CHAPTER 5

METHODS
5.1. Overview of the Study

- The first aim of the study is to find out the Radiation induced Telomere Damage and genomic Instability in Breast cancer patient: This part of the study was conducted at Manipal Hospital Bangalore, Department of Genetics in collaboration with Department of Radiation Oncology. The damage due to radiation was studied using Peripheral Blood lymphocytes of the Patients Pre and Post Radiotherapy. The parameter used was Micronuclei technique using Fluorescent In situ Hybridisation technique (FISH).

- The second aim of the study is to recruit another cohort of patients which was a randomized control trial. And then divide the patients into two groups (Yoga) and (Supportive Counselling Group). The patients were randomly recruited from there recruiting Hospitals in Bangalore. Psychological and DNA damage parameters were studied pre and post Radiation therapy and they were correlated for significance and association. The major aim was to study the effect of Yoga in modulating the Genotoxic and Psychological stress in the Breast cancer patients.
5.2. Overview of the Study I:-

The first aim of the study is to find out the Radiation induced Telomere Damage and genomic Instability in Breast cancer patient: - This part of the study was conducted at Manipal Hospital Bangalore, Department of Genetics in collaboration with Department of Radiation Oncology. The damage due to radiation was studied using Peripheral Blood lymphocytes of the Patients Pre and Post Radiotherapy. The parameter used was Micronuclei technique using Fluorescent *Insitu* Hybridisation technique (*FISH*)
5.4. Patient recruitment (COHORT- I) Source

Fifty five female patients with age group of 36-63 years were recruited using a random allotment chart that fitted the inclusion criteria. The Staff Nurses and attendants were educated about the study and informed consent form was taken at the directive of Atomic Energy regulatory Board (AERB) Govt of India. The project was approved by Institutional Review Board (IRB) of the Manipal Hospital, Bangalore, India.

5.4.1. Inclusion Criteria

1. Females with age not less than 35 years and not more than 70 years.

2. Early breast cancer patients who are undergoing Radiation therapy or concurrent Chemo - Radiation therapy.

3. Having a performance status of 0 - 3 in accordance with Zubrods performance status 0-3. [0 = Asymptomatic, fully ambulatory; 1 = Symptomatic, fully ambulatory; 2 = Symptomatic, ambulatory > 50 % of the time. 3 = Symptomatic, ambulatory < 50% of the time]. [Kennealey and Mitchell, 1977]
4. The patients with no exposure to other mutagens, smoking or alcohol for at least 3 months prior to pre-radiation blood donation.

5. The patients who did not receive chemotherapy before radiation and all the patients were recruited at the Department of Radiotherapy, Manipal Hospital, Bangalore, India.

5.4.2. Exclusion Criteria

1. Adults with age less than 35 years and more than 70 years.


3. Those in Zubrod’s performance status of 4 or severely ill.

   [3 = Symptomatic, ambulatory < 50% of the time. 4 = Bed ridden]


5.5. Informed Consent form: - They were all counselled and consent forms were taken prior to recruitment into the study. The project was approved by Institutional Review Board (IRB) of the Manipal Hospital, Bangalore, India.
5.6. Ethical clearance:- The project was approved by Institutional Review Board (IRB) of the Manipal Hospital, Bangalore, India.

5.6. Work Flow of Methodology I:-

- Blood collection Pre and post radiotherapy treatment
- Cell culture of Cytokinesis blocked Micronuclei (CBMN) Assay
- Fluorescent Hybridisation technique FISH of Telomeric Probes.
- Microscopy and photomicrography of Binucleates.
- Analysis of images
- Statistical analysis and Data interpretation

5.7. Blood sampling for Micronuclei FISH:-

Five ml of peripheral blood from breast cancer patients (both pre- and post-radiotherapy schedule) were collected by venous puncture vacutainer method. The blood samples were coded and despatched to the laboratory for blind-analysis.
5.8. Cytokinesis blocked micronucleus assay (CBMN assay):

The method essentially followed the protocol described by Fenech and Morley [23]. Briefly, one ml of freshly collected heparinised peripheral blood was added to 5 ml of RPMI-1640 (Sigma Aldrich) media containing 10% foetal bovine serum (Gibco BRL) and 200 μl of 1% phytohaemoagglutinin (Gibco BRL). The culture was incubated in a CO₂ incubator for 69 h. After 44 h of culturing, 100 μl of Cytochalasin B (6 μg/ml; Sigma) was added to all the cultures and harvested at 69 h post culture initiation. Cell suspension was centrifuged at 1500 RPM for 10 min and supernatant was discarded. The pellet was subjected to 0.075 M KCl (hypotonic solution). After 10 min, the cells were centrifuged and washed twice with Carnoy’s fixative (3:1, Methanol and Acetic Acid). The cells were carefully dropped on to pre-cleaned slides. Two slides from each sample were prepared for Giemsa staining and FISH with telomere probes. The Giemsa stained slides were analysed under OlympusBX 60 bright field upright microscope. An average of 1000-1500 binucleated cells was scored per patient, pre- and post-radiotherapy.
5.9. Fluorescence in situ Hybridisation (FISH) on Micronuclei

Slides prepared from PBLs from patients pre- and post-radiotherapy were taken and hybridised with PNA (Peptide Nucleic Acid probe from DAKO (cat no K532611). The FISH procedure was followed according to the manual instruction of DAKO. The counterstained slides were analysed under Zeiss Axioiplan Fluorescence microscope with appropriate filters for fluorescence imaging.

One thousand bi-nucleated cells were scored and all the micronuclei containing red telomere signals were recorded in the image processing software attached with the microscope.

5.10. Overview of the Study -II:-

The aim of the study is to recruit another cohort of patients which was a randomized control trial. And then divide the patients into two groups (Yoga) and (Supportive Counselling Group). The patients were randomly recruited from there recruiting Hospitals in Bangalore. Psychological and DNA damage parameters were studied pre and post Radiation therapy and they were correlated for significance and association. The major aim was
to study the effect of Yoga in modulating the Genotoxic and Psychological stress in the Breast cancer patients.

This study seeks to evaluate efficacy and compare the effects of a purely stress reduction intervention such as yoga versus supportive therapy and counselling as controls in breast cancer patients receiving radiotherapy and how Yoga modulates the DNA damage and Psychological stress.

Figure 5.2
5.11. Methods of Study II (Randomized control Trial)

5.11.2. Recruitment of Subjects. (Source)

A randomized controlled study was initiated and a convenient sampling strategy was used to enrol patients in the study. The patients were recruited from three cancer hospitals in India, Bangalore Institute of Oncology (BIO), Manipal Hospital Bangalore India, and Bharat Cancer hospital Mysore, India. Clinical staff/Research assistants were informed of the study and invited to refer patients. Posters and leaflets announcing the study and inviting patient participation were posted in public areas of the clinic.

A total of 78 patients were recruited from January 2004 till December 2005 who met the inclusion criteria.

5.11.3. Inclusion Criteria

i. Females with age not less than 35 years and not more than 70 years.

ii. Breast cancer patients who are undergoing Radiation therapy

iii. Having a performance status of 0 - 3 in accordance with Zubrods performance status 0-3. [0 = Asymptomatic, fully ambulatory; 1 = Symptomatic, fully ambulatory; 2 =
Symptomatic, ambulatory > 50% of the time. 3 = Symptomatic, ambulatory < 50% of the time. [Kennealey and Mitchell, 1977]

**Justification:** the inclusion criteria are intended to enrol as many subjects as possible.

### 5.11.4. Exclusion Criteria

5. Adults with age less than 35 years and more than 70 years.


7. Who are on hydrocortisone medications and anti-depressants.

8. Those in Zubrod’s performance status of 4 or severely ill.

   [3 = Symptomatic, ambulatory < 50% of the time. 4 = Bed ridden]

9. Illiterate patients.

10. Major psychiatric/ neurological and autoimmune diseases.

**Justification:** Participants with psychiatric illnesses, cognitive impairment, or substance abuse and illiterates that may impede their ability to follow directions for the clinical visits or provide informed consent and fill up questionnaires were excluded (5,6). Medical conditions
and other factors that can confound endocrine variables are also excluded (2, 3, and 6). Those who conform to the selection criteria were chosen for the study consent to participate in the study. Subjects were excluded if they had any concurrent medical condition likely to interfere with the treatment, major psychiatric, neurological illness or autoimmune disorders, cardiovascular illness and any known metastases. The patients did not have any exposure to other mutagens, smoking or alcohol for at least 3 months prior to pre-radiation blood donation.

5.11.5. Target Population: Patients diagnosed with breast cancer of stage (II-III) who are registered in the hospital based cancer registries in Manipal Hospital/Bangalore Institute of Oncology/Bharath Cancer Hospital undergoing radiotherapy.

5.11.6. Sampling: A convenience sample of all subjects registered in radiation oncology department for radiotherapy and who qualify for the entry criteria and give their consent to participate in the study were recruited.
5.11.7. Subjects and sample size: Participants who meet the selection criteria will be recruited by referrals from the radiation oncologist at the study centres with a request for participation in a study of yoga to improve health. As there is no other studies done else where we followed the standard sample size of 35 patients in each arm (according to Raghavendra et al 2006)

5.11.8. Ethical approval: - The study was approved by the Institutional review board of all the three participating institutions (Bangalore Institute of Oncology (BIO), Manipal Hospital Bangalore India, and Bharat Cancer hospital Mysore, India.) Finally It was also approved by the Institutional review committee of Swami Vivekananda Yoga Anusandhana Samisthana.

5.11.9. Randomization:

Subjects were randomized using random numbers generated by a computer for a 2 group assignment using sealed envelopes. The sealed envelopes were kept in the order of their assignment to check and tally with the analysis.
5.11.10. **Blood sampling**: - Five ml of peripheral blood from breast cancer patients (both pre- and post-radiotherapy schedule) were collected by venous puncture vacutainer method. The blood samples were coded and despatched to the laboratory for blind-analysis.

5.12. **Demographic information**: -  
During initial visit demographic information including age, marital status, education, occupation, obstetric and gynaecologic history, medical history and intake of medications were obtained and clinical data was abstracted on the history of breast cancer, investigative notes and radiotherapy and chemotherapy treatment regimen.

5.13. **Intervention**: -  
Both the groups received their conventional treatment. They also received counselling and education materials to allay their fears of treatment and help them take an active part in their own health care concerns. Counselling was directed to reinforce social support in both groups and was used to nullify variables such as education, attention and support which could have confounded the results in this study. Patients in
intervention group were made to undergo intensive yoga therapy training in hospital set-up imparted by a trained instructor. Patients were asked to maintain a diary noting their daily activity, daily yoga schedule, duration of practice, intake of medications, and distressing symptoms etc.

5.14. Counselling about the concept of YOGA:-

The most important part of the study is to counsel the patients and make them realise the basic goal of Yoga intervention.

The patients were taught in the following concepts of YOGA:-

**Introduction on Yoga**

Yoga is becoming popular in all parts of the world. For the restless mind it gives solace. For the sick, it is a boon. For the common man it is the fashion of the day to keep him fit and beautiful. Some use it for developing memory, intelligence and

Because of its rational basis, the modern medical system has replaced almost all the traditional systems of medicine in different parts of the globe. It has proved itself most effective in saving man from the fatal hands of contagious and infectious diseases. However, new widespread
psychosomatic ailments and psychiatric problems are posing a great challenge to the modern medical system. It is here that yoga is making a vital contribution to the modern medical system.

Extensive research on Yoga therapy over the last few decades has brought out the usefulness of Yoga for dealing with these ailments as an effective adjunct to medical management and also for long term rehabilitation.

‘Prevention is better than cure’ a proverbial saying is kept only as an accepted proverb in modern medicare delivery system. Hardly 1% of the budget is allotted in any country. Yoga could play a vital role in preventing diseases. All health clubs have started including yoga as part of their schedule and many go only for yoga in these health clubs.

Promotion of positive health is being nurtured by many who do not want to be the victims of modern ailments. Yoga is playing a vital role in this aspect in the new millennium.

**Understanding Yoga**

The term Yoga has its verbal root as yuj! (Yuj) in Sanskrit. Yuj means joining, yuJyte Anen #it yaeg> (Yujayete anena iti yogaù). Yoga is that
which joins. In the traditional terminology it is joining of jIvaTma (jévātmā) with prmaTma (paramātmā) the individual self with the universal self. It is an expansion of the narrow, constricted, egoistic personality to an all pervasive, eternal and blissful state of \textit{REALITY}.

Patanjali Yoga is one among the six systems of Indian philosophy known as ‘\textit{Ñat darçanas’}. One of the great \textit{Åñis} [Seer], Patanjali compiled the essential features and principles of Yoga in the form of ‘\textit{Süträs’} [aphorisms] and made a vital contribution in the field of Yoga, nearly 4000 years ago. Accordingly, Yoga is a conscious process of gaining mastery over the mind.

In general, there is a growth process due to interactions with nature in all creation. But it may take thousands and millions of years for this natural growth; that is the long, instinctive way in animals. Man, endowed with discriminative power, conscious thinking faculty the buIī [buddhi] and well-developed voluntary control systems, aspires to accelerate growth. Yoga is that systematic conscious process which can greatly compress the process of man’s growth.
Thus, Yoga is a systematic process for accelerating the growth of a man in his entirety. With this growth, man learns to live at higher states of consciousness. Key to this all-round personality development and growth is the culturing of mind.

**Definition of Yoga**

A. **Mastery over mind**

Patanjali defines ‘Yoga Citta Vâtti Nirodhah’ (Yoga Sûträ: 1.2) Yoga is a process of gaining control over the mind. By so controlling the mind we reach our original state; tda d+qu> svarUpe AvSwanm! ‘Tadä Drañtuû Svarüpe Avasthänam’ (Yoga Sutras: 1.3) Then the Seer establishes himself in his causal state. This is the technique of ‘mind control’ prescribed by Patanjali. Control involves two aspects – a power to concentrate on any desired subject or object and a capacity to remain quiet any time. Rarely, the second capacity of man, to remain calm and silent, has been harnessed. Hence, Yoga mainly emphasizes, this second aspect. Yoga is thus a %pay> [Upâyaû], a skilful, subtle process and not a brutal, mechanical gross effort to stop the thoughts in the mind and thereby becomes a process for elevating oneself through calming of mind.
In action, The dexterity is in maintaining relaxation and awareness in action. Relaxed action is the process. Efficiency in action is an outcome. Thus, Yoga is a skilful science of gaining mastery over the mind. Yoga is normally and traditionally conjectured and popularly known as a process or a technique to reach the ultimate state of perfection. However, yoga is found defined even as ultimate state of silence. Further, yoga is also described as the power of all creative endeavors and creation itself.

B. A state

Yoga is a state of great steadiness at emotional level; balance between concentration and detachment at mental level and homeostasis at body level. It integrates the personality by bringing body-mind co-ordination in a well balanced way. Hence yoga is the very states of higher, subtler layers of mind.

C. A power of creation

Yoga is conceived as a creative power in man and that of the reality itself.
The Four Streams of Yoga

There are a large number of methods of yoga catering to the needs of different persons in society to bring about the transformation of the individual. They are broadly classified into four streams. Swami Vivekananda puts them as work and worship, philosophy and psychic control.

1. The path of work (Karma Yoga) involves doing action with an attitude of detachment to the fruits of action. This makes man release himself from the strong attachments and thereby brings in him a steadiness of mind which verily is Yoga – ‘Samatvam Yoga Ucyate’ (Gēta 2.48). Instruments of action and understanding (karmendriyās and jnanendriyās) get cleansed.

2. The control of emotions is the key in the path of worship (Bhakti Yoga). In this modern world, man is tossed up and down due to emotional onslaughts. The path of Bhakti is a boon to gain control over emotional instabilities by properly harnessing the energy involved in it.

3. The age of science has made man a rational being. Intellectual sharpness is imminent. Analysis forms the tool. The path of philosophy (Jnana Yoga) is apt for the keen intellectuals and is centered on the analysis of ‘happiness’, the vital contribution of Upaniñads. Also many other fundamental questions regarding the mind, the outside and inside
world and the reality are taken up. Basic questions are raised even involving the intellect itself to reach the very basis of intellect.

4. Culturing of mind is the key for success in almost all endeavors in our lives. The yoga of mind culture or psychic control (Rāja Yoga) gives a practical and easy approach to reach higher states of consciousness. It is based on the Aṅtāṅga Yoga of Patanjali’s Yoga system.

Aṅtāṅga Yoga

One of the major contributions of Patanjali’s Yoga Sutrās is the eight-limbed Yoga, popularly known as ‘Aṅtāṅga Yoga’, which gives a comprehensive and systematic approach for developing the mind. The eight limbs are;

1. ym - Yama (the disciplines, ‘DONT’S’: Niṣedhas)
2. inym - Niyama (the injunctions, ‘DO’S’: Vidhis)
3. Aasn - Āsana (the posture of the body)
4. P+aa[ayam - Prāēāyāma (the control of Prāēa, the life force)
5. P+āTyahar - Pratyāhāra (restraint of senses from their objects of enjoyment)
6. Xar[a - Dhāraēa (focusing of mind)
7. Xyan - Dhyāna (deconcentration)
8. Samaix - Samādhi (super consciousness)
The first five limbs come under Bahiranga Yoga. In this the Bahirindriyās are used for indirect control of mind. It includes:

a) **Karmendriyās**: Hands, feet, organs of speech, excretion and procreation.

b) **Jñānendriyās**: Eyes, ears, organs of smell (nose), taste (tongue), and touch (skin).

The last three limbs are referred to as Antaranga Yoga; the mind is used directly for culturing itself.

Thus, the four streams of Yoga help man to develop the personality at four different levels – physical, mental, intellectual and emotional and simultaneously bring about spiritual progress. Most of the other methods of Yoga – Laya yoga, Japa yoga, Mantra yoga, Hatha yoga, Kundalini yoga, etc., are permutations and combinations of these basic methods of yoga.

**Unity in Diversity**

The four streams of Yoga have a basic unity among them in that all these paths lead independently to the same goal and there is the same structural transformation that takes place in the mind. This ‘Unity in Diversity’ forming the core of Indian culture offers a grand note of cohesiveness
among various practices. With this catholicity in understanding, when persons follow any one or more of these paths, they allow a harmonious and total growth of the personality. Thus, ‘Yoga’ is a vital tool for the development of man, probably more relevant in the modern scientific era than ever before.

A. Practices at annamaya koça (the physical layer)

A healthy yogic diet, kriyäs, loosening exercises and yogāsanas are used to operate at the annamaya koça level and to remove the physical symptoms of the ailments.

Kriyäs: These are yogic processes described in Hatha Yoga to cleanse the inner organs of our body. They bring about the following effects;

- Activating and revitalising the organs
- Toning up their functions
- Desensitization and
- Development of deep internal awareness.

Among the major kriyäs enumerated in the texts of yoga, simplified versions of a few kriyäs like catheter Neti, Jala Neti, Kapālabhäti, Agnisāra, Vamana Dhouti (Kunjal kriya), etc are used extensively.
Physical exercises and Movements - Čithilékaraëa Vyāyāma: Very simple physical movements to mobilise and activate the affected parts of the body are used. Some easy physical exercises are adopted to fulfil the needs of the particular ailments to;

- Loosen the joints
- Stretch and relax the muscles
- Improve the power and
- Develop stamina.

Yogāsanas – Postures: Yogāsanas are physical postures often imitating the natural positions of the animals meant to make the mind tranquil. Through these postures, the physical revitalisation and deep relaxation and mental calmness are achieved.

B. Practices at Prāṇamaya Koça (The layer of Prāēa)

Prāēa is the basic life principle. Prāēyāma is a process for gaining control over Prāēa. The five manifestations of Prāēa and the corresponding most comprehensive definition of Prāēyāma in the human system are
described in praṇopaniñat. Also the conventional Prāēyāma through regulation of breath is described therein.

Through the practice of proper breathing, kriyās and prāēyāma, we start operating on the Prāṇamaya Koça. Suitable types of prāēyāma and breathing help to remove the random agitations in prānic flows in the Prāṇamaya Koça. Thus, the ailments are handled at this Prāṇamaya Koça level.

C. Practices at the Manomaya Koça (The mental layer)

Dhāraēa and Dhayāna: A direct operation on this level is made possible by the last three limbs of Aṅtāṅga Yoga of Patanjali – Dhāraēa, Dhayāna, and Samādhi. The culturing of mind is accomplished by focussing of the mind (Dhāraēa) initially, followed by relaxed dwelling of the mind in a single thought (Dhayāna) for longer and longer durations leading ultimately to superconsciousness (Samādhi). A progressive habitation allows the mind to remain relaxed during the period of meditation (Dhayāna). The benefits of Transcendental Meditation, a simple standardised technique, are numerous interesting and noteworthy. Its application to treat many psychosomatic ailments has become popular.
**Emotion culture:** to handle and gain control over the basic cause for mental agitations, we use the yoga techniques that control our emotions.

A devotional session containing Prayers, Chants, Bhajans, Nāmavali, Dhuns, Stotras etc, help to build a congenial atmosphere to evoke, recognise, attenuate and dissipate the emotions. Thus, control over emotions is obtained through the devotional session. The emotional imbalances and upsurges are eliminated by such control.

**D. Practices at the Vijnānamaya Koça (The layer of wisdom)**

A basic understanding is the key to operate from vijnānamaya koça. **Upaniñads** are the treasury of such knowledge which is the redeemer of all miseries and obsessions. It is the lack of that inner jnāna which is responsible for many wrong habits, agitations, etc. The Happiness Analysis - Ānanda Mimāmsa of the Taitteriya upaniñad handles the most fundamental problem relevent to all living creatures. The analysis systematically leads the reader to the substratum from which prāēa and mind emerge – the Ānandamaya Koça. It helps the person to change his attitude of greed and deep attachment to material possessions and enjoyments towards the realisation that happiness is within and ‘each one of us’ in our causal state is ‘Ananda’ embodied. As a result, man’s outlook in life changes. Knowledge burns the strong attachments, obsessions, likes
and dislikes which are the basic reasons for the agitations of mind. The sära type of ädhis can only be removed by this knowledge (ätma jnäna or self-realisation).

MIND SOUND RESONANCE TECHNIQUE (M S R T)

STARTING POSITION:

- Sit in any meditative comfortable position or lie down in Śavāsana with legs apart, hands away from the body, head and neck in a very convenient position. The whole body is completely collapsed on the ground.
- Let us start the session with the prayer ‘Mṛtyunjaya Mantra.’

Om Trayambakam yajamahe
Sugandhim Pustivaradhanam
Urvarukamivabndhanat
Mṛtyormukiya mamrtat
   . Om Shanti, Shanti, Shanti.

STEP-I: A-KĀRA Chanting (9-Rounds)

Maintain calmness of your mind and let us slowly proceed to the practice of M S R T, recognizing all the subtle changes during chanting. Let us
being with chanting 9 rounds of A-kāra Synchronizing with the whole group and chanting very smoothly and try to feel the vibration in the lower parts of the body. Inhale A……… very carefully observe the changes, all the vibrations smoothly settling down. Very slowly and leisurely awaken the energies and chant another A-kāra. Inhale A……… Once again recognize all the vibrations settling down, gradually merging into the inner calmness, and taking you very naturally into that inner quietude. Recognize the sublime state of energies. Learn to effortlessly remain in that peaceful state for longer and longer duration. Inhale A……………..very carefully observe all the changes within Inhale again A……….., Inhale A……………., Fine vibrations of A kāra engulfing your whole being and smoothly taking you into inner calmness, recognize the subtle and sublime state of energies at all levels. Once again inhale deeply A……….., Every chanting taking you into deeper and deeper level of calmness, softer and softer states of your being. Try to produce a very rich sound and every chanting giving full expression to your energies. Inhale A………Recognize, the energies very smoothly subsiding, taking you into inner quietude. Inhale A……….. Let go all inhibitions of your energies. Recognize a very tranquil flow. Final round of A-kāra. Inhale A……….. learn the subtle technique of producing resonance by perfectly matching the sound vibrations and that of the body vibrations followed by quietude.
STEP –II: U-KĀRA Chanting (9-rounds)

Let us now move on to U-kāra chanting. Inhale U……. Try to produce very powerful sound, the flutter, the buzzing sound as you exhale. Inhale U……. Feel the pleasant resonance in the chest cavity very peacefully subsiding. Again inhale U……. Maintaining all the alertness of the mind and keen sensitivity. Recognize all the subtle changes. Inhale U……., Inhale U……., Breathe In for the sixth round U…….. Again inhale U…….. Recognize the smooth and relaxed state of energies. Inhale U……., Inhale U…….. Last round Inhale U…….. Appreciate the inner calmness.

STEP –III: M-KĀRA Chanting (9-rounds)

Let us move on to produce the finest vibration of M-kāra. Inhale M……. Feel the blossoming of energies particularly in the head region, giving you the wonderful feeling of expansion. Again inhale M……., Inhale M……., inhale M……., Inhale M……., inhale M……., Inhale M……., inhale M……., Last round Inhale M……. allow the resonance to diffuse in the head region.
STEP –IV: OM-KĀRA Chanting (9-rounds)

Let us now proceed to chant OM-kāra by combining all the three syllables, A U M, giving a sublime release of energies. Feel the flow of energy during chanting starting from A-kāra and ending with M-kāra, wonderful feeling of expansion in the whole body. Inhale A...U...M...... in the ration of 1: 1: 2. Inhale A...U...M...... Recognize the blissful feeling of lightness and expansion of your energies, the wonderful calmness and tranquility of the mind. Again inhale A...U...M...... Check your position. Allow all the vibrations to completely quiét down. Inhale A...U...M......, Inhale A...U...M...... Merge into the divine vibration of OM. Inhale A...U...M......, Inhale A...U...M...... Inhale A...U...M...... Last round inhale A...U...M...... Allow the resonance and the subtle vibrations to diffuse and merge into silence.

E. Practices at the Änandamaya Koṣa (The layer of bliss)

To bring the bliss of our causal body (Kāraṇa Çarēra) called Änandamaya Koṣa in all our actions is the key for a very happy and healthy life. This also brings our innate healing power to effect, a complete cure of our ailments. The techniques used come under the heading Karma Yoga, the secret of action. The secret lies in maintaining an inner silence, equipoise
the mental level as we perform all our actions. Normally we get upset, or excited over things which we do not like or we like. But we have to learn to maintain equipoise (samatva). The next step is to have a deep silence and a blissful awareness in the inner subtler layers of our mind while we are in action.

This is accomplished by self awareness, constant drive to change oneself and auto-suggestions. To recognise that ‘I am getting tensed’ is the first step. Correct by withdrawing to the inner compartment of total bliss, peace and rest. Remember this by repeated inner silence several times in the day. Retain a smiling relaxed face during all the yoga practices.

5.15. Summary of YOGA intervention:-

The randomly allotted intervention group was assigned under a group of expert yoga trainers for 6 weeks. In the beginning only meditative practice as well as slow stretching and loosening exercises were taught to the patients. They were motivated and counselled at the beginning and the various postures (asanas) were meticulously taught. The special techniques designed for the cancer patients included guided imageries of cancer cells, positive thought provocation, chanting of various sounds
according to the respective religious beliefs of the patients. During the middle period of the trial, group awareness practices were given. They were also provided with the audio and video tools to practice at home and were followed up via telephone during the weekends to ensure continuity of practice. Special care was taken for patients who suffered from surgery associated side effects such as numbness or pain. The patients were familiarized with various breathing practices called Prāṇayama (voluntary regulated nostril breathing). Each session was of 90 minutes duration with full time breath awareness and complete relaxation. At the end of each session deep relaxation was given in the form of soothing sound vibrations and guided imageries called (yoga nidra). These practices are known to build inner awareness and attention of mental phenomena. This is known to alter the perceptions and mental responses to both external and internal stimuli, slow down reactivity and responses to such stimuli and instil a greater control over stressful situations which promotes physical wellbeing and mental calmness(also see Appendix I)
5.16. Data Extraction, Variables

**Predictor:**
Randomization to receive a yoga intervention or controls receiving only supportive therapy and counselling during radiotherapy.

**Confounders:**
Age, education level and motivation scores for intervention/treatment, stage of cancer, size of tumour.

**Primary outcome measures:**
DNA damage profile pre and post Yoga intervention.

**Secondary outcomes measures:**
1. Hospital Anxiety and Depression Scale.
2. Perceived Stress Scale.

*See Appendix IV for details regarding reliability and validity of the questionnaires.*
5.16.1. Data Analysis

Demographic and medical characteristics of the study population were summarized using descriptive statistics. Tests for Normality (Shapiro Wilk’s) carried out for all the data variables showed a normally distributed data. Paired t test was used to analyze within group differences in the yoga and control groups and Intervention effects were compared across groups using ANCOVA on post measures adjusted for their respective baseline differences.

5.16.2. Quality Control and Data Management

5.17. DNA Damage Study

Alkaline single-cell gel electrophoresis (Comet) assay. Peripheral blood lymphocytes were isolated by Ficol Histopaque method from the blood collected from the patients pre and post radiotherapy. The cells were washed in ice-cold 1x PBS, and re-suspended in HBSS with 10% DMSO with EDTA. The cells were then suspended in (0.75%) molten low melting point agarose (at 37°C) and immediately pipetted onto the comet slides (Trevigen, Gaithersburg, MD). Electrophoresis was done as per vendor's suggestions. After electrophoresis, slides were briefly rinsed in
neutralization buffer (500 mmol/L Tris-HCl, pH 7.5), air-dried, and stained with propidium iodide dye. Three hundred to four hundred randomly chosen comets were analyzed per sample. The extent of DNA damage observed was expressed as number of comets analysed per 100 cells, which corresponded to the fraction of the DNA damage in the peripheral blood lymphocytes of the patients and the data was compared using suitable statistics (SPSS software version 10) between pre and post radiotherapy in yoga and control group of patients.

**Questionnaires:** The patients were asked to fill the questionnaires at various assessment points and were assisted by the field personnel if they sought any clarification. The research assistants were trained in imparting questionnaires.

**5.16.3. Hospital Anxiety and Depression Scale:** It is a 14 item questionnaire developed by Snaith and Zigmond and used for screening for depression and anxiety in hospital patients. This has a high reliability 0.62 to 0.8 and correlates strongly with DSM IV criteria for depression and anxiety.
The hospital anxiety and depression scale (HADS) is a widely used and popular self-report measure that has been extensively translated and utilized in a broad variety of clinical populations. This 14-item measure has been subject to two previous reviews exploring a number of psychometric aspects of this tool. A relatively consistent finding of previous reviews of this instrument is that it is a reliable and valid measure of two independent and separable dimensions of anxiety and depression; indeed, this aspect of the HADS is crucial to the validity of the measure in clinical practice. The current review examines contemporary research reports that use factor analytic techniques, which suggest that the assumed bi-dimensionality of the HADS is, in fact, erroneous. The findings suggest that the HADS is underpinned by a tridimensional factor structure comprising dimensions of anhedonia, negative affectivity and autonomic arousal. Implications for the use of the HADS in light of these observations are discussed and recommendations made within the context of screening practice for the referral to liaison psychiatry services. The HAD scale is a questionnaire commonly used by Doctors and Therapists to assess levels of Anxiety and Depression.

The HADS comprises statements which the patient rates based on their experience over the past week. The 14 statements are relevant to
generalised anxiety (7 statements) or 'depression' (again 7), the latter being largely (but not entirely) composed of reflections of the state of anhedonia (inability to enjoy oneself or take pleasure in everyday things enjoyed normally).

Even-numbered questions relate to depression and odd-numbered questions relate to anxiety. Each question has 4 possible responses. Responses are scored on a scale from 3 to 0. The maximum score is therefore 21 for depression and 21 for anxiety. A score of 11 or higher indicates the probable presence of the mood disorder with a score of 8 to 10 being just suggestive of the presence of the respective state. The two subscales, anxiety and depression, have been found to be independent measures.

Advantages of HADS:-

- Contains an easy-to-use questionnaire, which allows to establish the presence and severity of both anxiety and depression simultaneously, whilst giving a separate score for each
- Gives cut-off points to indicate whether someone is ‘within the normal range’, or in a ‘mildly’, ‘moderately’ or ‘severely’ disordered state
- The manual contains two scales that give an estimate of irritable mood disorders and help identify particular areas of anxious concern
- All ages from adolescent upwards. It can be used with hospital outpatients, primary care and community settings. It is used by researchers and clinicians.

5.16.4. Perceived Stress Scale, PSS (Sheldon Cohen et al. 1983)

It is a 14-item, self-reported one-dimensional instrument developed to measure a perceived stress in response to situations in a person’s life. Respondents report the prevalence of an item within the last month on a 5-point scale, ranging from never to very often. A 4-item version is available for telephone interviews, and a 10-item version has been psychometrically tested.

The Perceived Stress Scale (PSS) is the most widely used psychological instrument for measuring the perception of stress. It is a measure of the
degree to which situations in one’s life are appraised as stressful. Items were designed to tap how unpredictable, uncontrollable, and overloaded respondents find their lives. The scale also includes a number of direct queries about current levels of experienced stress. Moreover, the questions are of a general nature and hence are relatively free of content specific to any sub-population group. The questions in the PSS ask about feelings and thoughts during the last month. In each case, respondents are asked how often they felt a certain way. Although developed for a general population, the tool has been used with caregivers of people with dementia/Alzheimer’s and spinal cord injuries, and cancer patients undergoing Radiotherapy and Chemotherapy.

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thoughts during the last month. In each case, respondents are asked how often they felt a certain way.

5.16.5. Perceived Stress Scale Scoring

PSS-10 scores are obtained by reversing the scores on the four positive items, e.g., 0=4, 1=3, 2=2, etc. and then summing across all 10 items. Items 4, 5, 7, and 8 are the positively stated items.

Advantages:-

- The PSS was designed for use with community samples with at least a junior high school education.
- The items are easy to understand and the response alternatives are simple to grasp.
- The questions are quite general in nature and hence relatively free of content specific to any sub population group.
- In light of the generality of scale content and simplicity of language and response alternatives, the data from representative samples of the general population would not differ significantly from other population worldwide.