CHAPTER 11

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

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

11.1 Rationale

Age-related decline in neural, immune and endocrine mediators and compensatory mechanisms contributes to the progression of reproductive aging in women and predisposes them to develop female-specific age-associated diseases like autoimmunity, infectious diseases and cancer. The level of psychosocial stress is very high in women with breast cancer and alternative therapies focusing on alleviating it have been shown to have beneficial effects. Hence, it is relevant to study the effect of spirituality as a neuroendocrine-immune modulator on the humoral immune responses of women with breast cancer.
11.2 Methods

11.2.1 Study design

As explained in the previous chapter, peripheral blood samples was collected from Indian women suffering from metastatic breast cancer coming to the SRM University Hospital and healthy volunteers as per ethical committee norms. Subjects were briefly interviewed prior to blood sample collection using the FACIT Spirituality assessment questionnaire. Samples from the participants were collected during the same season. Information was collected about the alternative therapeutic options they were currently undertaking such as Yoga, meditation, or none; stage of cancer, treatment undergone, medications taken and recurrences if any and religious inclination of the patients. Inclusion and exclusion criteria are already described in detailed previously.

11.2.2 Treatment

11.2.2.1 Experiment 1

PBMCs (2 X 10^5 cells/ml) were cultured with 0.01 to 1 µg/ml of LPS 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 LPS-induced proliferation of lymphocytes PBMCs (2 X 10^5 cells/well) were cultured in 24 well flat-bottom tissue culture plates with 0.1 µg/ml of LPS 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 measuring VEGF C production using ELISA kits (eBiosciences, San Diego, CA) and nitric oxide production using Greiss Reagent system.

11.2.2.2 Experiment 2

PBMCs (2 X 10^5 cells/ml) were cultured with 0.1 µg/ml of LPS 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-kB inhibitor BAY11-7082 (5 µM) for 72 hours. After 72 hours, MTT assay was performed to measure the proliferation of lymphocytes.
11.3 Results

11.3.1 Spirituality does not alter LPS-induced PBMC proliferation in women with metastatic breast cancer

LPS-induced proliferation of PBMCs was significantly (p<0.05) 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 LPS-induced PBMC proliferation in young, middle-aged controls and cancer patients between the spiritual and non-spiritual sub-groups (Fig. 11.1A).

Figure 11.1. Correlative effects of spirituality on LPS-induced PBMC proliferation. Spirituality does not affect LPS-induced proliferation of PBMCs in cancer patients LPS-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.*p<0.05 compared with young spiritual control. #p<0.05 compared with respective group without inhibitor.
11.3.2 Spirituality alters LPS-induced PBMC cytokine production in women with metastatic breast cancer

There was no significant change in the expression of TNF-α (Fig. 11.2A) and IL-6 (Fig. 11.2B) upon stimulation with LPS.

However, LPS-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 LPS-induced IL-2 production by PBMCs isolated from spiritual women with breast cancer compared with non-spiritual women with breast cancer (Fig. 11.2C).

There was a significant (p<0.01) decline in LPS-induced IFN-γ production by PBMCs isolated from women with breast cancer compared with young controls. Spirituality did not influence LPS-induced IFN-γ production compared to non-spiritual groups. (Fig. 11.2D).

11.3.3 Spirituality does not alter LPS-induced PBMC nitric oxide production in women with metastatic breast cancer

LPS-induced nitric oxide production by PBMCs was significantly unaltered 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 LPS-induced PBMC nitric oxide production in young, middle-aged controls and cancer patients between the spiritual and non-spiritual sub-groups (Fig. 11.3A). Similarly, LPS-induced VEGF C production by PBMCs was significantly unaltered with age, spirituality and disease state (Fig. 11.3B).
Effects of spirituality on LPS-induced cytokine production. Spirituality does not alter LPS-induced TNF-α (A) and IL-6 (B) production although there was a significant increase in IL-2 (C) and no change in IFN-γ (D) production. *p<0.05 compared with respective young control (B). #p<0.05 compared with respective non-spiritual group.
Figure 11.3 Correlative effects of spirituality on LPS-induced NO and VEGF C production in PBMCs. Spirituality did not affect LPS-induced nitric oxide production (A) or VEGF C production (B) by PBMCs in cancer patients. LPS-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. *p<0.05 compared with young spiritual control. #p<0.05 compared with respective group without inhibitor.
11.3.4 Spirituality does not alter resting PBMC antioxidant enzyme activities in women with metastatic breast cancer

There was a significant decline in the superoxide dismutase (SOD) activity of women with breast cancer compared to middle-aged and young controls. The spiritual status of the person did not affect the activity of SOD in the resting PBMCs (Fig. 11.4A).

The catalase (CAT) activity was significantly increased in spiritual young women compared with non-spiritual young women although no such differences were observed among middle-aged women and women with breast cancer. A significant age-related decline was observed in middle-aged spiritual and non-spiritual women compared with young spiritual and non-spiritual women. Similarly, there was a significant decrease in the CAT activity of spiritual women with breast cancer compared with young spiritual women. There was no significant difference between the CAT activity of resting PBMCs among middle-aged spiritual and non-spiritual women and spiritual and non-spiritual women with breast cancer (Fig. 11.4B).

Glutathione peroxidase activity was significantly decreased in PBMCs from middle-aged spiritual and non-spiritual women and spiritual and non-spiritual women with breast cancer compared with young spiritual and non-spiritual controls (Fig. 11.4C).
Antioxidant enzyme activities of resting PBMCs decline with age and disease state, but are unaltered with spiritual status. Age-related decline was observed in PBMC CAT (B) and GPx-1 (C) activities although SOD activity (A) did not decline with age. SOD, CAT and GPx-1 activities were decreased in spiritual and non-spiritual women with breast cancer compared to young women. **p<0.05 compared with young and middle-aged spiritual or non-spiritual women. *p<0.05 compared with young spiritual/ non-spiritual women. #p<0.05 compared to spiritual women of the respective group.

11.3.5 Spirituality does not alter extent of lipid peroxidation in resting PBMCs of women with metastatic breast cancer

The extent of lipid peroxidation was significantly enhanced in spiritual and non-spiritual women with breast cancer compared with young
and middle-aged spiritual and non-spiritual women. There was no significant effect of spirituality on the extent of lipid peroxidation among young and middle-aged women and women with breast cancer (Fig. 11.5A).

![Graph showing lipid peroxidation levels]

**Figure 11.5** *Spirituality does not affect the extent of lipid peroxidation in PBMCs from breast cancer patients.* A significant increase in the extent of lipid peroxidation was observed in PBMCs isolated from spiritual and non-spiritual women with breast cancer compared to young women. **p<0.05 compared with young and middle-aged women without breast cancer.

### 11.3.6 Spirituality alters LPS-induced PBMC GPX-1 activity alone in women with metastatic breast cancer

There was a significant decline in the superoxide dismutase (SOD) activity of LPS-stimulated PBMCs isolated from women with breast cancer compared with middle-aged spiritual and non-spiritual women. Age-related increase in SOD activity was also observed in LPS-stimulated PBMCs from spiritual and non-spiritual middle-aged women and spiritual and non-spiritual women with breast cancer compared with young. However, the spiritual status of the person did not affect the activity of SOD (Fig. 11.6A).

Similar to SOD, the catalase (CAT) activity was significantly decreased in LPS-stimulated PBMCs from non-spiritual young women compared with young spiritual
women. Also, there was a significant decline in spiritual and non-spiritual middle-aged women and spiritual and non-spiritual women with breast cancer compared to young women (Fig. 11.6B).

Glutathione peroxidase activity was significantly decreased with age and disease compared to young women who were spiritual and non-spiritual. There was a significant decrease in the GPx-1 activity of LPS-stimulated PBMCs isolated from non-spiritual young and middle-aged women and non-spiritual women with breast cancer compared to their respective spiritual counterparts (Fig. 11.6C).

Figure 11.6  Antioxidant enzyme activities of LPS-stimulated PBMCs alter 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 increased in spiritual young and middle-aged women compared with respective non-spiritual groups. Age and cancer-related decline was observed in spiritual women with cancer compared to young and middle-aged spiritual controls. \*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.

11.3.7 Effects of Spirituality on LPS-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 LPS-stimulated PBMCs isolated from spiritual and non-spiritual women with breast cancer compared to young and middle-aged spiritual and non-spiritual women (Fig. 11.7A).

There was no significant alteration in p-CREB expression among spiritual and non-spiritual young, middle-aged and women with breast cancer (Fig. 11.7B). p-Akt expression was not significantly altered in young, middle-aged and women with breast cancer (Fig. 11.7C).

11.3.8 Inhibition of PKA/PKC, ERK, Akt or NF-kB pathways on LPS-induced PBMC proliferation in spiritual and non-spiritual women

There was a significant age and disease-associated decline in LPS-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 LPS-induced PBMCs isolated from young spiritual and non-spiritual women. Inhibition of ERK signaling using PD98059 (Fig. 11.6B), Akt signaling using A6730 (Fig. 11.6C) or NF-kB pathway using Bay-11-7082 (Fig. 11.6D) significantly decreased proliferation of LPS-induced PBMCs isolated from young spiritual women alone although there was no significant difference in LPS-induced proliferation compared with non-spiritual patients with breast cancer.
**Fig. 11.7**  
*Spirituality did not alter LPS-induced p-ERK (A), p-CREB (B) and p-Akt expression in PBMCs isolated from women with breast cancer.* p-ERK expression alone was increased in the PBMCs from breast cancer patients compared with age-matched control, although no age or spirituality-related differences were observed in the expression of p-ERK, p-CREB and p-Akt in LPS-stimulated PBMCs. **p<0.05** from young control and middle-aged control.
Figure 11.8 Spirituality does not affect LPS-induced proliferation of PBMCs in cancer patients. LPS-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. *p<0.05 compared with young spiritual control. #p<0.05 compared with respective group without inhibitor.
11.4 Key Findings

There was a significant age and cancer-related decline in the LPS-induced proliferative capacity of PBMCs although it did not alter with spiritual status. Although LPS-induced expression of TNF-α and IL-6 was not altered with age, breast cancer or spiritual status, IFN-γ and IL-2 production declined with breast cancer while IL-2 production alone was reversed in spiritual women with breast cancer. LPS-induced nitric oxide and VEGF C production by PBMCs was not altered by age, breast cancer and spirituality in LPS-induced cells.

In resting lymphocytes, a breast cancer-related decline was observed in SOD activity, while CAT and GPx-1 activities declined with age and breast cancer and spiritual young women had elevated CAT activity compared with nonspiritual young women. The extent of lipid peroxidation in resting PBMCs was significantly enhanced in spiritual and non-spiritual women with breast cancer compared with young and middle-aged controls. In LPS-stimulated cells however, SOD activity was higher in middle-aged controls compared with young and declined in women with breast cancer while CAT and GPx activities declined with age and breast cancer. CAT activity was enhanced in spiritual young women alone while GPx-1 activity was enhanced in young, middle-aged and women with breast cancer compared to their respective non-spiritual controls (Fig. 11.6C).

p-ERK expression of LPS-stimulated PBMCs was elevated in women with breast cancer while p-CREB and p-Akt expression was not altered. Inhibition of PKA/PKC pathway using H89, ERK signaling using PD98059, Akt signaling using A6730 and NF-kB pathway using Bay-11-7082 significantly decreased proliferation of LPS-induced PBMCs isolated from young women alone.

In summary, there was a significant age and breast cancer-related modulation of LPS-induced lymphocyte functions. Spirituality altered the expression of antioxidant enzyme activities (CAT in young women alone and GPx-1 in young, middle-aged and women with breast cancer).