

Effects of Practicing Yoga and Meditation on Cortisol Hormone Rhythm and Immunoglobulin A among Medical Students at the University of Pécs: A Mixed Method Study

PhD Thesis

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Abbreviations

- APA: American Psychological Association
- BSI: Brief Symptom Inventory
- BMI: Body Mass Index
- **BWV: Brain Wave Vibration Procedure**
- CG: Control Group
- CONSORT: Consolidated Standards of Reporting Trials
- CT: Combined training group
- DASS-21: Depression Anxiety Stress Scales-21
- DERS: Difficulties in Emotion Regulation Scale
- ECLIA: Electrochemiluminescence Immunoassay
- EIA: Enzyme Immunoassay
- FBG: Fasting Blood Glucose
- GSY: Goodbye Stress with Yoga
- HbA1C: Hemoglobin A1c
- HbA1C_IFCC: Hemoglobin A1c (International Federation of Clinical Chemistry)
- HDL: High-Density Lipoprotein
- HPA: Hypothalamic-Pituitary-Adrenal
- IgA: Immunoglobulin A
- IgG: Immunoglobulin G
- IgM: Immunoglobulin M
- ISQ: Illness Symptoms Questionnaire
- LDL: Low-Density Lipoprotein
- MAAS: Mindfulness Attention Awareness Scale
- MET: Metabolic Equivalent of Task

MVPA: Moderate-to-Vigorous Physical Activity

- Non-HDL: Non-High-Density Lipoprotein
- PA: Physical Activity
- PNEIMED: Psychoneuroendocrinoimmunology-based meditation
- PSQI: Pittsburgh Sleep Quality Index
- PSS: Perceived Stress Scale
- RCT: Randomised Controlled Trial
- RIA: Radioimmunoassay
- SCN: Suprachiasmatic Nucleus
- SD: Standard Deviation
- SVS: Six-Item Subjective Vitality Scale
- T2DM: Type 2 Diabetes Mellitus
- WHO: World Health Organization
- WHOQOL: World Health Organization Quality of Life

Abstract

Introduction: Medical students consistently face elevated stress, impacting physiological (e.g., cortisol, immune function) and psychological well-being. Medical students consistently face elevated stress, impacting physiological (e.g., cortisol, immune function) and psychological well-being. This dissertation explores the comprehensive effects of a structured yoga intervention on stress biomarkers, immune parameters, metabolic health, and psychosocial outcomes in this high-risk population.

Methods/Designs: This dissertation encompasses five distinct studies and involved a total of 377 participants across four of its empirical investigations. In the First study, a systematic literature review following PRISMA guidelines, analyzing 17 studies to explore methodologies for assessing cortisol and immunoglobulin A (IgA) in yoga interventions. In the Second study, a pilot interventional study (N=28), utilized self-reported questionnaires to assess changes in depression, anxiety, and stress following a 10-week Goodbye Stress with Yoga (GSY) protocol. In the Third study, an experimental pre-post intervention study (N=37), investigated the GSY protocol's impact on immune (IgA, IgG, IgM) and metabolic (e.g., glucose, lipids) parameters via fasting blood samples and validated laboratory procedures. In the Fourth study, another experimental pre-post intervention study (N=212), assessed mental health outcomes, quality of life, sleep quality, and emotional regulation using validated self-report scales. Finally, the fifth study employed a single-center, two-arm Randomized Controlled Trial (RCT) (N=100) comparing the 10-week GSY intervention to a control group, measuring diurnal salivary cortisol, sleep quality, quality of life, and psychological distress using both objective (ELISA for cortisol) and subjective (questionnaires) assessments. Statistical analyses varied by study, including t-tests, Wilcoxon signed-rank tests, Cohen's d, regression, and ANCOVA, with p<0.05 as significance.

Results: In First study identified ELISA as a common method for salivary cortisol measurement, noting that yoga interventions consistently reduce salivary cortisol, with initial evidence suggesting improved IgA. In Second study demonstrated significant reductions in anxiety (p=0.049) and depression (p=0.019). In Third study found significant increases in IgA (p<0.001), IgM (p=0.001), and HDL cholesterol (p=0.005), with a significant decrease in IgG (p<0.01), suggesting favorable physiological adaptations. In Fourth study revealed significant improvements across stress, anxiety, depression, all quality of life domains, sleep quality, and emotional regulation (all p<0.001). In Fifth study (RCT) showed the yoga group achieved significant reductions in morning cortisol (-9.14%, p<0.001), evening cortisol (-1.15 ng/ml, p=0.014), depression (-28.94%, p<0.001), anxiety (-17.41%, p<0.001), and stress (-18.24%, p=0.007), alongside improved sleep quality (-17.07%, p=0.019) and overall quality of life (+32.01%, p<0.001) compared to controls, even during exam periods.

Conclusions: The systematic review highlights the potential of yoga to reduce stress biomarkers and improve immune markers, advocating for longer intervention studies. Pilot data confirmed the GSY protocol's efficacy in reducing anxiety and depression. Subsequent experimental studies demonstrated yoga's positive influence on immune parameters (IgA, IgM, HDL) and metabolic health, while also confirming significant improvements in mental health, sleep quality, quality of life, and emotional regulation. The RCT findings underscore yoga as a promising, accessible, and effective strategy for enhancing physiological resilience, mitigating psychological distress, and improving overall well-being in medical students, serving as a buffer against academic stressors.

Keywords: Yoga, Meditation, Cortisol, Immunoglobulin A, Medical Students, Stress, Anxiety, Depression, Quality of Life, Sleep Quality, Randomized Controlled Trial.

Introduction

University students face a pretty demanding transition from adolescence into early adulthood (Dai, Chen, & Sharma, 2023). This brings significant psychological and physiological changes, including elevated stress (Hogan & Astone, 1986). Among medical students, chronic stress is a critical concern, especially its effect on academic performance and the students' overall health (Abdulghani et al., 2011). Cortisol hormone is a form of hormone from the steroid family called Glucocorticoid. It is a neuroendocrine hormone which can be measured by saliva (Nicolson, 2008).

Cortisol hormones influence the body's immunity; the effect can be acute and chronic on the immune system, especially immunoglobulin A (Elenkov, Webster, Torpy, & Chrousos, 1999). However, acute stress improves immune function by releasing pro-inflammatory cytokines, temporarily improving immune defence (Daniela et al., 2022).

Yoga is classified as a combined mind-body intervention that integrates all the components of an individual's lifestyle, thus fostering overall well-being (Bhargav, 2018). In the traditional yogic text, there are mentioned some Yogic postures specifically impact on reproductive organs, the uterus and ovaries, and also on digestion (S. Mishra & Dash, 2017). The system does not primarily revolve around focusing on the purpose of the treatment objective (D. Singh, Kishore Chaturvedi, Singh, & Kandan, 2022). However, evidence shows that consistent yoga practice promotes and maintains health conditions and manages disease.

Overall, evidence indicates that regular yoga practice can positively impact hormonal and metabolic pathways, resulting in many health benefits for diverse populations and people who suffer from chronic stress.

Dissertation aims

The aim was to investigate the Effects of Yoga and Meditation on Cortisol Hormone Rhythm and Immunoglobulin A among medical students.

Sub-study 1- Systematic literature review of cortisol hormone and IgA measurement after yoga interventions: a qualitative synthesis

1.1 Introduction

The cortisol hormone is usually measured by using saliva samples and blood samples (El-Farhan et al., 2017). Similarly, the Immune parameter IgA is found in the mucosal area of the body and also plays a very important role in immune function and response; this parameter can reflect the effect of stress on the immune response of the individual (Staley, Conners, Hall, & Miller, 2018).

Moreover, yoga intervention studies focus on psychological parameters and have fewer measured biochemical parameters elucidating the mechanism of cortisol hormone and immune response (Bower et al., 2014). The current systematic literature review aims to explore the most appropriate measuring method of cortisol hormone and IgA in the context of yoga intervention studies.

1.2 Methods/Design

The current Literature review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (PRISMA) 2020, (Parums, 2021). The search was conducted in four databases: PubMed, Embase, Cochrane Trials, and Web of Science.

The search was limited to the English language with no timeline restriction. Inclusion and exclusion criteria with a self-built search strategy. Duplicates were removed using EndNote 21, and title abstract screening was conducted using Rayyan. The selection of the studies by title, abstract, and full-text screening was performed by two independent reviewers; in the case of conflicts or disagreements, the third reviewer resolved each step by discussion.

1.3 Results

In the Current Literature review, 733 citations were found after a systematic search in 6 databases (PubMed = 21, Embase = 45, Cochrane Trial = 54, Scopus = 83, Web of Science = 66, Clinical Trials = 6). No relevant studies have been found other than these included studies from Google search. After removing duplicates, 476 citations were left, and 438 citations were excluded after performing title abstract screening. 38 studies were left and considered for full-text screening. After the full-text

screening, 21 studies were excluded. In the end, 17 studies were included in the current review for qualitative synthesis. In total, 1007 participants were studied in the current review.

1.4 Discussion

The current literature review included 17 studies according to inclusion criteria, which included diverse 1007 participant. The included studies showed variability in the findings, which the higher level of heterogeneity in the study population and frequency of yoga intervention can understand. Salivary Cortisol level was measured in 12 studies using various measurement tools. However, an ELISA kit was used in most studies.

1.5 Conclusion

The present literature review examining the different measurement tools in the study focuses on the effect of different yoga interventions on the cortisol hormone and IgA levels. Most of the included studies used ELISA kits to measure salivary cortisol biomarkers, and most of the findings support that the effect of yoga intervention reduces the salivary cortisol level in the participants. Salivary IgA level is measured in two included studies, which showed significant improvement in the marker. However, the small duration of intervention can be explained as a temporary improvement in the IgA level. Addressing the potential benefits of the yoga intervention to reduce stress biomarkers and improve the immune marker highlights the need for further studies with a longer intervention duration to better understand the effects.

Sub-study 2- Effect of yoga in medical students to reduce the level of depression, anxiety, and stress: pilot study (Goodbye Stress with Yoga GSY)

2.1 Introduction

Stress is very common among medical students (Kjeldstadli et al., 2006). From different regions around the globe, medical students have been found at risk of mental disorders, psychological stress, and a decline in life satisfaction (Kjeldstadli et al., 2006). The overall aim of this pilot study is to examine the potential role of yoga in the medical student's life to reduce stress, depression, and anxiety levels and improve the quality of life.

2.2 Materials and methods

2.2.1 Study design

An interventional study was carried out with consecutive sampling using a self-administered questionnaire in paper-and-pencil format.

2.2.2 Sampling

Data collection was carried out of the medicine students of the University of Pécs, Hungary between February 15 to 13 of May 2022. The inclusion criteria for the study required participants to be currently enrolled in the Medicine Faculty of the University of Pécs. However, certain exclusion criteria were established. Students with specific health conditions, including recent injuries, chronic pain, congenital skeletal problems or significant arthritis, which could potentially pose safety concerns during participation in a yoga intervention, were excluded from the study.

2.2.3 Study participants

All participants who registered in the physical training course with the name "Indian Yoga" at the University of Pécs, Hungary during the month of February -May 2022 were invited to participate in this current research. The research specifically included those students who expressed interest actively in the current study (N=28) with an average age of 23.54 ± 4.36 years and a BMI of 22.42 ± 3.42 kg/m2. Each participant received comprehensive instruction and a detailed explanation of the study.

2.2.4 Intervention

The research spanned a period of 10 weeks, from February 2022 to May 2022, with 90 min of yoga sessions, occurring once a week. Each yoga session followed a structured format, consisting of a different activity. The development of the yoga protocol, named "GSY Goodbye Stress with Yoga Protocol," was a collaborative effort involving a certified yoga trainer, a medical researcher, and experienced yogis from India. conducted by a certified yoga instructor with 7 years of teaching experience.

2.2.5 Assessment scales

The variables of the research framework were analysed by a self-reported questionnaire. These questionnaires were filled out at the Yoga session before starting the intervention and after the 10 weeks of intervention. The questionnaire was used in paper and pencil form and was deeply explained before giving it to the participants to ensure more accurate responses.

2.2.5.1 Assessment of physical activity

To assess participants' physical activity levels, enable comparisons and analysis, using both subjective and objective assessment. Global Physical Activity Questionnaires were used for Subjective Assessment, and ActiGraph was used for Objective Assessment.

2.2.5.2 Assessment of stress

To investigate the variation in stress, depression, and anxiety levels before and after implementing a yoga intervention. The Depression Anxiety Stress Scale DASS-21 21 was applied in English (Norton, 2007)..

2.2.5.3 Assessment of quality of life

To analyse the Individual perception of quality of life in recent days before and after the Yoga Intervention. World Health Organization Quality of Life BREF WHOQOL BREF tool is used in the English version (Group, 1998).

2.2.6 Ethical approval

The study was reviewed and approved by the Regional Research Ethics Committee as the Institutional Review Board Record number 9117- PTE 2022 University of Pécs. Before the initiation of any study-related procedures, participants provided written informed consent, indicated by their signature on the Informed Consent Form. The current study adheres to the principles outlined in the Declaration of Helsinki.

2.2.7 Data analysis

Statistical analyses were performed using SPSS 26.0 software (SPSS Inc., Chicago, ILUSA). For the baseline data, descriptive statistics were used. Data were presented as percentage (%) and frequency (N) for categorical variables, while continuous variables were presented as mean \pm SD. Shapiro-Wilk test was used to test the normality of the data. The Wilcoxon Signed Ranks Test was utilized to assess the mean differences between the pre-and post-data. Paired sample correlation was used to investigate the correlation in the pre-and post- data, and Spearman's Rank Correlation test was also applied. The significance level of p<0.05 was considered in each case.

2.3 Results

The major socio-demographic data of the study participants are shown in Table 4. The mean age of participants is 23.54 ± 4.36 years and a BMI of 22.42 ± 3.42 kg/m2. The medium height of participants is 165 cm (155–188) and the medium weight of participants is 60 kg (48–98). 78.6% of females participated and 21.4% of male participated in the current study. Baseline physical activity assessments showed participants reported significantly more MVPA via questionnaire (483.39 min/week) than accelerometer measurements (275.25 min/week). Although self-reported physical activity increased across several domains after a yoga intervention, these changes were not statistically significant. However, the yoga intervention significantly improved physical health (p=0.001) and psychological health (p<0.001) domains of quality of life. While other quality of life domains also showed improvement, they were not statistically significant. Depression (p=0.019) and anxiety (p=0.049) scores

significantly decreased after the yoga intervention. Stress levels also decreased, though not statistically significantly (p=0.078). Overall, the 10-week yoga intervention positively impacted medical students' depression, anxiety, and perceived health.

2.4 Discussion

This study suggests a 10-week yoga intervention effectively reduces depression and anxiety and improves the quality of life in medical students. Notably, these benefits appear to stem from the yoga itself, as participants did not significantly alter other physical activity habits. While stress reduction was observed, it wasn't statistically significant, potentially due to data collection during exam periods. The findings align with previous research on yoga's positive mental health effects, emphasizing its potential in managing student well-being. However, limitations include a small, self-selected sample and the absence of a control group, indicating a need for further robust research.

2.6 Conclusion

Beyond the substantial interest in the intervention, our pilot study was able to provide validation for the efficacy and necessity of the yoga intervention with our preliminary survey. It demonstrated that 10 weeks of yoga intervention can result in a significant reduction in the perceived level of anxiety, depression, and stress.

Sub-study 3- Changes in immune and metabolic parameters by 10 weeks of yoga intervention among medical students

3.1 Introduction

Chronic stress in medical students can weaken the immune system by decreasing IgA, IgG, and IgM, making them more susceptible to illness. It also elevates lipid profiles and disrupts glucose, increasing risks for cardiovascular and metabolic diseases. Yoga has shown promise in mitigating these effects, positively impacting insulin resistance, lipid profiles, and glycaemic control. However, there's a research gap regarding yoga's combined impact on both immune and metabolic parameters in this population. This study aims to investigate whether a 10-week yoga intervention can improve medical students' immune (IgA, IgG, IgM) and metabolic health, thereby aiding stress management.

3.2 Methodology

The current study is an experimental study carried out in the framework of a pre-post-test yoga intervention at the University of Pécs, Hungary. It analyses the effect of yoga intervention on immune and metabolic, among medical students.

3.2.1 Sampling

This study conveniently sampled medical students at the University of Pécs, Hungary, from September to December 2022. Due to recruitment constraints, a pre-determined sample size was not used; participation was voluntary with informed consent. Both male and female students from various years were included, provided they were enrolled in the medical faculty and had no prior yoga experience or pre-existing health conditions. Two participants dropped out due to university transfer, but their data were included to maintain the reliability of the findings.

3.2.3 Population

Among the total participants, 81.1% were females and 18.9% were males in the current study. All medical students who registered with their interest in the physical education course named "Indian Yoga" were encouraged to participate in the current research. Out of 75 registered students, 37 students participated in the current research with a mean age of 21.84 ± 2.67 years and a BMI of 22.56 ± 3.85 kg/m2. Before participation, all students received thorough instruction and details of the study procedure and purpose.

3.3 Reported Outcome

We have used the immune parameters, which are IgA, IgG, and IgM, as the primary outcome, as chronic stress results in a decrease in the level of Immunoglobulin, which leads to a reduction in the individual's immunity⁵³. The secondary outcome measures were glucose, lactate, HbA1c, HbA1c_IFCC, cholesterol, non-HDL, HDL, LDL, triglycerides, depression, anxiety, stress, and sedentary behaviors. Fasting venous blood samples were collected from participants in the morning (7:00 AM to 8:00 AM); samples were drawn in suitable vacutainers

3.3.2 Ethical declaration

All included participants provided their informed consent before any study-related procedure by signature on the consent form, which was explained verbally to each participant. The investigation of the current study conforms to the principles outlined in the Declaration of Helsinki. The National Research Ethics Committee reviewed and approved the study as the Review Board Record number 26914-5/2021/EÜIG National Public Health Center, Hungary.

3.3.3 Statistical Analyses

Data distribution was assessed using the Shapiro-Wilk test, with paired sample T-tests and Cohen's d for normally distributed variables, and Wilcoxon signed-rank tests with effect size 'r' for non-normally distributed ones. A significance level of p<0.05 was applied, and all analyses were conducted using SPSS 26.0 software.

3.4 Results

The study included 35 medical students (mean age 21.84 years, majority female), with all baseline and post-intervention markers within normal ranges. Primary outcomes revealed a significant increase in IgA (p<0.001) and IgM (p=0.001), alongside a slight but significant decrease in IgG (p<0.01). Among secondary metabolic outcomes, HDL cholesterol significantly increased (p=0.005). Other metabolic markers showed non-significant changes: HbA1c increased (p=0.360), HbA1c_IFCC increased (p=0.293), fasting blood glucose increased (p=0.10), total cholesterol decreased (p=0.554), triglycerides decreased (p=0.298), LDL increased (p=0.14), and non-HDL decreased (p=0.214).

3.5 Discussion

The present study on medical students showed that a 10-week yoga intervention significantly improved IgA and HDL levels, even with post-intervention data collected during high-stress exam periods. While IgA and IgM saw beneficial increases, IgG decreased, though remaining within normal ranges, possibly reflecting short-term intervention effects on long-term immune memory. Metabolic markers like HbA1c, glucose, cholesterol, and triglycerides showed non-significant changes, likely influenced by the intervention's duration, student demographics, and the challenging exam period. Despite some positive trends, limitations like a small, non-controlled sample and data collection during exams suggest cautious interpretation.

3.7 Conclusion

The current study shows the significant impact of 10 weeks of yoga intervention on immune and metabolic parameters among medical students. Regular yoga practice for 10 weeks improved medical IgA level, blood glucose control, and favourable lipid parameters. The current finding underscores the meaningful impact of yoga intervention can positively influence certain aspects of medical students' physiological health.

Sub-study 4- Impact of yoga intervention on mental health and overall well-being among medical students: GSY study

4.1 Introduction

In the last decade, more attention has been given to the mental health issues faced by medical students (Jafari, Loghmani, & Montazeri, 2012). Evidence shows that medical student's mental health deteriorates throughout medical education and training (Goebert et al., 2009; Lomis, Carpenter, & Miller, 2009; Niemz, Griffiths, & Banyard, 2005). Research shows that yoga and meditation interventions positively impact individuals' overall well-being (Descilo et al., 2010; Elder, Nidich, Moriarty, & Nidich, 2014; Thordardottir, Gudmundsdottir, Zoëga, Valdimarsdottir, & Gudmundsdottir, 2014; Woodyard, 2011). The current chapter's primary aim is to explore the effect of a 10-week yoga

intervention on mental health outcomes (stress, anxiety, and depression), quality of life, emotional regulation, and quality of sleep of medical students.

4.2 Methods

The study, approved by the Regional Research Ethics Committee (Record number 9117- PTE 2022), involved medical students from the University of Pécs, Hungary, selected via non-random convenient sampling due to voluntary registration for a yoga course. An active control group was not included for ethical reasons, as students voluntarily sought stress reduction through yoga. Conducted from February 15 to May 15, 2023.

4.2.1 Study Population

In total, 212 medical students participated with a mean age of 21.36 ± 2.20 years in the current study. 89.2% were female, and 10.8% were male out of total participants. Students registered for the course with the name "Indian Yoga" were encouraged to take part in the research, and the research team took their written and verbal consent. To ensure the safety of the participants, we build inclusion and exclusion criteria to select the participants to take part in the yoga intervention.

4.2.2 Intervention

The current study's yoga intervention took place between 15 February and 15 May 2023. It was a 10-week (90-minute-per-week) program specifically designed to reduce participants' stress levels.

Instruction—Before participating, all the students were instructed not to drink or eat for two hours before the session.

4.2.3 Outcome Reported

In the present study, we have used mental health outcomes and quality of life scores as the primary objective of the study. Additionally, the secondary outcome was quality of sleep and emotional regulation.

4.2.4 Measures

Self-reported questionnaires were used to collect the data from the participants. All questionnaires were provided in paper-and-pencil form, and data were calculated before and after the 10-week yoga intervention. We used the Depression Anxiety Stress Scale DASS (Norton, 2007), Pittsburgh Sleep Quality Index PSQI (C. Smyth, 1999), and World Health Organization Quality of Life BREF WHOQoL BREF (Group, 1998), Emotion Regulation Scale (DERS) (Grant, Salsman, & Berking, 2018) tools in the English version.

4.2.5 Statistical Analyses

We used SPSS 26.0 software (SPSS Inc., Chicago, ILUSA) to conduct statistical analyses. The distribution of the data was tested using the Kolmogorov-Smirnov test. Based on the distribution of the data, a paired sample T-test was conducted to compare the mean of continuous variables. The association between continuous variables was tested using Pearson's correlation. Based on the significance of these correlations, we conducted a stepwise linear regression analysis to define the association of changes in stress level, anxiety level, and depression level with psychosocial factors. Data was demonstrated in mean and SD for the continuous variable as well as percentage and frequency for the categorical variable. The p<0.05 is considered significant in each case.

4.3 Results

In total, 212 medical students participated in the current study, averaging 21.36 ± 2.20 years; 89.2 % were female, and 10.8% were male. A 10-week yoga intervention significantly improved participants' mental health, with substantial decreases in depression (mean difference: -8.00, p<0.001), anxiety (mean difference: -8.90, p<0.001), and stress (mean difference: 8.52, p<0.001) scores. Participants also reported significant improvements across all quality of life domains, including overall (mean difference: 2.74, p<0.001), psychological, physical, social, and environmental scores (all p<0.001). Quality of sleep significantly improved (mean difference: -13.19, p<0.001). Furthermore, participants showed significant improvements in most emotional regulation domains ($p \le 0.001$). Regression analyses revealed that an increase in depression $(\beta=0.626, p<0.001)$ and anxiety $(\beta=0.608, p<0.001)$ showed a strong positive relation to higher stress levels. Conversely, improved environmental conditions (β =-0.302, p<0.001) were associated with decreased stress levels. The models demonstrated statistical significance and no multicollinearity.

4.4 Discussion

We explore the 10-week yoga intervention's impact on mental health and well-being using the experimental pre-post-test intervention method. Our findings showed that a 10-week yoga intervention has a significant decrease in stress, anxiety, and depression, which leads to improvement in the mental health of medical students. Participants reported enhancements in emotional regulation, sleep quality, and overall quality of life, demonstrating yoga's holistic benefits regardless of gender. The study highlights the importance of yoga in promoting mental health by fostering a calming environment and improving emotional awareness. However, limitations such as the absence of a control group and potential confounding factors suggest caution in interpreting the results. Future research should focus on randomized control trials to validate these findings and explore long-term effects.

4.5 Conclusion

The current chapter shows that yoga intervention used in GSY protocol can significantly improve medical students' mental health and overall well-being. The findings highlight that yoga is a holistic tool to enhance the quality of sleep, quality of life, emotional regulation, and mental health outcomes. However, the present study does not have a control group, which limits the ability of the findings to show that all observed outcomes are solely due to the yoga intervention. Future studies should focus on long-term intervention with randomized control trials.

Sub-study 5- Effect of 10 weeks of yoga intervention on Evening and Morning salivary cortisol of medical students: Randomised control trial

5.1 Introduction

The cortisol hormone, also called as "stress hormone," plays a vital role in the physiological response of the body to stress (Holsboer & Ising, 2010). It is a steroid hormone released and synthesized by the adrenal cortex. A systematic review showed that yoga practice can stabilize the salivary cortisol rhythm(Ryan, Booth, Spathis, Mollart, & Clow, 2016), although there is very limited evidence in the case of medical students(Moreno, Becerra, Ortega, Suarez-Ortegón, & Moreno, 2023). The primary aim of the current study was to investigate the effect of 10 weeks of yoga intervention on evening and morning salivary cortisol. Additionally, it also investigates the association between cortisol hormone, sleep quality, and overall quality of life after 10 weeks of yoga practice among medical students.

5.2 Methods

5.2.1 Trial design

The current study is a single-centered, two-arm simple randomisation control trial (RCT) with an equal allocation of participants to an experimental group, and a passive control group was pre-registered at Clinical Trial.gov (NCT06661603). The Current RCT was consistent with the Consolidated Standards of Reporting Trials (CONSORT) guidelines (Moher et al., 2010) (Figure 4). The passive control group was advised not to engage in any other structured physical activity and to follow the usual daily routine.

5.2.2 Participants and procedures

Medical students from all the years of study were invited to the seminar at the Medical School, University of Pécs, Hungary, where the principal investigator of the current study presented a research plan to the medical students. Participants allocated to the yoga group were provided once a week, for a 10-week yoga session with 90 minutes duration of each session from February 2024 to May 2024. To minimize the bias, participants from the control group were advised to report any significant change they may have made during the duration of the study, especially in the case of physical activity habits, although there were no participants who reported any change in their habits during the duration of the study. Sociodemographic data, including age, height, weight, relationship status, and financial support, questionnaire data, morning saliva sample, and evening saliva sample were gathered before the intervention from both groups.

5.2.3 Randomization

In the current study, participants were allocated in a 1:1 ratio to the intervention group and control group using a computer-generated simple random sequence number. The generated numbers were printed out on paper and later put inside of envelope. Due to the nature of the intervention used in the current study, double blinding was not possible, this study used single-blinding methods. The data collectors, statisticians, and laboratory experts were blinded to the participants' group allocation and assignment, ensuring the outcome assessment remained free from potential bias.

5.2.4 Intervention

A detailed 20-minute in-person information session was provided to the participants allocated to the yoga group about the goal, topic, data collection procedure, and structure of the intervention. After all the participants agreed to all the detailed information provided by verbal and written consent, the predata collection intervention began. The intervention was given, once a week, a 90-minute session for 10 weeks to Intervention participants.

5.2.5 Outcome Measurement

This Randomized Controlled Trial (RCT) employed both subjective and objective measures to assess a 10-week yoga intervention. Validated questionnaires (DASS-21, WHOQOL-BREF, PSQI) captured mental health, quality of life, and sleep quality subjectively. Objective data involved saliva collection in the evening and morning to measure cortisol levels using ELISA. All assessments were conducted at baseline and post-intervention.

5.2.6 Sample Size

The target sample size of 100 participants, with 50 allocated to the yoga intervention group and 50 to the control group. This sample size was considered a pragmatic and achievable goal within the study's constraints, allowing for an initial exploration of potential differences between the groups on the outcome variables.

5.2.7 Ethical Approval

The study was carried out following the Declaration of Helsinki. The study was reviewed and approved by the Regional Research Ethics Committee as the Institutional Review Board Record number 9117-PTE 2022, University of Pecs, Hungary.

5.2.8 Statistical methods

In the current study, statistical analyses were performed using SPSS 26.0 software (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test determined the data distribution. Paired and independent samples T-tests compared within- and between-group means, with Cohen's d calculating effect sizes. ANCOVA was utilized to compare post-intervention differences between groups, controlling for baseline variations, with partial eta squared evaluating its effect size. A significance level of p<0.05 was applied.

5.3 Results

In the current RCT, 100 medical students participated, with 50 students allocated to the yoga group and 50 to the control group. There were no significant differences in age (p = 0.135), weight (p = 0.810), height (p = 0.190), or gender distribution (p = 0.656). Morning salivary cortisol significantly decreased in the intervention group (p = 0.027) but significantly increased in the control group (p = 0.021). The DASS 21 revealed significant improvements in depression (p < 0.001), anxiety (p = 0.007), and stress (p = 0.061) scores in the intervention group, while the control group showed significant increases (p < 0.001). The overall quality of life significantly increased in the intervention group (p < 0.001) and significantly decreased in the control group (p < 0.001). The Pittsburgh Sleep Quality Index (PSQI) global score significantly decreased in the intervention group (p = 0.021).

5.4 Discussion

The current RCT found that 10 weeks of yoga significantly decreased morning and evening salivary cortisol levels, and improved depression, anxiety, and stress scores in medical students compared to a control group. The yoga group also demonstrated improvements in overall quality of life, physical health, psychological health, and social relations, along with improved quality of sleep. Notably, these benefits were observed despite post-intervention data collection occurring during a high-stress academic exam period.

5.5 Conclusion

This randomized controlled trial demonstrates that a 10-week yoga intervention using a structured GSY protocol significantly reduces morning cortisol levels and improves psychological well-being, sleep quality, and quality of life in medical students. However, it is suggested as a future recommendation to explore the long-term effects of using the current yoga protocol among medical students.

Summary of novel findings

- Sub Study 1. As per the author's knowledge this dissertation topic is the first study conducted on the effect of yoga intervention on cortisol hormone and immunoglobulin A. A review of 17 studies (1007 participants) found most used ELISA kits to measure salivary cortisol biomarkers, with findings supporting yoga's reduction of salivary cortisol. Two studies on salivary IgA showed significant improvement, though brief intervention durations suggest temporary effects. Further longer-duration studies are needed to understand these effects on stress biomarkers and immune markers.
- 2. Sub Study 2. After reviewing the literature, we conducted a pilot study using the GSY protocol as an intervention for 10 weeks among medical students to analyze changes in levels of stress, anxiety, and depression. In the pilot survey only, validated questionnaires were used. The finding of this chapter not only showed that the current intervention significantly reduced the level of anxiety and depression (p = 0.019 and p = 0.049) but also provided efficacy and necessity of yoga intervention.
- 3. Sub Study 3. We conducted an experimental study using a pre-post intervention to analyse the effect of a 10-week yoga intervention on the immune markers and metabolic parameters among medical students. Standard laboratory procedures were followed during the collection of fasting blood samples from the participants. The results from pre- and post-blood marker parameters fell within the normal range. Regular yoga practice for 10 weeks improved medical IgA level, blood glucose control, and favourable lipid parameters. Future studies should focus on the higher frequency of yoga intervention per week and its long-term impact on the clinical outcomes, including mental health outcomes of medical students.
- 4. Sub Study 4. We conducted an experimental study focusing on the effect of 10 weeks of practice of GSY protocol among medical students. Data was collected before and after the intervention using validated questionnaires DASS-21, WHOQoL-BREF, PSQI, and DERS. The present chapter findings show a significant stress reduction (p < 0.001), depression (p < 0.001), and anxiety (p < 0.001) of participants, overall quality of life (p < 0.001), quality of sleep (p < 0.001) and emotional regulation (p < 0.001) significantly improved after the intervention. By including yoga and meditation practice in the daily lives of medical students, educational institutes can provide a more supportive environment that focuses on students' overall health.
- 5. Sub Study 5. We conducted a randomised controlled trial focusing on the effect of a 10-week yoga intervention on diurnal cortisol, sleep quality, and quality of life in medical students. A single-centre, two-arm randomized controlled trial (n=100, mean age 22.27 ± 3.00 years) was conducted. The yoga group exhibited a significant 9.14% reduction in morning cortisol levels (, p<0.001), improved sleep quality (p=0.019), and enhanced overall quality of life (p<0.001) compared to the control group. Significant improvements were also observed in depression (p<0.001), anxiety (p<0.001), and stress (p=0.007) scores in the yoga group. Yoga offers a promising, accessible, and effective strategy for managing stress and enhancing well-being in this high-risk population.</p>

List of Publications

Published articles related to the thesis

Chauhan, S., Babu, A. M., Galgalo, D. A., Melczer, C., Prémusz, V., & Karsai, I. (2024). Effect of yoga in medical students to reduce the level of depression, anxiety, and stress: pilot study (Goodbye Stress with Yoga GSY). *BMC Complementary Medicine and Therapies*, *24*(1), 203.

Chauhan, S., Najaf, S. S., Gergely, L., Kinga, K. A., Karsai, I., & Prémusz, V. (2025). Impact of 10 Weeks of Yoga Intervention on Mental Health and Overall Well-Being Among Medical Students: GSY Study. *Sports*, *13*(4), 114.

Other published articles

Galgalo, D. A., Mokaya, P., Chauhan, S., Kiptulon, E. K., Wami, G. A., Várnagy, Á., & Prémusz, V. (2024). Utilization of maternal health care services among pastoralist communities in Marsabit County, Kenya: a cross-sectional survey. *Reproductive Health*, *21*(1), 126.

Prémusz, V., Lendvai-Emmert, D., Makai, A., Amrein, K., Chauhan, S., Bódis, J., ... & Várnagy, Á. (2022). Pre-Treatment Physical Activity Could Positively Influence Pregnancy Rates in IVF despite the Induced Oxidative Stress: A Cohort Study on Salivary 8-Hydroxy-2'-deoxyguanosine. *Antioxidants*, *11*(8), 1586.

Articles under review

Chauhan, S., Najaf, S.S., Nagy, Z., Al-Jawarneh1, M., Nagy, T., Miseta, A., Prémusz, V., & Karsai, I. Changes in immune and metabolic parameters by yoga intervention among medical students.

Chauhan, S., Muka, T., Najaf, S.S., Galgalo, D.A., Atmaca, L., Karsai, I., Prémusz, V. Impact of yoga intervention on the Anti-Mullerian Hormone (AMH), androgen level, and metabolic parameters in women suffering from polycystic ovarian syndrome – A systematic review.

Chauhan, S., Najaf, S.S., Babu, A. M., Atmaca, L., Galgalo, D. A., Prémusz, V., & Karsai, I. Effect of 10 weeks of yoga intervention on Evening and Morning salivary cortisol of medical students: Randomised controlled trial.