Date: February 29, 2020 **NCT number**: Not Available

Official Project Title

Investigating the Role of Yoga in Alleviating Chromosomal Translocations in Security Guards of Chandigarh Police Who Are Responsible for the Safety of VIPs

Project summary:

Policemen recruited in the Chandigarh police who take care of the safety of VIPs most likely suffer from chronic stress, as they need to be vigilant round the clock. Since chronic stress has been shown to be responsible for multiple molecular changes at both the genomic and epigenomic levels, it can lead to various diseases like hypertension, diabetes and even cancer. Further, it has been shown that deregulation in DNA Damage Response (DDR) and DNA repair pathways is responsible for cancer. Though it is well known that yoga relieves stress and anxiety, literature lacks in studies that focus onto assessing the effect of yoga on DDR and DNA repair pathways at the molecular level. In the present study, we propose to investigate the role of yoga in modulating DDR and repair. For this, personnel of Chandigarh police who need to be extra vigilant could be the ideal cohorts. A sample size of around 230 security guards from Chandigarh police shall be recruited in the study with their consent, upon screening them for mild or severe stress on Cohen's Perceived Stress scale. 10 ml of blood will be withdrawn from the participants followed by separation of serum, plasma and WBCs from these subjects. Biochemical assays to measure the levels of cortisol (stress marker) will be performed and analyzed. This study proposes to analyze DDR markers like γH2AX, 53BP1, and ATM and the extent of damage in WBCs using comet and DNA repair assays. Post the baseline assessments, they will undergo Yoga intervention for a period of 3 months, and at the end of three months, they will be assessed again on the same parameters. The baseline and post intervention results will be compared for discussion and conclusion. This study will help us to understand the effect of yoga in maintaining genomic integrity and stability at the molecular level and can assist the Department of Chandigarh police in designing strategies to improve the overall well being of policemen.

Key words: Yoga exercises, DNA damage response (DDR), DNA double-strand break (DSB), DNA double-strand break repair (DSBR), Cancer

Introduction:

Origin of the proposal

It is well known that yoga relieves stress and anxiety. Since chronic stress has been shown to be responsible for multiple molecular changes at both the genomic and epigenomic levels, that can lead to various diseases like hypertension, diabetes and even cancer. Alterations in DNA damage response (DDR) and repair are considered as one of the major causative agents of cancer. As the effects of yoga on DNA damage response (DDR) and DNA repair are not known, studying the role of yoga will provide us with an insight on the role of yoga in modulating DDR in a way that can alleviate the effects of chronic stress on DDR and repair leading to cancer. DDR is a signaling event, which is activated upon DNA damage and encompasses DNA damage repair, cell-cycle checkpoint activation, apoptosis and senescence. Deregulation in DDR has been known to be responsible for carcinogenesis and it has been suggested that defects in DNA repair is the hallmark of most cancers (Gavande, 2016). Although preliminary studies have reported the effects of yoga in relieving oxidative stress, but no study has investigated the effects of common yoga practice on DDR and DNA repair. This study proposes to analyze the effect of yoga on DDR markers like γH2AX, 53BP1, and ATM and the extent of damage in WBCs using comet and DNA repair assays. Such analysis will uncover the effect of yoga on DDR and cell survival mechanisms. We will also estimate the extent of oxidative free radicals as well as other DNA repair machinery. We will further evaluate the possible mechanisms by which yoga affects DDR and DNA repair by studying the expression and activation of DDR proteins upon DNA damage. Hence, this study is designed to examine the effect of yoga on DDR and DNA repair and the associated molecular mechanisms. This data could be useful to not only examine the cancer preventive role of yoga but also could be extended to potentiate the therapeutic efficacy of commonly used anti-cancer drugs.

Stress at Molecular Level

Stress can induce responses commonly associated with infections and tissue damage and increase the levels of various biomarkers of inflammation (Black, 2002). Several

studies have reported stress induced immune dysfunction as it was found that receptors for stress neuropeptides and hormones are broadly expressed in immune cells (Glaser & Kiecolt-Glaser, 2005). The presence of long-term stress, anxiety and depression is a prelude to increased biological susceptibility to various psychiatric ailments, physical health problems as well as an increased risk of various chronic diseases like diabetes, hypertension and even life threatening diseases like cancers (Miller, Chen & Parker, 2011; Antonova, Aronson & Mueller, 2011; Fleshner & Klotz, 1998; Mariotti, 2015). According to a study, psychological stress induces oxidative damage to nuclear DNA (Moreno-Villanueva et al., 2018). Another study done in year 2000 showed involvement of psychological stress in the pathogenesis of cancer via oxidative DNA damage in rats (Irie et al., 2000), whereas a recent study concluded that stress mediated DNA damage plays an important role in the pathogenesis and development of breast cancer (Yasuda, Sakakibara & Shimoi, 2017). A study done on human population indicated that cells from the stressed population were more sensitive to the induction of DNA damage and had higher level of residual damage (Dimitroglou et al., 2003). Triggering curiosity further, a study demonstrated in detail, how stress promotes prostrate carcinogenesis in mice, in an adrenaline-dependent manner by evading apoptosis (Hassan et al., 2013), and another study showed that psychological stress can induce oxidative stress and is a well-known causative agent for cancer (Hayashi, 2015).

Yoga And DNA Damage

A growing interest has been seen, among the cancer survivors, in the use of various complementary therapies as adjuvants to conventional treatment in the anticipation of coping with the treatment (Buettner et al., 2006). A number of studies including randomized trials have reported positive therapeutic outcomes following yoga programs. This includes reported improvements in blood pressure, immune function and serum cortisol levels (Henderson & Donatelle, 2004). Banerjee et al. reported that integrated approach of yoga intervention modulates the stress and DNA damage levels in breast cancer patients during radiotherapy (Banerjee et al., 2007). Scientists today are driven towards understanding the mechanisms by which yoga and meditation achieve relaxation of mind and body, and its effects on various physiological processes and

oxidative stress (Chong, Tsunaka & Chan, 2011; Dhawan et al., 2018). It has been shown that yoga based lifestyle intervention not only reduces oxidative DNA damage, but also improves the integrity of sperm DNA and reduces mutation load (Dhawan et al., 2018). Though these studies describe the beneficial and biochemical aspects of yoga, the effects of yoga at the molecular level on DNA damage response and repair, deregulation in these processes leading to cancer still needs to be determined.

Yoga in Ancient Texts

Ancient texts talk about two kinds of diseases in accordance with the site where disease manifests: Physical and mental. The assessment of components of the physical body (doṣa, dhātu, mala, agni, etc.) and the treatment thereafter is covered by Ayurveda, whereas, assessment of the mind is covered by Yoga.

According to caraka, both body and mind are the locations of disorders as well as pleasures. Good health and pleasurable life are maintained and bestowed by balanced use of application of time, intelligence, whereas diseases occur by negative and excessive utilization of time, intelligence and sense objects. Thus, abnormalities in lifestyle may contribute to both psychic and somatic disorders.

According to suśruta sūtra 24.08, psychic diseases are thought to originate from aggravation of the rajasa and tamasa guṇa, while physical diseases originate from aggravation of vāta, pitta and kapha doṣa.

On the other hand, Yoga broadly divided diseases into two categories: Ādhijāvyādhi and anādhijāvyādhi. ādhijāvyādhi are the diseases that originate at the mind level, or psychosomatic, whereas, anādhijāvyādhi are the diseases, which do not originate at the mind level. anādhijāvyādhi may include infections and contagious diseases, which may be handled through conventional medicine, i.e., modern medicine, Ayurveda, etc. ādhijāvyādhi, however, is caused due to agitation at the mind level during one's interaction with the world around. These agitations cause violent fluctuations in the flow of prana (vital energy) within the nāḍī (energy channels). These disturbances at the

prāṇa level manifests into tridoṣa imbalance. Imbalance in tridoṣa disturb the metabolic activities of the body, consequently forming āma (metabolic waste), which further obstructs the srota (channels in the body). This obstruction, ultimately, manifests as disease at the physical level.

Management of Psychosomatic Disease According to Yoga

As discussed above, ādhi in manomaya kośa is the source for psychosomatic diseases, which manifest as imbalance tridoṣa at the physiological level. Hence, the treatment approach for psychosomatic medication should include such interventions that can correct the imbalance both at the mind level as well as body level. Following module based on ancient methods of Yoga is thus proposed, to control and prevent the psychosomatic disorders.

Key questions expected to be answered by this study:

a) What are the effects of yoga on biochemical and molecular markers of stress leading to changes in DNA damage response

b) Is there any change in the DNA repair capacity of yoga-practicing subjects as compared to matched controls?

c) What will be the mechanism by which yoga modulates DNA damage response and repair.

Definition of the problem

Evaluate the molecular impact of yoga on DNA damage response and repair on security guards of Chandigarh Police

Objectives

1. Analysis of molecular markers related to DNA damage response and repair before and after the practice of yoga on Chandigarh Police

2. To examine the molecular mechanism by which yoga may modulate DNA damage response and repair.

Review and status of Research and Development in the subject:

Chronic stress and anxiety leading to various lifestyle disorders have become a common problem in today's world and the current health care industry is unable to resolve this vicious cycle. This has led to development of alternative approaches to wellness that can improve the overall health of individuals and can even prevent various life-threatening diseases. Various studies have suggested that yoga can be beneficial for the treatment of many psychological disorders like anxiety; depression etc. and can prevent cognitive impairment of the aging individuals. Numerous studies have shown yoga as a holistic approach for the well being of an individual that affects the overall physiology. However, these effects have not been studied at the molecular level.

Policemen need to be alert for a long duration of time and hence remain in chronic stress. Many of them suffer from depression, stress and anxiety and have undergone the extreme step of committing suicide. Quite understandably, this distress can have a huge impact on their professional and personal life. Various studies have shown that chronic mental stress can further accentuate stress at the cellular and molecular level. This long-term stress and anxiety, thus, is a prelude to various chronic diseases like diabetes, hypertension and even life-threatening diseases like cancer (Mariotti et al., 2015). Cancer is a life-threatening disease due to rapid uncontrolled cell growth and most of the cancers show altered DNA damage response and repair capacity.

International status:

Yoga-based lifestyle intervention (YBLI) is being recognized as an adjunct to modern medicine in order to treat many medical conditions including stress and lifestyle-based disorders, chronic inflammatory disorders and metabolic syndromes (Yadav et al., 2012). Various clinical trials across the globe have shown the beneficial effects of yoga on cancer patients in terms of overall improvement in the quality of life and mood states (Rao et al., 2017). However, these trials are heterogeneous as various types of asanas were performed. Carlson et al. showed that telomeric length was maintained in the intervention group while it was decreased in the control participants, though no

associations were found between changes in telomeric length and mood or stress scores (Carlson et al., 2015). Lengacher et al. studied the effect of yoga intervention on breast cancer patients and found a steady increase in telomerase activity while the length of telomeres did not change (Lengacher et al., 2014). A similar clinical trial by Kiecolt et al. observed a reduction in inflammation and fatigue in breast cancer patients (Kiecolt- Glaser, 2014). Chandwani et al. integrated yoga regime in stage I-III breast cancer patients, and stage 0-III, breast cancer patients undergoing radiotherapy (Chandwani et al., 2014). They checked for the changes in fatigue, depression, sleep quality, and salivary cortisol. Interestingly, they found a significant improvement in the quality of life along with physiological changes associated with radiotherapy. Systematic evaluation of the effect of yoga as a complementary intervention in cancer therapy has proposed that yoga is associated with positive effects on psychological functioning (Smith & Pukall, 2009). A report demonstrated the potential effect of yoga intervention on telomeric length of breast cancer survivors (Carlson et al., 2015). This study thus supports association between genetic alterations, cancer initiation and survival, leading to an increase in data that shows an important role of disease-regulating processes and outcomes (Epel et al.,2009). Though Lotzke et al. did not find any significant difference between the intervention and control groups when the study was done in stage I-III breast cancer patients (Lotzke et al., 2016), a detailed molecular study has demonstrated that stress can evade apoptosis and can promote prostate carcinogenesis in mice in an epinephrine-dependent manner (Hassan et al., 2013).

National status:

Yoga may help in managing distress and modulate circadian rhythms in early breast cancer patients undergoing neoadjuvant radiotherapy (Vadiraja et al., 2009). However, various studies show heterogeneity in benefits of yoga depending on the extent of intervention, practice, duration and indications (Rao et al., 2017). A recent study demonstrated that YBLI protects sperms from oxidative stress, reduces sperm DNA damage that in turn leads to an improvement in sperm motility and count (Dhawan et al, 2018). A similar study showed a significant improvement in the markers of cellular aging in 96 healthy individuals after YBLI (Tolahunase et al., 2017). Recent studies have

shown that increase in the incidence of childhood cancers can be attributed to oxidative stress leading to DNA damage in sperms. YBLI led to a significant improvement in oxidative DNA damage markers in 131 fathers of children with Retinoblastoma (Dada et al., 2016).

Novelty of the study:

In this study, we are investigating the detailed molecular mechanisms by which yoga can modulate DNA damage response and repair. Although multiple studies have examined the effect of yoga on DNA damage repair, a detailed mechanism by which yoga improves high-fidelity DNA double strand break repair is lacking.

Human DNA is exposed to both endogenous and exogenous sources of DNA damage. Endogenous sources of DNA damage mainly include oxidative free radicals while exogenous sources like UV radiation, X-rays, chemicals etc are the sources of DNA strand breaks and major sources of cancer. Most of the studies have focused on effects of yoga on oxidative stress by using 8-OHdG as a marker. Few studies have used comet assays, expression of PARP1 and telomerase activity to evaluate DNA strand damage and repair. These preliminary studies provide for the platform to further evaluate the effect of yoga on DNA damage response and repair at the molecular level. In this proposed study, using various techniques, we will examine the detailed molecular mechanism by which yoga may tweak DNA damage response and repair mediated tumorigenesis using both in vitro (cell lines) and in vivo (human subjects)models.

Relevant references

Acharya, V. Y. T. (2004). Charaka Samhita of Charaka. Varanasi: Chaukambha Sanskrith Samsthana.

Adachi, S., Kawamura, K., & Takemoto, K. (1993). Oxidative damage of nuclear DNA in liver of rats exposed to psychological stress. Cancer Research, 53(18), 4153-4155.

Antonova, L., Aronson, K., & Mueller, C. R. (2011). Stress and breast cancer: from epidemiology to molecular biology. Breast Cancer Research, 13(2), 208.

Arbuthnott, K., & Frank, J. (2000). Trail making test, part B as a measure of executive control: validation using a set-switching paradigm. Journal of clinical and experimental neuropsychology, 22(4), 518-528.

Balakrishnan, R., Nanjundaiah, R. M., Nirwan, M., Sharma, M. K., Ganju, L., Saha, M., ... & Ramarao, N. H. (2019). Design and validation of integrated yoga therapy module for antarctic expeditioners. Journal of Ayurveda and integrative medicine, https://doi.org/10.1016/j.jaim.2017.11.005

Banerjee, B., Vadiraj, H. S., Ram, A., Rao, R., Jayapal, M., Gopinath, K. S., ... & Hegde, S. (2007). Effects of an integrated yoga program in modulating psychological stress and radiation-induced genotoxic stress in breast cancer patients undergoing radiotherapy. Integrative cancer therapies, 6(3), 242-250.

Bhavanani, Y. D. A. B. (2013). Psychosomatic mechanisms of yoga. Annals of SBV, 2(2), 27-31.

Black, P. H. (2002). Stress and the inflammatory response: a review of neurogenic inflammation. Brain, behavior, and immunity, 16(6), 622-653.

Buettner, C., Kroenke, C. H., Phillips, R. S., Davis, R. B., Eisenberg, D. M., & Holmes, M. D. (2006). Correlates of use of different types of complementary and alternative medicine by breast cancer survivors in the nurses' health study. Breast cancer research and treatment, 100(2), 219-227.

Carlson, L. E., Beattie, T. L., Giese-Davis, J., Faris, P., Tamagawa, R., Fick, L. J., ... & Speca, M. (2015). Mindfulness-based cancer recovery and supportive-expressive therapy maintain telomere length relative to controls in distressed breast cancer survivors. Cancer, 121(3), 476-484.

Chandwani, K. D., Perkins, G., Nagendra, H. R., Raghuram, N. V., Spelman, A., Nagarathna, R., ... & Kirschbaum, C. (2014). Randomized, controlled trial of yoga in women with breast cancer undergoing radiotherapy. Journal of Clinical Oncology, 32(10), 1058.

Chong, C. S., Tsunaka, M., & Chan, E. P. (2011). Effects of yoga on stress management in healthy adults: a systematic review. Alternative therapies in health and medicine, 17(1), 32.

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. Journal of health and social behavior, 385-396.

Dada, R., Kumar, S. B., Chawla, B., Bisht, S., & Khan, S. (2016). Oxidative stress induced damage to paternal genome and impact of meditation and yoga-can it reduce incidence of childhood cancer. *Asian Pacific Journal of Cancer Prevention*, *17*(9), 4517-4525.

Das, R. C. (1991). Standardization of the Gita inventory of personality. Journal of Indian Psychology.

Dhawan, V., Kumar, M., Deka, D., Malhotra, N., Dadhwal, V., Singh, N., & Dada, R. (2018). Meditation & yoga: Impact on oxidative DNA damage & dysregulated sperm transcripts in male partners of couples with recurrent pregnancy loss. The Indian journal of medical research, 148(Suppl 1), S134.

Dimitroglou, E., Zafiropoulou, M., Messini-Nikolaki, N., Doudounakis, S., Tsilimigaki, S., & Piperakis, S. M. (2003). DNA damage in a human population affected by chronic psychogenic stress. International journal of hygiene and environmental health, 206(1), 39-44.

Dölken, G., Dölken, L., Hirt, C., Fusch, C., Rabkin, C. S., & Schüler, F. (2008). Age-dependent prevalence and frequency of circulating t (14; 18)-positive cells in the peripheral blood of healthy individuals. Journal

of the National Cancer Institute Monographs, 2008(39), 44-47.

Fleshner, N. E., & Klotz, L. H. (1998). Diet, androgens, oxidative stress and prostate cancer susceptibility. Cancer and Metastasis Reviews, 17(4), 325-330.

Gavande, N. S., VanderVere-Carozza, P. S., Hinshaw, H. D., Jalal, S. I., Sears, C. R., Pawelczak, K. S., & Turchi, J. J. (2016). DNA repair targeted therapy: the past or future of cancer treatment?. Pharmacology & therapeutics, 160, 65-83.

Glaser, R., & Kiecolt-Glaser, J. K. (2005). Stress-induced immune dysfunction: implications for health. Nature Reviews Immunology, 5(3), 243-251.

Hassan, S., Karpova, Y., Baiz, D., Yancey, D., Pullikuth, A., Flores, A., ... & Datta, S. R. (2013). Behavioral stress accelerates prostate cancer development in mice. The Journal of clinical investigation, 123(2).

Hayashi, T. (2015). Conversion of psychological stress into cellular stress response: Roles of the sigma-1 receptor in the process. Psychiatry and clinical neurosciences, 69(4), 179-191.

Henderson, J. W., & Donatelle, R. J. (2004). Complementary and alternative medicine use by women after completion of allopathic treatment for breast cancer. Alternative Therapies in Health & Medicine, 10(1).

Irie, M., Asami, S., Nagata, S., Miyata, M., & Kasai, H. (2000). Classical conditioning of oxidative DNA damage in rats. Neuroscience letters, 288(1), 13-16.

Kiecolt-Glaser, J. K., Bennett, J. M., Andridge, R., Peng, J., Shapiro, C. L., Malarkey, W. B., ... & Glaser, R. (2014). Yoga's impact on inflammation, mood, and fatigue in breast cancer survivors: a randomized controlled trial. Journal of Clinical Oncology, 32(10), 1040.

Lengacher, C. A., Reich, R. R., Kip, K. E., Barta, M., Ramesar, S., Paterson, C. L., ... & Park, H. Y. (2014). Influence of mindfulness-based stress reduction (MBSR) on telomerase activity in women with breast cancer (BC). Biological research for nursing, 16(4), 438-447.

Lötzke, D., Wiedemann, F., Rodrigues Recchia, D., Ostermann, T., Sattler, D., Ettl, J., ... & Büssing, A. (2016). Iyengar-yoga compared to exercise as a therapeutic intervention during (neo) adjuvant therapy in women with stage I–III breast cancer: health-related quality of life, mindfulness, spirituality, life satisfaction, and cancer-related fatigue. *Evidence-Based Complementary and Alternative Medicine*, *2016*.

Mariotti, A. (2015). The effects of chronic stress on health: new insights into the molecular mechanisms of brain–body communication. Future science OA, 1(3).

Miller, G. E., Chen, E., & Parker, K. J. (2011). Psychological stress in childhood and susceptibility to the chronic diseases of aging: moving toward a model of behavioral and biological mechanisms. Psychological bulletin, 137(6), 959.

Moreno-Villanueva, M., von Scheven, G., Feiveson, A., Bürkle, A., Wu, H., & Goel, N. (2018). The degree of radiation-induced DNA strand breaks is altered by acute sleep deprivation and psychological stress and is associated with cognitive performance in humans. Sleep, 41(7), 067.

Nambiar, M., Kari, V., & Raghavan, S. C. (2008). Chromosomal translocations in cancer. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer, 1786(2), 139-152.

Nambiar, M., & Raghavan, S. C. (2010). Prevalence and analysis of t (14; 18) and t (11; 14) chromosomal translocations in healthy Indian population. Annals of hematology, 89(1), 35.

Nambiar, M., & Raghavan, S. C. (2013). Chromosomal translocations among the healthy human population: implications in oncogenesis. Cellular and Molecular Life Sciences, 70(8), 1381- 1392.

Natu, M. V., & Agarwal, A. K. (1997). Testing of stimulant effects of coffee on the psychomotor performance: An exercise in clinical pharmacology. Indian journal of pharmacology, 29(1), 11.

Ram, A., Banerjee, B., Hosakote, V. S., Rao, R. M., & Nagarathna, R. (2013). Comparison of lymphocyte apoptotic index and qualitative DNA damage in yoga practitioners and breast cancer patients: A pilot study. International journal of yoga, 6(1), 20.

Rao, R. M., Vadiraja, H. S., Nagaratna, R., Gopinath, K. S., Patil, S., Diwakar, R. B., ... & Nagendra, H. R. (2017). Effect of yoga on sleep quality and neuroendocrine immune response in metastatic breast cancer patients. Indian journal of palliative care, 23(3), 253.

Roulland, S., Navarro, J. M., Grenot, P., Milili, M., Agopian, J., Montpellier, B., ... & Nadel, B. (2006). Follicular lymphoma-like B cells in healthy individuals: a novel intermediate step in early lymphomagenesis. The Journal of experimental medicine, 203(11), 2425-2431.

Satsangi, A., & Kumar, S. (2018). YOGIC AND MODERN EDUCATION SYSTEM: GUNAS. International journal of Movement Education and Social Science, 7(1), 116-125.

Schüler, F., Hirt, C., & Dölken, G. (2003, June). Chromosomal translocation t (14; 18) in healthy individuals. In Seminars in cancer biology, 13(3), 203-209.

Shastri, A.D. (2008). Hindi commentary: Ayurveda-Tattva-Sandipika on Sushruta Samhita of Sushruta. Varanasi: Chaukambha Sanskrith Samsthana.

Smith, K. B., & Pukall, C. F. (2009). An evidence‐based review of yoga as a complementary intervention for patients with cancer. Psycho‐Oncology: Journal of the Psychological, Social and Behavioral Dimensions of Cancer, 18(5), 465-475.

Sudsuang, R., Chentanez, V., & Veluvan, K. (1991). Effect of Buddhist meditation on serum cortisol and total protein levels, blood pressure, pulse rate, lung volume and reaction time. Physiology & behavior, 50(3), 543-548.

Tiwari, A., Khatik, R., Kanungo, N., & Kanungo, V. (2019). Agni Dushti and Associated Diseases: An Ayurveda Perspective. Journal of Drug Delivery and Therapeutics, 9(3), 881-884.

Tolahunase, M., Sagar, R., & Dada, R. (2017). Impact of yoga and meditation on cellular aging in apparently healthy individuals: a prospective, open-label single-arm exploratory study. *Oxidative medicine and cellular longevity*, *2017*.

Vadiraja, H. S., Rao, M. R., Nagarathna, R., Nagendra, H. R., Rekha, M., Vanitha, N., ... & Ajaikumar, B. S. (2009). Effects of yoga program on quality of life and affect in early breast cancer patients undergoing adjuvant radiotherapy: a randomized controlled trial. *Complementary therapies in medicine*, *17*(5-6), 274-280.

Yadav, R. K., Magan, D., Mehta, N., Sharma, R., & Mahapatra, S. C. (2012). Efficacy of a short-term yoga-based lifestyle intervention in reducing stress and inflammation: preliminary results. *The journal of alternative and complementary medicine*, *18*(7), 662-667.

Yasuda, M. T., Sakakibara, H., & Shimoi, K. (2017). Estrogen-and stress-induced DNA damage in breast cancer and chemoprevention with dietary flavonoid. Genes and Environment, 39(1), 10.

Importance of the proposed project in the context of current status

We already have evidence of association of psychological stress with DNA damage. A lot of studies also suggest that Yoga has a positive effect on stress and DNA damage. However, the mechanism of how yoga affects the DNA damage response and DNA repair pathways is not known. To the best of our knowledge, no study in literature has tried to assess the effect of yoga on DDR and DNA repair pathways at the molecular level. Therefore, in the present study we propose to investigate the role of yoga in modulating DNA damage response and repair which will further help in understanding if yoga can prevent life-threatening diseases like cancer. This study can further be extended to evaluate if yoga can potentiate the therapeutic efficacy of anti-cancer drugs.

Review of expertise available with proposed investigating group/institution in the subject of the project

Principal investigator (Dr. Sheetal Sharma) is working as an Additional Professor at Department of Experimental medicine and Biotechnology, PGIMER, Chandigarh. She is a molecular biologist with more than ten years of research experience in biochemistry and cell biology. She has been involved in both basic and pre-clinical research in cancer biology with a major focus on studying molecular mechanisms and targeting DNA damage response and repair. Thus, it is clear that PI has appropriate competency to carry out this research.

Co-investigator Dr Akshay Anand is certified QCI level 1 yoga trainer and is professor of Neurosciences at PGIMER, Chandigarh, and has a certified lab for assessing molecular biomarkers. Also, he is involved in many yoga trials including AYUSH projects.

Work plan:

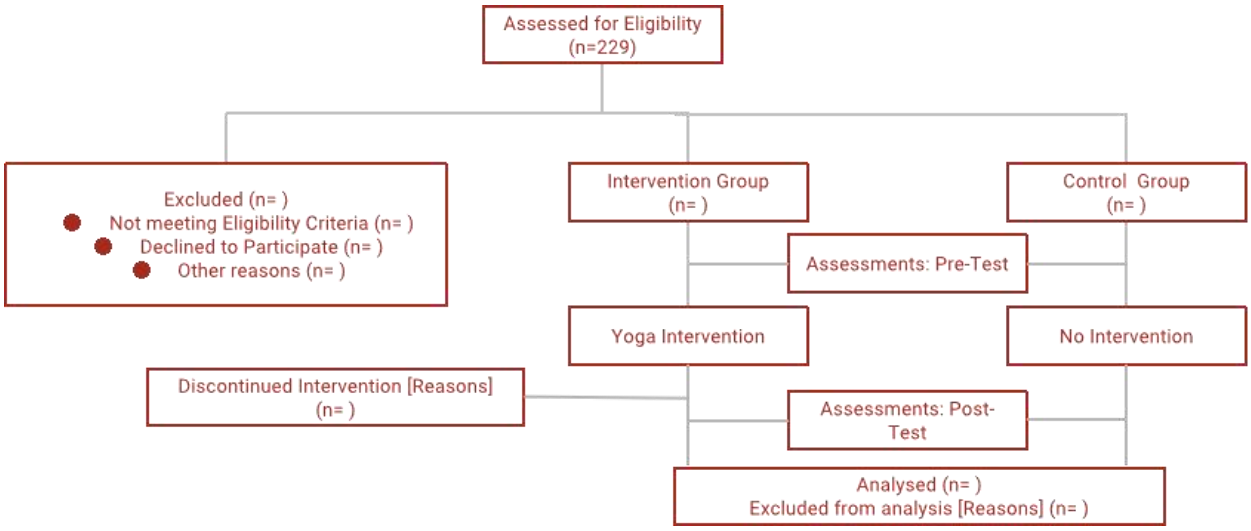
Methodology

Recruitment of individuals: Department of Police, Chandigarh has agreed to help us on this aspect. Policemen working for Chandigarh Police will be chosen based on the inclusion and exclusion criteria.

Subjects: Those security guards (age: >20 and <60) who are willing to consent to participate in this study will be screened for moderate and higher stress on Cohen's PSS, according to inclusion criteria and randomly divided between Intervention (Yoga Group) and Control Group.

Sample Size: The sample size of 206 was calculated for 99% confidence level (reliability coefficient = 2.58), at the alpha level of 0.05, a power of 0.95, and a relative precision of 0.2, derived from a pilot study done on breast cancer patients and yoga practitioners (Ram et al, 2013). Assuming a 10% drop out rate, a total of 229 subjects will be recruited.

Flow Chart 3: Allocation of Sample Size According to Consort Guidelines



-

Inclusion criteria

i. Guards scoring more than 15 points on Cohen's scale. Once these subjects' consent to enroll, each one of them will be coded to maintain anonymity.
ii. Both males and female guards of age 20 to 60 years.

Exclusion criteria

i. Guards with complications in co-morbid conditions like Diabetes, Cirrhosis, *etc.*

ii. Guards of age less than 20 or more than 60 years.

iii. Guards who do not consent to be a part of this study.

iv. Guards who fail to comply with the yoga protocol in Intervention Group.

Design of the study

In a randomized control trial (RCT), participants are allocated randomly between intervention and control groups to compare the treatments, which enables statistical control over the influences imparted by the aforementioned treatments. In the present study, the policemen who satisfy our eligibility criteria and willing to consent to participate in our study will be enrolled, grouped randomly in intervention and control groups, and assessed for the dependent variables of the study before and after the Integrated Yoga intervention period.

Variables

- Socio-demographic details – age, diet, alcohol consumption, etc.
- Evaluation of stress level on Cohen's Perceived Stress Scale (pre and post intervention) (Cohen, Kamarck & Mermelstein, 1983)
- Evaluation of stress level using vital parameters: Blood pressure and resting heart rate of participants will be recorded pre and post intervention.

- Evaluation of stress level using molecular parameters: (pre and post intervention) 10 ml of blood will be withdrawn from the subjects by trained phlebotomists, and coded. WBCs and plasma will be separated using standard protocols and stored at -80℃. Plasma will be utilized to assess the levels of cortisol.
- Assessment of chromosomal translocations: (pre and post intervention) Genomic DNA will be extracted from WBCs isolated, and tested for chromosomal translocations using PCR assay. Isolated genomic DNA will be subjected to nested PCR using primers specifically designed to check for t(14;18) and t(11;14) translocations.
- Assessment of DDR and DNA repair:
- *Immunofluorescence*: Isolated PBMCs will be subjected to DDR analysis by immunofluorescence. For this, these cells will be fixed and stained with various DDR markers like γH2AX, 53BP1, pATM.
- *Gene expression analysis*: Total RNA will be isolated from these cells; cDNA will be synthesized and the expression of following DDR and DNA repair genes will be performed using real-time PCR: RAD51, MRE11, RAD50, NBS1, KU70, LIGASE IV, PARP1
- *Comet assay*: Cells will be used to perform both the neutral as well as alkaline comet assay to assess for the number of single- and double-strand breaks.
- Telomeric length: Relative telomere length (TL) using quantitative polymerase chain reaction (qPCR) will be performed on genomic DNA isolated from control and group blood samples and will be compared.

Time Line for experiments:

S.No.	**Experiments**	**Time point**
1.	Cortisol levels	0 day
2.	DDR: Immunofluorescence for γH2AX, 53BP1, and ATM. Gene expression analysis for KU70, LIGASE IV, RAD51,	0 day

	PARP1, MRE11, RAD50, NBS1	
3.	Nested PCR for Chromosomal Translocations	0 day
4.	DNA repair: Comet assay	0 day
5.	Cortisol levels	3 months
6.	DDR: Immunofluorescence for γH2AX, 53BP1, and ATM. Gene expression analysis for KU70, LIGASE IV, RAD51, PARP1, MRE11, RAD50, NBS1	3 months
7.	Nested PCR for Chromosomal Translocations	3 months
8.	DNA repair: Comet assay	3 months

Intervention: Specific Yoga module designed for proposed work

'Practice Time
*Transition Time

Week		1	2	3	4	5	6	7	8	9	10	11	12	13	Duration
Starting Prayer		*Mahāmṛtyuñjaya-mantra*													1 Min'
Cleansing technique (śūddhi kriyā)		Kapālabhātī													2 Min' + 1 Min*
		30 strokes			40 strokes			50 strokes			60 strokes				
Loosening practices (sūkṣmavyāyāma)															5 Min' + 1 Min*
	Griva-shakti vikasak kriya														
	Anguli-shakti vikasak kriya														
	Manibandh-shakti vikasak kriya														
	Karaprashtha-shakti vikasak kriya														
	Kati-shakti vikasak kriya i & ii														
	Janu-shakti vikasak kriya														
	Pindali-shakti vikasak kriya														
	Gulpha-pada-prstha-pada-tala-shakti vikasak kriya														
Relaxation		Instant Relaxation Technique													30 Sec' + 30 Sec*
Sun salutations (sūryanamaskāra)		1 round			2 rounds			4 rounds			6 rounds				10 Min' + 1 Min*
Relaxation		Quick Relaxation Technique													2 Min' + 1 Min*
Postures (Āsana)															14 Min' + 6 Min*
Standing Postures	*Ardhakati Cakrāsana*														
	Trikoṇāsana														
	Parivṛtta Trikoṇāsana														
	Pārśvakoṇāsana														
Sitting Postures	*Vajrāsana*														
	Uṣṭrāsana														
	Vakrāsana														
	Ardhamatsyendrāsana														
Prone Postures	*Bhujaṃgāsana*														
	Ardha-dhanurāsana														
	Dhanurāsana														
	Ardha-śalabhāsana,														
	Śalabhāsana														
Supine Postures	*Sētubaṁdhāsana*														
	Matsyāsana														
	Uttānpādāsana														
	Sarvāṁgāsana														
	Halāsana														
Breathing Exercises (Prāṇāyāma)															
	Vibhāgīya svasana	8 rounds + 8 rounds + 8 rounds													3 Min' + 30 Sec*
	Bhrāmarī	9 rounds													2 Min' + 30 Sec*
	Nāḍī Śodhana	2 rounds		4 rounds				6 rounds							4 Min' + 1 Min*
Meditation (Dhyāna)		(Guided)													7 Min' + 1 Min*
Closing Prayer		*Śantī Mantra*													1 Min'
Duration per Session															**65 Minutes**

This module was designed and validated for stress among Antarctic expeditioners (Balakrishnan et al., 2019). The classical Yoga texts does not specify symptom based guidelines to practice Yoga, because the primary objective of Yoga is to gain mastery over mind. The observed physical and mental benefits are by-products of yoga practice. Therefore, the practices included in this module were selected from the ancient texts based on their approximating descriptions of mental and physical health benefits and the feasibility of practice by commoners. This Yoga module was validated by 30 experts who agreed to most of the practices and the final module was used as an intervention in the 35th Indian Scientific Expedition to Antarctica.

Total duration in weeks/months for yoga therapy: 3 months (13 Weeks: 5 days a week)

Time schedule of activities giving milestones

First year:

A. Recruit policemen and get them to fill a questionnaire in order to identify most stressed individuals.
B. Obtain blood samples, isolate PBMCs, serum and plasma. Biochemical analysis to test for cortisol levels will be performed.
C. The subjects will be given yoga training for 3 months along with studying DNA damage response and repair using comet assay, immunofluorescence and real-time PCR.
D. Follow up of the subjects to ensure compliance for yoga protocol.

Second year:

A. Continue with isolation of plasma, serum and PBMCs for studying DDR and DNA repair for the next group.
B. Analysis of data from the first year will be performed

Third year:

Compiling of data, interpretation and analysis.