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ORIGINAL PAPER

Higher Serum Antioxidant Capacity Levels and Its Association with Serum NOx Levels Among Long-term Experienced Meditators in Sri Lanka

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Abstract

Objectives Recent medical research into meditation based on stress, pain, coping, and quality of life has shown an overall positive impact on health and immunological outcomes including oxidative stress. This study was aimed to assess the total nitric oxide, nitrite levels, and antioxidant capacity in experienced meditators compared to an age-, gender-, and education level–matched non-meditating group and to determine relationship between these parameters.

Methods The total serum nitric oxide ($NOx{:}NO_3^- + NO_2^-$) and nitrite (NO_2^-) levels of long-term, experienced meditators ($n = 12$), recruited using a validated interview, and age-, gender-, and educational level–matched control subjects ($n = 12$) who had never practiced meditation, were determined using the modified Griess and Griess assay respectively. The Trolox equivalent antioxidant capacity (TEAC) was determined using the ABTS assay using Trolox as a standard.

Results Serum NOx 5.03 ± 0.31 (mean ± SD) and nitrite levels 0.52 ± 0.05 of the meditators were significantly lower and TEAC values 424.35 ± 41.53 of the meditators were significantly higher compared to control group who had serum NOx levels of 5.42 ± 0.42 ($p = 0.016$, $d = -1.05$), nitrite levels of 0.92 ± 0.52 ($p = 0.014$, $d = -1.08$), and TEAC values of 376.15 ± 12.69 ($p = 0.001$, $d = 1.57$). There was a correlation of the TEAC levels with NO_2^- ($r = 0.562$; $d = 0.316$) and NOx ($r = 0.664$; $d = 0.441$).

Conclusions These findings indicate a lower production of nitric oxide and a higher serum antioxidant capacity in the long-term meditators with potential beneficial effects against oxidative stress.

Keywords Meditation · Oxidative stress · Nitric oxide · Antioxidant capacity

Meditation and mindfulness have been practiced throughout the history in diverse Asian cultures. This long history has given rise to multiple techniques, forms, and definitions of both meditation and mindfulness (Ott, 2002). Meditation is defined as a deliberate approach to achieve mindfulness by continuous practice (Black & Slavich, 2016), and 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 (Kabat-Zinn, 2003).

Theravada Buddhism is the main religion in Sri Lanka, practiced by around 70% of the population, and meditation is a common practice among the Buddhist community. Of the two stages of meditation practiced in Theravada Buddhism, *Samatha* (Tranquility) and *Vipassana* (Insight) meditation, the former stage is used to achieve calmness of the mind while the latter is essential to attain *nibbana*, which is a state of complete mindfulness and inner peace (Batchelor, 2011). Out of the two stages, *Vipassana* (Insight) stage is considered unique to Buddhism which describes a path to further mental development, both mundane and supra mundane (Hart et al., 2013). Various programs were developed for mindfulness meditation throughout, but it was only in 1979 that these mindfulness-based interventions (MBI) were standardized and introduced as mindfulness-based stress

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reduction (MBSR) programs (Kabat-Zinn et al., 1985). These programs were based on mindfulness meditation and therefore have strong roots to Buddhist philosophy (Simkin & Black, 2014). In recent years, mindfulness meditation has gained much attention as a complementary treatment for various clinical modalities. Although the initial focus of medical research into meditation was based on stress, pain, coping, and quality of life, it has shown to have an overall positive impact on health, especially on immunological outcomes such as circulating and stimulated inflammatory proteins, cellular transcription factors and gene expression, antibody response, immune cell count, and oxidative stress (Black & Slavich, 2016).

Oxidative stress is caused by the imbalance between the production of oxidants and their elimination by antioxidants (Halliwell, 1997). This imbalance is created as the oxidizing agents are generated at a rapid pace and at increased levels which are beyond the detoxifying capacity of the antioxidants present (Grune & Berger, 2007) resulting in oxidative damage of target molecules such as protein, lipid structures, and DNA (Halliwell, 2007). Oxidative stress is implicated in the pathogenesis of several diseases such as cancer, cardiovascular disease, diabetes mellitus, Alzheimer's disease, and Parkinson disease (Valko et al., 2007).

The oxidants or free radicals that are widely known as reactive species (RS) are molecules comprising of an unpaired electron (s) in atomic or molecular orbitals which make them highly unstable (Valko et al., 2007). RS are mainly made of two groups, the reactive oxygen species (ROS) which include superoxide radicals, hydroxyl radicals, and hydrogen peroxide, and the reactive nitrogen species (RNS) which include nitric oxide radicals, nitrogen dioxide radicals, and nitrous acid. Naturally, free radicals are generated endogenously from mitochondrial electron transport, immune response, detoxification, and protein folding (Bashan et al., 2009). Exposure to pollutants, chemicals, radiation, and physical and mental stresses can also accelerate the production of RS (Lachance et al., 2001). RS are unstable molecules which rapidly donate their unpaired electron to other biological molecules in order to reach the ground state. Thus, it is difficult to measure and directly quantify the ROS and RNS due to their high reactivity and short half-life. Therefore, oxidative stress is generally measured by the secondary products of oxidation reactions within the cell which are considered the indicators of oxidative stress. Alternately, anti-oxidant capacity (AOC) can be measured in serum and is an indirect indicator of both ROS and RNS production and the potential for overall protection against oxidative damage (Arguelles et al., 2004).

Nitric oxide (NO) is one of the key oxidants as it is capable of diffusing through almost all types of cells and tissues (Gil et al., 2004). NO is a highly reactive and unstable molecule that rapidly converts into the more stable forms, nitrite (NO_2^-) and nitrate (NO_3^-) collectively known as NOx. Overproduction of RS results in oxidative damage causing annihilation of biomolecules (Kurutas, 2016). Hence, the balance between pro-oxidants like ROS and RNS with antioxidants in the body is vital for health (Rahal et al., 2014).

The effects of meditation associated with oxidative stress have been a focus of research in recent times (Mahagita, 2010). Recent studies on different meditation and yoga practices have shown to cause significant reduction of oxidative stress and enhancement of the antioxidant system (Ingole et al., 2015; Kiecolt-Glaser et al., 2010; Lim & Cheong, 2015; Mahagita, 2010; Sinha et al., 2007). The relaxation induced by diaphragmatic breathing which is a fundamental procedure in Pranayama, Zen, transcendental meditation, and other meditation practices has also shown to lower the levels of oxidative stress (Martarelli et al., 2011). Even though there are several studies focused on various types of indicators of oxidative stress, studies on specific parameters such as serum levels of RNS are limited. NO represents a double-edged sword where small quantities produced by the constitutive enzymes are essential to make its bioavailability for normal physiological functions whereas inducible NO synthases may lead to larger quantities that results in detrimental effects.

The finding from studies on Zen and Loving-Kindness meditation indicating higher levels of serum RNS levels and possible effects on vascular physiology requires further studies to understand the underlying mechanisms (Kemper et al., 2014; Kim et al., 2005). Further, there is limited knowledge on the effects of Theravada mindfulness meditation techniques on reducing the levels of oxidative stress and human health. Therefore, this study was aimed to determine the serum levels of RNS and antioxidant capacity among long-term experienced meditators in Sri Lanka in comparison with its age-, gender-, and education level–matched controls.

Methods

Participants

Initially, 98 long-term, experienced meditators were recruited for this study from two main meditation centers in Sri Lanka. Out of these meditators, only 55 fulfilled the inclusion and exclusion criteria. The inclusion criteria were as follows: (i) above 18 years of age, (ii) practiced meditation at least cumulatively 1 week in formal sessions of meditation (retreat or temple based), and (iii) practiced meditation 6 h per week for the last 3 years. The exclusion criteria were the following: (i) having autoimmune diseases or suffering from non-communicable diseases, (ii) having mental illness or diagnosed with neuropsychiatric disorders, (iii) on immunomodulating and anti-inflammatory medications, (iv) monks, (v) pregnant or breastfeeding women, (vi) smokers, and (vii) participating in other stress management techniques such as Yoga and *Sudarshan Kriya*.

These 55 participants were further screened using an 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, 12 long-term experienced, skilled meditators were eligible for the study as experienced meditators and informed consent was obtained to participate in this study. Twelve age-, gender-, and education level/occupation–matched participants who were also above 18 years of age and who had never practiced any form of mediation or mindfulness exercise were recruited as controls.

The total sample size for the study comprising of 60 participants (30 each for meditators and controls) was calculated using G Power Software by providing the effect size as 0.7, and the power as 0.8 for alpha error at 0.05. The preliminary data of 12 meditators and 12 controls (effect size as 1.2, and the power as 0.8 for alpha error at 0.05) were analyzed and reported herein.

All the participants involved in the current study were of the same ethnicity, Sinhalese, and their religion was Theravada Buddhism. Among 24 participants, 18 (75%) were males. Average age (mean ± SD) of participants was 39.0 ± 10.52 and 38.6 ± 9.49 years for meditators and controls respectively. When considering the meditator group, their mean duration of the meditation practice was 6.46 ± 2.89 years and their meditation frequency was 8.91 ± 4.57 h per week.

Procedures

This is a case–control matched study where the cases (long-term meditators) were matched with controls (non-meditators) for age (± 2 years), gender, and highest educational level. The cases (long-term meditators) were matched one to one with a non-meditator control.

Participants were required to fast for 12 h after an overnight sleep before the collection of the blood sample to minimize possible dietary effect. Ten milliliters of peripheral venous blood was collected into a sterile plain tube. Serum was separated after incubating the blood sample for 1 h at 37 °C and for 2 h at 4 °C followed by centrifugation at 900 *g* for 10 min. Serum samples were stored frozen in aliquots at – 20 °C until used for assays.

Assay for Measurement of Trolox Equivalent Antioxidant Capacity

The antioxidant capacity levels were measured in duplicate using the 2, 2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) decolorization method, expressed as serum 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) equivalent antioxidant capacity (TEAC) (Fernando et al., 2016; Re et al., 1999). The ABTS solution was freshly prepared by mixing 7 mM ABTS stock solution and 2.4 mM $K_2S_2O_8$ in equal quantities and kept at room temperature for 4 h in the dark, allowing them to generate radical cations by the oxidation of ABTS with potassium persulfate ($K_2S_2O_8$). The working ABTS solution was then diluted 40 times using 5 mM phosphate buffered saline (PBS, pH 7.2). Serum samples were mixed with ABTS working solution in 1:9 ratio and kept for 1 min at dark to complete the scavenging process. The reagent blank was prepared by mixing equal volumes (10 µL each) of both distilled water and $K_2S_2O_8$ with 800 µL of 5 mM PBS. Test samples were analyzed in duplicate and absorbance was measured at 734 nm against the reagent blank using spectrophotometer (Synergy HT multimode microplate reader, Biotek, USA) (Arumugam et al., 2006; Kambayashi et al., 2009). A series of twofold Trolox dilutions (12.5–400 µM) were mixed at the same ratio (1:9) with ABTS working solution and the standard curve was plotted using absorbance values. TEAC was calculated using the Trolox standard curve.

Assay for Measurement of Serum NO_2^- and NOx Levels

Deproteinization of Serum The serum samples were first deproteinized before measuring for serum NO_2^- and NOx levels (Kalugalage et al., 2013). Ten microliters of 1.5 g/mL $ZnSO_4$ solution was added to 1 mL of serum. Mixture was thoroughly vortexed for 1 min and centrifuged at 10,000 *g* for 15 min at room temperature (RT, 25 °C). The supernatant was centrifuged again for 10 min. This step was repeated until clear deproteinized serum was obtained. The deproteinized serum was immediately used for the assessment of NO_2^- and NOx.

Assay for Serum NO_2^- Levels Serum NO_2^- levels were measured in duplicate using the Griess assay (Ghasemi et al., 2007; Kalugalage et al., 2013). The Griess reagent was prepared by mixing 1% sulfanilamide in 5% H3PO4 acid and 0.1% N-(naphthyl) ethylene diamine hydrochloride. Serum NO_2^- concentrations of test samples were measured by adding an equal volume (100 µL each) of deproteinized serum sample and Griess reagent in triplicate into wells in a 96-well plate, followed by incubation for 15 min at RT in dark.

Assay for Serum NOx Levels The total serum levels of NO derivatives (NO_2^- and NO_3^-) referred to as NOx were measured in duplicate using the modified Griess assay (Ghasemi et al., 2007; Kalugalage et al., 2013). Briefly, equal volumes of deproteinized serum samples, 8 mg/mL

VCl_3, and Griess reagent were mixed, incubated for 30 min at RT in dark which allowed the conversion of NO_3^- into NO_2^- by VCl_3. The absorbance was measured at 540 nm using spectrophotometer (Synergy HT multimode microplate reader, Biotek, USA). A series of twofold dilutions of $NaNO_2$ (0.193–100 µM) were mixed at equal volumes with the Griess reagent and the standard curve was plotted using absorbance values.

Measures

An interviewer-administered questionnaire (Supplementary file) was used to collect data from the participants on their age, gender, educational level, marital status, body mass index, sleeping hours, working hours, and healthy habits including alcohol consumption, diet type, and exercise hours per day. These scales were administered among both long-term meditators and control groups.

Data Analyses

Statistical analysis was performed using Statistical Package for the Social Sciences/Statistical Product and Service Solutions (SPSS) version 20.0. The Shapiro–Wilk test was used for testing the normality of the data as it is considered appropriate for testing normality in sample sizes less than 50 (Ghasemi & Zahediasl, 2012). Descriptive statistics were summarized as percentages and mean ± SD. Since data was normally distributed, parametric tests were performed to analyze the data. Independent samples *t*-test was used to compare the data of two study groups (meditator group and control group). One-way analysis of variance (ANOVA) and Pearson correlation were performed to evaluate the relationship between study groups with sociodemographic data and also to correlate between TEAC, NO_2^-, and NOx among the study participants. Statistical significance was defined as $p < 0.05$ at confidence interval of 95%.

The effect sizes (Cohen's *d*) and effect size correlation (*r*) were calculated for the serum NO_2^-, NO_x levels, and serum TEAC levels between the meditator group and control group. Cohen's *d* was calculated by subtracting the mean of one group from the other $[M_{1(Experimental)} - M_{2(Control)}]$ and divide by the pooled SD of two groups. Cut-off values for Cohen's *d* and *r* were considered $d < 0.19$ as trivial effect size; $0.20 < d < 0.49$ or $r = 0.10$ as small effect size; $0.50 < d < 0.79$ or $r = 0.30$ as medium effect size; and $d > 0.80$ or 0.50 large effect size (Cohen, 1992).

Results

Serum RNS Levels in Long-term Meditators

Serum NO_2^- level of long-term, experienced meditators (0.52 µM ± 0.05) was significantly lower compared to that of the non-meditator controls (0.92 µM ± 0.52; $p = 0.014$) with a large effect size (Cohen's *d*: − 1.08; coefficient *r*: − 0.48) (Fig. 1). Similarly, the serum NOx levels of the long-term, experienced meditators (5.03 µM ± 0.31) were significantly lower compared to that of the respective controls (5.42 µM ± 0.42; $p = 0.016$) with a large effect size (Cohen's *d*: − 1.05; coefficient *r*: − 0.47) (Fig. 1).

Serum Anti-oxidant Levels in Long-term Meditators

Serum TEAC levels of long-term experienced meditators (424.35 µM ± 41.53) were significantly higher than that of the non-meditator controls (376.15 µM ± 12.69; $p = 0.001$)

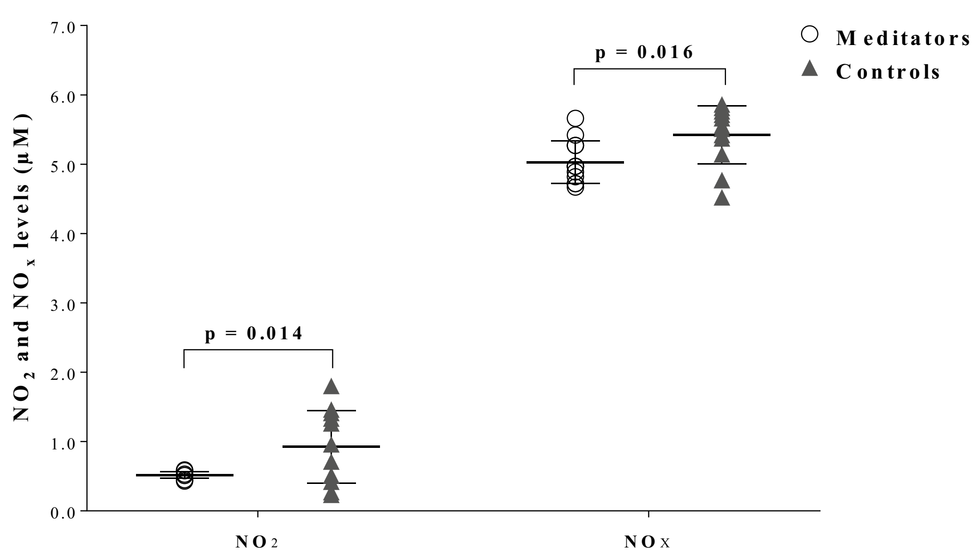

Fig. 1 Serum NO_2^- and NOx levels (µM) of meditator ($n = 12$) and control group ($n = 12$). Horizontal lines represent mean ± SD of each data set

with a large effect size (Cohen's *d*: 1.57; coefficient *r*: 0.62) (Fig. 2).

Association Between Serum NO_2^- and NOx Levels with TEAC

The analysis of correlation between serum NO_2^- and NOx levels with the TEAC values of participants showed a negative association. The TEAC levels showed a non-linear correlation with the NO_2^- levels ($r = 0.562$) with a medium effect size of 0.316 (Fig. 3a) and with NOx levels ($r = 0.664$) with a medium effect size of 0.441 (Fig. 3b).

Relationship of Socio-demographic Factors of Study Population with Serum RNS and TEAC Levels

Analysis of the relationship between socio-demographic parameters and the serum factors studies, i.e., NO_2^-, NOx,

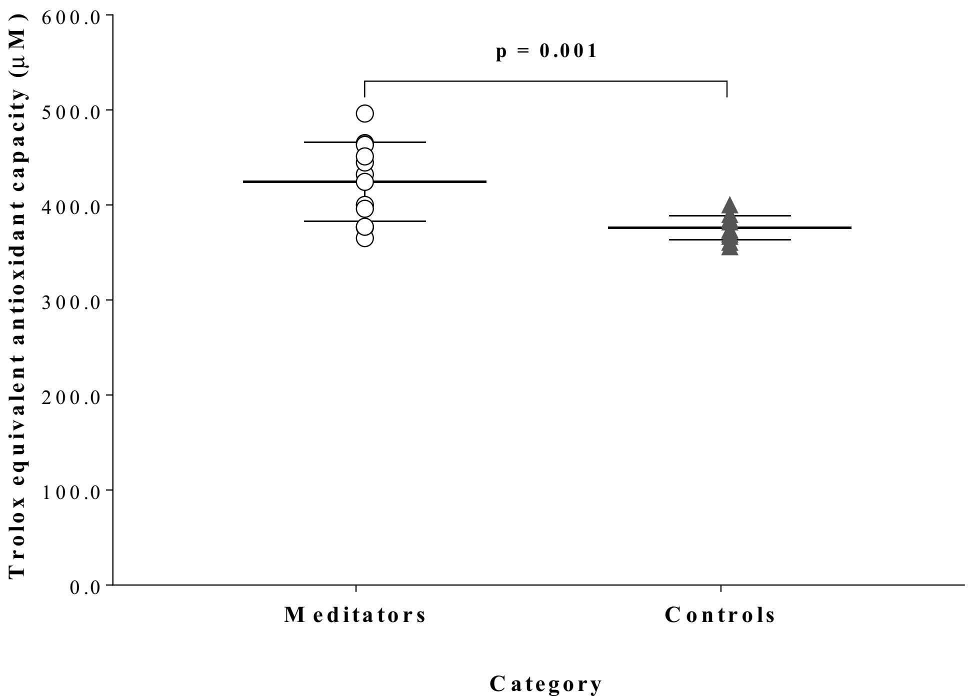

Fig. 2 Serum Trolox equivalent antioxidant capacity (µM) of meditator ($n = 12$) and control group ($n = 12$). Horizontal lines represent mean ± SD of each category

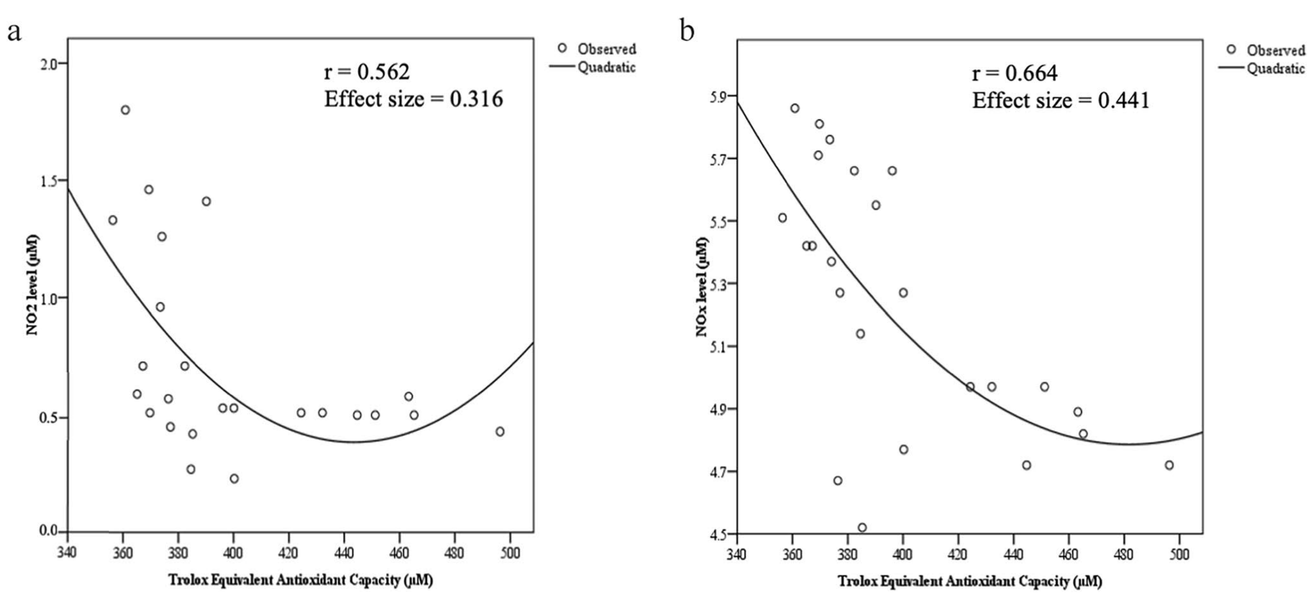

Fig. 3 Association between NO_2^- level with TEAC (**a**) and NOx level with TEAC (**b**) among the study participants ($n = 24$)

and the TEAC levels, did not show any significant association with any of the socio-demographic factors such as gender, age weight, height, BMI, marital status, and educational status (Table 1). The factors related to habits such as alcohol consumption, sleeping hours, or exercising hours were also not significantly associated with serum NO_2^-, NOx, and TEAC levels of the studied groups.

Discussion

The present study shows that the total antioxidant capacity (TEAC) levels were higher with a large effect size and the serum nitrite and NOx levels were significantly lower with a large effect size among the experienced, long-term meditators compared to the age-, gender-, and education level–matched controls. The latter finding reflects a significantly lower production of nitric oxide in experienced meditators. Further, there was a negative correlation between the serum antioxidant capacity levels and the reactive nitrogen species representing nitric oxide levels.

The findings of the present study on levels of serum total antioxidants corroborate with previous studies that had shown the effects of Buddhist meditation techniques which reduce oxidative stress (Ingole et al., 2015). There is adequate evidence that proves the relationship between psychological stress and free radical activity and its contribution to oxidative stress (Adachi et al., 1993; Scarpellini et al., 1994). Different meditation techniques have shown to decrease both psychological stress and oxidative stress (Castillo-Richmond et al., 2000; Eppley et al., 1989; Schneider et al., 1995, 1998). A significantly decreased oxidative stress level was observed in a group of meditators practicing Zen meditation and Transcendental meditation, when compared to pre- and post-meditation. The findings of this study are consistent with a previous study on diaphragmatic breathing and how it significantly increased biological antioxidant potential and decreased reactive oxygen metabolites (Martarelli et al., 2011). Further, mindfulness activities like *Sudharshana Kriya* (Sharma et al., 2003) and Yoga (Sinha et al., 2007) have improved the antioxidant system by increasing the activity of superoxide dismutase (SOD) and catalase.

Studies on meditation have shown that hormonal reaction to stressors is affected where cortisol levels are decreased and melatonin levels are increased in meditators indicating that meditation is capable of modulating the neuroendocrine system through neurological pathways (Mahagita, 2010). Melatonin is a neurohormone that acts as a strong antioxidant because it increases several intracellular enzymatic antioxidant enzymes, such as SOD, catalase, and glutathione peroxidase (GSH-Px) (Reiter et al., 2005; Rodriguez et al., 2004) and induces the activity of γ-glutamyl cysteine synthetase, thereby stimulating the production of the intracellular antioxidant GSH (Winiarska et al., 2006) thus increasing antioxidant capacity.

The findings of the current study showing lower levels of serum nitrite and NOx among long-term, experienced meditators compared to age-, gender-, and education

Table 1 Association between socio-demographic factors with NO_2^-, NOx, and TEAC levels

Variable	Meditators	Non-meditators	Significance levels for		
			NO_2^-	NOx	TEAC
Socio-demographics					
Gender ratio (% of males)[a]	9/12 (75%)	9/12 (75%)	$r=-0.296$ $p=0.160$	$r=0.274$ $p=0.195$	$r=-0.112$ $p=0.603$
Age (mean ± SD)[a]	39.00 ± 10.52	38.58 ± 9.49	$r=-0.004$ $p=0.985$	$r=-0.026$ $p=0.903$	$r=-0.305$ $p=0.148$
Marital status (% married)	6/12 (50%)	8/12 (66.7%)	$r=0.094$ $p=0.661$	$r=0.176$ $p=0.411$	$r=-0.466$ $p=0.404$
Educational level[a]	-	-	$r=-0.060$ $p=0.781$	$r=0.152$ $p=0.478$	$r=-0.151$ $p=0.480$
Body mass index (mean, SD)	27.50 ± 5.23	24.39 ± 2.61	$r=-0.039$ $p=0.878$	$r=-0.003$ $p=0.990$	$r=0.142$ $p=0.573$
Habits					
Non-vegetarian diet	11/12 (92%)	12/12 (100%)	$r=0.138$ $p=0.522$	$r=-0.023$ $p=0.917$	$r=0.127$ $p=0.555$
Alcohol use[b] (%)	3/12 (25%)	4/12 (33.4%)	$r=-0.378$ $p=0.069$	$r=-0.249$ $p=0.240$	$r=0.169$ $p=0.429$
Sleep (> 6 h/day)	12/12 (100%)	12/12 (100%)	$r=-0.529$ $p=0.137$	$r=-0.214$ $p=0.315$	$r=0.273$ $p=0.197$
Exercise (> 3 h/week)	8/12 (66.7%)	3/12 (25%)	$r=-0.225$ $p=0.290$	$r=-0.029$ $p=0.895$	$r=-0.229$ $p=0.282$

[a]Matched variables. [b]Consume alcohol occasionally

level–matched non-meditators differ from those of two previous studies on Loving-Kindness meditation (Kemper et al., 2014) and Zen meditation (Kim et al., 2005). In the present study, meditators had significantly lower levels of serum nitrites, which is the first intermediate molecule generated from NO, as well as lower serum NOx (nitrite + nitrate) levels. It is also interesting to note that the basal serum nitrite levels of the controls of the current study were similar to those observed in meditators who practiced the Loving-Kindness meditation (Kemper et al., 2014) and the nitrite levels observed in healthy controls in other previous studies in Sri Lanka (Kalugalage et al., 2013; Mapalagamage et al., 2018). Our observation on a lower serum nitrite level may reflect a lower NO generation, especially via inducible nitric oxide synthase. Mindfulness and yoga meditation is known to significantly reduce pro-inflammatory gene expression including NF-kB which in turn may downregulate the expression of iNOS (Black et al., 2019; Kasala et al., 2014) that would lead to minimizing the serum RNS levels. Our findings emphasize on further investigations on the expression of iNOS for elucidation of exact mechanism involved.

In addition to the lower levels of serum nitrites observed in the present study, the serum NOx levels were also observed as significantly lower in the long-term, experienced meditators. Compared to nitrite levels, the serum nitrate levels are less reliable measures of NO bioavailability (Lauer et al., 2002). Serum nitrate levels may depend on dietary factors (Kobayashi et al., 2015) and therefore 12-h fasting blood samples were taken from all participants in the present study to minimize any dietary involvements. In addition to endogenous NO generation through the L-arginine-NO synthase (NOS) pathway, NO is also generated through the NOS-independent nitrate-nitrite-NO pathway. The two previous studies (Kemper et al., 2014; Kim et al., 2005) which observed higher levels of NOx in meditators have considered it as intriguing and emphasize that larger and controlled studies are required for further verification. The observed higher levels of NOx (Kemper et al., 2014) could be due to the fact that NO production/levels depend on the consummation of NO-rich foods as dietary habits vary from country to country (Kobayashi et al., 2015). It is interesting to note that there are other criteria such as high altitude that may contribute to higher nitric oxide levels. It has been shown that NOx upregulation is a common physiological response to hypoxia among Tibetans and lowlanders moving to high altitudes (He et al., 2018).

Another important observation in this study is that the graphs showing each participant's serum NO_2^- and NOx levels (Fig. 1) and serum TEAC (Fig. 2) illustrate that the variation is much smaller in the group of long-term meditators compared to the control group. This demonstrates that meditation could be quite effective in reducing serum RNS levels and in increasing antioxidant levels predominantly in those with extreme serum NO_2^-, NOx levels, and TEAC levels.

The present study reports an association between the total serum antioxidant levels and the serum RNS levels among the study participants. Although the effect size was comparatively small, the TEAC levels had a non-linear correlation with NO_2^- levels ($r = 0.562$) and with NOx levels ($r = 0.664$). There was no significant correlation between the total serum antioxidant capacity levels and RNS levels with the socio-demographic factors such as gender, age, weight, height, body mass index (BMI), marital status, highest educational level, non-vegetarian diet, alcohol consumption, sleeping (hours per day), and exercising (hours per day) in this study.

Limitations and Future Research

This study has certain limitations. This is a cross-sectional comparative study with a relatively small sample size which compares the RNS and TEAC levels between the long-term, experienced meditators and controls. The lack of longitudinal measurements on the effects of meditation in participants pre- and post-meditation is a limitation. Another limitation is that we have assessed serum TEAC level as an overall indicator of oxidative stress and not individual antioxidants such as malondialdehyde (MDA), glutathione peroxidase (GPX), and superoxide dismutase (SOD), and glutathione reductase (GR). Further, there is no detailed data gathered on the dietary habits of the participants other than being non-vegetarians to exclude other possible factors that would contribute to serum NOx levels.

In conclusion, this study has shown that long-term, experienced meditators had a large effect of significantly lower serum NO_2^- and NOx levels and a large effect of significantly higher serum antioxidant levels when compared to age-, gender-, and education level–matched non-meditators. Further, a significant negative association was demonstrated between NO_2^- and NOx levels with TEAC levels. These finding suggests a lower production of nitric oxide and a higher serum antioxidant capacity in the long-term, experienced meditators with potential beneficial effects against oxidative stress.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12671-022-01840-8.

Author Contribution JCT, SMH, and DWND designed the experiments. JCT carried out experimental work and SMH, DWND, and HWD supervised the research. JCT, SMH, DWND, and HWD analyzed the data. JCT drafted the manuscript, which was improved by SMH, DWND, and HWD. All four authors contributed to the final version of the manuscript. The manuscript has been read and approved by all the authors.

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Data availability The data used to support the findings of this study are available from the corresponding author upon request.

Declarations

Ethics Approval and Consent to Participate Ethical approval for this study was obtained from the Ethics Review Committee, Faculty of Medicine, Colombo, Sri Lanka (EC-19–068). Informed written consent was obtained from each participant prior to sample collection.

Conflict of Interest The authors declare no competing interests.

Disclaimer The funder played no role in role in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

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