

Research Article

Exploring the Impact of Pranayama on Emotional Stability in Post-Graduate Students: Correlation with Serum Cortisol and Haemoglobin Levels

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A B S T R A C T

Background: Postgraduate students (PGs) are often facing extreme pressure to excel academically and produce high-quality research, by which they often become the victim of emotional instability, stress and anxiety states. Pranayama breathing exercise (PBE), a traditional form of breathing exercises has been recommended to optimise psycho-physiological health, i.e., body, mind and consciousness.

Aim: Hence, this research is meant to investigate the potential impact of PBE on serum cortisol (SC) and haemoglobin (Hb) levels of PGs; those are directly related to emotional instability.

Methods: Sixty participants with ages ranging from 21 to 24, meeting the eligibility requirements were randomly distributed into two equal groups i.e., 30 in the pranayama and control groups, respectively. The pranayama group received 45 minutes of PBE daily for three months. Data collected at pre-phase, post-phase 1 and post-phase 2 by using the ESQ questionnaire and SC and Hb levels measured through lab tests. The SPSS version 2.5.11 (110) was applied for data analysis.

Results: No significant changes were recorded between the groups at pre-phase, whereas participants in the pranayama group (i) indicated a notably higher emotional stability (ES) at post-phase 1 ($P=.019$) and post-phase 2 ($P<.001$), (ii) significant reduction of SC level at post-phase 1 ($P<.001$) and post-phase 2 ($P<.001$) and (iii) also confirmed a significantly higher Hb level at post-phase 1 ($P<.001$) and post-phase 2 ($P<.001$) as vis-a-vis to the control group.

Conclusion: The result confirmed that PBE improved ES by minimising the SC level and increasing Hb level efficiency, benefiting oxygen transport to body cells, which indicates stress reduction, parasympathetic dominance and enhanced physiological well-being.

Keywords: Pranayama, Emotional Stability, Serum Cortisol, Haemoglobin

Introduction

Emotional instability is a common concern that can affect individuals, including post graduate students (PGs) at various stages of their lives.^{1,2} Pursuing postgraduate studies is a challenging and demanding experience often characterised by rigorous academic work, high expectations and increased responsibilities.³ The demanding nature of the present education system, employment complexity and distorted lifestyle frequently lead to greater stress and emotional instability among university students.⁴ Academic workload is one of the most significant stressors that influence the mental wellness and emotional stability (ES) of university students.⁵

In 2020-2021, over 60% of students were found to have one or more mental health issues marking a nearly 50% surge compared to 2013. Notably, college students witnessed the most substantial rise in depression, anxiety and suicidal propensity during their study period.⁶ A Chinese cross-sectional study also revealed 13.24% of master's students with moderate to severe depression. Among them, specific life events like academic struggles and interpersonal tensions were linked to a higher likelihood of emotional disorders.⁷ Thus, in light of the significant economic costs associated with mental disorders, including healthcare expenses and productivity loss,⁸ there is a current demand for a cost-free and side-effect-free treatment method to preserve mental health.^{8,9} Keeping in view this burning necessity, the present study was undertaken.

Pranayama breathing exercise (PBE) is a traditional breathing practice that originated from the centuries-old traditions of India. It involves controlled and conscious manipulation of breath to regulate and enhance the circulation of "prana" or vital force within the human body.¹⁰ Studies indicate that PBE have a significant favourable influence on both psychological and physiological health.¹¹ It includes controlled breathing techniques, which have produced favourable outcomes in terms of encouraging ES and stress reduction.¹² This practice is believed to have a profound effect on physical, mental and emotional well-being.^{12,13}

PBE have proven stress and anxiety reducing effects.¹⁴ It was postulated that ongoing and enhanced sympathetic nervous system (SNS) activity would correspond with diminished parasympathetic vagal activity and impairment of the sympathetic-adrenal-medullary system.^{13,15} It leads to the adrenal cortex releasing cortisol into the circulatory system, interfering with cognitive processes and increasing the timeframe necessary for effective recovery.^{13,16,17} PBE has also been found to have a beneficial impact on the removal of negative emotions in cancer patients.¹⁵ The cortisol ratio serves as an indicator of training progress and sympathetic recovery, potentially linked to chronic stress and burnout.¹⁷ Monitoring haemoglobin (Hb) level is vital

for overall health assessment. PBE contributes to increasing oxygen saturation in the bloodstream, which is essential for efficient oxygenation and by extension, cognitive function and emotional regulation.¹⁸ PBE during academic stress can influence ES and overall health by modulating lipid profiles and blood cell parameters.¹⁹ Hence, this research is significant in understanding the potential benefits of incorporating PBE into academic settings, offering a holistic approach to stress management and emotional resilience among PGs. Hence, the aim of this research work was to explore the potential effect of pranayama breathing exercise (PBE) on serum cortisol (SC) and haemoglobin (Hb) levels that are directly related to emotional stability (ES) of post graduate students (PGs).

Methodology

Participants

The primary focus of the study is on PGs exhibiting low ES scores. The research sample consisted of PGs from multiple departments of H.N.B. Garhwal University, Srinagar Garhwal (Uttarakhand). Following the recruitment of 200 subjects via the ESQ assessment, 60 participants who met the eligibility requirements were randomly allocated, with 30 participants assigned to the pranayama group and the remaining 30 to the control group. The individuals allocated to the pranayama group were exposed to PBE for three months regularly for 45 minutes except on Sunday and gazetted holidays, with the direct supervision of the investigators. The subjects of the control group remained free of specific tasks; they continued with their routine's normal activity as usual. Data were obtained in three phases: pre-phase (before giving the treatment), post-phase 1 (after forty-five days of treatment) and post-phase 2 (after ninety days of treatment). Prior to the assignment of treatments, each subject was requested to complete a signed Informed Consent Agreement [Figure-1].

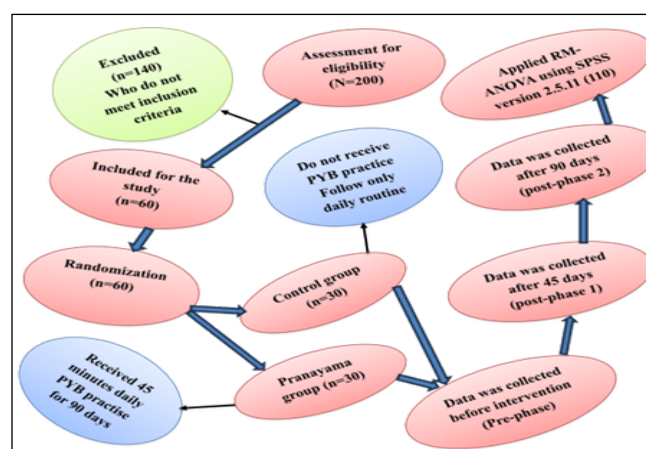


Figure 1. Outlines of the research procedure, featuring participant selection, allocation, data gathering and analysis

Inclusion/ Exclusion Criteria

The study included PGs aged between 21 and 24 years (average age: 22.95 ± 1.15), who had low ES scores and were in good general health without prior exposure to PBE practice. However, PGs with major psychological disorders, high ES levels and participants who expressed unwillingness to engage in the PBE session were eliminated from this research study. Participants from both groups were strictly prohibited from taking any form of medicine, counselling, or psychotherapy or engaging in physical workouts or physical therapy to ensure that the result of the treatment variable remained uncontaminated, thus allowing the experiment to be conducted under completely controlled conditions.

Ethical Approval

The research received clearance from the Board of Studies (BoS) Committee, and written consent was obtained from each participant as per the ethical guidelines of the Helsinki Declaration.

Assignment of Treatment

The investigators provided a three-day introductory training session for the subjects in the pranayama group before commencing the PBE session. Following the completion of the pre-phase data collection and the introductory PBE training, subjects of the pranayama group underwent PBE therapy as outlined in Table-1.²⁰

Measurements

The Emotional Stability Questionnaire (ESQ), developed by Sanjay Vohra from the National Psychological Corporation, Agra (U.P.) was utilised to gather data on the ES of the PGs. Similarly, SC levels in $\mu\text{g/dl}$ were assessed using the Electro chemiluminescent Immunoassay (ECLIA) method in the pathology testing centre. Samples of blood were

obtained from both the pranayama group and the control group during the period of 8:00 AM to 10:00 AM and swiftly delivered to the testing centre for additional evaluation. Hb levels were assessed in g/dL utilising the same blood sample collected for SC testing.

Analysis of Data

Using SPSS version 2.5.11 (110), a repeated-measures ANOVA was used, accounting for two factors: groups (pranayama and control) and phases (pre-phase, post-phase 1 and post-phase 2). To account for multiple comparisons, a post-hoc Bonferroni-corrected analysis was conducted, and the degrees of freedom were adjusted using the Huynh-Feldt epsilon method.

Study Findings Based on Repeated-Measures ANOVA

Basic features of the study group, including 27 male subjects and 33 female subjects aged between 21 and 24 years (average age: 22.95 ± 1.15) are provided in Table-2. During the commencement of the investigation, all variables displayed no substantial disparities between the groups. The study was successfully completed by all 60 subjects, and no harmful incidents occurred during the treatment phase.

The RM-ANOVA Yielded the Following Outcomes:

Significant differences between the groups for Emotional Stability (ES) ($p=0.002$), Serum Cortisol (SC) ($p<0.001$) and Haemoglobin (Hb) ($p<0.001$) were revealed by RM-ANOVA. Significant phase-wise differences were also observed for ES ($p<0.001$), SC ($p<0.001$) and Hb ($p<0.001$). It is noticeable that interactions existed between the groups and phases for ES ($p<0.001$), SC ($p<0.001$) and Hb ($p<0.001$), demonstrating the interrelatedness of these variables. Specific F values, degrees of freedom (df), Huynh-Feldt epsilon and p-values are provided in Table-3.

Table 1. Sequence of pranayama breathing exercise (PBE) treatment in the pranayama group

S.no	PBE practice title	Time Duration (Minutes)	Practice Technique	Benefits
1.	Sahaja PBE	05	Mindfully observe natural breath without control it, shifting awareness to different parts of body.	Increases mindfulness, focus, innovation, vitality, tranquillity, mental discipline, inner harmony and emotional steadiness.
2.	Anuloma-viloma PBE	10	Breathing through alternate nostrils with equal air intake and release	Synchronizes breath with brain function, eases anxiety and aids in the healing of heart and nervous system ailments.
3.	Bhramari PBE	10	Involves deep inhalation and humming exhalation	Reduces stress, anger, anxiety, insomnia; increases healing ability; fortifies the voice

4.	Ujjayi PBE	10	Inhale using your throat, producing a gentle snoring noise; take relaxed, deep inhalations	Calming practice that soothes the nervous system, calms the mind, treats insomnia, benefits hypertension
5.	Bhastrika PBE	10	Strong inhalation and exhalation using diaphragm and abdominal movements for breathing.	Eliminates toxins, assists women during childbirth, enhances oxygen exchange and metabolism, boosts the nervous system.

Table 2. Mean Scores and SD values of the subjects of Experimental and Control Group at Pre-experimental (baseline) Stage

Items	Experimental (Pranayama) Group (n=30)	Control Group (n=30)	Total
Age group (mean±SD)	22.93 (1.04)	22.96 (1.27)	22.95 (1.15)
Gender [n (%)], Male	16 (53.33%)	11 (36.66%)	27 (45%)
Female	14 (46.66%)	19 (63.33%)	33 (55%)
-	Pre (mean±SD)	Pre (mean±SD)	P value
Emotional Stability	46.73(5.56)	47.10(3.34)	0.76
Serum Cortisol (µg/dl)	23.17(1.80)	22.85(1.59)	0.47
Haemoglobin (gm/dl)	11.57(1.03)	11.15(1.05)	0.12

Table 3. Overview of RM-ANOVA for emotional stability, serum cortisol & haemoglobin.

Variables	Factors	F(df)	Huynh-Feldt (ε)	P	ηp2
Emotional Stability (ES)	Group	10.71 (1, 58)	0.881	0.002	0.15
	Phases	19.02 (1.76, 102.20)	0.881	<0.001	0.24
	Group X Phases	21.01(1,58 X 1.76,102.20)	0.881	<0.001	0.26
Serum Cortisol (SC)	Group	32.35(1, 58)	0.983	<0.001	0.35
	Phases	126.37(1.96, 113.99)	0.983	<0.001	0.68
	Group X Phases	126.89(1,58 X 1.96,113.99)	0.983	<0.001	0.68
Haemoglobin (Hb)	Group	28.07(1, 58)	1.000	<0.001	0.32
	Phases	161.49(2.00, 116.00)	1.000	<0.001	0.73
	Group X Phases	136.71(1,58 X 2.00,116.00)	1.000	<0.001	0.70

The Results of the Within-Groups Post Hoc Analysis are as Follows:

1. In the pranayama group, ES scores demonstrated a significant increase from the pre-phase to the post-phase 1 ($P=0.004$, 95% CI [-4.10, -0.63]) and from the pre-phase to the post-phase 2 ($P<0.001$, 95% CI [-9.70, -4.82]). Conversely, the control group displayed no significant differences.
2. Significantly lower SC levels were noted in the pranayama group between the pre-phase to post-phase 1 ($P<0.001$, 95% CI [1.51, 2.49]) and from the pre-phase to post-phase 2 ($P<0.001$, 95% CI [4.17, 5.35]). However, the control group showed no significant differences.
3. Likewise, the Hb level in the pranayama group was significantly increased from the pre-phase to the post-phase 1 ($P<0.001$, 95% CI [-1.07, -0.57]) and from the pre-phase to the post-phase 2 ($P<0.001$, 95% CI [-2.72, -2.24]). Although the control group displayed no significant changes.

The Following Findings Emerged from the Post Hoc Analysis Between the Groups:

1. At the post-phase 1 ($P=0.019$, 95% CI [0.41, 4.52]) and post-phase 2 ($P<0.001$, 95% CI [4.64, 9.69]), the pranayama group revealed significantly higher ES scores than the control group. Nonetheless, both the groups displayed no significant differences at the pre-phase.
2. At both the post-phase 1 ($P<0.001$, 95% CI [-2.72, -1.17]) and post-phase 2 ($P<0.001$, 95% CI [-5.19, -3.75]), the pranayama group demonstrated significantly lower SC levels than the control group. But both the groups displayed no significant differences at the pre-phase.
3. Similarly, the pranayama group demonstrated significantly greater Hb levels in comparison with the control group at the post-phase 1 ($P<0.001$, 95% CI [0.47, 1.59]) and post-phase 2 ($P<0.001$, 95% CI [2.18, 3.34]). Yet, both the groups did not show any significant differences at their pre-phase.

Table-4 displays the post hoc comparisons for both within-group and between-group analyses of which graphical representations are illustrated in Figure-2.

Table 4. The findings for the outcome measures at the pre-phase, post-phase 1 and post-phase 2 for both the pranayama and control groups are summarized, with values presented as mean \pm standard deviation

Variables	Pranayama Group (n=30)			Control Group (n=30)		
	Pre-phase	Post-phase1	Post-phase2	Pre-phase	Post-phase1	Post-phase2
ES	46.73(5.56)	49.10(4.52) **\$	54.00(6.10) ***\$\$\$	47.10(3.34)	46.63(3.32)	46.83(3.23)
SC ($\mu\text{g/dl}$)	23.17(1.80)	21.16(1.33) ***\$\$\$	18.40(1.28) ***\$\$\$	22.85(1.59)	23.11(1.66)	22.88(1.49)
Hb (gm/dl)	11.57(1.03)	12.39(1.05) ***\$\$\$	14.05(1.07) ***\$\$\$	11.15(1.05)	11.36(1.10)	11.29(1.15)

Note: ESQ-Emotional Stability; SC-Serum Cortisol; Hb -Haemoglobin

Note: (**= $P<0.01$, ***= $P<0.001$, *= $P<0.05$) displayed significance as per Bonferroni-adjusted post hoc analysis while comparing post-phase 1 and post-phase 2 with their respective Pre-phase values. (\$\$= $P<0.001$, \$\$= $P<0.01$, \$= $P<0.05$) is based on Bonferroni-adjusted post hoc analysis of the Pre-phase, post-phase 1 and post-phase 2 of Pranayama Group compared to corresponding states of the Control Group.

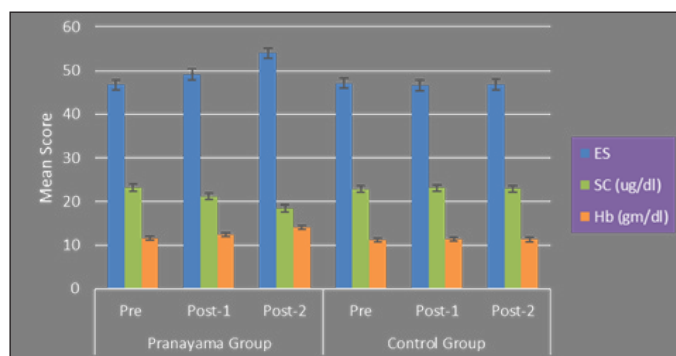


Figure 2. Visual illustration of emotional stability (ES), serum cortisol (SC) and haemoglobin (Hb) levels among post-graduate students (PGs)

Discussion

After the analysis of collected data, it has been made conspicuous that significant effects of pranayama breathing exercise (PBE) were observed on both psychological and physiological variables. The psychological variable like emotional stability (ES) showed group differences, changed over phases and had an interaction effect. Similarly, physiological variables like serum cortisol (SC) and haemoglobin (Hb) levels displayed significant group differences, changes over phases and interaction effects.

In within-group comparison, post hoc analysis indicated a notably positive effect of PBE. The pranayama group showed improved ES scores at post-phase 1 and post-phase 2, while the control group showed no changes. Additionally, the pranayama group had reduced SC levels and increased Hb levels at post-phase 1 and post-phase 2, whereas the control group did not display any noteworthy differences. In between group comparison, the pranayama group exhibited higher ES scores, lower SC and higher Hb levels at post-phase 1 and post-phase 2 in comparison with the control group. These outcomes revealed that PBE improved ES, reduced SC level (indication of lower stress) and increased Hb level, which is associated with better oxygen-carrying capacity.

The findings of this study aligned with previous research on the positive effects of PBE on ES, SC levels and Hb levels. The observed improvements within the pranayama group over 45 and 90 days are consistent with a growing ES supporting the benefits of PBE across the selected post-graduation students (PGs) population. This study's emphasis on ES aligns with the work of Deshmukh (2021) and Kumari et al. (2015), who also reported highly enhanced ES through PBE interventions.^{21,22} Additionally, a four-week Bhastrika PBE found reduced anxiety and negative effect, correlating with brain changes in emotion processing²³ and six weeks of PBE lessened negative emotions in cancer patients; those results are similar to present findings.²⁴ The highly significant reduction in SC levels in the pranayama group of this study corresponds with the findings reported by Liang et al. (2023) and Maheshkumar et al. (2022).^{25,26} Additionally, another previous investigation reported a slightly smaller reduction in cortisol levels ($P < 0.05$) compared to the outcomes of the present research investigation.¹⁴ Besides, the increase in Hb level within the pranayama group is consistent with the outcomes of Sahu and Kishore (2015) and Bhardwaj (2012), which also highlighted the potentiality of PBE for its positive influence on oxygen transport and overall physiological well-being.^{27,28}

Furthermore, the present study delves into the potential neurological mechanisms underlying these observed effects. The amygdala, a limbic system structure is highly

associated with negative emotions. The fMRI studies show increased amygdala responses to emotional stimuli.²⁹ Bilateral amygdala lesions lead to fear recognition and expression problems.³⁰ The right amygdala is implicated in the arousal of undesirable emotions, while the left amygdala participates in processing a range of positive and negative emotions.³¹ The PBE calming effects may reduce amygdala responses to negative emotions, promoting ES and reducing stress (salivary cortisol levels). Regular practice may enhance positive emotional associations in the left amygdala and thus contribute to emotional balance.^{23,32}

The discussion also touched upon the sympathetic and parasympathetic systems and how PBE potentially achieved a parasympathetic condition. The PBE is believed to reset the autonomic nervous system via the activation of both inhibitory signals and hyperpolarisation currents through the tissue stretching. This synchronisation of neural elements induced a parasympathetic state. During PBE activation, the pulmonary stretch receptors lead to increased inhibitory neural impulses, promoting vasodilatation, which further indicates parasympathetic activation. The slow and controlled breathing technique involves inhaling and retaining breath to generate these effects in the central and peripheral nervous systems, ultimately shifting the autonomic balance towards parasympathetic dominance.^{33,34} The PBE practice increased alpha wave activity, indicating calmness and reduced stress. Findings suggested a negative correlation between SC and alpha waves, possibly implying a psycho-neuro-endocrine link.³⁵

Apart from these, the present study emphasised the impact of PBE on haematological parameters, specifically highlighting the role of improved oxygen transportation in enhancing Hb levels. The PBE enhanced the efficiency of Hb in transporting oxygen to body cells. The retention phase of PBE increased the lungs' alveoli surface area, improving oxygen transportation to cells, boosting tissue functions and increasing Hb production.³⁶ Breathing is linked to the brain and nervous system, affecting emotions and neurotransmitter secretion.³⁷ By practising breath control, the system becomes more controlled, positively impacting the brain and respiratory system. Better oxygen supply due to PBE practice may lead to improved blood indices like Hb, and red blood cell counts as well as haematocrit levels.

Limitations and Future Direction

This study was conducted with a small sample size at a single campus of H.N.B. Garhwal University, located in Srinagar Garhwal (Uttarakhand). The patterns of ES could be influenced by factors such as geographical location, institutional management and social structures. This study's second limitation is its confinement to a population of PGs only. Consequently, the inadequate subjects for

study diminish the capacity to generalise results to larger population demographics. Circadian variations in SC levels additionally restrict the generalisability of the findings. Thirdly, the research focused exclusively on ES, SC and Hb as psycho-physiological parameters among the PGs, neglecting the effects of age, gender and various types of exercises. The fourth drawback is that insufficient physiological appraisal prevents a strong relationship between emotional changes and levels of SC and Hb. Thus, further comprehensive research is required for definitive insights into the effects of PBE, involving more extensive sample sizes and the integration of yoga postures, meditation and various other yogic exercises. The study's strengths are concealed in its randomised controlled design, incorporating objective measures (SC and Hb levels) and utilising the ESQ questionnaire for ES assessment. Future studies should be integrated as additional psychological parameters, collaborate across disciplines for a holistic approach and explore practical applications of PBE in educational programmes for PGs.

Conclusion

Pranayama breathing exercise (PBE) practice has shown significant beneficial effect on emotional stability (ES), as evidenced by higher ES scores and reduced serum cortisol (SC) levels which are the indication of lower stress. Its' calming effects on the amygdala may contribute to emotional balance by reducing responses to negative emotions and enhancing positive emotional associations. The PBE also promotes parasympathetic dominance, as it activates pulmonary stretch receptors leading to vasodilation and increased inhibitory neural impulses which are associated with reduced stress and increased alpha wave activity. Moreover, PBE improved haemoglobin efficiency by increasing oxygen transport to body cells, positively impacted the respiratory system and overall physiological well-being. Therefore, PBE can be considered as a most potent and promising technique of treating emotional instability of post graduate students.

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