTERT gene expression, perceived stress and emotional wellness has not been reported. The present cross-sectional study aimed to assess the expression of hTERT gene, Perceived stress and Emotional wellness in long-term Heartfulness meditators in a real-world environment.
**Methods**: Self-reported healthy, age and gender matched long-term Heartfulness meditators and non-meditators were assessed for hTERT gene expression, perceived stress and emotional wellness.
**Results**: Meditators demonstrated an overall higher hTERT, lesser perceived stress ($p<=0.002$), greater emotional wellness ($p<0.005$) and lesser sleep hours ($p<0.000$). Significantly higher ($p=0.022$) hTERT was noted in meditators of >40 years age. Duration of daily meditation correlated positively with hTERT expression.
**Conclusion**: Our results are suggestive of a significant healthier aging response along with better mental and emotional levels in the meditators in a real-world environment. Also, duration of daily meditation might play a role in determining health and longevity.

**Keywords:** Heartfulness, hTERT, Meditation, Stress, Mental and Emotional wellness

[1*]Heartfulness Research centre, Department of Philosophy, Recognized by University of Mysore, Mysuru. 571130. E-mail: parmita.contractor@gmail.com
[2,3]Cancer Biology Department, The Gujarat Cancer & Research Institute, Civil Hospital Campus, Asarwa, Ahmedabad. 380 016.
[4]Former Professor and Head, Cancer Biology Department, The Gujarat Cancer & Research Institute, Asarwa, Ahmedabad 380 016.

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**Abbreviations**: cDNA- complementary DNA; DNA- deoxyribo nucleic acid; DOM- duration of daily meditation; EDTA- Ethylenediaminetetraacetic acid; Est1- Telomere elongation protein; EWA- Emotional wellness assessment; hTERT- human telomerase reverse transcriptase; hTERC- human telomerase RNA component; mRNA- messenger RNA; NCD- Non-communicable disease; PCR- Polymerase chain reaction; PSS- Perceived stress scale; RNA- Ribonucleic acid; RT- Reverse transcription; TA- telomerase activity; YOP- years of meditation practice.

## 1. Introduction

Meditation helps people live longer and better by reducing the wear and tear on both the mind and the body (Yogananda, 2013). Lowly spiritual health poses increased risk of poor mental and social health which ultimately leads to poor physical health in turn leading to diseases through involvement of the autonomic nervous system (Singh et al., 2016). Better lifestyle choices reducing oxidative stress can influence telomerase activity, prevent telomere shortening, delay onset of age-associated diseases and increase lifespan. Meditation practice is among the chief factors important for human health and lifestyle disorders (Rathore & Abraham, 2018).

The modern society shares a commonality in goal i.e. of wellbeing and healthy aging (Lara et al., 2015). The enzyme telomerase has been linked with health and death through its effect on telomere length (Schute & Malouf, 2014). Short telomeres link to several risk factors of aging, early mortality, non-communicable diseases (NCDs) and psychological stresses across prenatal to adulthood period (de Punder et al., 2019). Partial or total loss of telomerase accelerates aging and is associated with age-linked disorders and its re-activation might act as an assuring means for reversal/delay of cell senescence resulting in extension of health span (Boccardi & Paolisso, 2014). Telomerase is a DNA polymerase made up of two central units- hTERT and hTERC plus auxillary factors- Est1 proteins and dyskerin. The hTERT gene (loci- 5p15.33) is the catalytic subunit reverse transcriptase whereas hTERC gene (loci-3q26) is the RNA subunit which provides the RNA template for hTERT to cater de novo addition of telomeric DNA (Cifuentes-Rojas & Shippen, 2012; Leao et al., 2018). hTERT is the rate limiting factor for telomerase activity (TA) control since it silences telomerase due to its stringent repression (Yuan & Xu, 2019). TA in normal individuals is seen only in those cells which exhibit requirement of proliferative potential and are mitotically active- such as germ, stem, embryonic tissues, endometrium tissue, hair follicles, intestine, skin basal layer and hematopoietic system (Cifuentes-Rojas & Shippen, 2012; Leao et al., 2018; Mitchell & Collins, 2000).

hTERT expression has been reported to be the mediator of telomere maintenance and lifespan extension in a number of genetic and biochemical studies (Sharma et al., 2003). Apart from lengthening the telomeres, telomerase/hTERT extends its functioning in crucial cellular functions like gene expression, signalling pathways, mitochondrial mechanisms, cell survival, regulation of ubiquitin proteasomal functions, expression of microRNA, DNA damage repair, reduction of oxidative stress, neuroprotection, RNA dependent RNA polymerase activity, long-term immune function, cellular health, blocking apoptosis, genome stabilisation, stress resistance thus exerting active effects in physiological activities and eventually aging (Boccardi et al., 2016; Cheung et al., 2019; Epel & Prather, 2018; Kumar et al., 2015; Pirzio et al., 2004; Yuan & Xu, 2019). Telomerase has been accounted to be the psycho-biomarker which predicts human age (Dasanayaka et al., 2022).

The cellular and systemic levels experience gradual wear and tear from emotional, psychological and physical demands impacting telomere biology (Rentscher et al., 2020). Depending on the type, time and severity, stress can produce wide range of alterations in the body from affecting homeostasis to endangering life, acting as a trigger or aggravating agent for pathological conditions, various diseases and even death (Yaribeygi et al., 2017). Individual attributes such as one's ability of managing thoughts, emotions, interactions and behaviour in addition to social, cultural, economic, political and environmental along with particular psychological/ personality/ genetic factors, determine mental health (World Health Organization, 2022a). Mental health conditions- mental disorders (significant disturbances in thinking, emotional regulation/ behaviour), psychosocial disabilities and other mental states are associated with significant distress, impairment in functioning, or risk of self-harm (World Health Organization, 2022b). The recent 2019 consensus as per World Health Organization, reports 1 in 8 individual to be living with a mental disorder globally (World Health Organization, 2022c). Perceived stress, negative mental and emotional states have been found to be associated to health adversities (Chu, 2010). Strong evidences back the association of relationships and emotions on one's resultant physical health. Moreover, the perception of lifespan points towards a wide encompassing developmentally-grounded vision of interwoven networking of social relationships, emotions and health over the life of an individual (Uchino & Rook, 2020)

Meditative practices have been found to be highly effective in reducing the levels of perceived stress and negative emotions. The Prevention, management and treatment of stress and related diseases by meditation training have become increasingly popular (Lane et al., 2007). Yogic meditation practices, intensive meditation training, comprehensive lifestyle and health promoting behavioural changes following interventions have been reported to be resulting in increased TA and wellbeing (Cheung et al., 2019; Ho et al., 2012). Expression of both the hTERT gene and hTR gene is promoted by meditation (Dasanayaka et al., 2022). Nonetheless improvement of TA by different mind-body techniques is still to be illuminated (Ho et al., 2012). Though telomeric maintenance by pursuing meditation has been evidenced and postulated in the existing literature, the conditions resulting in this positive impact and the length of time until which these impacts are perseverant is not clear (Conklin et al., 2019). No study has been reported on TA measured by hTERT gene expression along with perceived stress and emotional wellness in the long-term Heartfulness meditators with >10 years of meditation practice. Heartfulness meditation practice is a system designed vigilantly with 3 essentials to maintain the meditative state and its effects throughout the day and farther. First, heart based meditation aided by transmission which allows one to be absorbed within. Second, Heartfulness cleaning practised at the end of one's day's work, cleanses one's system of accrued heaviness and results in revival. Third, heartfulness prayer connects one with the source/God before going to sleep (The way of the heart, 2019). The present cross-sectional, study was performed to assess the difference in hTERT gene expression, perceived stress and emotional wellness between adept self-reported healthy Heartfulness meditators and self-reported healthy non-meditators living in a real-world environment.

## 2. Methods

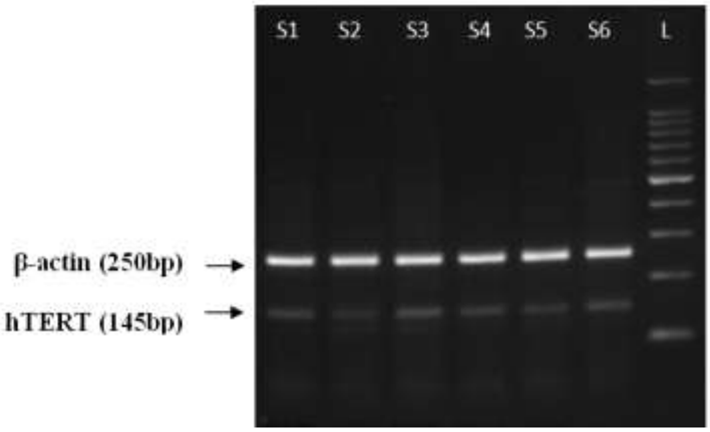
This study was approved by Heartfulness Institute Medical Research wing-India, Ethics Committee (approval no. 001/HMRT/IEC/2019). Participants were identified as befitting the criteria. Inclusion criteria (Meditators): Heartfulness meditators practising meditation for ≥10 years, healthy and substantial regularity of meditation practice. Inclusion criteria (Controls): healthy individuals not having prior or present meditation experience. Exclusion criteria: Individuals with current illness/ recent x-rays/ recent blood transfusion, genetic diseases, diabetes, thyroid, hyperlipidemia, hypertension, kidney disease, cardiac diseases, pathological conditions, mood disorders, psychotic disorders, suicidal tendencies, alcoholic, smoker and individuals taking frequent medications or health supplements.

The meditator group participants were approached via advertisement and distribution of a basic questionnaire on regularity of meditation practice, medical history, routine medication, supplements and demographics, at a local heartfulness meditation centre in Ahmedabad, Gujarat, India. Meditators following considerably regular Heartfulness meditation practice ≥10 years were identified with screening of the basic questionnaire. Years of meditation practice (YOP) of the participants ranged from 11.08 – 39 years, duration of daily meditation (DOM) ranged from 40-60 minutes and their age range was 31.02 -83 years. Whereas the control group participants were approached via word of mouth to friends, relatives, colleagues and advertisement in whats-app groups followed by administration of the basic questionnaire on medical history, routine medication, supplements, prior or current meditation experience and demographics. Accordingly, age and gender matched control group participants were identified and enlisted for the study. The age range of control group participants was 31.09 – 81.08 years. All the 60 participants were age (0 ± 16 months, except for 1 participant +33 months) and gender matched. None of the participants were made to go through any sort of intervention on or before participating in the study. The meditator group participants were involved in their regular life and also practising meditation as prescribed under Heartfulness system of meditation, i.e. Meditation, Cleaning, Prayer, one weekly guided meditation session with Heartfulness trainer and one group meditation session in the weekend whereas the non-meditator group participants were only performing their usual day to day activities. Demographic details (Table -1), blood samples and self-administered questionnaires for Perceived stress and Emotional wellness were collected on obtaining written consent from the participants. Blood sample testing was performed at Cancer biology department, Gujarat Cancer and Research Institute, Ahmedabad, Gujarat, India.

**Table 1**. Socio-demographic characteristics of the participants

| Characteristics | Groups | Meditators: N(%) | Non-meditators: N(%) |
|---|---|---|---|
| **Gender** | Male | 18(60) | 18(60) |
| | Female | 12(40) | 12(40) |
| **Body mass index (Asian cut-off)** | Underweight | 1(3.3) | 0(0) |
| | Normal | 7(23.3) | 12(40) |
| | Overweight | 5(16.7) | 5(16.66) |
| | Obese | 17(53.3) | 13(36.66) |
| **Age (in years)** | 31-40 | 9(30) | 9(30) |
| | 41-50 | 13(43.3) | 13(43.33) |
| | 51-83 | 8(26.6) | 8(26.66) |
| **Marital Status** | Unmarried | 1(3.3) | 3(10) |
| | Married | 26(86.7) | 25(83.33) |
| | Widowed | 3(10) | 2(6.66) |
| **Socio-economic status** | Middle | 25(83.3) | 22(73.33) |
| | High | 5(16.7) | 8(26.66) |
| **Physical Activity (Self-reported)** | Low | 21(70) | 15(50) |
| | Moderate | 9(30) | 15(50) |
| **Diet** | Vegetarian | 30(100) | 30(100) |
| **Locality** | Urban | 30(100) | 30(100) |
| **Health** | Illness/Disease/Disorder | 0(0) | 0(0) |
| **Alcoholic/Smoker** | Habitual/ Occasional | 0(0) | 0(0) |
| **Family type** | Joint | 13(43.3) | 13(43.3) |
| | Nuclear | 17(56.7) | 17(56.7) |
| **Education** | Secondary school | 1(3.3) | 2(6.7) |
| | Higher Secondary school | 3(10) | 3(10) |
| | Graduate | 14(46.7) | 16(53.3) |
| | Post graduate | 10(33.3) | 7(23.3) |
| | Professional degree | 2(6.7) | 2(6.7) |

hTERT assay was performed with reference to primers from Nakamura et al., (1997) via semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR). Blood samples were collected in EDTA vacutainer, stored at $4^0$C and were processed for RNA isolation within 3-4 hours of collection. RNA isolation was performed as per the manufacturer's protocol using QIAmp RNA Blood mini kit (Qiagen, Germany). Extracted RNA was stored at -$80^0$C until further processing. cDNA was prepared as per the manufacturers protocol using high capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Lithuania) and stored at -$20^0$C until further use. Sequence of primers used for PCR amplification of hTERT and β-actin were, hTERT (145bp) - forward: 5'-CGGAAGAGTGTCTGGAGCAA-3', reverse: 5'-GGATGAAGCGGAGTCTGGA-3' and β-actin (250bp) - forward: 5'CATGTACGTTGCTATCCAGGC-3', reverse: 5'-CTCCTTAATGTCACGCACGAT-3'. Cycling conditions for both hTERT and β-actin were $94^0$C 1 min, 40 cycles of $94^0$C 1 min, $60^0$C 1 min and $72^0$C 30sec, final extension of $72^0C$ 5 min. TopTaq master mix kit (Qiagen, Valencia, California) was used for amplification reaction in 12.5ul total reaction volume. cDNA and PCR reactions were performed on Proflex PCR system (Applied Biosystems, Life Technologies, USA). The PCR products were examined on 2% agarose gel (Electrophoresis unit- GeNei, India) and quantified on Gel Documentation as obtained integrated density values (Alpha Innotech, USA & Alpha Imager EP, USA). Expression index of hTERT gene was quantified as the ratio of hTERT gene to β-actin gene. Perceived stress was measured by a 10-item Perceived Stress Scale (Cohen et al., 1988) and Emotional wellness was assessed by a 22-item Emotional Wellness Assessment (EWA) questionnaire (Thimmapuram et al., 2017).




S1-S6: sample, L: 100bp ladder

**Figure 1**. Representative pattern for hTERT expression

**Statistical Analysis**: Data were analysed using SPSS (version 23) software applying independent samples t-test and Pearson's correlation at significance level <0.05.

## 3. Results

**hTERT gene Expression:** hTERT gene expression was higher in the meditators than the non-meditators (p=0.081). A Significant (p=0.022) difference in hTERT gene expression was observed in the meditators in the age of >40 years (Mean age= 51.86 years) compared to the age and gender matched non-meditators (Table 2). No significant differences in hTERT expression were noted among male and female participants between both the groups (Table 2). Expression of hTERT gene did not show any correlation with BMI and age in the meditators [r(28)= -0.024, .898; r(28)= -0.010, 0.956] and the non-meditators [r(28)= -0.010, 0.959; r(28)= -0.116, 0.543], respectively. Increase in DOM was significantly associated with increase in hTERT gene expression (Table 4). A non-decreasing trend of hTERT gene expression relative to age was observed in the meditators (Figure 2).

**Table 2.** Comparison of study parameters between Meditators and Non-meditators

| Parameters | Group (N) | Mean (SD) | Mean Difference | p-value |
|---|---|---|---|---|
| **Age (years)** | Meditators (30) | 47.24 (11.39) | 0.32 | 0.912 |
| | Non-Meditators (30) | 47.92 (11.21) | | |
| **BMI (kg/m²)** | Meditators (30) | 24.75 (2.97) | 0.5 | 0.532 |
| | Non-Meditators (30) | 24.25 (3.14) | | |
| **YOP (years)** | Meditators (30) | 22.48 (0.08) | - | - |
| **DOM (minutes)** | Meditators (30) | 52.50 (7.40) | - | - |
| **hTERT (31 – 83 years)** | Meditators (30) | 0.23 (0.04) | 0.02 | 0.081 |
| | Non-Meditators (30) | 0.21 (0.05) | | |
| **hTERT (≤40 years)** | Meditators (9) | 0.22 (0.03) | 0.01 | 0.394 |
| | Non-Meditators (9) | 0.23 (0.04) | | |
| **hTERT (>40 years)** | Meditators (21) | 0.23 (0.05) | 0.04 | 0.022* |
| | Non-Meditators (21) | 0.20 (0.05) | | |
| **hTERT Female** | Meditators (12) | 0.23 (0.05) | 0.03 | 0.156 |
| | Non-Meditators (12) | 0.20 (0.06) | | |
| **hTERT Male** | Meditators (18) | 0.23 (0.04) | 0.01 | 0.327 |
| | Non-Meditators (18) | 0.21 (0.04) | | |
| **Perceived stress scale** | Meditators (30) | 10.63 (6.12) | -5.77 | 0.002* |
| | Non-Meditators (30) | 16.40 (7.43) | | |
| **Hours of Sleep** | Meditators (30) | 6.57 (0.82) | -0.97 | 0.000* |
| | Non-Meditators (30) | 7.53 (0.80) | | |

*p<0.05, SD-Standard deviation, BMI-Body Mass Index, N-numbers, YOP-years of meditation practice, DOM-duration of daily meditation

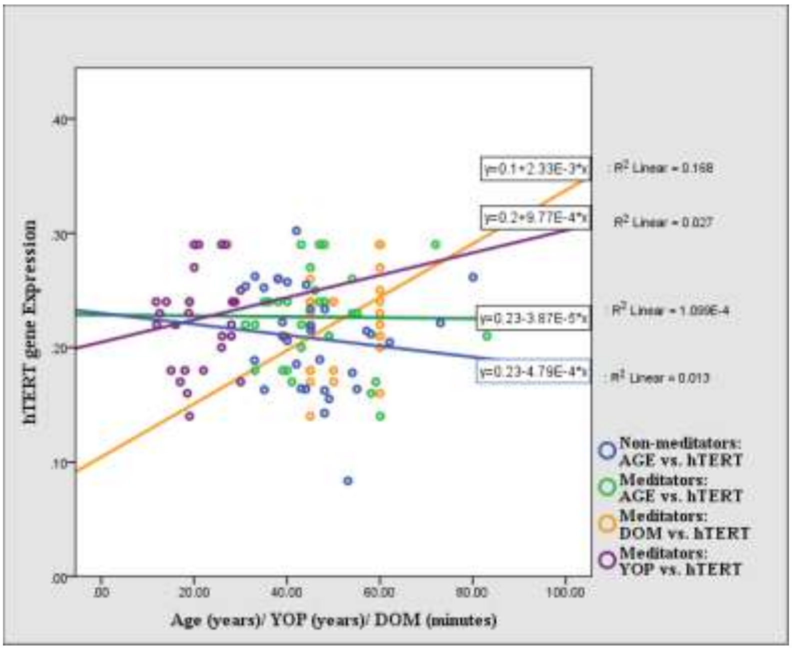



**Figure 2**. hTERT expression with Age and Duration of meditation practice in Meditators and Non-meditators

**Perceived Stress Scale (PSS) and Emotional Wellness Assessment (EWA):** Meditators reported significantly lower (p=0.002) perceived stress and significantly greater (p<0.05) emotional wellness in all the parameters of EWA section-A and section-B compared to the non-meditators (Table 3). Perceived stress was significantly correlated with decrease of all the positive attributes and increase of majority of the negative attributes of emotional wellness in the non-meditators. Whereas in the meditators, significant correlation between perceived stress and EWA attributes were much fewer (Table 5). DOM showed significant positive correlation with concentration and negative correlation with anxiety and apathy (Table 4). YOP was found to have significant positive correlation with calmness, clarity and quality of sleep and significant negative association with anxiety, sorrow and irritability (Table 4). Hours of sleep were found to be significantly (p= 0.000) lower in the meditators (Table 2).

**Table 3**. Comparison of EWA between Meditators and Non-meditators

| EWA | | Mean (SD) | | Mean Difference | p-value |
|---|---|---|---|---|---|
| | | **Meditators** | **Non-meditators** | | |
| **Section-A (Positive attributes)** | Concentration | 7.67 (1.27) | 6.30 (2.09) | 1.37 | 0.0048* |
| | Calmness | 8.80 (0.76) | 6.67 (2.14) | 1.37 | 0.000* |
| | Clarity of goal | 8.40 (1.69) | 6.77 (2.14) | 1.63 | 0.002* |
| | Harmony | 8.77 (0.97) | 7.67 (2.12) | 1.10 | 0.014* |
| | Quality of Sleep | 8.60 (1.48) | 7.40 (2.24) | 1.20 | 0.017* |
| | Joy | 8.80 (0.96) | 6.73 (1.96) | 2.07 | 0.000* |
| | Positive thinking | 9.03 (0.89) | 7.50 (1.98) | 1.53 | 0.000* |
| | Self confidence | 8.57 (1.52) | 7.79 (1.13) | 0.78 | 0.030* |
| | Empathy | 9.11 (0.64) | 7.23 (1.99) | 1.88 | 0.000* |
| | Tolerance | 8.43 (1.10) | 7.10 (1.83) | 1.33 | 0.001* |
| | Honesty to self | 9.17 (0.79) | 7.97 (1.87) | 1.20 | 0.002* |
| **Section-B (Negative attributes)** | Anger | 2.70 (1.62) | 3.93 (2.75) | -1.23 | 0.040* |
| | Stress | 2.57 (1.65) | 4.83 (2.57) | -2.27 | 0.000* |
| | Anxiety | 2.53 (1.83) | 4.70 (2.37) | -2.17 | 0.000* |
| | Fear | 2.53 (1.80) | 3.70 (2.22) | -1.17 | 0.029* |
| | Sorrow | 2.23 (1.25) | 4.10 (2.22) | -1.87 | 0.000* |
| | Irritability | 2.47 (1.80) | 3.70 (2.07) | -1.23 | 0.017* |
| | Jealousy | 1.60 (1.13) | 2.50 (2.03) | -0.90 | 0.039* |
| | Addiction | 1.53 (0.86) | 3.33 (2.97) | -1.80 | 0.003* |
| | Apathy | 2.47 (2.06) | 3.73 (2.39) | -1.27 | 0.032* |
| | Cynicism | 1.83 (0.89) | 4.10 (2.62) | -2.27 | 0.000* |
| | Impulsiveness | 2.93 (2.18) | 4.50 (2.78) | -1.57 | 0.018* |

**Table 4.** Correlation of YOP and DOM with hTERT, EWA and PSS.

| Pearson's Correlation | | hTERT | Concentration | Calmness | Clarity of goal | Quality of Sleep | Anxiety | Sorrow | Irritability | Apathy | PSS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **YOP** | Pearson's r | 0.171 | 0.352 | 0.496* | 0.376* | 0.394* | -0.499** | -0.433* | -0.434* | -0.285 | -0.234 |
| | p-value | 0.366 | 0.057 | 0.005 | 0.041 | 0.031 | 0.005 | 0.017 | 0.017 | 0.127 | 0.214 |
| **DOM** | Pearson's r | 0.410* | 0.423* | 0.153 | 0.193 | 0.142 | -0.445* | -0.140 | -0.091 | -0.418* | -0.226 |
| | p-value | 0.024 | 0.020 | 0.419 | 0.308 | 0.454 | 0.014 | 0.461 | 0.633 | 0.022 | 0.229 |

YOP- years of meditation practice; DOM- duration of daily meditation

**Table 5**. Correlation of PSS with Emotional Wellness in Meditators and Non-meditators

| Pearson's Correlation | **Meditators** | | **Non-meditators** | |
|---|---|---|---|---|
| | Pearson's r | p-value | Pearson's r | p-value |
| Hours of Sleep | 0.091 | 0.632 | -0.372* | 0.043 |
| **Concentration** | -0.323 | 0.082 | -0.475* | 0.008 |
| **Calmness** | -0.349 | 0.059 | -0.605* | 0.000 |
| **Clarity of goal** | -.0501* | 0.005 | -0.561* | 0.001 |
| **Harmony** | -0.166 | 0.382 | -0.523* | 0.003 |
| **Quality of Sleep** | -0.425* | 0.019 | -0.520* | 0.003 |
| **Innerjoy** | -0.241 | 0.199 | -0.805* | 0.000 |
| **Positive thinking** | -0.548* | 0.002 | -0.737* | 0.000 |
| **Self confidence** | -0.650* | 0.000 | -0.442* | 0.019 |
| **Empathy** | -0.091 | 0.651 | -0.735* | 0.000 |
| **Tolerance** | -0.282 | 0.132 | -0.514* | 0.004 |
| **Honesty to self** | -0.286 | 0.126 | -0.529* | 0.003 |
| **Anger** | 0.343 | 0.064 | 0.532* | 0.002 |
| **Stress** | 0.593* | 0.001 | 0.593* | 0.001 |
| **Anxiety** | 0.599* | 0.000 | 0.650* | 0.000 |
| **Fear** | 0.542* | 0.002 | 0.552* | 0.002 |
| **Sorrow** | 0.705* | 0.000 | 0.583* | 0.001 |
| **Irritability** | 0.603* | 0.000 | 0.640* | 0.000 |
| **Jealousy** | 0.416* | 0.022 | 0.439* | 0.015 |
| **Addiction** | -0.112 | 0.555 | 0.142 | 0.454 |
| **Apathy** | 0.350 | 0.058 | 0.257 | 0.171 |
| **Cynicism** | 0.255 | 0.181 | 0.372* | 0.043 |
| **Impulsiveness** | -0.190 | 0.313 | 0.405* | 0.027 |

## 4. Discussion

This is the first study to report hTERT gene expression along with perceived stress and emotional wellness in long-term Heartfulness meditation practitioners. The adept Heartfulness meditators reported an overall higher hTERT gene expression (p= 0.081) compared to age and gender matched non-meditators. On the other hand, significantly higher (p=0.022) hTERT gene expression was seen in the meditator participants >40 years of age (Age: 41-83; YOP: 17-39; N=21) compared to the age and gender matched non-meditators. A cross-sectional study among 42 (20 males and 22 females) healthy individuals, age (22-64 years) & BMI matched, vegetarian, non-smokers, consuming no supplements by Sharma and co-workers (2008) also reported non-significant alterations in hTERT among sudarshan kriya practitioners (1.33±0.28) with daily 1 hour practice compared to controls (0.95±0.21). Another cross-sectional study by Dasanayaka et al. (2022) including 30 expert healthy meditators (>3 years of meditation practice- loving kindness, breathing meditation and body scan meditation) with average age 43.83±9.92 years, 6.8±3.27 years of meditation practice and average daily meditation hours 5.82±3.45 and 30 matched healthy non-meditators with average age 43.51 ± 9.92 years, reported significantly higher (p= 0.002) plasma telomerase in advanced meditators compared to non-meditators.

There have been fewer cross-sectional studies in this particular area but many interventional studies have reported significantly increased TA as a result of meditation and related mind-body practices in healthy and diseased individuals. Significantly higher ($p < 0.05$) TA was reported in the intensive meditation retreat participants post 3 months with ∼6 hours/day meditation compared to age, gender, BMI and meditation experience matched controls (Jacobs et al., 2011). Duan et al. (2016) reported increased TA (p=0.000) in middle aged (55-65 years) healthy individuals followed by 6 months tai chi intervention compared to the controls. Chaix et al. (2017) reported significant protective impact of years of meditation on intrinsic epigenetic aging acceleration only in the ≥52 years age group suggesting a progressive cumulative effect of meditation on epigenetic aging. The presence of significantly higher hTERT gene expression particularly in the self-reported healthy long-term Heartfulness meditators >40 years of age (Age: 41-83 years; YOP: 17-39 years) might represent an enhanced genomic stability, telomeric maintenance and a favourable ageing process in the meditators compared to the non-meditators.

Telomerase activity is influenced by age and BMI since both produce a negative influence on telomeres and are associated with oxidative stress and DNA damage (Andreu-Sánchez et al., 2022; Poljsak & Milisav, 2013; Shammas, 2011). Both the groups in this study did not show any significant correlation of hTERT expression with age and BMI but the long-term Heartfulness meditators displayed a non lowering trend of hTERT expression with age which was not apparent in the non-meditators. Likewise, in a study by Conklin et al. (2019) TA was not predicted by age, gender and BMI whereas Dasanayaka and co-workers reported significant negative correlation between age and plasma telomerase ($r = -0.666$, $p < 0.001$), and none ($p > 0.05$) with BMI (Dasanayaka et al., 2022).

Premature aging and death can result as an adverse effect of inadequate sleep quality and quantity due to its negative influence on mental and physical health. For daily rejuvenation of mind and body, sleep is considered to be an essential component which is influenced positively by meditation (Amarnath et al., 2017). Meditation also claims decrease in time of sleep requirements. We observed significantly (p=.000) lesser sleep hours and significantly better sleep quality in long-term Heartfulness meditators compared to non-meditator participants. Similarly, Kaul and co-workers (2010) reported lower duration of sleep in long term experienced meditators compared to controls. A yogic meditation intervention of 8 weeks among healthy healthcare professionals reported both subjective and objective improvements in sleep quality in the meditation group (n=32) compared to control group (n=32) (Guerra et al., 2020).

Significant decrease of TA with increase in perceived stress as well as increase of TA with decrease in negative affectivity and increase of perceived control has been observed (Epel et al., 2004; Jacobs et al., 2011). In this study, the long-term Heartfulness meditation practitioners showed significantly ($p<0.05$) lower perceived stress scores and greater emotional wellness for both the negative and positive attributes of emotion than the non-meditators. Likewise, a 12 week study involving 35 healthcare professionals reported a significant improvement in burnout and mostly all EWA attributes followed by Heartfulness meditation intervention compared to the control group participants who did not show significant changes (Thimmapuram et al., 2017). Chu (2010), reported higher emotional intelligence, lower perceived stress and lesser negative mental health in experienced meditators compared to those with lesser meditation experience and controls in a cross-sectional study of 351 adults. In a survey among 541 collegians, Lo and Wu (2007) reported significantly lower ($p<0.001$) negative emotional states like depression, anxiety and stress/ tension in experienced (>0.5 years meditation experience) zen meditators compared to the controls. Decrease of perceived stress and improvement of different emotional states have been reported post various meditation interventions (Lane et al., 2007; Valosek et al., 2018).

Many studies have proven the association of stress with negative emotions (Du et al., 2018). In this study, we found significant positive correlations of perceived stress with the negative emotional attributes and negative correlations with the positive emotional attributes. Increase in perceived stress had an inverse association with all the positive emotional attributes and a positive correlation with most of the negative emotional attributes in the non-meditators. Perceived stress presented significant correlations with decrease of certain positive attributes (clarity of goal, quality of sleep, positive thinking and self-confidence) and increase of certain negative attributes (stress, anxiety, fear, sorrow, irritation and jealousy) between both the groups in common. But the intensities of the correlation ought to differ because of the significant differences in the levels of perceived stress and emotional wellness attributes.

Perceived stress via central nervous system leads to release of cortisol which can in turn reduce TA (Burke et al., 2005; Price et al., 2013) while lower levels of stress and anxiety have been associated with increased telomerase levels and telomere length which might reduce cellular aging (Dasanayaka et al., 2022). Boccardi et al. (2013) reported negative regulation of TA in presence of oxidative stress and inflammation and that higher peripheral blood mononuclear cell TA relates with better health position. Higher TA can result into telomere lengthening, increase mitochondrial health and help in neurogenesis via growth factor stimulation and might perchance better mental well-being (Epel & Prather, 2018) on the contrary reduction in TA is linked with faster cellular senescence (Dasanayaka et al., 2022). However, both high and low extremes in presence of telomerase does not confer benefits since very high TA increases chances of cancer and very low TA can hamper body's healthy regenerative capacity (Rehman, 2014). Fundamental epigenetics of TERT gene regulation is unclear but methylation of DNA, histone methylation–acetylation, and non-coding RNAs regulate TERT expression for different biological processes in ageing and cancer (Dogan & Forsyth, 2021). Elicitation of TERT in response to physiological signals is seen in a subgroup of few human cells viz. stem cells, highly regenerative tissues and activated lymphocytes indicating its role in biology of stem cell, homeostasis of tissues and immune-modulation, ultimately having an impact on the aging process. Hence the appropriate expression of TERT/telomerase is warranted (Yuan & Xu, 2019).

Additionally, YOP and DOM in the present study were differently correlated with few parameters of EWA and with hTERT expression. In this study the long-term Heartfulness meditators reported significant ($p = 0.024$) increase in hTERT with increase in DOM with a daily practice duration of 40-60 minutes. A similar cross-sectional study by Dasanayaka and co-workers (2022) reported significantly ($r = 0.450$, $p = 0.011$) increased plasma telomerase activity with respect to duration of meditation (average daily meditation 5.82±3.45).

Growing number of researches support the multi-dimensional relationship of meditation with healthy aging (Klimeki et al., 2019). Researches on epigenetic profiles of long-term/ continual meditators are indicative of a healthier aging pattern with physiological benefits in advanced meditators. Rate of epigenetic aging assessed among 5 – 30 years of continual meditation practicants and naive meditators revealed increased intrinsic epigenetic aging acceleration with age in the control group and not in the meditators indicating protective effect of a daily routine meditation practice reflecting in their epigenetic aging over the length of time (Chaix et al., 2017). Dusek et al., 2008 suggested that relaxation response related gene expression transformation is consistent and constitutive and might identify with long term physiological outcomes. The results of this study are in concurrence with the researches so far. All the participants in this study were self-reported healthy, age and gender matched and resided in the same city which also reduces the lifestyle and/or environmental bias between the participants. For all the participants, sampling was performed in fasting condition in the morning hours between 8.00 AM – 10.00 AM in the month of march-may thereby reducing sampling time, seasonal bias and any possible diurnal changes.

This study documented significantly higher hTERT expression in a subgroup comprising middle aged and elderly long-term Heartfulness meditation practitioners and an overall significantly better mental and emotional status compared to the non-meditators in real world environment. Strikingly, even in the smaller sample size there was a difference in hTERT expression. Increase of hTERT with DOM implies that duration of daily meditation is an important factor for overall wellbeing.

## 5. Conclusions

In this real-world study, the long-term Heartfulness meditators reported an overall greater hTERT gene expression which was significant in the age >40 years compared to age and gender matched non-meditators. Significantly lesser perceived stress, greater emotional wellness and lesser required hours of sleep are evident with long-term practice of Heartfulness meditation. Duration of daily meditation is an important factor associated with increase of hTERT gene expression.

The present study is suggestive of a healthier aging response as well as healthier mental and emotional levels in the long-term HFN meditation practitioners. Further large scale and longitudinal studies would help provide better understanding and confirmation of these findings.

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