CHAPTER 10

CORRELATIVE ASSESSMENT OF SPIRITUALITY-INDUCED EFFECTS ON THE NEUROENDOCRINE-IMMUNE NETWORK: EFFECTS ON CELL-MEDIATED IMMUNE FUNCTIONS IN INDIAN WOMEN WITH METASTATIC BREAST CANCER

Specific Objective 3 Correlative assessment of spirituality-induced effects on the neuroendocrine-immune network

10.1 Rationale

Majority of Indians, irrespective of their religious background, are spiritual in nature. Yogic exercises, meditation, chants and prayers are stringent daily rituals followed by many Indian women. This is unlike Western countries where such populations are in minority and are taught to adopt certain practices. Hence, it is relevant to study the effect of spirituality in modulating the neural-endocrine-immune interactions in mammary cancers in the context of Indian women.

10.2 Methods

10.2.1 Study design

Peripheral blood samples was collected from Indian women suffering from metastatic breast cancer coming to the SRM University Hospital and from young and middle-aged volunteers, from SRM University, Chennai, Guduvancheri and
Chengalpettu areas. Subjects were briefly interviewed prior to blood sample collection using the FACIT Spirituality assessment questionnaire according to the instructions specified (A5.1, A5.2, A5.3). Samples from the participants was collected during the same season. Sample collection was done at 08:00 am from volunteering patients and healthy controls from the anterior cubital vein. Both volunteers and patients were given a detailed account of the study one day before sample collection and the sampling was scheduled with informed consent as per the guidelines of the human ethical committee (A6.2). Apart from the FACIT questionnaire, information was also collected about the alternative therapeutic options they were currently undertaking such as Yoga, meditation, or none; exercise habits, stage of cancer, treatment undergone, medications taken and recurrences if any and religious inclination of the patients to be used as additional inclusion or exclusion criteria for the study upon sub-grouping.

The criteria for inclusion or exclusion were as follows: Persons with pre-existing endocrinological, neurological or infectious diseases or those undergoing treatment, pregnancy, lactation, abnormal vaginal bleeding, contraception or hormone therapies/antihistamine therapies were excluded from the study. Healthy female volunteers meeting age-specifications discussed below and do not fall under exclusion criteria were recruited as controls. Volunteering patients suffering from metastatic breast cancer without any pre-existing or known additional complications were recruited for the study.

10.2.2 Groups

1. **Young Control**: 18-22 year old women volunteers were recruited and categorized into two groups based on spiritual inclination (on the basis of FACIT Spirituality Questionnaire Scores): Spiritual and Non-spiritual; (n=30 per group).

2. **Middle-aged Control**: Middle-aged women volunteers (35-60 years) were recruited and categorized into two groups based on spiritual inclination (on the basis of FACIT Spirituality Questionnaire Scores): Spiritual and Non-spiritual; (n=30 per group).

3. **Breast Cancer Patients** (35-60 years): Volunteers from SRM Hospital were recruited and categorized into two groups based on spiritual inclination (on the basis of FACIT Spirituality Questionnaire Scores): Spiritual and Non-spiritual; (n=30 per group).
10.2.3 Experiment 1

PBMCs (2 X 10^5 cells/ml) were cultured with 0.5 to 5 µg/ml of Con A in 96-well, flat-bottom tissue culture plates (Falcon, Becton Dickinson, Oxnard, CA) at 37°C and 5% CO₂. After 72 hours, MTT assay was performed to measure Con A-induced proliferation of lymphocytes PBMCs (2 X 10^5 cells/well) were cultured in 24 well flat-bottom tissue culture plates with 1.25 µg/ml of Con A in supplemented RPMI medium and kept at 37°C in an incubator with 5% CO₂ for 24 hours. After 24 hours, supernatants were collected for assessing VEGF C production using ELISA (eBiosciences, San Diego, CA) and nitric oxide production using Griess Reagent system.

10.2.4 Experiment 2

PBMCs (2 X 10^5 cells/ml) were cultured with 1.25 µg/ml of Con A in 96-well, flat-bottom tissue culture plates (Falcon, Becton Dickinson, Oxnard, CA) at 37°C and 5% CO₂ with the PKA and PKC inhibitor H89 (10 µM) or the ERK inhibitor PD98059 (5 µM), or the Akt inhibitor A6730 (10 µM) or the NF-κB inhibitor BAY11-7082 (5 µM) for 72 hours. After 72 hours, MTT assay was performed to measure the proliferation of lymphocytes.

10.3 Results

10.3.1 Spirituality alters con A-induced PBMC proliferation in women with metastatic breast cancer

Con A-induced proliferation of PBMCs was significantly declined in middle-aged controls (spiritual and non-spiritual) and in cancer patients (spiritual and non-spiritual) compared with Young spiritual controls.

There was no significant difference in con A-induced PBMC proliferation in young and middle-aged controls between the spiritual and non-spiritual sub-groups. However, in cancer patients, the spiritual group showed significantly enhanced con A-induced proliferation compared with the non-spiritual group (Fig. 10.1A).
**10.3.2 Spirituality alters con A-induced PBMC cytokine production in women with metastatic breast cancer**

There was no significant change in the expression of TNF-α (Fig. 10.2A) and IL-6 (Fig. 10.2B) upon stimulation with Con A. However, Con A-induced IL-2 production was significantly decreased in PBMCs isolated from non-spiritual women with breast cancer compared to non-spiritual middle-aged and young controls. There was a significant increase in Con A-induced IL-2 production by PBMCs isolated from spiritual women with breast cancer compared with non-spiritual women with breast cancer (Fig. 10.2C).
There was a significant (p<0.01) age-related decline in Con A-induced IFN-γ production by PBMCs isolated from middle-aged controls and women with breast cancer compared with young controls. Spirituality induced a significant increase in Con A-induced IFN-γ production in both groups compared to respective non-spiritual groups. (Fig. 10.2D).

**Fig. 10.2** Spirituality alters IL-2 and IFN-γ production in breast cancer patients. Spirituality does not alter con A-induced TNF-α (A) and IL-6 (B) production although there was a significant increase in IL-2 (C) and IFN-γ (D) production. *p<0.05 compared
with respective young control (B). #p<0.05 compared with respective non-spiritual group. §p<0.05 compared with respective young control and non-spiritual group.

10.3.3 Spirituality alters con A-induced PBMC nitric oxide production but not VEGF C production in women with metastatic breast cancer

Con A-induced nitric oxide production by PBMCs was significantly declined in middle-aged cancer patients (spiritual and non-spiritual) compared with Young and Middle-aged spiritual controls.

There was a significant (p<0.01) increase in con A-induced PBMC nitric oxide production in middle-aged spiritual group and cancer spiritual group compared with middle-aged non-spiritual group and cancer non-spiritual group respectively. However, there was no significant difference in Con A-induced nitric oxide production among young spiritual and non-spiritual women (Fig. 10.3A).

However, there was no significant change in VEGF C production by Con A-induced PBMCs with age, spiritual status or disease (Fig. 10.3B).

10.3.4 Correlation analysis between spirituality and physical, emotional and functional well-being in women with metastatic breast cancer

A scatter plot analysis of spirituality scores versus physical well-being of women with breast cancer revealed a significant positive correlation (r = 0.54, p < 0.001) between spiritual inclination and the emotional well-being of the patient (Fig. 10.4A). Similarly, a significant positive correlation was observed between spiritual inclination and emotional well-being of women with breast cancer (r = 0.52, p < 0.001; Fig. 10.4B) and spiritual inclination and functional well-being of women with breast cancer (r = 0.52, p < 0.001; Fig. 10.4C).
Fig. 10.3  *Spirituality enhanced Con A-induced NO production alone.* Spirituality enhances con A-induced nitric oxide production (A) but does not alter VEGF C production (B) by PBMCs in women with breast cancer. **p<0.05 compared with respective young and middle-aged Control (B). #p<0.05 compared with respective non-spiritual group.
Fig. 10.4  Spiritual inclination correlates with physical, emotional and functional well-being in cancer patients. Linear regression analysis showed a significant positive correlation between spirituality and physical (A), emotional (B) and functional well-being (C) in women with breast cancer. *p<0.001

10.3.5 Spirituality alters Con A-induced PBMC GPX-1 activity alone in women with metastatic breast cancer
There was a significant decline in the superoxide dismutase (SOD) activity of Con A-stimulated PBMCs isolated from women with breast cancer compared to middle-aged spiritual women and young controls. Age-related decline in SOD activity was also observed in Con A-stimulated PBMCs from spiritual middle-aged women compared with
young. However, the spiritual status of the person did not affect the activity of SOD (Fig. 10.5A).

The catalase (CAT) activity was significantly decreased in Con A-stimulated PBMCs from middle-aged spiritual women compared with young spiritual women although no disease-related alteration was observed. Also, there was no significant difference between the CAT activity of Con A-stimulated PBMCs from spiritual and non-spiritual women with breast cancer, young and middle aged women (Fig. 10.5B).

Glutathione peroxidase activity was significantly decreased with age compared to young women who were spiritual and non-spiritual. There was a significant decrease in the GPx-1 activity of Con A-stimulated PBMCs isolated from non-spiritual middle-aged women and non-spiritual women with breast cancer compared to their respective spiritual counterparts (Fig. 10.5C).

10.3.6 Correlation analysis between spirituality and Con A-induced proliferation and nitric oxide production in women with metastatic breast cancer

A scatter plot analysis of spirituality scores versus Con A-induced proliferation of PBMCs isolated from women with breast cancer revealed a significant positive correlation ($r = 0.45$, $p < 0.001$) between spiritual inclination and the Con A-induced proliferative capacity of PBMCs from breast cancer patients (Fig. 10.6A).

Similarly, a significant positive correlation was observed between spiritual inclination and Con A-induced nitric oxide production from the PBMCs of women with breast cancer ($r = 0.46$, $p < 0.001$; Fig. 10.6B).

Linear regression analysis of spirituality scores against Con A-induced Glutathione peroxidase activity from PBMCs of women with breast cancer showed a significant ($r = 0.51$, $p < 0.001$; Fig. 10.6C) positive correlation between spirituality and Con A-induced PBMC GPx-1 activities.
Figure 10.5  Antioxidant enzyme activities of Con A-stimulated PBMCs decline with age and disease state while GPx-1 alone was altered with spiritual status. Age-related decline was observed in PBMC SOD (A), CAT (B) and GPx-1 (C) activities. SOD activity alone was decreased in spiritual and non-spiritual women with breast cancer compared to young and middle-aged women. GPx-1 activity alone was decreased in non-spiritual middle-aged women and women with breast cancer compared with respective spiritual groups. *p<0.05 compared with young spiritual/ non-spiritual women. #p<0.05
compared to spiritual women of the respective group. §p<0.05 compared with young and middle-aged spiritual women. **p<0.05 compared with young spiritual and respective non-spiritual group).

Fig. 10.6  

_Spirituality correlates with Con A-induced proliferation and GPx-1 activity in cancer patients_  

Linear regression analysis and Pearson’s correlation coefficient obtained showed a significant positive correlation between spirituality and Con A-induced PBMC proliferation (A), Con A-induced PBMC nitric oxide production (B) and Con A-induced GPx-1 activity in PBMCs isolated from women with breast cancer. *p<0.001
10.3.7 Effects of Spirituality on Con A-induced p-ERK, p-CREB and p-Akt expression in PBMCs from spiritual and non-spiritual women

There was a significant increase in the p-ERK expression of Con A-stimulated PBMCs isolated from young spiritual women compared to young non-spiritual women. Breast cancer-related decline in p-ERK expression was also observed in Con A-stimulated PBMCs from non-spiritual women with breast cancer compared with young. Also, spirituality enhanced Con A-induced p-ERK expression in women with breast cancer compared to non-spiritual women with breast cancer (Fig. 10.7A).

The p-CREB expression was significantly decreased in Con A-stimulated PBMCs from spiritual and non-spiritual women with breast cancer compared with young and middle-aged spiritual and non-spiritual women. Also, there was no significant increase in p-CREB expression by spiritual women with breast cancer compared with non-spiritual women with breast cancer (Fig. 10.7B).

p-Akt expression was significantly increased in young spiritual women compared with young non-spiritual women. There was a significant decrease in the p-Akt expression of Con A-stimulated PBMCs isolated from non-spiritual and spiritual middle-aged women and spiritual women with breast cancer compared to their respective young controls (Fig. 10.7C).

![Graph showing p-ERK expression levels in different groups](image)
10.3.8 Inhibition of PKA/PKC, ERK, Akt or NF-κB pathways on Con A-induced PBMC proliferation in spiritual and non-spiritual women

There was a significant age and disease-associated decline in Con A-induced PBMC proliferation in spiritual and non-spiritual middle-aged women with or without breast cancer compared with young spiritual women.

Inhibition of PKA/PKC pathway using H89 significantly decreased proliferation of Con A-induced PBMCs isolated from young spiritual and non-spiritual women and spiritual women with breast cancer (Fig. 10.8A).

Inhibition of ERK signaling using PD98059 (Fig. 10.8B), Akt signaling using A6730 (Fig. 10.8C) or NF-κB pathway using Bay-11-7082 (Fig. 10.8D) significantly decreased proliferation of Con A-induced PBMCs isolated from young spiritual women alone.
Figure 10.8 Spirituality enhances Con A-induced proliferation of PBMCs in cancer patients through activation of PKA pathway. Age-related decline in Con A-induced PBMC proliferation was observed. PKA/PKC blockade using H89 alone decreased proliferation of PBMCs from spiritual women with breast cancer (A) while ERK (B), Akt (C) and NF-kB (D) blockade using PD98059, A6730 and Bay 11-7082 significantly decreased proliferation of PBMCs from spiritual and non-spiritual young women alone.
*p<0.05 compared with young spiritual control (B). §p<0.05 compared with respective spiritual group. #p<0.05 compared with respective group without inhibitor.

10.4 Key Findings:

There was a significant decline in the Con A-induced proliferative capacity of PBMCs isolated from nonspiritual women with breast cancer compared to their spiritual counterparts suggesting that spirituality enhances con A-induced proliferation of PBMCs in cancer patients. Although Con A-induced TNF-α and IL-6 production was not altered with age, disease or spiritual inclination, there was a significant increase in Con A-induced IL-2 and IFN-γ production by PBMCs isolated from spiritual women with breast cancer compared with non-spiritual women with breast cancer.

Also, there was a significant increase in the Con A-induced nitric oxide production in spiritual women compared to non-spiritual women. VEGF C production by PBMCs did not alter with age or disease or spiritual status. Although SOD and CAT activities did not alter with spiritual status, there was a significant decrease in PBMC Gpx-1 activities in non-spiritual women with breast cancer compared to spiritual women with breast cancer.

Correlation analysis using linear regression on scatterplot data showed a significant positive correlation between spirituality and physical (A), emotional (B) and functional well-being (C) as reported by the survey collected from the patients themselves. Also, a significant positive correlation was obtained between Spirituality and Con A-induced PBMC proliferation, Con A-induced PBMC nitric oxide production and Con A-induced GPx-1 activity in PBMCs isolated from women with breast cancer.

There was a significant increase in the p-ERK and p-CREB expression of Con A-stimulated PBMCs isolated from spiritual women with breast cancer suggesting that spirituality enhanced Con A-induced p-ERK and p-CREB expression. Pathway inhibitor data suggests that the beneficial effects of spirituality in cancer may be transduced through the PKA/PKC pathway.
Thus, taken together these results suggest that apart from the beneficial psychosocial effects on physical well-being, emotional well-being and functional well-being of cancer patients, spirituality also enhances cell mediated immune functions as measured by Con A-induced proliferation of PBMCs in cancer patients through activation of PKA pathway, enhanced NO production and GPx-1 activities.