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Original Research

Effect of a Yogic Breathing Technique on Immune Parameters among Healthcare Workers: A Randomized Controlled Pilot Study

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Abstract

Healthcare workers (HCWs) have faced challenging situations globally with increased stress and its related inflammatory/immune disorders, leaving behind a deficit in the number of HCWs worldwide. An increased focus on their physical and mental well-being is strongly warranted. Relaxation techniques such as yoga, pranayama, and meditation have great potential to promote health and prevent diseases. This study aims to determine the effects of Sudarshan Kriya Yoga (SKY) on inflammatory and immune parameters among HCWs. This pilot randomized controlled trial includes recruiting HCWs from a single centre in South India in 2022. We excluded any form of medical illness and recruited regular volunteers. The SKY intervention was delivered and followed up for 12 weeks. Baseline and post-SKY intervention blood investigations were compared between study and control groups (1:1 ratio) and analyzed. Thirty-eight subjects were recruited for the pilot study. Among them, 27 were females (71%) and 11 males (29%). Pro-inflammatory markers such as erythrocyte sedimentation rate, C-reactive protein and neutrophil-to-lymphocyte ratio showed a decrease in mean values after 12 weeks in both groups, which was not statistically significant. A slight increase in interleukin (IL)-6 values and a higher IL-4 mean values was observed in the study group compared to controls. CD56 and CD8 counts were higher in the study group compared to controls. However, no such difference was noted in cortisol levels. This pilot study provides data for further large-scale studies to examine the effect of easily deliverable mindfulness-based interventions among the healthcare community to improve their health and well-being.

Keywords: Sudarshan kriya; Breathing; Immunity; Yoga; Inflammation; Healthcare workers

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Introduction

The human immune system is vital for survival, and it has evolved to control pathogens, thereby resisting infections and inflammatory diseases. It plays a dual role by resisting the body from the harmful effects of pathogens and also by tolerating and preventing an exaggerated response. Thus, disorders of the immune system may occur due to too much or too little immunologic reactivity, and an equilibrium needs to be maintained to keep the immune system under check, avoiding a hyper-vigilant response vs. a feeble response [1].

Health care workers (HCWs) are frontline warriors crucial for any health care system. In a recent publication, the World Health Organization (WHO) has predicted a shortage of > 18 million HCWs by 2030 [2]. The number of healthcare providers is less than 25% of the WHO's benchmark. The excess shortage has been exacerbated by the unfortunate loss of HCWs due to the contagious COVID-19 infection during the pandemic. A high prevalence of anxiety (24%), depression (28%) and sleep disorders (44%), resulting in frequent illness, depressed immunity and recurrent infections, was observed during the pandemic. HCWs have a higher risk of infection exposure with lesser recovery times, resulting in a pro-inflammatory state [3]. Smoldering inflammation is implicated in obesity, cancer, diabetes, autoimmune diseases, allergic disorders, heart diseases and ageing [4]. Mental stress, anxiety, addictions, poor quality of nutrition and inadequate sleep among HCWs may conversely result in a depressed immune status. Therefore, reducing stress and building immunity would help individuals, particularly HCWs, fight future waves of infections, alleviate stress-related psychosomatic illnesses and control chronic inflammation-related metabolic and endocrine diseases.

There are multiple ways to modulate immunity, like adopting changes in diet, exercise, sleep patterns, avoiding addictions and consuming health supplements. Along with these, Mind-body therapies (MBTs), such as hatha yoga, pranayama and meditation, are ancient yet globally famous and modulate their effects via the autonomic nervous system and the hypothalamic pituitary adrenal (HPA) axis, leading to changes in the immune system. There is growing evidence to support the fact that these interventions exert beneficial immunological effects like inducing changes in lymphocytes and inflammatory cytokines, thus having the potential to prevent a variety of diseases [5,6]. Meditation techniques have been found to improve T cell counts in Human Immunodeficiency Virus (HIV)-positive people [7]. Techniques such as Qi-Gong that combines meditative practices were also found to demonstrate elevated Cluster of differentiation (CD)4 T cells and increased CD4/CD8 ratio in healthy practitioners compared to their controls [8]. Some studies evaluating the role of yoga-based lifestyle intervention on inflammatory cytokines have shown decreased pro-inflammatory cytokine response after yoga training compared to the control group [9,10].

As described by Patanjali, yoga has eight limbs, some of which include 'hatha yoga' comprising asanas or postures held for a period of time, pranayama, which comprises effective breathing techniques and 'Dhyana' or meditation. Studies on many such yogic techniques have proven to be effective in clinical and non-clinical populations. One such productive, doable, self-empowering relaxation technique is the Sudarshan Kriya Yoga (SKY), a yogic cyclical rhythmic breathing technique adopted by millions of people across the world, which has been shown to have multiple favorable effects on various aspects of the body-mind complex, including the immune system [11].

Thus, there is a need for a methodological study to scientifically validate the effects of SKY, which is a simple, yet profound, remotely deliverable, mindfulness-based yogic breathing practice on circulating inflammatory markers and hematological parameters.

Objectives

This study aims to determine the effects of SKY on inflammatory and immune parameters among HCWs.

Materials and Methods

Study design

This study represents a subsample (n = 38) of a double-arm single-blinded randomized controlled trial (RCT) involving a single center. Thirty-eight (38) HCWs with equal sex distribution were recruited for the study. Institutional Ethics Committee (IEC) approval was obtained before the start of the study with the number IEC-NI/21/APR/78/70, and it conformed to the principles embodied in the Declaration of Helsinki. Clinical Trial Registry of India (CTRI) clearance was obtained with the reference number CTRI/2022/03/041417, and Good Clinical Practice Training (GCP) was also completed before the start of the study. Consolidated Standards of Reporting Trials (CONSORT) guidelines for RCT were followed for the reporting of data (Figure 1).

Sample size

The sample size was calculated based on a previous similar study done by Nuamtanung Y, et al. [12]. This was done using the mean and standard deviation values of CD4 count being 41.97 ± 7.92 in the con-

trol group and 50.33±7.42 in the study group, with an alpha error of 5%, power of 90%, considering the attrition to be 20%, using the formula.

$$n = \frac{2s_p^2\left[z_{1-\frac{\alpha}{2}} + z_{1-\beta}\right]^2}{\mu_d^2}$$

$$s_p^2 = \frac{s_1^2 + s_2^2}{2}$$

Where,

s_1^2 : Standard deviation in the first group

s_1^2 : Standard deviation in the second group

μ_d^2 : Mean difference between the samples

α : Significance level

1 - β : Power

By applying the formula, the sample size was 18 per group. A total sample of 38 was collected with 19 participants in each group.

Participant recruitment and eligibility

This was a single-center study. HCWs were recruited from the campus via poster advertisement, WhatsApp messages, and multiple introductory talks given in small groups across departments. Those interested in enrolling had to fill out a Google form created by the investigator. The Google form had information on the primary demographic profile analyzed in table 1.

The recruitment process was completed in two months.

Inclusion criteria: Age of 25-40years, health care worker, practicing mild to moderate physical exercise (International physical activity questionnaire), not regularly practicing any yoga/relaxation techniques, never had a history of intake of alcohol or smoking and free from acute infection (4 weeks after complete recovery from illness).

Exclusion criteria: Absence of underlying medical conditions like neurological disorders such as epilepsy, endocrine disorders, metabolic disorders, cardiovascular disease, inflammatory conditions, respiratory diseases, pregnancy (antepartum and post-partum (6months), psychiatric disorders like schizophrenia, maniac depressive disorders, those taking lithium, those on health supplements or severe form of exercises.

All participants were clearly explained about the study, and written informed consent was obtained from all the participants in the language they could comprehend.

Randomization and blinding

The principal investigator used the block randomization method to ensure an equal number of subjects in each group (1:1 ratio). Each block size was fixed to 6. Another investigator performed allocation concealment using the envelope method. Computer-assisted software (studyrandomizer.com) was used for randomization. It was a single-blinded study, and participants were not blinded for the study. However, those assessing outcomes were blinded for the study.

Data collection

All parameters were assessed at baseline and after 12 weeks.

Materials

All samples were collected at the host institute by 8.30 am after an overnight fast of 12 hours. In the case of menstruation, the sample was collected after the cycle was completed. Regarding antibiotic intake, the sample was collected after the washout period of 2 weeks. Blood samples were collected in appropriate vacutainers for analysis. Samples were processed within 24 hours of collection. A coded sample was given for analysis and blinded to the analyzer. The following was estimated;

- Cell counts were estimated in Mindray BC-5130.
- Lymphocyte subsets like CD56, which serves as a marker for innate immunity, and CD4, eight counts for adaptive immunity, were determined by flow cytometric analysis in the flow cytometer.
- The total Interferon Gamma (IFN-γ) T count was assayed using the Diaclone kit. IFN-γ correlates with cell activation, chemotaxis, and cytotoxicity (functional activity).
- The Interleukin (IL)-6 (a pro-inflammatory cytokine) and IL-4 (an anti-inflammatory cytokine) assays were performed using the Enzyme-Linked Immunosorbent Assay (ELISA) technique.

Intervention

A total of thirty-eight participants were randomized into a control group (CG) or intervention group (IG). Participants in the study group had to attend a 4-day module, which was 2 hours each. A synchronous method of teaching was adopted via videoconferencing. Participants were instructed to keep their videos on throughout the session. This module was organized in multiple (morning 6 am-8 am/ evening 6 pm-8 pm) batches. SKY, taught at the internationally acclaimed Art of Living Foundation, was the interventional tool used in this study. Each session was a total of 30 minutes of practice. SKY comprised of 5 steps that include;

1) Ujjayi breath pranayama- 3 stages, eight cycles each.

Each cycle consists of breathing in for four counts, holding the breath for four counts, breathing out for six counts, and holding for two counts.
2) Bhastrika pranayama- 20 times,3 rounds each.
3) Om chanting three times.
4) Kriya (cyclical rhythmic breathing) consists of counts of 20 long breaths, 40 medium breaths, and 40 fast breaths for three continuous cycles.
5) Rest for 10 minutes.
Following this, participants had to practice the SKY breathing technique for 180 minutes/week (30 minutes per day) for 12 weeks. Intention-to-treat analysis was followed. To ensure compliance, daily online group sessions were organized via the online platform, along with weekly online booster sessions on Sundays. Asynchronous teaching methods, like cartoons and videos, were provided to guide them in their daily practice. Mentors were allotted to monitor the study participants every week to ensure further compliance with practice. Study subjects were instructed to maintain their usual physical activity and dietary habits.

Statistical analysis

Analyzes were performed by an independent statistician using the Statistical Package for Social Sciences (SPSS) software. A paired t-test was done between and within groups and presented as mean and standard deviation. The normality of data was checked, and mean difference values at the end of the study period were calculated and represented as the mean and standard error of the mean. Statistical significance was set to $p < 0.05$.

Results

Subjects

Figure 1 depicts the CONSORT flowchart of subject recruitment and participation. One person dropped out of the CG, and no harmful effects were observed in the IG.

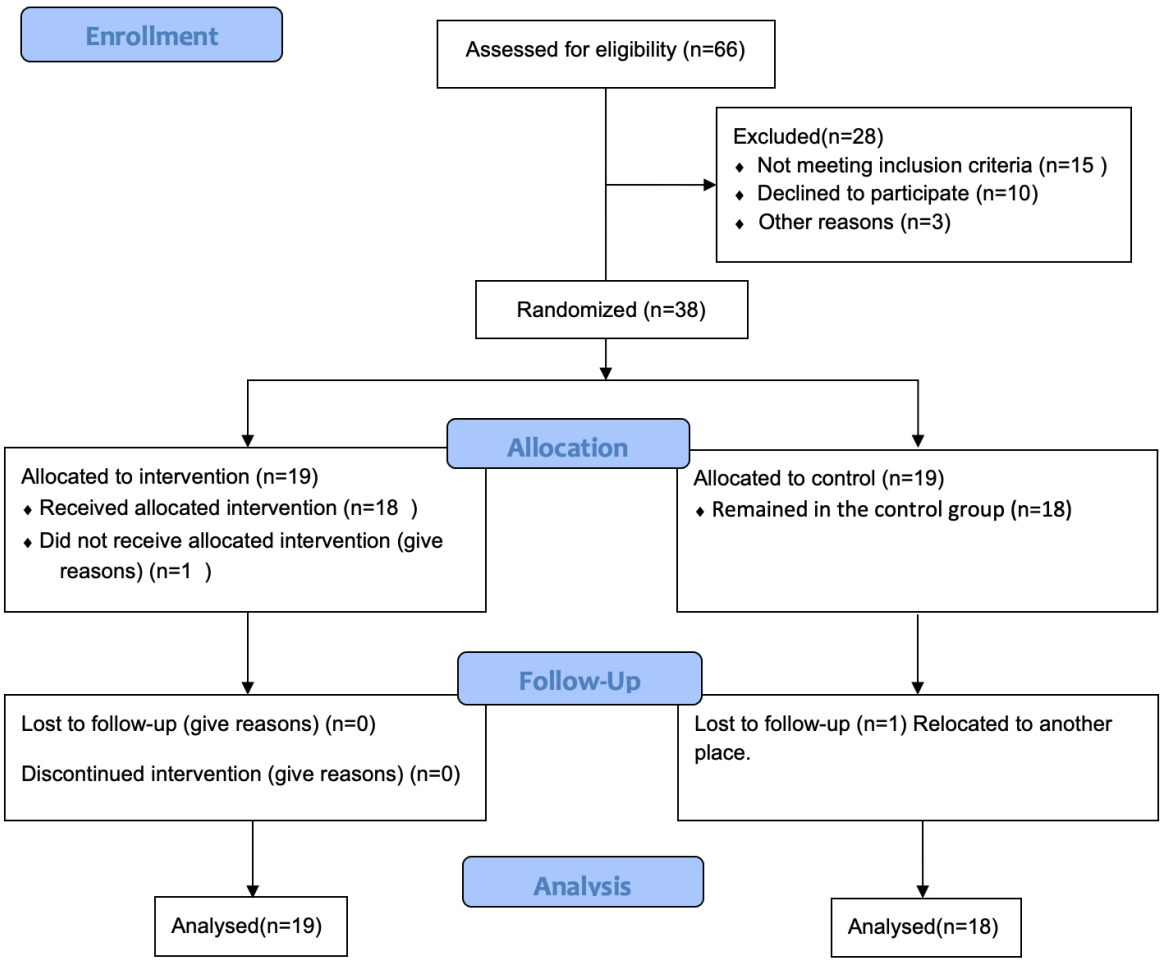

Figure 1. CONSORT flow diagram

Demographic profile

Demographic characteristics of the 38 participants eligible to participate in the study at baseline are presented in table 1. Participants included were in the age group of 25-40, with the mean age being 32, falling into the middle age category, who generally have more responsibilities at work and family. Male: female ratio was 1:2.45, with females predominating in enrolment for the study. Most participants were married (68%) at enrolment; while others were unmarried, widowed or divorced. Participants were generally well-educated, and most were post-graduates (89.5%) from middle-class families living in Urban areas (92%). Work shifts were taken into account based on the timing of their postings two weeks before the baseline evaluation.

Compliance

A single SKY session is 30 minutes in duration. Participants were required to perform the SKY technique for 180 minutes/week, i.e., at least six days per week for 12 weeks. Among the 19 participants assigned to the IG,14(74%) showed compliance by practicing 180 minutes/week, 30-minute sessions per day for 12 weeks. Five participants (26%) needed to be more compliant as they performed the intervention for 150 minutes in some weeks.

Table 1. Demographic characteristics of the participants between groups

Characteristic	Intervention group (n=19)	Control group (n=19)
Age		
25-30	5(26.3%)	8(42.1%)
31-40	14(73.7%)	11(57.9%)
Sex		
Female	12(63.2%)	15(78.9%)
Male	7(36.8%)	4(21.1%)
Place of residence		
Urban	18(94.7%)	17(89.5%)
Rural	1(5.3%)	2(10.5%)
Marital status		
Married	13(68.4%)	13(68.4%)
Unmarried	4(21.1%)	5(26.3%)
Widow	1(5.3%)	1(5.3%)
Divorce	1(5.3%)	-
Level of Education		
Post graduate	17(89.5%)	17(89.5%)
Under graduate	2(10.5%)	2(10.5%)
Profession/Job title		
Doctor	13(68.4%)	11(57.9%)
Nursing/Paramedical	2(10.5%)	4(21.1%)
staff	1(5.3%)	2(10.5%)
Administration	3(15.8%)	2(10.5%)
Student		
Type of shift		
Day shift	16(84.2%)	14(73.7%)
Day and **night shift**	3(15.8%)	5(26.3%)
Level of activity		
Mild	14(73.7%)	14(73.7%)
Moderate	5(26.3%)	5(26.3%)

Outcomes

The statistical analysis results have been depicted in table 2 and table 3. Pro-inflammatory markers such as ESR, CRP and N:L ratio showed a decrease in mean values after 12 weeks in the IG and the CG. However, this was not statistically significant. The neutrophil-to-lymphocyte (N:L) ratio, an inflammatory/stress marker, showed a lower mean value in IG than in CG. A slight increase was noted in IL-6 levels in both groups at the end of the study. The mean values of IL-4, an anti-inflammatory marker, showed a rise in both the groups (IG and CG), which was not statistically significant. Higher IL-4 mean values were observed in the IG group compared to CG at the end of 12 weeks. IFN-γ, a cytokine and a marker of lymphocyte function, was boosted in both IG and CG at the end of 12 weeks compared to the baseline. However, there was no statistical significance nor any difference between groups.

CD56, one Natural Killer (NK) cell subset, are innate immunity markers. They showed an increase in both groups after 12 weeks compared to the baseline with a statistically significant value (p=0.001 in IG, 0.003 in CG). Mean values were higher in the IG compared to the CG. CD8 cell count was elevated in both IG and CG groups after 12 weeks with a statistical significance (IG group p value of 0.016, CG group p value of 0.012). There was, however, no statistical significance between the groups. CD4 values were slightly elevated in both groups, with no statistical significance. Serum cortisol levels, total leukocyte count (TLC), hemoglobin and platelet counts did not change significantly.

*Indicates $p<0.05$

Table 3 compares the average mean difference between groups. However, it does not show statistical significance, possibly due to the effect of time, compliance to practice, or the intrinsic motivation of control participants to adopt other non-intervention methods.

Discussion

Over the last few decades, scientific work on MBTs like yoga, deep breathing, and meditation have received increasing attention from the scientific community as it has been shown to regulate the immune system. They also reduce inflammation, strengthen anti-viral immune responses and improve immune parameters. Yoga involves the modulation of sympa-

Table 2. Inflammatory markers and hematological parameters at baseline and after 12 weeks in both groups

Variable	Intervention group		Control group	
	Baseline	After 12 weeks	Baseline	After 12 weeks
Pro-inflammatory markers				
IL-6	1.75±1.45	2.4±2.1	1.3±0.6	1.8±1.3
ESR	19.6±9.3	18.3±7.8	18.7±9.5	16.6±8.1
CRP	8.9±7.5	8.2±8.6	10.7±9.2	*7±5.3
N:L ratio	1.8±0.6	1.7±0.6	1.8±0.6	1.8±0.7
Anti-inflammatory markers				
IL-4	0.1±0	0.2±0.4	0.1±0.21	0.1±0.07
Immune parameters				
CD56	1353.4±356.9	1926.4±828.6*	1380.2±480.6	1840.2±504.5*
CD4	1011.7±256.9	1020.8±450.8	1013.6±343.7	996.6±255.9
CD8	532.5±215.5	687.4±388*	580.35±307.4	737.35±307.4*
IFN-γ	0.21±0.3	0.7±1.2	0.5±1.3	1±1
TLC	6852.6±1180	6631.6±1457	6682.4±1266.5	7017.6±1190
Stress parameters				
Serum Cortisol	93.7±44.6	101.6±42.1	105.3±53.6	97.6±50.7
Other parameters				
Hemoglobin	13±1.7	13±2	13±1.5	13±1.8
Platelet count	2.7±0.5	2.6±0.6	2.7±0.5	2.69±0.5
Eosinophil (%)	2.8±1.7	3±2.1	2.3±1.3	2.2±1.3
FBS	95.9±20.5	96.1±17.6	98.9±13.9	94.8±6.2

Abbreviations: IL-Interleukins-Erythrocyte Sedimentation Rate, CRP- C Reactive protein, IFN-γ-Interferon-gamma, N:L-Neutrophil: Lymphocyte ratio, TLC-Total Leukocyte Count, FBS-Fasting Blood Sugar

thovagal, neuroendocrine and psycho-neuro-immunological balance, restoring good health. The benefits of MBTs are known to occur via effector pathways like the autonomic nervous system and the HPA. Thus, Psychoneuroimmunology has gained popularity over the last few decades. This preliminary study provides insight into the effects of a 12-week rhythmic breathing technique intervention on inflammation and immune cell counts in healthy participants from a healthcare setup.

The study's main findings showed a slight decrease in some pro-inflammatory markers, such as ESR, CRP, and N: L ratio, in both groups. However, no such change was observed with IL-6, a cytokine that mediates inflammatory response, and the circulating levels showed a mild increase in values in both groups, which were not statistically significant. Results of a previous study showed the effect of an 8-week Hatha yoga training with no impact on the circulating levels of IL-6 but a significant reduction in cultured whole blood [10]. The present study's findings were similar to a study that found no significant differences in IL-6 levels between yoga and control groups among healthy individuals [13]. This may be considered due to a non-clinical population in whom circulating inflammatory markers are usually low and a potential dose-response relationship of the duration of the intervention. In diseased conditions like Rheumatoid arthritis, levels of IL-6 have been reported to decrease after yoga practice [14]. However, it is increasingly recognized and noteworthy that IL-6 also plays a role in healing and regenerative processes contributing to the improvement of the immune microenvironment [15].

IL-4, an anti-inflammatory cytokine assessed, was found to increase post-intervention but was not sta-

Table 3. Average mean difference between pre and post among the groups

Variable	Intervention group n=19 (Mean±SE)	Control group n=17 (Mean±SE)	p value
Pro inflammatory markers			
IL-6	0.64±0.5	0.55±0.35	0.9
ESR	-1.32±1.4	-2.0±1.6	0.7
CRP	-0.63±1.2	-3.8±1.2	0.07
N:L ratio	-0.16±0.15	0±0.15	0.5
Anti-inflammatory markers			
IL-4	0.12±0.1	0.01±0.01	0.2
Immune parameters			
CD56	572.9±151.9	459.7±129.6	0.6
CD4	9.1±65.9	-17±55.7	0.7
CD8	154.9±58.0	157±55.6	0.9
IFN-γ	0.52±0.3	0.53±0.3	0.9
TLC	-221±309.4	335.3±268.7	0.2
Stress parameters			
Serum Cortisol	7.83±8.7	-7.7±13.2	0.3
Other parameters	-0.09±0.12	-0.09±0.2	0.9
Hemoglobin	-0.13±0.1	-0.06±0.06	0.5
Platelet count	0.26±0.4	-0.06±0.3	0.5
Eosinophil (%)	0.26±1.7	-4.12±3.5	0.2
FBS			

tistically significant, similar to a study by Gopal et al., 2011 in healthy students with exam stress [16]. A study done on another anti-inflammatory cytokine, IL-10, reported increased levels in industrial workers with a risk of sustained inflammation [17].

A review by Bower et al., 2015 showed mixed effects of MBT on circulatory inflammatory markers, requiring genomic markers to understand the potential mechanisms [18]. The findings of a previous study by Cahn et al., 2017, also observed unusual non-stereotypic patterns of increases in both pro-inflammatory and anti-inflammatory cytokines in healthy populations after yoga intervention, thus adding complexity to understanding the effects they modulate [19].

IFN-γ, a cytokine primarily involved in cell-mediated immunity, though not statistically significant, was found to have a slight increase in both the groups in our study. A similar study by Lim et al., 2015 reported an increase in IFN-γ levels with statistical significance in the yoga group compared to controls in healthy students [20]. Gopal et al., 2011, however, reported a slight decrease in IFN-γ in healthy students during exam stress in the yoga group, pointing to a probable buffering effect of the intervention [16]. These results concord with the study by Cahn et al., 2017 who also found that IFN-γ levels, which are T helper cell-mediated, increased after a yoga retreat program [19]. These modulations are hypothesized to be adaptive based on the setting, and mixed findings suggest theories of parallel regulatory pathways. Earlier studies needed to be made more explicit on how the effects of MBTs are carried out, and more research on the molecular level was required for a better understanding. Among the very few studies on molecular profiling, gene expression profiling in practitioners of SKY suggested that SKY attenuates the effects of stress on cells by increasing the expression of a gene that inhibits cell death, thereby enhancing the immune system [21].

Studies on gene expression analysis have recently shed light on these pathways and found that the down-regulation of NF- NF-targeted genes reverses the effects of chronic stress [22].

Earlier studies have assessed the effect of yoga and

MBTs on immune system cells. Nuamtanung et al. studied the effects of meditation on volunteers. They found increased CD3, CD8 and CD56 levels by flow cytometry at the end of four months, consistent with our study, which showed a significant increase in CD56 and CD8 levels before and after SKY [12]. However, this increase was not statistically significant compared to the control group, possibly due to a smaller sample size. Earlier findings indicated a potential role of SKY in modulating immune function. In a study by Subramanian et al., SKY intervention significantly increased the lymphocyte count by more than fivefold compared to controls in participants with exam stress, suggesting an enhanced immune function [23]. The effect of SKY was studied in the NK cell counts in cancer patients at AIIMS, Delhi, demonstrating that NK cells significantly increased in the SKY group compared to cancer patients who did not practice SKY [24]. This is particularly encouraging since cancer patients have low immunity that can predispose them to infections and new/recurring cancers. No significant difference in CD4 count and eosinophil % was noted between groups in our study; however, a substantial increase in CD4+ cell count was noted in the yoga group compared to controls among HIV individuals in a study done by Naoroibam et al., 2016 [25]. Besides this, evidence from previous RCT's have indicated the beneficial effects of yoga to reduce eosinophil counts in asthma patients; thus, alleviating their symptoms [26,27]. Based on the last molecular findings of yogic practices, a study by Qu et al., 2013, demonstrated significant gene expression changes rapidly with long-term effects in peripheral blood mononuclear cells among healthy people who practiced SKY and related practices compared to the control group [28]. Sharma et al. studied the gene expression profiling of antioxidant enzymes from immune cells in SKY practitioners. They found a better antioxidant status at the enzyme level and their gene expression patterns [29]. A study done on SKY and serum cortisol levels before exams among normal medical students in India showed significantly reduced levels compared to the control group [30]. Among other similar healthcare communities, such as business executives, it was noted that SKY intervention greatly improved emotional health subjectively evident by simultaneously reduced blood cortisol levels, thereby encouraging incorporation of SKY in improving health and wellness among all communities [31]. These encouraging results can help the progress of further advancements in this field, thereby encouraging the implementation of cost-effective alternate strategies for enhancing the health and well-being of HCWs. The results of further studies may form a basis to step up alternate solutions towards challenges faced by the healthcare community.

Limitations and suggestions for further research

This study is subject to some limitations, some of which may be attributed to the study of absence of available literature or prior research studies on healthy volunteers. Also, further studies to assess the therapeutic potential of SKY in other inflammatory or autoimmune conditions may be beneficial. A small sample size decreased the effect. Hence, it is recommended that the sample size be increased to improve the reliability of the result. Though practicable, online intervention delivery may have heterogeneity and a lesser impact than in-person training. Interventions of short duration may not be enough to produce the desired results, and a longer duration of practice with a follow-up assessment may be required to bring consistent changes. Unintentional bias in data collection by applying strict exclusion criteria increased female subjects' enrolment. Lack of funding led to the inability to incorporate gene expression profiling in estimating inflammatory/immune parameters, thereby limiting the study's in-depth analysis of the mechanistic pathways involved.

Conclusions

Although yogic practices have gained popularity, there still exists a barrier to their adoption and implementation in the healthcare community. Reducing stress and building immunity would help HCWs combat challenging situations in the future, and they would aim to protect and promote the overall health and well-being of HCWs. The role of yogic breathing practices and other MBTs in changing inflammatory and immune markers must be understood by additional mechanistic pathways. Further studies incorporating larger sample sizes and encompassing advanced molecular methods would pave the way and aid in developing tools and techniques to implement traditional yet scientific therapies to enhance the health of global communities.

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Conflict of Interests

None.

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