

Original Research

## Serum melatonin and serotonin levels in long-term skilled meditators

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### ABSTRACT

**Introduction:** Melatonin and its precursor serotonin are neurochemicals that play an important role in the physiological regulation of mood, sleep, and behavior. Studies have suggested the possibility of changes in the levels of melatonin and serotonin following meditation. However, the outcome of Buddhist meditation on both these two neurochemicals collectively have not been studied yet.

**Objective:** To assess the effect of Vipassana meditation on serum melatonin and serotonin levels in long-term meditators and to compare them with an age, gender, and education level matched, non-meditating control group.

**Methods:** The serum melatonin and serotonin levels of long-term meditators (n=30), recruited using a validated interview, and age, gender and educational level matched control subjects (n=30) who had never practiced meditation, were determined using commercial ELISA kits (LDN, Nordhorn, Germany).

**Results:** The median concentration of melatonin (18.3 pg/ml) and serotonin (149.0 ng/ml) in the meditator group, were significantly higher compared to the control group; melatonin (15.6 pg/ml; p = 0.006), serotonin (118.1 ng/ml; p < 0.001). The levels had no significant correlation with demographic factors but positively correlated with meditation factors in those who had meditated for <=10years (n=26, p < 0.05).

**Conclusion:** The findings indicate elevated melatonin and serotonin levels in the long-term meditators with potential beneficial effects in decreasing stress and improving relaxation in individuals.

### Introduction

Meditation and mindfulness practices have existed in diverse Asian cultures throughout history with multiple techniques, forms and definitions. Mindfulness is an intrinsic ability of the human mind which has been defined as “the awareness that develops by purposefully paying attention to the present and to the unfolding experiences moment by moment”<sup>12</sup>. These meditation and mindfulness practices integrate brain functions with various physiological mechanisms to preserve the mental and physical wellbeing<sup>23</sup>

Transcendental meditation, Buddhist and Dhammakaya meditation, Mindfulness Based Stress Reduction (MBSR), Progressive Muscle Relaxation, and multiple types of yoga are some of the practices that follow the core concept of being aware of the self and surroundings, focusing on the present moment by blocking all distraction while purposefully controlling the breath and physical movements<sup>7</sup>. Meditation is a common practice among the Theravada Buddhist community in Sri

Lanka. *Samatha* (Tranquility) and *Vipassana* (Insight) are the two main stages of Theravada Buddhism which are practiced respectively to attain calmness of the mind and *nibbana*, a state of complete inner peace.

The initial research interest of meditation was focused on stress, pain, coping, and quality of life but recently, it has gained attention as a complementary treatment for various clinical modalities. Research implies some of the benefits of meditation on the nervous system are positive changes in autonomic activity, cognition, sensory perception, hormones, and neurotransmitters<sup>26</sup>. Growing evidence demonstrates that mindfulness and meditation practices modulate various psycho-physiological processes<sup>13</sup>.

Recent research has found that mindfulness and meditation practices are involved in maintaining a proper sleep-wake cycle facilitating to enhance the quality of sleep. Sleep quality is an important determining factor of health and wellbeing<sup>16</sup>. The neurotransmitter, melatonin, sometimes referred to as the ‘sleep hormone’ or ‘hormone of darkness’ which is produced by the pineal gland in the brain regulates the

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sleep-wake cycle<sup>36</sup>. Melatonin is synthesized endogenously from the amino acid tryptophan (derived from serotonin) by the enzyme 5-hydroxyindole-Omethyltransferase. Melatonin biosynthesis is influenced by the day-night cycle and is involved in the regulation of circadian rhythm<sup>14,38</sup>. Melatonin reaches its peak levels between midnight and 4 am. Melatonin has found to reduce pain sensitivity and also acts as an antioxidant and immunomodulator, stimulating the immune system and the antioxidative defense system, thus delaying aging<sup>4,20</sup>. Some studies have shown that melatonin can even inhibit the growth of certain types of cancer<sup>6,20</sup> and prevent coronary atherosclerosis, while upregulating the immune system<sup>2,30,31</sup>. Melatonin secretion is altered in patients suffering from affective disorders, eating disorders, and schizophrenia, indicating that low melatonin levels cause depressive phases<sup>27</sup>.

Previous studies have shown that different mindfulness and meditation types have effects on melatonin levels of different biological fluids in the body. Massion et al.,<sup>20</sup> tested the hypothesis that the regular practice of mindfulness meditation is associated with increased physiological levels of melatonin and have obtained some positive results. Transcendental meditation (TM)- Sidhi or yoga showed significantly higher plasma melatonin levels in the period immediately following meditation compared with the same period at the same time on a control night<sup>35</sup>. Harinath et al.,<sup>10</sup> evaluated the effect of Omkar meditation and Hatha yoga on melatonin secretion and found melatonin levels at 2:00 AM, 3:00 AM and 4:00 AM after 3 months of yoga and meditation were significantly higher compared to that of control. They also found that the rise of melatonin level in the yoga group showed a significant correlation with well-being score. Solberg et al.,<sup>32</sup> measured plasma melatonin secretion levels during ACEM meditation (ACEM is a meditation organization that originated in Oslo, Norway), and found that advanced meditators have higher melatonin levels than non-meditators, although melatonin levels decrease during long-term meditation. Chinese Original Quiet Sitting (COQS) elevated the nighttime salivary melatonin levels by showing statistically higher melatonin level in the COQS meditation group and the level was unchanged in the control group after nighttime meditation<sup>17</sup>. Another study showed that, diurnal serum melatonin levels were significantly higher in Vipassana meditators compared to non-meditating controls<sup>24</sup> and the morning melatonin level showed a positive correlation with the N3 sleep stage.

Serotonin, the precursor of melatonin and a powerful neurotransmitter in the central nervous system acts on the digestive tract and regulates the physiological mechanisms such as body temperature, motor control, and circadian rhythm<sup>8</sup>. Serotonin affects mood by producing a general sense of satisfaction and relaxation<sup>7</sup> which helps to maintain the emotional wellbeing and overall health. Serotonin also plays a role in regulating sleep-wake cycles and circadian rhythms<sup>29</sup>. It is an important neurotransmitter in the descending analgesic pathway from the brain stem to the dorsal horn of the spinal cord, which is important in pain relief<sup>33</sup>.

Several studies have shown associations between low levels of serotonin and serotonin receptors with depression and related conditions. Low levels of serotonin in plasma and serum are correlated with feelings of depression, anxiety and higher sensitivity to noxious stimuli<sup>18</sup>. Lower levels of serotonin in the cerebrospinal fluids have been shown to correlate with depression<sup>1</sup>. Patients diagnosed with panic disorder had about one-third lower level of serotonin 1A receptors compared to healthy individuals indicating that depression is associated with deficiency of serotonin activity on receptors<sup>15</sup>. Studies with 5-HT1A (5-Hydroxytryptamine receptor 1A) serotonin receptor knockout mice have indicated that they have increased avoidance, decreased locomotor activity, and increased autonomic arousal which suggests increased anxiety-like behavior<sup>9,28</sup>. However, a recent review article by Moncrieff et al.,<sup>22</sup> on the association of depression with serotonin levels revealed that there was no consistent evidence supporting the hypothesis that depression is caused by lowered serotonin activity or concentrations. Although, the same review article identified multiple evidence showing

that antidepressants were strongly associated with lower serotonin levels<sup>22</sup>.

Several studies have shown that after meditation, the breakdown products of serotonin (5-HT) in urine are significantly increased, suggesting an overall elevation in serotonin during meditation<sup>26</sup>. Studies have found that following the transcendental meditation, the main breakdown product of serotonin, 5-hydroxyindole-3-acetic acid (5-HIAA) in urine was significantly increased<sup>5,37</sup>. In contrast,<sup>32</sup> found that blood serotonin concentrations decreased in meditation and reference groups after one hour of meditation.

Serotonin has been closely linked to melatonin; both play an important role in mood stabilization (including depression), positive affect, stress-prevention and aging<sup>27</sup>. The physiological effects of meditation suggest the possibility of a role played by either melatonin or serotonin or by both in influencing mood changes and relaxation<sup>21</sup>.

Even though, there are early studies showing the effect of different mindfulness practices and meditation types on melatonin and serotonin levels, there is limited knowledge on the effects of Vipassana meditation technique on serum serotonin and melatonin levels collectively, especially in Sri Lanka. Therefore, this study was aimed to determine the serum levels (concentrations) of two different neurochemicals, serotonin and melatonin among long-term Vipassana meditators in Sri Lanka in comparison with age, gender and education level matched non-meditator controls.

## Methodology

### Study Design

This is a comparative cross-sectional study. Long-term vipassana meditators were grouped as the cases and age, gender, educational level matched non-meditators were grouped as controls. The cases (long-term meditators) were matched one to one with a non-meditator control.

### Ethics approval

Ethical approval for the study was obtained from the Ethics Review Committee, Faculty of Medicine, Colombo, Sri Lanka (EC-19-068). Informed written consent was obtained from the eligible participants prior to data and sample collection.

### Meditators

Long-term meditators were recruited from two main meditation centers in Sri Lanka, International Vipassana Meditation Centre, Colombo Sri Lanka and Siyane Vipassana Meditation Centre, Kanduboda, Sri Lanka and were screened using a validated intake interview which determined their duration of meditation practice, details of meditation practice, heightened peripheral awareness, stable attention, alertness and emotional stability (Supplementary file). Based on the intake interview, 30 long-term vipassana meditators were eligible for the study as skilled meditators. The meditators were above 18 years of age and had practiced meditation at least one week cumulatively in formal sessions of meditation (retreat or temple based) and six hours per week for the last three years.

### Controls

Thirty age, gender and education level matched participants who were also above 18 years of age and who had never practiced any form of meditation or mindfulness exercise were recruited as control subjects for the study.

Participants who were having autoimmune diseases, non-communicable diseases, mental illness or neuropsychiatric disorders or those who were on immunomodulating or anti-inflammatory medications and medications that include Selective Serotonin Reuptake

Inhibitors (SSRIs) were excluded from the study. Monks, pregnant women, smokers and those who participated in other stress management techniques such as guided imagery, breath focus, yoga, Sudarshan kriya and tai chi were also excluded from this study (Fig. 1).

*Socio-demographics factors*

All participants (from both long-term meditator and control groups) were requested to fill a questionnaire (Supplementary file) prepared to collect data on their age, gender, educational level, marital status, body mass index, sleeping hours, working hours, healthy habits, alcohol consumption, type of diet and exercise hours per day.

*Sample collection*

Participants were asked to avoid foods or liquids containing pineapple, eggplant, avocados, bananas, currants, melon, plums, chocolate, tomatoes, or walnuts for 2 days before and including the day of the sample collection. Participants were also requested to fast for 12 hours and to get sufficient and undisturbed rest during the night before the day of sample collection. Ten milliliter (10 ml) of peripheral venous blood was collected into plain tubes and kept on ice. Blood was collected from all study participants in the morning between 8 AM – 9 AM.

*Serum and separation*

Blood was centrifuged at 900 g for 10 minutes at 4 C. Serum was separated and stored at -20 °C until used for assays.

*Determination of serum serotonin levels*

Serotonin was analyzed in the serum samples using an enzymatic immunoassay kit (Serotonin ELISA<sup>Fast Track</sup>, LDN, Nordhorn, Germany). The enzyme linked immunosorbent assay (ELISA) was performed on samples in duplicate according to the manufacturer’s instructions. All samples and standards were analyzed on a microplate reader set at 450 nm. The analytical measurement range of the ELISA is 10.2 - 2500 ng/ml with a CV of 9.7% and the reference range for serum serotonin is 70 - 270 ng/ml. Quality control samples at two levels were analysed in each and every run to assess the performance of the assay.

*Determination of serum melatonin concentration*

Melatonin levels were analyzed in the serum samples using an enzymatic immunoassay kit (Melatonin ELISA, LDN, Nordhorn, Germany). ELISA was performed according to manufacturer’s instructions and each sample was tested in duplicates. Optical densities (OD) of all samples were read at the wavelength of 450 nm. The analytical measurement range of the assay is 5.6 - 134.3 pg/ml with a CV of 6.4% and the reference range for serum melatonin is 3.8 - 80.4 pg/ml. Quality control samples at two levels were analysed in each and every run to assess the performance of the assay.

*Statistical Analysis*

Statistical analysis was performed using Statistical Package for the Social Sciences/ Statistical Product and Service Solutions (SPSS) version 20.0. The Shapiro-Wilk and Kolmogorov-Smirnov tests was used for testing the normality of the data distribution. Since the distribution of

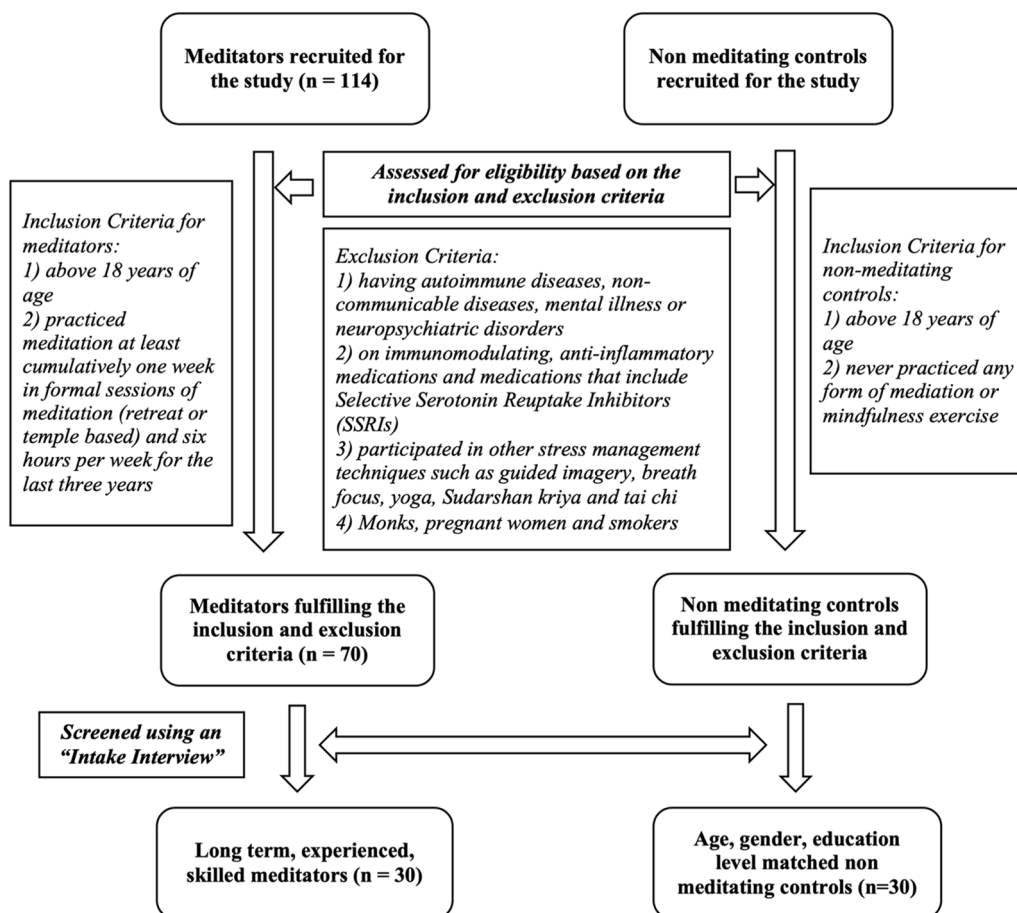


Fig. 1. The process of recruiting participants.

both serotonin and melatonin was skewed, non-parametric tests were used for inter-group analysis. Mann-Whitney U test was performed to compare the levels of neurotransmitters between meditator and the control group and results are presented as median values. Spearman correlation coefficient was performed to evaluate correlations between the levels of neurotransmitters of both study groups with socio-demographic factors and to evaluate the correlation between neurotransmitters, serotonin and melatonin. Statistical significance was defined as  $p < 0.05$  at confidence interval of 95%.

## Results

### Socio-demographic factors of the study groups

All the participants involved in the current study were Sinhalese and their religion was Theravada Buddhism. Among the 30 participants in each group, 19 (63.33%) were males. Average age (mean  $\pm$  SD) of participants was  $43.83 \pm 9.92$  and  $43.51 \pm 9.92$  years for meditators and controls respectively. The total sample size for the study was calculated as 60 (30 each for meditators and controls), using G power software to provide an effect size of 0.8 and 80% power for an alpha error of 0.05 (Kang, 2021). When considering the meditator group, their mean duration of the meditation practice was  $6.80 \pm 3.27$  (median 5.25 with a range of 16.0) years, and their mean meditation frequency was  $5.82 \pm 3.45$  (median 4.75 with a range of 19.0) hours per week.

### Serotonin concentrations

Median serotonin concentration in serum was 149.0 ng/ml (C25: 119.6; C75: 152.3) in the meditation group. Control group showed a median value of 118.1 ng/ml (C25: 105.5; C75: 139.1) for serum serotonin concentration (Fig. 2). Serum serotonin concentration was significantly higher in meditators compared to the control group ( $p < 0.001$ ).

### Melatonin concentrations

The median serum melatonin concentration in meditator group was of 18.3 pg/ml (C25: 16.9; C75: 21.5) and in control group it was 15.6 pg/mL (C25: 14.2; C75: 20.7) (Fig. 3). Meditator group showed

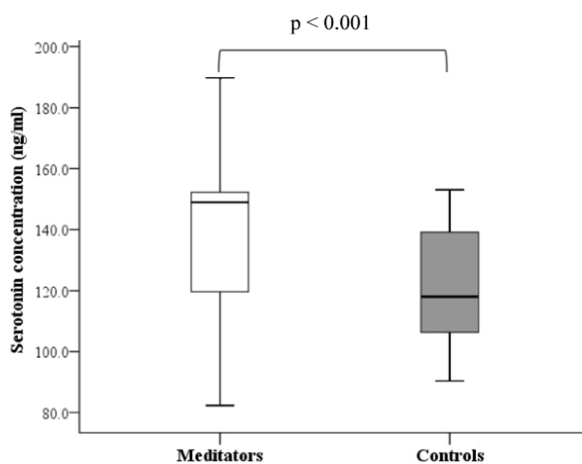


Fig. 2. Serum serotonin concentration of meditator and control groups (n = 29\* per group).

Data is presented as median and inter-quartile ranges, C25: C75); (Significance level  $p < 0.05$ ).

\*The non-meditating control participant 51 was an outlier who was the age, gender and education level matched control participant for the meditating group participant 21, therefore participant 51 and the corresponding participant 21 were excluded from the analysis.

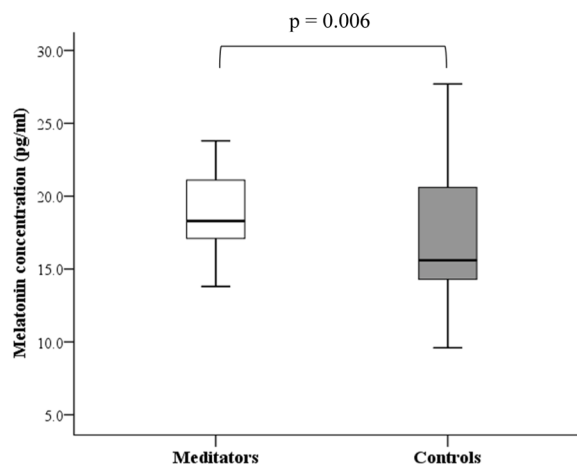


Fig. 3. Serum melatonin concentration of meditator and control groups (n = 29\* per group).

Data is presented as median and inter-quartile ranges, C25: C75); (Significance level  $p < 0.05$ ).

\*The non-meditating control participant 49 was an outlier who was the age, gender and education level matched control participant for the meditating group participant 19, therefore participant 49 and the corresponding participant 19 were excluded from the analysis.

significantly higher ( $p = 0.006$ ) melatonin concentrations in serum compared to the controls.

### Association between serum melatonin and serotonin

The analysis of correlation between serum melatonin and serum serotonin of participants showed a positive association (Fig. 4). Even though, the serum melatonin concentration showed a non-linear correlation with the serum serotonin concentration, the correlation was not significant ( $r = 0.206$ ;  $p = 0.114$ ).

### Relationship of socio-demographic factors of study population with serum serotonin and melatonin levels

There was no significant relationship observed between the studied parameters (serum serotonin and serum melatonin concentration) and socio-demographic parameters such as gender, age weight, height, body

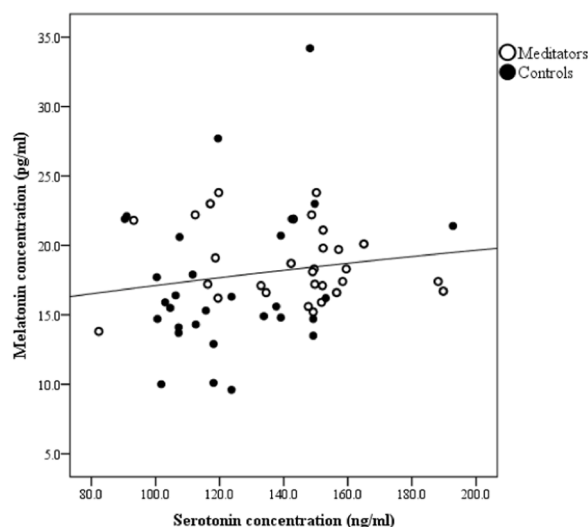


Fig. 4. Association of serum melatonin with serotonin among the study participants (n = 30 per group).

mass index (BMI), marital status, educational status ( $p > 0.05$ ). The factors related to habits such as alcohol consumption or sleeping hours were not significantly ( $p > 0.05$ ) associated with serum serotonin and melatonin levels of the studied groups.

*Relationship of duration of meditation with serum serotonin and melatonin levels*

The meditational parameters such as the years of meditational practice (duration of meditation) and the number of hours meditation was practiced in a week (meditation frequency) had no significant relationship when analyzing all 30 meditators (Fig. 5). However, there was a significant relationship of the duration of meditation between both the serotonin and melatonin levels when considering only those who practiced meditation for 10 years or less ( $n=26$ ). Further, when we looked at those who have practiced meditation for more than 5 years but less than 10 years ( $n=16$ ) there was a significant relationship with melatonin levels but not with serotonin levels. When considering only those who practiced meditation for less than 5 years ( $n=10$ ) serotonin levels were significant with the duration of meditation but not melatonin levels (Table 1).

**Discussion**

This study showed significantly higher serum concentrations of melatonin and serotonin among the long-term Vipassana meditators compared to their age, gender and education level matched controls. There was no significant correlation between the serum melatonin and serotonin concentrations with none of the socio-demographic factors considered such as gender, age, weight, height, BMI, marital status, highest educational level, non-vegetarian diet, alcohol consumption and sleeping (hours per day) in this study. These indicate that all the socio-demographic parameters were not possible confounding factors and difference in the serum serotonin and melatonin levels between the meditators and controls could be attributed to the meditation practice.

According to the current study, serum melatonin levels were significantly higher than that of the controls. Previous research findings indicated that different meditation and yogic types increased plasma or salivary or blood melatonin levels. TM, Omkar meditation, Hatha yoga and COQS practitioners had high levels of melatonin compared non-meditator controls<sup>20,35,10</sup>. ACEM meditators had higher plasma melatonin levels initially compared to non-meditator controls, but the melatonin levels decreased after 1 hour and 3 hours in both the groups.

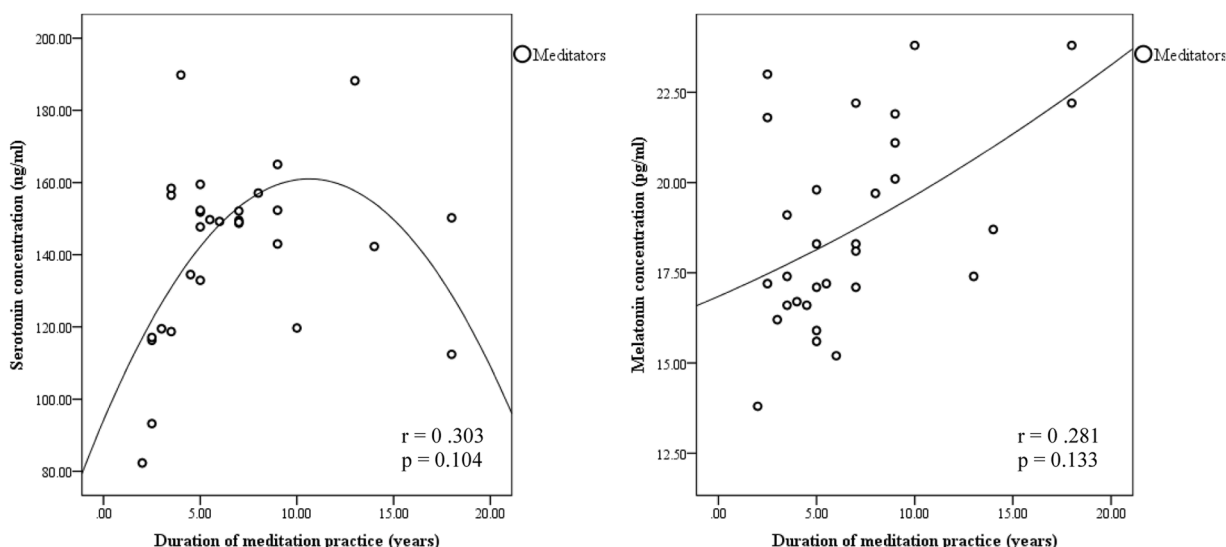
**Table 1**

Association between duration of meditation with serotonin and melatonin levels.

Duration of Meditation	Serotonin Correlation (r)	Significance (p)	Melatonin Correlation (r)	Significance (p)
All meditators (n = 30)	0.303	0.104	0.281	0.133
Meditators who practiced meditation for more than 10 years (n = 4)	0.105	0.895	-0.056	0.944
Meditators who practiced meditation between 5 – 10 years (n = 16)	-0.038	0.890	0.691	0.003
Meditators who practiced meditation for less than 5 years (n = 10)	0.864	0.001	-0.087	0.811
Meditators who practiced meditation for 10 years or less (n = 26)	0.426	0.030	0.395	0.046

A previous study on Vipassana meditators had indicated diurnal serum melatonin levels were higher in meditators compared to that of controls<sup>24</sup>. The current study further supports the results of the previous study indicating that Vipassana meditation significantly ( $p = 0.006$ ) increased serum melatonin levels.

Previous literature indicates that results were varied depending on the time of the day when the melatonin measurement was done. Tooley et al.,<sup>35</sup> and Liou et al.,<sup>17</sup> have measured the melatonin concentrations secreted during night where<sup>32</sup> measured morning melatonin levels. During the current study blood was collected from all participants between 8AM - 9AM and measured morning melatonin levels of Vipassana meditators. The current can be partially corroborated with the earlier study done by<sup>24</sup> where they have measured both morning and evening melatonin levels of Vipassana meditators. Duration of meditation might differently influence melatonin levels<sup>24</sup>. Solberg et al.,<sup>32</sup> stated that melatonin levels could be declined during the long meditation session, however authors had not provided an exact explanation for the finding.



**Fig. 5.** Association between duration of meditation and neurotransmitters (n=30).

They have suggested an alternative explanation as that meditation practiced over time may delay the phase of melatonin secretion. This could be a possible reason for the that the current study showed a significant correlation between the duration of meditation practice and melatonin level only after excluding the meditators practiced meditation for more than 10 years.

The exact mechanism by which melatonin increases during meditation still remains unclear<sup>7</sup>. However, the biosynthesis of melatonin is inhibited by strong light probably to match the sleep cycle with the day-night cycle<sup>7</sup>. Since most meditation techniques are practiced with eyes closed it may have an influence on the increased production of melatonin. Further, it is also possible that the decrease in the hepatic blood circulation during meditation Jevning et al.,<sup>11</sup> could result in the slowing down of melatonin metabolism leading to higher concentrations of melatonin in blood<sup>35</sup>. In addition, melatonin levels depend on its precursor, serotonin and several studies have shown that increase in the metabolite of serotonin, 5-HIAA, could directly lead to the increase in melatonin levels<sup>5,35,37</sup>. The increase in serotonin, combined with hypothalamic innervation of the pineal gland during meditation, may cause the pineal gland to increase production of melatonin through a conversion of serotonin<sup>20</sup>.

Melatonin level also depends on the type of the biological specimen and the methodology used to measure it. Melatonin readily passes through the blood-brain-barrier and accumulates in the central nervous system (CNS). Therefore, it accumulates in a substantially higher levels in CNS than it exists in blood<sup>34</sup>. It is also found that melatonin levels in plasma is ten times more compared to other biological fluids. In the current study, serum melatonin levels of meditators and controls were measured using the sandwich-ELISA method. Therefore, further studies can be designed to measure plasma melatonin levels or directly by measuring melatonin levels in CNS by more sensitive and accurate methods.

Literature indicates that long term practitioners of Vipassana meditators had an enhanced states of slow wave sleep (SWS) and rapid eye movement (REM) sleep compared to that of non-meditating control group<sup>23</sup>. Another study showed that morning melatonin level is having a positive correlation with the N3 sleep stage<sup>24</sup>. The significantly higher levels of serum melatonin (the neurochemical regulates the sleep-wake cycle) in meditators of the current study may suggest that Vipassana meditation could help in having a proper sleep or enhancing the quality of sleep which may relax the mind and the body by reducing stress. This relaxed state would have positive correlation with the wellbeing score. Future studies are needed to determine whether Vipassana meditation affects the correlation between melatonin levels and wellbeing score.

This is the first study to look at serotonin and melatonin levels in meditators in Sri Lanka and to the best of our knowledge, this is the first cross-sectional comparative study on the effect of Vipassana meditation on both serum melatonin and serum serotonin levels collectively within the same study. According to the results of the current study, serotonin levels were significantly higher ( $p < 0.001$ ) in the mediator group compared to the non-mediator control group. Vipassana meditation may have an effect of increasing serum serotonin levels. Several studies have shown serotonin levels or the main breakdown product of serotonin, 5-HIAA to be higher in TM group compared to non-mediator control group<sup>5,26,37</sup>. As this is the first study which studied the effect of Vipassana meditation on serum serotonin levels, the resulted significant increase of serotonin levels in Vipassana meditators laid a foundation for further studies on the same effects using different biological samples and also to measure its levels in CNS of Vipassana meditators. A review on the biochemical effects of meditation by Daube & Jakobsche,<sup>7</sup> suggest that these serotonin levels could remain elevated in meditators irrespective of the time of day as well as whether the participants were tested immediately after a meditation session or not. Solberg et al.,<sup>32</sup> observed a decline of serotonin levels after both one-hour meditation and rest, therefore they have suggested that serotonin may be a marker of general rest and not a meditation-specific relaxation. The results of

the current study indicate that serotonin levels are increased in Vipassana meditators. Since serotonin mediates the sense of satisfaction and also regulates sleep-wake cycle, the increase in serotonin levels in the current study suggests that Vipassana meditation could contribute to maintain the emotional wellbeing and overall health in a favorable manner.

The reason for the increase in serotonin levels could be speculated to the association of mindfulness meditation with the stimulation of lateral hypothalamus and prefrontal cortex, which is thought to induce changes in the serotogenic activity<sup>26</sup>. Stimulation of the lateral hypothalamus and prefrontal cortex results in the increased production and distribution of 5-HT by the cells of the dorsal raphe<sup>19</sup>. Studies have shown that 5-HT which is the breakdown products of serotonin has significantly increased following meditation<sup>37</sup>. These finding corroborates the findings of the current cross-sectional study as the long-term meditators of the current study had significantly higher serum serotonin levels compared to the non-meditators.

The results of the current study with Vipassana meditators indicate that there was a positive association between serum melatonin and serotonin concentrations suggesting that the increased melatonin levels may have been influenced by the increased serotonin levels. Further, the finding of increased serum serotonin and melatonin levels support the hypothesis that Vipassana meditation could influence the levels of the neurochemicals, serotonin and melatonin in blood and could lead to an increased production of serotonin and melatonin.

Solberg et al.,<sup>32</sup> stated that melatonin levels could decline during the long meditation session (3 hours or more meditation sessions), however authors had not provided an explanation for the finding. They have suggested that meditation practiced over time may delay the phase of melatonin secretion which could possibly be a reason that the current study showed a significant correlation between the duration of meditation practice and both melatonin levels and serotonin levels only after excluding the meditators who practiced meditation for more than 10 years. A recent study carried out by Bowles et al.,<sup>3</sup> indicates that the relationship between accumulated meditation practice hours and favorable psychological outcomes may be non-linear, with the relationship being strongest for approximately the first 500 hours of practice before plateauing. This plateauing possibly explains the findings of this study as to why serotonin and melatonin levels had a significant correlation with the duration of meditation in those who have been meditating for less than 10 years and not in those who have been meditating for more than 10 years.

There has been very little focus towards the serum concentrations of melatonin and serotonin levels in previous studies. These existing studies are not conclusive on elevation of serotonin and melatonin in the same cohort as most studies looked at solely on serotonin or melatonin levels. Although the mechanism by how the neurotransmitters, serotonin and melatonin is increased is still unclear these results could be interpreted as the potential beneficial effects of meditation in decreasing stress and improving relaxation in individuals. Since, this is a cross sectional comparative study further research is needed with interventions of meditational practices to identify the changes in the melatonin and serotonin levels immediately after a meditation session. An Indian study on Brahma Kumaris Rajayoga (BKRY) practitioners found that it was competence, not duration of practice, that determined whether meditation resulted in improved well-being<sup>25</sup>.

The findings of this study are expected to enhance the knowledge on neurochemical basis of effects of Vipassana meditation. The findings may also emphasize on the potential beneficial effects of meditation in facilitating a quality sleep and to have a good mood, decreased stress and improved relaxation in individuals which may be helpful for well-being and good health.

#### Limitations of the study and future directions

This was a comparative cross-sectional study with only 30 meditators

and 30 age, gender, and education level matched controls. Therefore, further studies involving a larger population of mediator-control pairs together with an intervention are required to comprehensively conclude the effect of Vipassana meditation on these neurochemicals and to evaluate the correlation between Vipassana meditation and wellbeing score.

Since the number of meditators who practiced meditation for more than 10 years in the current study is small, future studies with a larger mixed cohort of short-term and long-term meditators would be required to prove and comprehensively explain whether melatonin and serotonin levels have a significant correlation with the years of meditational practice. Future studies with interventions of meditational practice would be helpful to further strengthen these aspects and to explain possible mechanisms encountering these findings.

**Conclusion**

The results of this study provide strong evidence related to the effects of meditation on serum serotonin and melatonin levels. These findings support the hypothesis that Vipassana meditation could induce synthesis of serotonin and melatonin. This study has also shown that the levels of the neurochemicals, serotonin and melatonin correlated with the duration of meditation which emphasizes the importance of continuing meditation as a long-term practice. The therapeutic potential of the current findings needs to be explored further via interventional studies.

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**Supplementary materials**

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.explore.2023.03.006](https://doi.org/10.1016/j.explore.2023.03.006).

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