Comparison of lymphocyte apoptotic index and qualitative DNA damage in yoga practitioners and breast cancer patients: A pilot study

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INTRODUCTION

Cancer appears to be an ever growing disease and a leading cause of death worldwide. It accounted for 7.4 million deaths (or about 13% of all deaths worldwide) in 2008. There has been a constant increase in the incidence of cancer. World statistics indicate that in India alone 22.2% of women presently suffer from cancer, which is expected to increase to almost 30% in the next 5 years. Research to understand the etiology and eradicate the tumor burden without harming the host has progressed with many success stories that have resulted in cure (in a few cancers), improved longevity and quality life. In spite of these fascinating advances, treatment of cancer is laden with multiple side effects. Some degree of damage to normal healthy tissues is an expected side effect of both chemotherapeutic and radiation therapies. Continuing attempts to reduce these effects have had many success stories, although not yet complete. Radiation therapy is associated with known imbalances that result in increased apoptosis[3] and other chromosomal abnormalities.[4]

ABSTRACT

Background: Yoga is found to be effective in reducing stress levels and radiation-induced DNA damage, and improving the quality of life, in breast cancer patients. The present study was aimed at comparing the apoptotic index (AI) and DNA damage of advanced yoga practitioners with those of breast cancer patients.

Materials and Methods: This cross-sectional pilot study compared three groups (n = 9 each) of age-matched subjects viz. (1) Carcinoma breast patients in stage II or III undergoing radiation therapy after completing three cycles of chemotherapy; (2) Senior yoga practitioners who were practicing asanas, pranayama and meditation daily for more than 10 years; and (3) Normal healthy volunteers. Peripheral blood lymphocytes were isolated, and qualitative DNA damage (QDD) and AI were evaluated by single-cell gel electrophoresis assay. Approximately 500 cells were counted in each case. Number of cells that were normal, undergoing apoptosis, and with DNA damage were categorized and percentages were calculated.

Results: Data being normally distributed, one-way analysis of variance (ANOVA) showed significant interaction between groups in AI (P = 0.016) and QDD (P = 0.045). On post-hoc analysis using Scheffe test, AI was significantly lower in non-yoga volunteers as compared with the breast cancer group (P = 0.019) and QDD was significantly lower in yoga practitioners when compared with non-yoga volunteers (P = 0.047).

Conclusion: Cellular dysfunction requires restorative mechanisms to restore the system to a balance. The results of this pilot study show trends, which indicate that in ill-health, there is inadequate restorative mechanisms although dysfunction is high. Through regular practice of yoga, cellular dysfunction can be lowered, thus necessitating reduced restorative mechanisms. AI and QDD could also be useful indicators for predicting the three zones of health viz. disease, health, and positive health.

Key words: Apoptotic index; breast cancer; comet assay; DNA damage; yoga.
Apoptosis or programmed cell death in the tissues is a normal phenomenon, which is a very important and inevitable event in the remodeling of tissues during development and aging.[15] It is a crucial process for eliminating cancer cells.[16] Most carcinogens appear to induce tumors by damaging cellular DNA that results in abnormal cells.[7] Apoptosis is one of the protective mechanisms by which cells undergo self-suicide in response to DNA damage. Given that faulty DNA repair is associated with an increased incidence of abnormal cells,[7] the processes for repair or destruction of damaged cellular DNA are critical when it comes to defending the body against carcinogens. The studies on liver damage and neoplastic lesions suggest an extremely important role for apoptosis in controlling cancer.[16] It was seen in various studies that DNA damage and apoptosis tended to increase with the grade of the tumor.[16] Key steps crucial to progress of tumor progression are genomic instability and escape from apoptosis.[16] Studies also indicate that the older population shows higher basal levels of DNA damage and more sensitivity to DNA-damaging agents than the younger population.[11]

The possibility of a linkage between emotional distress and DNA repair was explored in a study using peripheral blood lymphocytes (PBLs) obtained from patients in a psychiatric hospital.[12] The results showed that lymphocytes from psychiatric patients demonstrated greater impairments relative to controls, in their ability to repair damaged cellular DNA, and those who were more depressed showed significantly poorer repair of damaged DNA than their less depressed counterparts. In another study[13] 45 rats, half of which were assigned to a rotational stress condition, were fed a carcinogen. The levels of the DNA repair enzyme (methyltransferase) induced in response to carcinogen damage were significantly lower in the stressed animals’ splenic lymphocytes.[11]

Research has also documented inhibition of apoptosis by stress,[14] which in turn could result in suppression of immune function. Tomei et al.[14] showed that examination stress in medical students enhanced the inhibition of radiation-induced apoptosis in PBLs. Thus it appears that psychosocial stressors could ultimately lead to progressive accumulation of errors within cell genomes as well as reducing tumor-specific and innate immune responses.[15]

A number of researchers have shown that stress-reducing interventions can improve immune functions.[16] The first well-controlled demonstration of immune enhancement via behavioral intervention came from a study on normal healthy adults that showed significant enhancement in natural killer (NK) cell activity, with concomitant decreases in distress-related symptomatology after 1 month of relaxation training.[17] One of the comprehensive intervention studies in cancer research evaluated both the immediate and longer term effects of a 6-week structured group intervention that consisted of health education and stress management techniques such as relaxation and psychological support[18,19] in patients with stage-I or -II malignant melanoma. A 6-year follow-up showed a trend toward greater recurrence, as well as higher mortality rates, among patients in the control group when compared with the patients in the intervention group.[16] In a randomized control study, Vadiraja et al.[20] compared the effects of an integrated yoga program with brief supportive therapy in 88 breast cancer outpatients undergoing adjuvant radiotherapy and showed decreases in anxiety, depression, perceived stress, 6 a.m. salivary cortisol, and pooled mean cortisol levels in the yoga group compared with controls. We studied the effects of an integrated yoga program in modulating perceived stress levels, anxiety, as well as depression levels and radiation-induced DNA damage, in 68 breast cancer patients undergoing radiotherapy. Radiation-induced DNA damage after radiotherapy was significantly elevated in both the yoga and the control groups, with a trend of a lesser level of DNA damage in the yoga group. There was also significant decrease in perceived stress and negative affect, with increase in positive affect after yoga.[21]

**MATERIALS AND METHODS**

This was a three-armed cross-sectional design that compared the apoptotic index (AI) and qualitative DNA damage (QDD) in three groups of age-matched subjects: (1) Carcinoma breast patients; (2) Advanced yoga practitioners; and (3) Normal healthy volunteers. Recruited for the study were 13 women with breast cancer in stage II or III who were referred to the radiology department for radiation therapy after completing three cycles of chemotherapy at Manipal Hospital and Bangalore Institute of Oncology. The inclusion criteria were (a) adult females in the age range 35-70 years with carcinoma of the breast in stage II or III undergoing radiation therapy and (b) performance status of 0-3 on Zubrod’s scale. Those with metastatic breast cancer; those who were on steroids; and those with other major medical conditions such as diabetes, coronary heart disease, and/or a major psychiatric illness were excluded.

The second group consisted of 11 age-matched (± 2 years) individuals from two yoga institutions (VYASA and Yogashree (the yoga wing of Hindu Seva Pratishthana)) with experience in regular practice of yoga for at least 1 h per day for more than 10 years. The third group of 10 normal healthy volunteers who did not have experience of yoga was selected from the staff of both the hospitals. Those who had any symptoms, illnesses, were on any form of medication, smoked, or consumed alcohol were not included in groups 2 and 3. This study was approved by the institutional ethical review committee and consent was
sought from all the subjects of the study. Five milliliters of fasting blood was drawn from the antecubital vein into heparinized vacutainers between 8:00 a.m. to 10:00 a.m. in the hospital premises by a laboratory technician. The samples were coded and analyzed for AI and QDD at Manipal Hospital by a blinded investigator.

Blinding

The PBL samples were coded to blind for groups and age.

Yoga practices

The advanced yoga practitioners included in this study were all senior yoga teachers who were teaching and practicing yoga daily regularly (5-7 days/week) for several years (>10 years). All of them had a routine of doing integrated yoga that included a few asanas, pranayama, and meditation.

Assessments and data extraction

The PBLs were isolated from the blood samples by the Ficoll density-gradient method using Histopaque 1077 (Sigma Aldrich, St Louis, MO, USA). Single-cell gel electrophoresis or comet assay was conducted according to the prescribed protocol. Cells embedded in agarose were lysed, subjected briefly to an electric field, stained with a fluorescent DNA-binding stain, and viewed using a fluorescence microscope. Fragmented DNA migrates farther in the electric field, and the cell then resembles a “comet” with a brightly fluorescent head and a tail region, which increases as damage increases. Slides were treated with the DNA-binding dye propidium iodide (1 mg/ml) (Sigma Aldrich) and viewed with appropriate filters at ×40 [Figure 1]. No standardization was necessary as this was a qualitative test and the comet cells, apoptotic cells, and normal cells were clearly distinguishable by the trained researcher who counted the cells in each of the coded slides. Approximately 500 cells were scored in total for each of the samples. The number of apoptotic cells and comets was expressed as a percentage of the total number of cells counted.

Data analysis

Data were cumulated and descriptive statistics were calculated. Data being normally distributed, comparisons were made using one-way analysis of variance (ANOVA) to evaluate the interactions between the three groups. Post-hoc tests (Scheffe test) were conducted to isolate the groups with significant differences.

RESULTS

The final numbers available for analysis were nine in each group. Demographic data showed that mean age was 46.67 ± 10.79 years in women with breast cancer (BC), 48.44 ± 10.91 in senior yoga (SY), and 47.11 ± 9.99 in non-yoga volunteers (NV). The values for QDD and AI were normally distributed. Table 1 shows the mean and standard deviation values for each of the groups followed by one-way ANOVA and Scheffe test for group differences.

Apoptotic index

One-way ANOVA showed that there was significant group interaction between the three groups ($F_{(2,24)} = 4.973, P = 0.016$). Post-hoc analyses using Scheffe test revealed that percentage apoptosis was significantly lower in the yoga group as compared with non-yoga volunteers ($P = 0.019$).

DNA damage

Percentage of comet cells was highest in the cancer patients and least in the senior yoga practitioners. One-way ANOVA showed significant group interaction between groups ($F_{(2,24)} = 3.534, P = 0.045$). On post-hoc analyses using Scheffe test, significantly lower comet percentages were seen in the senior yoga practitioners as compared with the breast cancer group ($P = 0.047$) [Table 1].

Thus, percentage apoptosis and levels of DNA damage showed significant group interactions with significant differences between the yoga and non-yoga groups and the breast cancer and yoga groups for percentage apoptosis and DNA damage, respectively.

| Group | Number of cells counted | % Apoptosis (AI) ANOVA, $P=0.016$ | % Comet (QDD) ANOVA, $P=0.045$ |
|-------|-------------------------|----------------------------------|-------------------------------|
|       |                         | % Post-hoc* sig. | % Post-hoc* sig. |
| BC    | 406.22±177.23           | 10.05±3.24 | BC:SY 3.13±1.74 | BC:SY 0.47† |
| SY    | 510.55±49.27            | 8.79±3.08 | SY:NV 1.53±1.00 | SY:NV 0.313 |
| NV    | 512.88±82.92            | 13.17±2.77 | NV:BC 2.47±0.93 | NV:BC 0.564 |

AI: Apoptotic index; ANOVA: Analysis of variance; BC: Breast cancer; NV: Non-yoga volunteers; QDD: Qualitative DNA damage; SY: Senior yoga practitioners

*Post-hoc analysis by Scheffe test † $P<0.05$ Observations: (1) Significant group interaction in both variables. (2) Least QDD in SY group. (3) Highest AI in NV group.
DISCUSSION

In this cross-sectional pilot study three groups of subjects viz. senior yoga practitioners (SY) \((n = 9)\), healthy non-yoga volunteers (NV) \((n = 9)\), and patients with carcinoma of breast undergoing radiotherapy after three cycles of chemotherapy (BC) \((n = 9)\) were selected. The results showed that the percentage apoptosis and DNA damage were least in the SY group. Percentage apoptosis was highest in the NV group and percentage comet was highest in the breast cancer group. Significant group interactions were observed as tested by one-way ANOVA.

Apoptosis is a process of genetically programmed alternations of cell structure that leads to failure of proliferation and differentiation, and eventual cell death. Apoptosis is induced by a variety of toxic cellular insults and is crucial for recognition and disposal of toxins and unhealthy cells. It provides an indication of the body’s response to physical and chemical stresses on the tissues. The need for apoptosis arises when regular functions like aging, protein profiles, genetic integrity, and inter-cellular signaling pathways are dysregulated to the extent that it deviates from normal homeostasis. This process may function to protect against the appearance of heritable phenotypic changes in cells and may be a critical factor in normal cellular immune function. Therefore AI is an indicator of the rate of toxin build-up at the cellular level. A high AI observed in the NV group indicates that the cellular environment required frequent “housekeeping”. A low AI in the yoga group would therefore indicate that the rate of cellular toxin build-up was low.

DNA is a repository of genetic information in each living cell, its integrity and stability being essential to life. It is subject to assault from the environment, and the resulting damage, if not repaired, leads to mutation and possibly disease. DNA damage could be the result of excessive exposure to UV radiation, tobacco smoke, mutations during DNA replication, byproducts of metabolism, and oxidative stress amongst others. In the present study, breast cancer patients who underwent therapeutic strategies (radiotherapy and chemotherapy) showed significantly higher DNA damage levels as compared with yoga practitioners. This could be due to treatment-related insult to the DNA. Psychological stress responses affect metabolic byproducts and oxidative stress, which could have contributed to the higher values of DNA damage in this group. The trend of low values of DNA damage in the yoga group as compared with the non-yoga group, although non-significant, could indicate a reversal of these stress-induced physiological and cellular changes. Hence, regular yoga practice may help to keep up the integrity of the DNA in breast cancer patients during conventional treatment modalities.

The authors have also tried to suggest a model of healthy aging utilizing both values of AI and QDD. This model works on the premise that QDD is an index of ill-health and AI is the ability to restore health in the body. The values for breast cancer patients have high levels of QDD with low values of AI, indicating illness with the inability to heal. In comparison, normal individuals without exposure to yoga show moderate levels of QDD but a high AI, indicating that the system is in a state of “high alert,” with restorative mechanisms at heightened levels. The third group of senior yoga practitioners (SY) however had low values for both AI and QDD, suggesting that regular long-term practice of awareness building and internalization achieved through yoga practice could improve the efficiency of the system. Mindful awareness of yoga brings about stress reduction and hence metabolic and oxidative homeostasis, which would percolate into cellular processes such as preserving the integrity of the DNA, resulting in reduced requirement of restorative mechanisms. This is represented figuratively [Figure 2] as a linear progression from disease, through health, toward positive health.

In conclusion, we may state that when one adopts a yogic way of life with minimal or no abuse to the body and mind, it tends toward a healthy body, which reflects in the cellular parameters of AI and DNA damage. Regular yoga practice could also be the key to healthy senescence as it could have a buffering effect on age-dependant DNA damage and repair capacity. Thus this pilot study paves the road map for future study of the synergistic effects of yoga and conventional therapeutic strategies on cellular health and aging.
for designing more robust studies using these variables.

Limitations of the study

This was a pilot experiment to look for directional differences between the three cohorts and hence, small yet heterogeneous cohorts were involved. Data from a fourth group consisting of cancer patients who had prolonged exposure to yoga practice would have been more effective in understanding the differences. Also, newly diagnosed and advanced-stage breast cancer groups would have added to the evidence of the hypothesis. Gender differences could have confounded the comparisons, although an attempt was made to match the age between the three groups. The technique of estimating the AI and QDD was manual observation of the morphology of cells at low magnification. More objective and accurate measures are advised if this study is not exploratory in nature.

Strength of the study

There are several studies on brain processes in senior meditators of vipassana, transcendental meditation, etc. But, to the best of our knowledge, this is the first study that has looked at cellular functions like AI in yoga practitioners and compared it with those of cancer patients undergoing radiation. This study provides direction for further investigations in order to understand fundamental differences between health and disease. The results of this study helped us to propose a new hypothesis of disease, health, and positive health, which needs validation by well-designed studies in future.

Suggestions for future research

As initiation, progression, and therapy of cancer are laden with many cellular, immunological, and psychological factors, it is important to have a comprehensive set of measures to understand the impact of yoga in cancer. In addition to apoptosis, the role of the complex components of the immune system such as cytokines and their respective transcription factors such as nuclear factor-kB (NF-kB)\(^{27}\) in disease and health are recognized. We propose future studies using a comprehensive battery of these cellular and immune measures. A four-armed study to compare the immune variables and NF-kB in age- and sex-matched patients of breast cancer with and without yoga, and normal volunteers with and without yoga, is presently underway.

REFERENCES

1. World Health Organization. World health Report factsheet [Internet]. Factsheet, 2012. Available from: http://www.who.int/mediacentre/factsheets/fs297/en/index.html [Last cited on 2012 Apr 24].
2. Ferlay J, Shin H, Bray F, Forman D, Mathers C, Parkin D. GLOBOCAN 2008 v1.2, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 10. International Agency for Research on Cancer. 2010. Available from: http://globocan.iarc.fr [Last cited on 2011 Jun 12].
3. Burke MA, Goodkin K. Stress and the development of breast cancer: A persistent and popular link despite contrary evidence. Cancer 1997;79:1055-9.
4. Janakiramaiah N, Gangadhar BN, Naga Venkatesha Murthy PJ, Harish MG, Subbakrishna DK, Vedamurthachar A. Antidepressant efficacy of Sudarshan Kriya Yoga (SKY) in melancholia: A randomized comparison with electroconvulsive therapy (ECT) and imipramine. J Affect Disord 2000;57:255-9.
5. Wyllie AH, Kerr JF, Currie AR. Cell death: The significance of apoptosis. Int Rev Cytol 1980;68:251-306.
6. Nicholson DW, Ali A, Thornberry NA, Vaillancourt JP, Ding CK, Gallant M, et al. Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. Nature 1995;376:37-43.
7. Setlow RB. Repair deficient human disorders and cancer. Nature 1978;271:713-7.
8. Warner HR. Aging and regulation of apoptosis. Curr Top Cell Regul 1997;35:107-21.
9. Gajecka M, Rydzanicz M, Jaskula-Sztul R, Wierzbicka M, Szyfter W, Szyfter K. Reduced DNA repair capacity in laryngeal cancer subjects. A comparison of phenotypic and genotypic results. Adv Otorhinolaryngol 2005;62:25-37.
10. Tripathi P, Aggarwal A. NF-kB transcription factor: A key player in the generation of immune response. Curr Sci India 2006;90:519-31.
11. Piperakis SM, Kontogianni K, Karanastasi G, Iakovidou-Kritsi Z, Piperakis MM. The use of comet assay in measuring DNA damage and repair efficiency in child, adult, and old age populations. Cell Biol Toxicol 2009;25:65-71.
12. Kiecolt-Glaser JK, Stephens RE, Lipetz PD, Speicher CE, Glaser R. Distress and DNA repair in human lymphocytes. J Behav Med 1985;8:311-20.
13. Glaser R, Thorn BE, Tarr KL, Kiecolt-Glaser JK, D’Ambrosio SM. Effects of stress on methyltransferase synthesis: An important DNA repair enzyme. Health Psychol 1985;4:403-12.
14. Tomei LD, Kiecolt-Glaser JK, Kennedy S, Glaser R. Psychological stress and phorbol ester inhibition of radiation-induced apoptosis in human peripheral blood leukocytes. Psychiatry Res 1990;33:59-71.
15. Kiecolt-Glaser JK, Robles TF, Heffner KL, Loving TJ, Glaser R. Psychoneuroimmunology and cancer: Psychoneuroimmunology and cancer. Ann Oncol 2002;13:165-9.
16. Kiecolt-Glaser JK, Glaser R. Psychoneuroimmunology: Can psychological interventions modulate immunity? J Consult Clin Psychol 1992;60:569-75.
17. Kiecolt-Glaser JK, Glaser R, Williger D, Stout J, Messick G, Sheppard S, et al. Psychosocial enhancement of immunocompetence in a geriatric population. Health Psychol 1985;4:25-41.
18. Fawzy FI, Kemeny ME, Fawzy NW, Elashoff R, Morton D, Cousins N, et al. A structured psychiatric intervention for cancer patients. II. Changes over time in immunological measures. Arch Gen Psychiatry 1990;47:729-35.
19. Fawzy FI, Fawzy NW, Hylan CS, Elashoff R, Guthrie D, Fahey JL, et al.

Figure 2: Difference in Apoptosis% and Comet% for the three cross-sectional groups (BC, breast cancer; NV, normal; SY: Yoga group)
Malignant melanoma. Effects of an early structured psychiatric intervention, coping, and affective state on recurrence and survival 6 years later. Arch Gen Psychiatry 1993;50:681-9.

20. Vadiraja HS, Raghavendra RM, Nagarathna R, Nagendra HR, Rekha M, Vanitha N, et al. Effects of a yoga program on cortisol rhythm and mood states in early breast cancer patients undergoing adjuvant radiotherapy: A randomized controlled trial. Integr Cancer Ther 2009;8:37-46.

21. Banerjee B, Vadiraj HS, Ram A, Rao R, Jayapal M, Gopinath KS, et al. Effects of an integrated yoga program in modulating psychological stress and radiation-induced genotoxic stress in breast cancer patients undergoing radiotherapy. Integr Cancer Ther 2007;6:242-50.

22. Bøyum A. Isolation of lymphocytes, granulocytes and macrophages. Scand J Immunol 1976;5:9-15.

23. Singh NP. A simple method for accurate estimation of apoptotic cells. Experimental cell research. 2000;256(1):328-37.

24. Rojas E, Lopez MC, Valverde M. Single cell gel electrophoresis assay: Methodology and applications. J Chromatogr B Biomed Sci Appl 1999;722:225-54.

25. Andersen BL, Kiecolt-Glaser JK, Glaser R. A biobehavioral model of cancer stress and disease course. Am Psychol 1994;49:389-404.

26. Clancy S. DNA damage and repair: Mechanisms for maintaining DNA integrity. In: Moss B, editor. Nature Education. 1st ed. Cambridge: Nature Publishing Group; 2008.

27. Biswas DK, Shi Q, Baily S, Strickland I, Ghosh S, Pardee AB, et al. NF-kappa B activation in human breast cancer specimens and its role in cell proliferation and apoptosis. Proc Natl Acad Sci USA 2004;101:10137-42.

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