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Antiaging Effects of an Intensive Mind and Body Therapeutic Program through Enhancement of Telomerase Activity and Adult Stem Cell Counts

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Abstract

Objective: Key modalities of integrative medicine known to rejuvenate the mind and body are meditation, yoga, and controlled diet. It has been shown previously that intensive or prolonged mind and body therapies (MBT) may have beneficial effects on the well-being of healthy people and in patients. Telomerase activity and levels of peripheral blood adult pluripotent stem cells (PB-APSC) are reliable markers of long-term well-being that are known to decrease with age. The objective of this study is to understand the effect of our MBT program on telomerase activity and stem cells in blood collected from the participants.
Design: Here, we have investigated the effects of an intensive three weeks MBT retreat on telomerase activity and the peripheral blood stem cells in participants before and after the MBT. A total of 108 people were enrolled in the study; 38 men and 70 women (aged 18-90) randomly assigned for the study.
Results: Telomerase activity was greater in retreat participants at the end of the MBT retreat. About 45% of people showed more than one-fold increase of telomerase activity after our MBT program. Furthermore, about 27% of people showed more pronounced fold increase (2-fold) in telomerase activity after the MBT. In addition, a substantial percentage of people (about 90%) exhibited increased stem cell counts after the MBT.
Conclusions: The data suggest increased telomerase activity and stem cells count in peripheral blood from MBT retreat participants that may lead to increased longevity and better quality of life at latter age.

Introduction

Aging represents the accumulation of physiological, biological and associated alterations in a person for a longer period of time [1, 2]. Aging is one of the most critical known risk factors for many human diseases [3]. Roughly 0.1 million people

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worldwide die each day of age-related complications [4].

Integrative mind-body therapies (MBTs), which include meditation, pranayama, other kinds of breath control exercises, and yoga have gained importance and intense attention from the scientific community over the last two decades. During these years the scientific community has been consistently trying to understand the safety and efficacy of meditation on physical and mental well-being. Among the various types of MBTs, meditation has still been considered as one of the most common practiced techniques. Many scientific studies have been carried out to understand the efficacy of meditation on physical and mental well-being [5]. Meditation, yoga with breath control exercises, and controlled diet with right nutritional supplementation exert the best anti-aging effects including reduced anxiety and illness, slow-down of the aging process, and improved cognitive function [6, 7, 8].

Recent studies show that meditation with breath and diet control, can lead to stable changes in brain pattern for a longer period of time as compared to unmediated human beings, thereby indicating the importance of intentional practices. These studies may help in understanding the basic unifying mechanisms of the brain, mind and body that enhance our capacity to adapt to stressful and uncertain conditions [9]. There are several cellular, molecular, biochemical, and clinical markers to evaluate the rate of aging in an individual. These markers are extensively being used to evaluate anti-aging potential of any MBT program. Telomerase activity levels in a cell and evaluation of the circulating adult stem cells in an individual are two such molecular and cellular parameters of detecting aging [10].

Telomeres are DNA sequences that are essential for protecting chromosome ends and ensure chromosome stability during replication and also allow cells to distinguish chromosome ends. However, their lengths get reduced with each cell division and further shortened during oxidative stress [11]. Below a critical telomere length, cell division can no longer occur which causes cells to enter in a state of senescence that can further enhance the tissue aging process [12, 13, 14, 15, 16, 17]. Nevertheless, a recent longitudinal study indicates that telomere length of peripheral blood mononuclear cells can increase over time [18-20]. This recent finding thus emphasizing the need for identifying potential regulator(s) that control telomere length shortening. The rate of telomere shortening or lengthening depends primarily on the activity and the levels of telomerase [21], which adds DNA sequences back to telomeres and thereby increasing their length and restore normal cellular function [22].

Lately telomere length has been proposed as a useful "psychobiomarkers" [18, 21, 23, 24] and the new findings indicate that telomere length can be regulated in part by psychological stress [25, 26]. Additionally, literatures on Buddhist tradition indicating a role of meditation on the reduction of psychological stress which may be due to upregulation of telomerase activity [27].

Adult stem cells are capable of renewal and facilitate regeneration of lost tissues and also repair damaged tissues in the body and during stress/insult [28]. Importantly, maintenance of stem cell quiescence is essential for preserving the long-term self-renewal potential of the stem cell pool [28] such as in the brain, bone marrow, musculoskeletal system, and the skin. Furthermore, there is an emerging body of evidence that signals such as metabolic stress can lead to impairment of adult stem cells function *in vivo* [29].

Materials and Methods

Study Design and Subject

The study determined effect of telomere shortening on aging by detecting peripheral blood adult regenerative stem cell (ARSS) and telomerase activity in peripheral blood mononuclear cells (PBMC). All samples were collected at the site of the study. The study originally included 110 participants. The samples from 2 participants could not be used since either they missed a session or were not good enough for further analysis. Hence total of 108 patients were enrolled in the study, 38 men and 70 women (aged 18-90).

Mind and Body Therapy (MBT) Program

Our proprietary MBT program consists of 5 components; (1) Breath control exercises (twice a

day), (2) Yoga program, (3) Meditation program for mind control, (4) Controlled diet program, and (5) Herbal nutraceutical regimen for better hematopoiesis. The MBT program is a residential 21 days retreat at Harid war and consisted of one hour of Hatha Yoga every morning. Two hours of meditation techniques was specially designed for the program every day. Breathing exercise pranayama techniques was used as part of the yoga session. Interactive sessions were held between mentor and participants throughout the day. Diet was strictly vegetarian with no calorie restriction.

All participants were recruited in the MBT program nationally and internationally through awareness programs at meditation and wellness centers etc. Participants were mostly from different social backgrounds and had different lifestyles, with stress, and physiological problems. Most of them had never undergone any similar or the same MBT programs previously. Participants' bloods were assessed before and after the MBT. Inclusion criteria were: age 18–90 years, no chronic diseases such as CMV infection, heart diseases, diabetes, HIV etc, and good psychological health for example lack of anxiety and depression, lack of bipolar disorders or schizophrenia.

Ethics Statement

The study protocol and the design have been approved by Institutional Ethics Committee. Informed consents in writings were obtained from all subjects prior to start-date of experimentation.

Intervention

On the day 1 of the program, 5ml of peripheral blood was collected from each subject in anticoagulant EDTA vacutainer (BD Biosciences, San Jose, USA). After 21 days (i.e., end of the program) again 5ml blood was collected in anticoagulant vacutainer and forward through validated clinical cold chain.

Stem Cell Isolation Process

Viability measurement was done by using Trypan blue (0·4% Trypan blue stain, Invitrogen, Grand Island, NY, USA). Trypan blue exclusion method [30] was used to count viable PBMCs. Stem cell count was done using hemocytometry. Adult regenerative stem cell (ARSS) was collected from the peripheral blood by density gradient centrifugation [31] and sedimentation process [32]. Our approved protocol on the isolation of PMBC was initially established by examining key pluripotency markers such as like OCT4, SOX2, NANOG by reverse transcriptase–polymerase chain reaction (RT-PCR) [33].

Telomerase Activity Measurement in PBMCs

Telomerase relative activity assessment was done as per the instruction of Millipore's TRAPeze telomerase detection kit (Billerica, MA, USA) with each sample being analyzed in triplicate. These assays were done in the peripheral blood mononuclear cell (PBMC) counts. Protein estimation assay was done using Lowry method [34] and equal amount of protein lysate (0.4μg) was used for TRAP reaction. PBMCs were isolated by centrifugation using Lympho-Ficoll (Ficoll-Paque Plus; GE Healthcare, Little Chalfont, UK) gradient density centrifugation for 30 minutes at 400g. The mononuclear layer was collected and washed 3 times in PBS supplemented with 2% FBS (Stemcell Technologies, Vancouver, BC. Canada), and finally an aliquot containing 0.2 million cells were transferred into an Eppendorf tube (Eppendorf, Hamburg, Germany) and centrifuged at 3000*g* for 5 min. The pelleted cells were preserved at -80°C until use. Heat-denatured telomerase was used as a negative control for TRAP reaction. The positive control was done using one million telomerase positive cells supplied in the kit. The relative telomerase activity was calculated by taking negative control values as 1 and the data were normalized based on exact protein content of the samples.

Statistical Analysis

Each patient served as their own control. We difference pre- and post- values for stem cell and telomerase. We tested for differences in pre versus post using the Wilcoxon Signed Rank Test. We found that for both changes in stem cells and telomerase, there was a significant increase, $P < 0.01$.

Results

A Proper Randomization of Patients Was Done Before the Start of the Treatment

As shown in Table 1, all patients participated in this study were randomized in terms of age, sex, stem cells, and telomerase activity prior to meditation.

The randomization was successful as evidenced by age-range, sex of the patients just to be sure increased telomerase activity in the treated group were not due to inclusion of more young people.

Mind-Body Therapy (MBT) Program Did Not Affect the Viability of Stem Cells

Out of 110 patients, 2 patients' samples found to be contaminated and hence rejected for the study. Using Trypan blue exclusion method 80-90% viability was observed in all subjects (101- 208). The results are presented in Table 2. A close examination of the table revealed that retreat did not affect the viability of the stem cells among all subjects. A substantially large numbers of stem cells (80-90%) were found to be viable before and after the meditation.

Table 1. Pre-treatment patient data

Sr. No	Gender	Age	Stem Cell Count Millions/ml (Pre)	Telomerase Relative Activity (Pre)
101	F	49	43	0.5
102	F	41	28	0.66
103	M	40	20	0.54
104	F	25	22	0.65
105	F	46	45	0.9
106	F	15	24	0.45
107	F	62	38	0.4
108	F	65	18	0.6
109	M	43	33	0.55
110	F	44	32	0.46
111	F	48	34	0.7
112	F	53	46	0.9
113	M	70	41	0.56
114	F	45	36	0.6
115	F	59	34	0.6
116	M	31	25	0.57
117	F	64	25	0.77
118	F	29	35	1
119	F	59	21	0.4
120	F	63	29	0.33
121	M	36	22	0.5
122	F	34	16	0.6
123	M	47	21	0.6
124	M	48	34	0.7
125	F	29	24	0.4
126	F	36	51	0.3
127	F	34	45	0.5
128	M	36	34	0.7
129	M	35	39	0.4
130	F	28	49	0.5
131	F	72	21	0.6
132	F	60	24	0.8
133	F	45	29	0.33
134	M	36	39	0.45
135	M	56	21	0.5

Sr. No	Gender	Age	Stem Cell Count Millions/ml (Pre)	Telomerase Relative Activity (Pre)
136	F	20	38	0.11
137	M	23	54	0.7
138	F	56	25	0.5
139	F	32	26	0.4
140	F	48	23	0.8
141	M	44	34	0.34
142	M	32	33	0.8
143	M	59	21	0.7
144	F	42	29	0.6
145	F	30	25	0.5
146	M	51	37	0.6
147	M	32	25	0.7
148	F	45	31	1
149	M	48	32	0.9
150	M	48	18	0.6
151	F	55	21	0.7
152	F	32	29	0.7
153	F	39	34	0.8
154	M	54	16	0.9
155	F	40	18	1.2
156	F	36	41	1
157	M	44	10	0.66
158	M	33	31	0.7
159	F	31	35	0.6
160	F	52	21	0.8
161	M	56	21	0.35
162	M	66	18	0.5
163	F	34	34	0.7
164	F	34	39	1
165	F	14	29	0.7
166	F	68	21	1.2
167	F	25	31	1
168	F	18	41	0.8
169	F	41	38	0.8
170	M	20	39	0.5
171	M	43	21	0.56
172	F	42	32	0.3
173	F	42	31	1.2
174	F	41	35	1.1
175	F	68	29	0.9
176	F	38	29	0.6
177	F	15	29	0.5
178	M	44	21	0.6
179	F	73	19	0.9
180	M	78	25	0.6
181	F	48	23	0.7
182	F	48	31	0.3
183	F	67	27	0.5
184	M	48	-	---
185	F	49	25	1.5
186	M	34	39	1
187	F	31	33	1.1
188	F	31	38	1.2

Table 1. (Continued)

Sr. No	Gender	Age	Stem Cell Counts Millions/ml (Pre)	Telomerase Relative Activity (Pre)
189	M	43	21	0.8
190	F	39	36	0.9
191	M	54	24	1.1
192	F	38	21	0.8
193	F	38	34	0.7
194	F	51	21	0.5
195	M	16	45	0.8
196	F	38	35	0.7
197	M	36	41	0.4
198	F	64	23	0.6
199	M	51	33	0.6
200	M	29	39	0.4
201	F	29	41	0.9
202	F	67	26	0.8
203	M	39	37	0.4
204	F	43	41	1.2
205	F	45	48	0.9
206	M	19	35	0.9
207	F	41	53	0.5
208	F	50	33	0.8

Table 2. Stem cell count and viability data

Sr. No	Gender	Age	Stem Cell Count Millions/ml (Pre-Treatment)	Viability % (Pre-Treatment)	Stem Cell Count Millions/ml (Post-Treatment)	Viability % (Post-Treatment)
101	F	49	43	80-90%	52	80-90%
102	F	41	28	80-90%	42	80-90%
103	M	40	20	80-90%	37	80-90%
104	F	25	22	80-90%	34	80-90%
105	F	46	45	80-90%	54	80-90%
106	F	15	24	80-90%	39	80-90%
107	F	62	38	80-90%	94	80-90%
108	F	65	18	80-90%	36	80-90%
109	M	43	33	80-90%	47	80-90%
110	F	44	32	80-90%	39	80-90%
111	F	48	34	80-90%	77	80-90%
112	F	53	46	80-90%	33	80-90%
113	M	70	41	80-90%	50	80-90%
114	F	45	36	80-90%	46	80-90%
115	F	59	34	80-90%	21	80-90%
116	M	31	25	80-90%	38	80-90%
117	F	64	25	80-90%	45	80-90%
118	F	29	35	80-90%	No cells	80-90%
119	F	59	21	80-90%	No cells	80-90%
120	F	63	29	80-90%	31	80-90%
121	M	36	22	80-90%	39	80-90%
122	F	34	16	80-90%	22	80-90%
123	M	47	21	80-90%	34	80-90%
124	M	48	34	80-90%	45	80-90%
125	F	29	24	80-90%	38	80-90%

Sr. No	Gender	Age	Stem Cell Count Millions/ml (Pre-Treatment)	Viability % (Pre-Treatment)	Stem Cell Count Millions/ml (Post-Treatment)	Viability % (Post-Treatment)
126	F	36	51	80-90%	77	80-90%
127	F	34	45	80-90%	50	80-90%
128	M	36	34	80-90%	No cells	80-90%
129	M	35	39	80-90%	47	80-90%
130	F	28	49	80-90%	53	80-90%
131	F	72	21	80-90%	42	80-90%
132	F	60	24	80-90%	30	80-90%
133	F	45	29	80-90%	No sample	80-90%
134	M	36	39	80-90%	42	80-90%
135	M	56	21	80-90%	35	80-90%
136	F	20	38	80-90%	46	80-90%
137	M	23	54	80-90%	35	80-90%
138	F	56	25	80-90%	39	80-90%
139	F	32	26	80-90%	45	80-90%
140	F	48	23	80-90%	No sample	80-90%
141	M	44	34	80-90%	42	80-90%
142	M	32	33	80-90%	No cells	80-90%
143	M	59	21	80-90%	34	80-90%
144	F	42	29	80-90%	No sample	80-90%
145	F	30	25	80-90%	38	80-90%
146	M	51	37	80-90%	39	80-90%
147	M	32	25	80-90%	13	80-90%
148	F	45	31	80-90%	42	80-90%
149	M	48	32	80-90%	39	80-90%
150	M	48	18	80-90%	29	80-90%
151	F	55	21	80-90%	32	80-90%
152	F	32	29	80-90%	41	80-90%
153	F	39	34	80-90%	40	80-90%
154	M	54	16	80-90%	23	80-90%
155	F	40	18	80-90%	28	80-90%
156	F	36	41	80-90%	50	80-90%
157	M	44	10	80-90%	19	80-90%
158	M	33	31	80-90%	26	80-90%
159	F	31	35	80-90%	40	80-90%
160	F	52	21	80-90%	35	80-90%
161	M	56	21	80-90%	34	80-90%
162	M	66	18	80-90%	20	80-90%
163	F	34	34	80-90%	40	80-90%
164	F	34	39	80-90%	48	80-90%
165	F	14	29	80-90%	No sample	80-90%
166	F	68	21	80-90%	30	80-90%
167	F	25	31	80-90%	40	80-90%
168	F	18	41	80-90%	50	80-90%
169	F	41	38	80-90%	40	80-90%
170	M	20	39	80-90%	45	80-90%
171	M	43	21	80-90%	34	80-90%
172	F	42	32	80-90%	13	80-90%
173	F	42	31	80-90%	42	80-90%
174	F	41	35	80-90%	46	80-90%
175	F	68	29	80-90%	37	80-90%

Table 2. (Continued)

Sr. No	Gender	Age	Stem Cell Count Millions/ml (Pre-Treatment)	Viability % (Pre-Treatment)	Stem Cell Count Millions/ml (Post-Treatment)	Viability % (Post-Treatment)
176	F	38	29	80-90%	38	80-90%
177	F	15	29	80-90%	34	80-90%
178	M	44	21	80-90%	30	80-90%
179	F	73	19	80-90%	32	80-90%
180	M	78	25	80-90%	55	80-90%
181	F	48	23	80-90%	30	80-90%
182	F	48	31	80-90%	39	80-90%
183	F	67	27	80-90%	No sample	80-90%
184	M	48	-	80-90%	No sample	80-90%
185	F	49	25	80-90%	34	80-90%
186	M	34	39	80-90%	50	80-90%
187	F	31	33	80-90%	44	80-90%
188	F	31	38	80-90%	48	80-90%
189	M	43	21	80-90%	30	80-90%
190	F	39	36	80-90%	44	80-90%
191	M	54	24	80-90%	18	80-90%
192	F	38	21	80-90%	32	80-90%
193	F	38	34	80-90%	42	80-90%
194	F	51	21	80-90%	25	80-90%
195	M	16	45	80-90%	52	80-90%
196	F	38	35	80-90%	47	80-90%
197	M	36	41	80-90%	32	80-90%
198	F	64	23	80-90%	34	80-90%
199	M	51	33	80-90%	42	80-90%
200	M	29	39	80-90%	41	80-90%
201	F	29	41	80-90%	50	80-90%
202	F	67	26	80-90%	32	80-90%
203	M	39	37	80-90%	46	80-90%
204	F	43	41	80-90%	23	80-90%
205	F	45	48	80-90%	52	80-90%
206	M	19	35	80-90%	40	80-90%
207	F	41	53	80-90%	62	80-90%
208	F	50	33	80-90%	43	80-90%

MBT Augmented Stem Cells Numbers in Peripheral Blood Isolated from the Participants

Having established that retreat did not have much effect on the viability of stem cells, we asked the question whether meditation affected the number of total stem cell in subjects. Table 3 shows the stem cell fold changes in subjects before and after the meditation. Thus retreat group participants showed a higher fold changes in stem cell numbers, indicating a possible role of stem cells on the well-being of the subjects undergoing meditation. In addition, substantial percentage of subjects (about 90%) exhibited increased stem cell counts after meditation as shown in Table 5.

MBT Augmented Telomerase Activity in Stem Cells Isolated from Peripheral Blood from the Participants

Furthermore, having established that meditation had marked effect on the stem cell fold changes among majority of the people undergone MBT as opposed to pre-MBT group, we went on to investigate

a key molecular parameter of human-well-being; telomerase activity. Table 4 represents the telomerase activity fold changes in subjects before and after the meditation.

Thus telomerase activity was augmented in majority of the subjects after MBT, thereby indicating better well-being status. In addition, as shown in Table 5, about 45% of people showed more than one-fold of telomerase activity after MBT. Furthermore, about 27% of people showed higher fold changes (2-fold) in telomerase activity after MBT retreat.

Table 3. Stem cell count with fold change

Sr. No	Pre-Treatment	Post-Treatment	Fold Change
101	43	52	Less than 1 fold change
102	28	42	Less than 1 fold change
103	20	37	Less than 1 fold change
104	22	34	Less than 1 fold change
105	45	54	Less than 0.5 fold change
106	24	39	Less than 1 fold change
107	38	94	1.5 fold change
108	18	36	1 fold change
109	33	47	Less than 0.5 fold change
110	32	39	Less than 0.5 fold change
111	34	77	1 fold change
112	46	33	No change
113	41	50	Less than 0.5 fold change
114	36	46	Less than 0.5 fold change
115	34	21	No change
116	25	38	Less than 0.5 fold change
117	25	45	Less than 1 fold change
118	35	No cells	No change
119	21	No cells	No change
120	29	31	No change
121	22	39	Less than 0.5 fold change
122	16	22	Less than 0.5 fold change
123	21	34	Less than 0.5 fold change
124	34	45	Less than 0.5 fold change
125	24	38	Less than 0.5 fold change
126	51	77	Less than 0.5 fold change
127	45	50	Less than 0.5 fold change
128	34	No cells	No change
129	39	47	Less than 0.5 fold change
130	49	53	Less than 0.5 fold change

Table 3. (Continued)

Sr. No	Pre-Treatment	Post-Treatment	Fold Change
131	21	42	1 fold change
132	24	30	Less than 0.5 fold change
133	29	No sample	No change
134	39	42	No change
135	21	35	Less than 1 fold change
136	38	46	Less than 0.5 fold change
137	54	35	No change
138	25	39	Less than 0.5 fold change
139	26	45	Less than 1 fold change
140	23	No sample	No change
141	34	42	Less than 0.5 fold change
142	33	No cells	No change
143	21	34	Less than 1 fold change
144	29	No sample	No change
145	25	38	Less than 0.5 fold change
146	37	39	No change
147	25	13	No change
148	31	42	Less than 0.5 fold change
149	32	39	Less than 0.5 fold change
150	18	29	Less than 1 fold change
151	21	32	Less than 0.5 fold change
152	29	41	Less than 0.5 fold change
153	34	40	Less than 0.5 fold change
154	16	23	Less than 1 fold change
155	18	28	Less than 1 fold change
156	41	50	Less than 0.5 fold change
157	10	19	Less than 1 fold change
158	31	26	No change
159	35	40	Less than 0.5 fold change
160	21	35	Less than 1 fold change
161	21	34	Less than 1 fold change
162	18	20	Less than 0.5 fold change
163	34	40	Less than 0.5 fold change
164	39	48	Less than 0.5 fold change
165	29	No sample	No change

Sr. No	Pre-Treatment	Post-Treatment	Fold Change
166	21	30	Less than 0.5 fold change
167	31	40	Less than 0.5 fold change
168	41	50	Less than 0.5 fold change
169	38	40	Less than 0.5 fold change
170	39	45	Less than 0.5 fold change
171	21	34	Less than 1 fold change
172	32	13	No change
173	31	42	No change
174	35	46	Less than 0.5 fold change
175	29	37	Less than 0.5 fold change
176	29	38	Less than 0.5 fold change
177	29	34	Less than 0.5 fold change
178	21	30	Less than 0.5 fold change
179	19	32	Less than 1 fold change
180	25	55	1 fold change
181	23	30	Less than 0.5 fold change
182	31	39	Less than 0.5 fold change
183	27	No sample	No change
184	-	No sample	No change
185	25	34	Less than 0.5 fold change
186	39	50	Less than 0.5 fold change
187	33	44	Less than 0.5 fold change
188	38	48	Less than 0.5 fold change
189	21	30	Less than 0.5 fold change
190	36	44	Less than 0.5 fold change
191	24	18	No change
192	21	32	Less than 0.5 fold change
193	34	42	Less than 0.5 fold change
194	21	25	Less than 0.5 fold change
195	45	52	Less than 0.5 fold change
196	35	47	Less than 0.5 fold change
197	41	32	No change
198	23	34	Less than 0.5 fold change
199	33	42	Less than 0.5 fold change
200	39	41	Less than 0.5 fold change
201	41	50	Less than 0.5 fold change

Table 3. (Continued)

Sr. No	Pre-Treatment	Post-Treatment	Fold Change
202	26	32	Less than 0.5 fold change
203	37	46	Less than 0.5 fold change
204	41	23	No change
205	48	52	Less than 0.5 fold change
206	35	40	Less than 0.5 fold change
207	53	62	Less than 0.5 fold change
208	33	43	Less than 0.5 fold change

Table 4. Telomerase activity with fold change

Sr .No	Pre-Treatment	Post-Treatment	Fold Change Increase
101	0.5	1.2	1 fold change
102	0.66	0.9	0.5 fold change
103	0.54	1.4	1 fold change
104	0.65	1.34	1 fold change
105	0.9	1.6	0.5 fold change
106	0.45	0.97	1 fold change
107	0.4	1.5	1.5 fold change
108	0.6	1.4	1 fold change
109	0.55	1.25	1 fold change
110	0.46	0.9	1 fold change
111	0.7	1.7	1 fold change
112	0.9	0.95	No change
113	0.56	1.2	1 fold change
114	0.6	0.8	Less than 0.5 fold change
115	0.6	1.7	1 fold change
116	0.57	1.4	1 fold change
117	0.77	1	Less than 0.5 fold change
118	1	1	No change
119	0.4	1.2	1.5 fold change
120	0.33	0.9	1.5 fold change
121	0.5	1.6	1.5 fold change
122	0.6	1.3	1 fold change
123	0.6	0.9	Less than 0.5 fold change
124	0.7	1	Less than 0.5 fold change
125	0.4	1.4	1.5 fold change

Sr. No	Pre-Treatment	Post-Treatment	Fold Change Increase
126	0.3	1.2	2 fold change
127	0.5	1	1 fold change
128	0.7	1.7	1 fold change
129	0.4	1.7	2 fold change
130	0.5	1.8	1.5 fold change
131	0.6	1.4	1 fold change
132	0.8	1.2	0.5 fold change
133	0.33	---	No change
134	0.45	1.2	1.5 fold change
135	0.5	1.6	1.5 fold change
136	0.11	0.76	Less than 1 fold change
137	0.7	0.9	Less than 0.5 fold change
138	0.5	0.9	Less than 0.5 fold change
139	0.4	0.8	1 fold change
140	0.8	---	No change
141	0.34	1.4	More than 2.5 fold change
142	0.8	0.8	No change
143	0.7	1.7	1 fold change
144	0.6	---	No change
145	0.5	1.34	1 fold change
146	0.6	1.5	1 fold change
147	0.7	1.2	0.5 fold change
148	1	1.8	0.5 fold change
149	0.9	1.8	1 fold change
150	0.6	1.7	1 fold change
151	0.7	1.3	Less than 1 fold change
152	0.7	1.3	Less than 1 fold change
153	0.8	1.5	Less than 1 fold change
154	0.9	1.9	1 fold change
155	1.2	1.4	Less than 0.5 fold change
156	1	1.8	Less than 1 fold change
157	0.66	1.34	1 fold change
158	0.7	1.6	1 fold change
159	0.6	1.2	1 fold change
160	0.8	1	Less than 0.5 fold change
161	0.35	1.7	2.5 fold change

Table 4. (Continued)

Sr. No	Pre-Treatment	Post-Treatment	Fold Change Increase
162	0.5	0.9	Less than 1 fold change
163	0.7	1.2	Less than 1 fold change
164	1	1.5	0.5 fold change
165	0.7	---	No change
166	1.2	1.5	Less than 0.5 fold change
167	1	1.9	Less than 1 fold change
168	0.8	1.2	Less than 1 fold change
169	0.8	1.3	Less than 1 fold change
170	0.5	1.5	1.5 fold change
171	0.56	1.7	1.5 fold change
172	0.3	0.5	Less than 0.5 fold change
173	1.2	1.9	Less than 1 fold change
174	1.1	1.8	Less than 1 fold change
175	0.9	1.6	Less than 1 fold change
176	0.6	1.6	1 fold change
177	0.5	1.3	1 fold change
178	0.6	0.9	Less than 0.5 fold change
179	0.9	1.1	Less than 0.5 fold change
180	0.6	1.5	1 fold change
181	0.7	1.8	1 fold change
182	0.3	0.8	1.5 fold change
183	0.5	---	No change
184	---	----	No change
185	1.5	1.8	Less than 0.5 fold change
186	1	1.9	Less than 1 fold change
187	1.1	1.9	Less than 1 fold change
188	1.2	1.7	0.5 fold change
189	0.8	1.5	Less than 1 fold change
190	0.9	0.9	No change
191	1.1	1.5	0.5 fold change
192	0.8	0.9	No change
193	0.7	1	No change
194	0.5	0.8	No change
195	0.8	1.5	Less than 1 fold change
196	0.7	1.5	1 fold change

Sr. No	Pre-Treatment	Post-Treatment	Fold Change Increase
197	0.4	1.12	1 fold change
198	0.6	1.6	1 fold change
199	0.6	0.8	No change
200	0.4	0.9	0.5 fold change
201	0.9	1.6	Less than 1 fold change
202	0.8	1.6	1 fold change
203	0.4	0.5	No change
204	1.2	1.4	No change
205	0.9	1.8	1 fold change
206	0.9	1.7	Less than 1 fold change
207	0.5	1.5	1.5 fold change
208	0.8	0.9	No change

Table 5. Percentage of Post-MBT subjects showing overall fold changes in telomerase relative activity compared to Pre-MBT Subjects

Fold change in the total study group	% of subjects showing change
Greater than 6	0.92
Greater than 4	5.55
Equivalent 4	0.92
Greater than 3	5.55
Equivalent 3	2.77
Greater than 2	27.77
Equivalent 2	5.55
Greater than 1	45.37
Equivalent 1	2.77
No change	5.55

Discussion

Telomerase activity is critical to prevent early telomere shortening which can delay the aging process [21, 14, 15]. Interventions that can augment telomerase activity are clinically important because [27, 35, 36, 37, 38, 39] there are no known pharmacological or behavioral interventions that have this beneficial effect till to date. Also there is an inverse relationship between telomerase activity and perceived stress [25] that can be reduced by meditative practice [40, 41].

To our knowledge, ours is the first study to date where simultaneous investigation of the critical cellular and molecular parameters of measuring human well-being were investigated. Most earlier

studies had looked at the effect of meditation on telomerase activity only. However, in this particular study we looked at the effect of the MBT on stem cell population along with telomerase activity.

Furthermore, in this study, we examined if there is any interrelationship between the telomerase activity and the augmentation of stem cell counts in subjects. Since stem cells are responsible for new cell generation, higher stem cell counts could facilitate any age-related damaged tissue healing process. We found after intensive MBT training, retreat participants had significantly higher telomerase activity compared to premeditated group. Interestingly, increased telomerase activity among the retreat participants was associated with increased stem cells count in the blood, thereby indicating an interrelationship between increased stem cells numbers and telomerase activity.

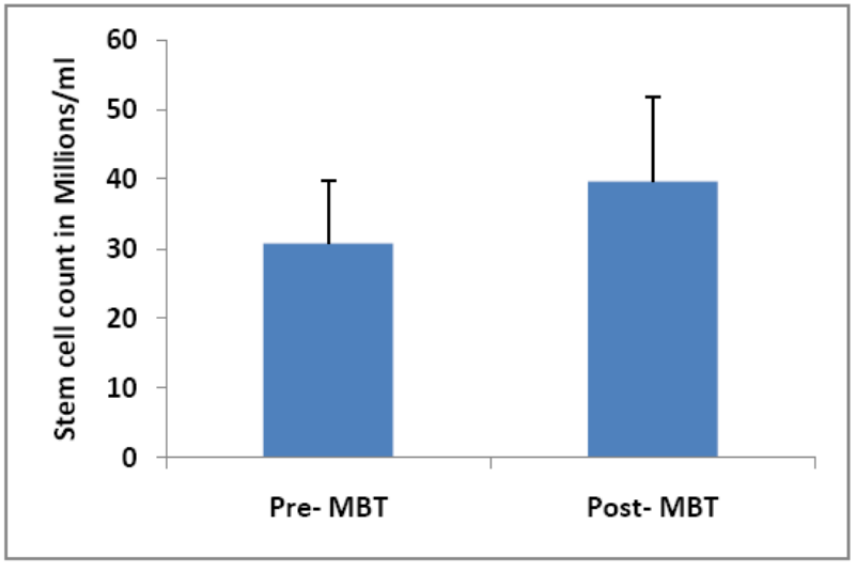

Figure 1. *Stem cells fold measurements in participants' blood before and after the MBT program.* Viable stem cells were counted from participants' blood using trypan blue measurement. Fold changes were determined by comparing viable stem cell counts before and after the meditation.

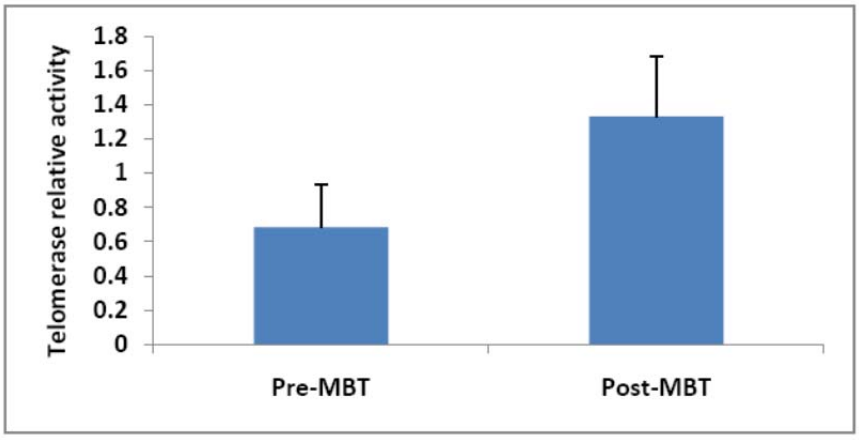

Figure 2. *Telomerase activity measurements in participants' blood before and after the MBT program.* Fold changes in activities were determined by comparing telomerase activity before and after the MBT program. Heat-denatured telomerase was used as a negative control for TRAP reaction. The positive control was done using one million telomerase positive cells supplied in the kit. The relative telomerase activity was calculated by taking negative control values as 1 and the data were normalized based on exact protein content of the samples.

The reasons for the positive correlation between the increased stem cell count and increased telomerase activity could be two-fold. First, increased telomerase activity could be due to intrinsic telomerase activity in the stem cells. So higher the stem cell number higher is the overall telomerase activity. Second, since the stem cells have the potent healing capacity, they could migrate and home to the damaged tissue due to age-related process and can stabilize the telomere length by activating telomerase enzyme through unknown mechanism. However, we don't know for sure whether the positive correlation between stem cell number and telomerase activity is direct or indirect. Future studies need to be done to address this.

However, increased telomerase activity has been associated with the occurrence of malignancy or carciongenesis [42]; the most prominent hypothesis is that maintenance of telomere stability is required for the long-term proliferation of tumors [43-45]. Therefore, it is possible that increased telomerase activity in the retreat patients can lead to cancer as a possible side effect. However, the enhancement of telomerase activity in the retreat patients was modest in majority of the post-MBT subjects; 45.37 % of MBT subjects exhibited greater than 1-fold and 27.77% of MBT subjects showed greater than 2-fold changes (Table 5) in this study and thus we presume that relatively modest increase of telomerase activity in majority of the post-MBT subjects may not cause cancer development in the retreat patients. A Higher level of telomerase activity (4.33-fold) enhancement was observed in colorectal cancer in humans [46].

Furthermore, a recent pilot study on early-stage prostate cancer patients [26] , in which an modest increase in telomerase activity (2.22 as opposed to 2.0 before MBT) occurred in response to lifestyle changes including a small amount of meditation or yoga is consistent with the positive effect of MBT on the enhancement of telomerase activity. This increase was due to a reduction in ‘‘intrusive thoughts’’ as reported by the patients. Given that meditative practice and negative impact of intrusive thoughts are inversely co-related, it is plausible that the increase in telomerase activity reported in that study might be in part due to meditation-induced changes. Importantly, the association between changes in control pre-MBT and post-retreat telomerase activity in the present study is consistent with the previous report showing an inverse relationship between perceived stress and immune cell telomerase activity in caregivers for their chronically ill children.

Conclusion

The present study focuses on how changes in psychological variables relate to post-retreat telomerase rather than how these variables relate to telomerase at a single time-point. Although the changes we observed in the study were modest, considering the short duration of the MBT program we believe that longer MBT process may reveal pronounced changes in stem cells numbers and the telomerase activity. Additionally, it is also important to determine how long the effects last in the post-retreat patients. Thus follow-up longitudinal studies will be beneficial to understand the minimum retreat period for a sustained effect on mental well-being.

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Disclosure Statement

None of the authors has any conflict of interest to declare.

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