"Evaluation of an integrated yoga intervention in modulating psychological stress and radiation induced genotoxic stress in breast cancer patients undergoing radiotherapy "

Dissertation submitted by

Birendra nath Banerjee

Under the guidance of

DR. SRIDEVI HEGDE

DR. R NAGARATHNA

DR. H.R NAGENDRA

Towards the partial fulfillment of

Doctor of Philosophy (Yoga)

TO

SWAMI VIVEKANANDA YOGA ANUSANDHĀNA SAMSTHĀNA (SVYASA)

(A University, established under Section 3 of the UGC Act, 1956 vide Notification No. F.9-45/2001-U.3 dated 8-5-2001 of the Government of India)

Eknath Bhavan, No.19, Gavipuram Circle, K G Nagar
Bangalore - 560 019, INDIA

Part - I

CONCEPT OF BHUJANGĀSANA IN INDIAN SCRIPTURES

Part - II

THE EFFECTS OF YOGA THERAPY ON LOW BACK PAIN IN A NON-RESIDENTIAL SETUP

Dissertation submitted by

PADMAVATI L

Under the Guidance of

R NAGARATHNA

H R NAGENDRA

Technical Support by

PADMINI T

towards partial fulfillment of

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CERTIFICATE

This is to certify that **Birendranath Banerjee** is submitting this dissertation titled**"*Evaluation of an integrated yoga intervention in modulating psychological stress and radiation induced genotoxic stress in breast cancer patients undergoing radiotherapy* ",** in partial fulfilment of the requirements for the Doctoral Degree in Yogic Sciences. He registered for the course on 12th January 2004 in Swami Vivekananda Yoga Anusandhana Samsthana under the division of Yoga and Life sciences and this dissertation is a record of the work carried out by him in this institute.

12th January 2008.

Guides:

Guide I

Dr. Sridevi Hegde

Guide II

Dr. Nagarathna Raghuram

Dr Venkataraman
Registrar
sVYASA University.

DECLARATION

I hereby declare that the work presented in this dissertation is done by me under the guidance of **Dr Sridevi Hegde, Dr R Nagarathna** and **Dr H.R Nagendra**. I also declare that this work entitled **"Evaluation of an integrated yoga intervention in modulating psychological stress and radiation induced genotoxic stress in breast cancer patients undergoing radiotherapy"**

This study has not previously formed the basis of any degree, diploma, membership or similar titles.

Place: Bangalore

Date: 12 January 2008

B.N. Banerjee

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Abstract:-

Background: Stress in any form both psychological and physiological has been known to be associated with cancer from the time of diagnosis. Yoga is an ancient eastern system which is being extensively used in research worldwide to study the effects in reducing the stress at the psychological and physiological levels.

Aim: In the current study an attempt has been made to find the effects of an integrated yoga program in modulating the perceived stress levels, the anxiety as well as depression levels and radiation induced DNA damage in breast cancer patients undergoing radiotherapy

Method: A total of 78 patients were recruited and randomized in the study cohort. The Yoga group had n=35 patients and control group had n=23 patients with 20drop outs

Two Psychological questionnaires (HADS and PSS) were taken pre and post radiotherapy. DNA damage was measured to find the effect of radiation on the peripheral blood lymphocytes (PBLs) of the patients both pre and post radiotherapy. The intervention group was given an integrated

Yoga program which included guided relaxation, meditation, breathing practices, asanas and imageries with positive group practices for six weeks. The control group was given supportive counseling and waitlisted to be given yoga after the trial period.

Results There was a significant decrease in the anxiety levels in yoga intervention group from Mean=8.5(baseline) to Mean=4.1 (48.2%) after the 6 weeks of yoga program, where as in the control group the Mean anxiety score increased to 10.5 (28%) $p<0.001$ for ANCOVA. The post depression score for the intervention group decreased from Mean= 8.0(baseline) to Mean= 3.4(57.5%) where as the in the control group the score increased from 7.8(baseline) to 9.7(24%). In the yoga group the mean perceived stress score (PSS) decreased from 20.4(baseline) to14.9 (26.9%) where as the control group showed no change in pre and post radiotherapy Mean= 19.0 and Mean=20.4. The DNA damage due to radiation was significantly high in both the yoga and control group after radiotherapy But the post radiotherapy DNA damage was slightly lesser Mean=24.3 when compared to the control group Mean=28.8.$p<0.001$.The baseline DNA damage being 2.6 and 2.8 respectively.

Conclusion: In our study we found that the integrated Yoga program was effective in reducing the stress significantly at both physiological and psychological levels when compared to the supportive counseling.

Key words: Yoga, meditation, radiotherapy, stress, DNA damage.

Abbreviations: HADs-Hospital Anxiety and Depression scale, PSS-Perceive Stress Scale, PBLs- Peripheral Blood Lymphocytes

Chapter 1

INTRODUCTION

Introduction:-

1.1 Breast cancer disease and incidence:-

All kinds of tissues are prone to develop tumours except hair, nails and tooth. But among women the Breast and cervix are the most commonly occurring tumours and in men prostrate and lung cancers are the most common. (Cancer facts and figure 2000). Over years due to a number of compounded effects of sedentary lifestyle, food habits stress and environmental toxicity the incidence of breast cancer has drastically increased world wide at an enormous rate of over 4 % rise in a worldwide survey. Cording to IARC Cancer database, Breast Cancer is increasing in endemic proportions in developed countries. U S A has the highest prevalence and mortality ratio from Breast Cancer followed by China, India, Russia, Germany, France, U K and Italy (WHO, IARC, 2001). It is the most common cancer in women, accounting for 16% of cancer-related deaths and ranking second only to lung cancer as a leading cause of cancer-related mortality (Landis S, Murry T, Bolden S, and Wingo PA, 1998).

1.2 Psychological effects of Breast cancer diagnosis and treatment:-

Breast cancer is a profoundly stressful disease posing both physical and psychological threats to the patient. Moreover, patients with breast cancer normally receive multimodal treatment over a long period of time .Psychological distress and trauma is often associated with the diagnosis of cancer and is common (Derogatis L R et al 1983, Stefanek M et al 1987, Farber JM et al 1983). Anxiety and depression are the commonest psychiatric problems encountered in cancer patients. It has been repeatedly acknowledged that many psychiatric disorders in cancer patients are not detected, diagnosed or treated (Fetting J, 1983).The prevalence of depression in cancer patients ranges from 4.5% to 58% (Lansky S.B, List M.A, & Herman C.A, 1985; Massie MJ & Holland JC, 1990). Patients with breast cancer undergoing radiation treatment also report anxiety and depression before, during and after the treatment (Chaturvedi SK et al., 1996; Wengstrom Y, Haggmark C, Strander H, & Forsberg C, 2000). There is an uncertainty about the prognosis of cancer, and social

isolation along with physical symptoms or functional losses resulting from the disease or its treatment are the most important factors. Due to these various difficulties (Spiegel D et al, 1995, Fox B .H et al 1995) many patients believe that stress, including that which is caused by their cancer experience, may contribute to poor coping as well as recurrence or progression of their disease. The prevalence of anxiety and depression in Indian cancer patients in Bangalore undergoing radiation treatment was 64% and 50% respectively (Chaturvedi SK et al., 1996). There is a very high correlation between anxiety and depression in cancer patients (Cassileth B.R, Lush E.J, Hutter R, Strouse T.B, & Brown L.I, 1984).

1.3. Radiation induced DNA damage and stress in breast cancer:-

Radiotherapy has become an indispensable tool in the effective management of most of the cancers. There have been efforts earlier to study the differential radio-sensitivity patterns in patients undergoing radiation treatment to correlate with treatment induced complications such as tissue injury, cell death, and chromosomal

aberration frequencies etc. (Johanson et al 1983). Lately with advent of better machines and innovative technology, individualisation of cancer radiotherapy is gaining greater grounds. There have been number of studies done earlier to prove the radio sensitivity of different individuals undergoing radiotherapy. (Oppitz *et al.*). In study by (Parshad R et al 1996) Women with breast cancer and a family history of breast cancer and some with sporadic breast cancer are deficient in the repair of radiation-induced DNA damage compared with normal donors with no family history of breast cancer. DNA repair was measured indirectly by quantifying chromatid breaks in phytohaemagglutinin (PHA)-stimulated blood lymphocytes after either X-irradiation or UV-C exposure, with or without post treatment with the DNA repair inhibitor, 1-beta-D-arabinofuranosylcytosine (ara-C). According to the authors deficient DNA repair appears was found to be a predisposing factor in familial breast cancer and in some sporadic breast cancers. In 14-year-long study (Pinar B et al 2007) makes a novel contribution to the debate on the relationship between the in vitro radio sensitivity of peripheral blood lymphocytes and normal tissue reactions after radiation therapy. They demonstrated a relationship between the sensitivity of in

vitro-irradiated peripheral blood lymphocytes and the risk of developing late toxic effects and opened up a possibility of predicting normal tissue response to radiation in individual patients, at least in high-dose non-conventional radiation therapy regimens.

Radio sensitivity has been extensively studied in breast cancer patients (Speit 1997 Scott 1998). In the study of Scott and colleagues, found that about 9% of healthy controls were radiosensitive. The deficient DNA repair capacity has been proposed to be a predisposing factor in familial breast cancer and in some sporadic breast cancer cases .Genomic instability has also been described for various, Scott 1997, 2004 1999,, Hussein 2005, P. Sanchez 1 2004and H Mozdarani 2005. It has been shown that about 40% of an unselected group of breast cancer cases were found to be radiosensitive (hereditary cancers including breast cancer (R. Parshad and A Bayenes 2002). These findings suggest that radio sensitivity could actually be a potential predisposing condition to breast cancer through mutations in low penetrance genes (Scott et, al 2004) and many genes may be involved in DNA damage processing and repair.

Robert W. Woodruff reviews that there is growing attention to the health benefits of mind/body interventions, particularly relaxation and

meditation. Biomedical research has provided undeniable evidence of the interconnectedness of the mind and body. The field of *psychoneuroimmunology* has defined the role of stress in reducing effectiveness of the immune system in combating infection and growth of malignant tumours. There are considerable evidences Kiecolt –Glasser et al 2001 et al (reviewed in the next chapter) that Yoga and meditation practices have been successful in managing various stress related effects due to cancer.

Breast cancer is a profoundly stressful disease posing both physical and psychological threats to the patient. Moreover, patients with breast cancer normally receive multimodal treatment over a long period of time .Psychological distress and trauma is often associated with the diagnosis of cancer and is common (Derogatis LR, Farber JM, 1983). There is an uncertainty about the prognosis of cancer, and social isolation along with physical symptoms or functional losses resulting from the disease or its treatment are the most important factors. Due to these various difficulties (Spiegel D., Fox B.H 1995) many patients believe that stress, including that which is caused by their cancer experience, may contribute to poor coping as well as recurrence or progression of their disease. In the last decade there is a growing interest amongst the cancer survivors to use

various complementary therapies adjuvant to the conventional treatment in the anticipation of reducing the burden of stress and better coping to the treatment. (Holmes MD 2006, Cassileth BR 1998) There is a considerable use of these therapies in recent times in approach to cancer treatment; therefore there is a need to understand the links between social, psychological, and physiological determinants of health (Brawley LR,2002). Yoga is an ancient eastern practice which has been used for therapeutic benefits world wide and is being scientifically studied by many clinicians (Gimbel MA 1991) It has been suggested that 'gentler' physical activities, such as yoga or tai chi, may help to promote regular participation, especially in chronic disease populations who face additional barriers to engaging in an active lifestyle (Johnson NA,1998 Brawley LR,2002). There have been a number of studies including randomized trials which reported positive therapeutic outcomes following Yoga program including our group Nagendra et al . There was also a wide range of benefits reported earlier such as in asthma(Nagarathna R 1985), increase in immune function(Henderson LE),hypertension (Schneider RH, -Raub JA 2002)improvement in cardiovascular effects(Johnson NA) ,decrease in blood pressure (Wenneberg SR diabetes(Sahay Bk 2002),and serum Cortisol levels(Sadsuang R 1991)The use of CAM as an adjuvant therapy in breast cancer patients have attracted the attention of many

researchers world wide (Homes M.D 2006). Burstein et al., reported that newly diagnosed early-stage breast cancer patients had stressful mental health 3 months after diagnosis in women who began using CAM. Meditation was basically used as a religious or spiritual practices, now it has been accepted world wide as a very effective tool to calm down the mind and harmonize the physiological and psychological parameters to have a balanced effect(Telles S 1998). Meditation based relaxation program have been implemented in a number of randomized and pilot studies particularly by Carlson et al(1999-2002) and they reported to have stress reduction effectively, reduced total mood disturbance and specific symptoms of Anxiety, Depression, Anger, and Confusion. In all these studies mentioned above the main aim was to improve the quality of life of either the breast cancer survivors or those who were undergoing treatment. There have been reports of improvement of quality of life (QOL) in breast cancer patients who under went Yoga based programs or supportive counseling along with relaxation and imageries. (Rosenbaum E, casso D 2004). Inspired by favorable out comes of these interventional studies Carson et al recently reported significant improvement in pain as well as psychological parameters of metastatic breast cancer patients. Recently there is a report where there is no physical improvement of breast cancer survivors over control patients after yoga intervention but

there was a significant improvement in the global quality of life scores and mood disturbance scores (Culos-Reed SN, 2006). In our recently study Raghavendra et al 2006- reported that yoga program has significant improvement in the chemotherapy induced nausea and emesis and the breast cancer survivors had significant improvement in the quality of life. The current study aims to study the effect of an intensive and integrated yoga program which is customized for the breast cancer patients in modulating the psychological and physiological stress. It is known that radiation causes DNA damage to the peripheral blood lymphocytes (PBLs) of the patients undergoing radiotherapy treatment (Scott D 1998). We also reported, banerjee et al**,** significant radiation induced DNA damage in breast cancer patients undergoing radiotherapy (Banerjee et al 2007). There was also a study in which DNA damage in the form of telomere shortening was linked to increased stress in the population of care givers by Blackburn et al, Our group also reported a significant increased in telomere associated DNA damage in breast cancer patients after radiotherapy. DNA repair capacity is also associated with psychological and physiological stress

Therefore the fact that breast cancer patients are under stress and they also undergo considerable radiation induced DNA damage, we set to investigate in the present study the effect of an intensive yoga program on

the Psychological parameters (HADs and PSS) as well as the radiation induced DNA damage in the PBLs derived from the breast cancer patients pre and post radiotherapy in both the intervention and supportive counseling group.

Why Yoga?

Any system or process will be accepted by the common man if it can prove its usefulness in his day-to-day aspects of life. In the past we have seen how the society accepted and adopted science as an integral part of its structure as technology solved the problem of providing the basic necessities of life and offering a more comfortable life to an individual. We have also seen, that now society is all set for Yoga as it offers man a conscious process to solve the menacing problems of unhappiness, restlessness, emotional upset, hyperactivity, etc., in the society and helps to evoke the hidden potentialities of man in a systematic and scientific way by which man becomes a fuller individual. All his faculties – physical, mental, intellectual and emotional – develop in a harmonious and integrated fashion to meet the all-round challenge of the modern technological era with its hectic speed.

Chapter 2

LITERATURE

SURVEY

2. Literature Survey

A comparative study of how perception and thoughts influences gene expression (*from current as well as ancient texts*)

2.1 Concept of Heredity, DNA, Genes and its functions:-

"Like begets like" and "chip of the old block" are two of the adages quoted often when we come across a situation to compare parents and children. These popular adages are indeed so commonplace that we may not be able to appreciate the complete truth behind it. Indeed why should a human being give birth to another human being and not any other organism. A mango seed sown in the soil produces only a mango tree and not a guava tree. What is it that is hidden inside the mango seed that makes it grow into a mango tree only. When children are born we often hear statements like "the nose is like the father's and the eyes are like the mother's but the hair and the mouth are different". This is shows that the new born in addition to having parental characters, has its own characters too. Again what is a mystery behind the fact is that, the child has sufficient similarity with the parents and yet sufficient differences also. **(Bateson, 1906)**

Genetics is a branch of biology that aims to find the answers to the above questions. In fact genetics is still trying to unravel the total mystery behind these very common statements. Even though most of the basic facts have been unearthed there still remain more unsolved questions and still more remain in the darkness of the unknown.

Organisms are capable of transforming their characters to their next progeny as a result of reproduction. Ever since life first arose on this earth, it has maintained itself today in myriad form only due to its capacity to reproduce. Until **1868** when **Mendel** working with pea plants came out with a convincing explanation regarding the mechanisms of **heredity**, it was largely speculative as to how organisms pass on their characters to their progeny. The term genetics was first used in 1906 by Bateson. Earlier to this the science of genetics had already made its firm beginnings from Mendel's epoch making work with pea plant. The rediscovery of Mendel's work individually by other scientists paved the way for founding the science of genetics. The term **'genetics**' was coined in 1906 by **Bateson**. Mendel speculated that our cells carry hereditary units which was later termed as **Genes** by Waldyene in 1937.

Figure 2.1. DNA Molecule

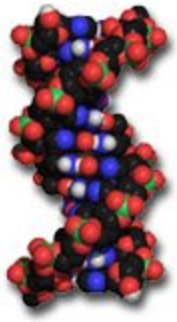
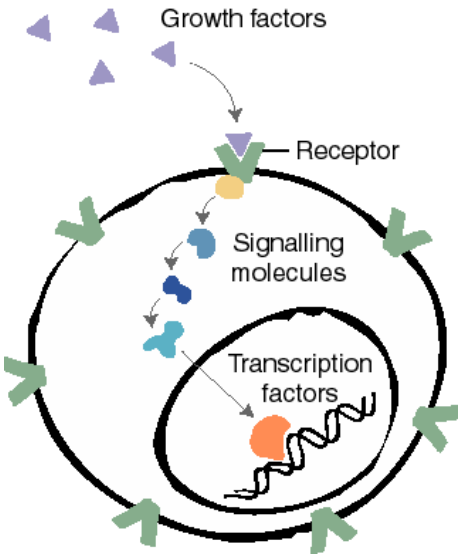


Figure 2.2. Showing a schematic diagram of functions of DNA.



DNA is a highly complex molecule manufactured in the cell nucleus and serves as the cell's "brain." DNA is the blueprint for everything the cell does. Nucleic acids are the basis of all life forms on earth. The Deoxy-ribonucleic acid or the DNA and Ribonucleic acid or RNA is the blue print of all life on earth. The DNA is a chemical compound which consists of a Deoxy Ribose Sugar a phosphate backbone and fours types of Nitrogenous bases. The bases (Adenine Guanine cytosine and thymine) are linked in a pattern. In a human cell, the DNA is arranged in 46 distinct sections called chromosomes. They are arranged in pairs, 23 chromosomes from each biological parent.

Together, the 46 chromosomes contain more than 40,000 genes. (Sanger UK update 2005).A gene is a segment of DNA that determines the structure of a protein, which is needed for development and growth as well as carrying out vital chemical functions in the body.(Watson et al 1953). Like the chromosomes, genes are arranged in pairs - one gene from the mother and one from the father.

There are 3.4 billion such bases which are linked in two helically coiled chains to form a double helix structure. In these vast sea of DNA

sequences there are few islands of meaningful sequence which functions or transcribes into RNA are called as **Genes. (**Kornberg, Molecular cell, 1978.**)**

Each gene occupies a specific location on a chromosome. Through a number of biochemical steps, each gene signals a cell to make a different protein. Some genes instruct the cell to manufacture structural proteins, which serve as building blocks. Other genes signal the cell to produce hormones, growth factors or cytokines, which exit the cell and communicate with other cells. Still other genes tell the cell to produce regulatory proteins that control the function of other proteins or tell other genes when to turn "on" or "off." When a gene is turned on, it transcribes another complex molecule called ribonucleic acid (RNA), which contains all the information the cell needs to make new proteins. **Central dogma**

Originally proposed by **F.H.C.Crick** in **1956**, the Central dogma of molecular biology states that genetic information flows uni-directionally from DNA through RNA into protein. The biosynthesis of proteins is under the direct control of DNA in most of the cases or else under the control of the genetic RNA where DNA is absent. The information for the structure of a polypeptide is stored in a polynucleotide chain. The

sequence of bases in particular segment of polynucleotide chain will determine the sequence of the amino acids in a particular polypeptide. This relationship is particularly known as Central dogma.

Cells divide only when they receive the proper signals from growth factors that circulate in the bloodstream or from a cell they directly contact. For example, if a person loses blood, a growth factor called erythropoietin which is produced in the kidneys circulates in the bloodstream and tells the bone marrow to manufacture more blood cells. (**Molecular biology of the gene, Watson 1971**.)

The understanding of the structure of a simple molecule, the DNA, stood at the gateway of a new field of study called genetics. Further more discoveries in the field of psychology; however, has added more understanding of life. In the current world there is a growing need to expand the understanding of this process of life. The researchers have realized that by localizing, any study of life does not lead to the proper knowledge and thus there is a cross talk between the studies to bring about a comprehensive understanding of life.

2.2. The process of perception and memory:-

Incoming **visual stimuli** are converted into neural signals by the retina and transmitted to the primary visual cortex. Often these visual stimuli are preserved in temporary memory traces so that the information is available for use for a few seconds after the stimulus is gone. The primate visual system has evolved two distinct pathways for serving this kind of visual memory. One is a dorsal pathway for spatial memory (the "where" pathway), and the other is a ventral pathway for object memory (the "what" pathway). The dorsal pathway carries visual information specialized for spatial location from the visual areas through the parietal cortex and to the dorsal lateral prefrontal cortex; in the monkey brain, this latter region is just anterior to the frontal eye field. The ventral visual memory pathway carries parallel information, specialized for object recognition, from the visual cortex through temporal regions and on to the middle and inferior frontal cortex. **(Courtney et, al 1999)**.

2.2.1 Mechanism of perception:-

Regulation of Ion Channel Function in CNS Neurons

CNS neurons express a variety of ion channels with specific roles in the generation and regulation of neuronal properties and functions.

Transmission of neuronal signals is similar to electrical signalling. The ion channels which are operated by mainly Calcium and Potassium ions facilitate the conductivity of nerevous signals. Of particular importance are the **voltage-gated calcium channels (VGCCs**). Although several types of these channels are expressed by neurons, calcium signaling through L-type VGCCs is particularly important in many fundamental neuronal processes, including neuronal development, neuronal excitability, and calcium homeostasis.

Extensive studies of VGCCs in cerebellar Purkinje neurons from adult animals, a neuronal type that plays a critical role in fine motor control, have indicated that the channels are essential for ***dendritic excitability***. The primary VGCCs involved in this function are the **P/Q-type VGCC**s, which are expressed in abundance in Purkinje neurons. Recent immuno - histochemical studies revealed that in addition to **P/Q-type VGCC**s, Purkinje neurons express **L-type VGCCs**, both in the mature state and at early stages of development. In the mature neurons, the L-type channels were located primarily in the somatic region, whereas the P/Q-type channels were prominent in both the somatic and the dendritic regions. (**Gruol, D et, al 2006**)

To examine the role of L-type VGCCs in Purkinje neurons, ***Gullette*** and his group used combined recordings of intracellular calcium and electrical activity in cultured Purkinje neurons at different developmental stages. The results showed that L-type VGCCs contribute to somatic excitability and calcium signaling both early in development, when each Purkinje neuron consists of just a soma and fine peri-somatic processes, and at mature stages, when dendrites are present. Interestingly, L-type VGCCs played a prominent role in the somatic excitability of Purkinje neurons. (**Gullette, et al 2006**).

2.2.2. Events that lead to memory

Memory stores patterns of activity in modular form in the brain's cortex. Different modules in the cortex process different kinds of information - sounds, sights, tastes, smells, etc. The cortex sends these networks of activity to a region called the hippocampus. The hippocampus then creates and assigns a tag, a kind of temporary bar code that is unique to every memory and sends that signal back to the cortex. (**Bruce McNaughton 2007**)

Each module in the cortex uses the tag to retrieve its own part of the activity. A memory of having lunch, for example, would involve a number of modules, each of which might record where the diner sat, what was

served, the noise level in the restaurant or the financial transaction to pay for the meal.

But while an actual dining experience might have taken up an hour of actual time, replaying the memory of it would only take 8 to 10 minutes. The reason, **McNaughton** said, is that the speed of the consolidation process isn't constrained by the real world physical laws that regulate activity in time and space.

The brain uses this biological trick because there is no way for all of its neurons to connect with and interact with every other neuron. It is still an expensive task for the hippocampus to make all of those connections. The retrieval tags the hippocampus generates are only temporary until the cortex can carry a given memory on its own.

"It's a slow process," according to **McNaughton.**

"The initial creation of the tag is made through existing connections. In order to do the rewiring necessary to have the inter modular connections carry the burden takes time. What you have to do is reinstate those memories multiple times. Every time you reinstate the memory, the modules make a little shift in the connection .something grows this way, grows that way, a connection gets made here, gets broken there. And eventually, after we do this multiple times, then an optimal set of connections gets constructed," **Mc Naughton** said.

The brain is generally thought to do all of this during sleep, specifically slow-wave sleep, when the brain is not busy with processing real-time inputs. McNaughton has developed the technology to record from multiple probes, each of which can track the activity of a dozen or more brain cells. McNaughton said that" We need groups of cells because in order to identify a pattern, we have to look at the collected activity of many neurons,". His previous research has showed that cells that fired during activity prior to sleep, also fired in the same sequential patterns during sleep. During sleep, the hippocampus sends little, 100-millisecond bursts of activity to the cortex as much as three times per second.

2.3. Emotional Memory and its Impact on Genes:-

The Swiss study by: John Timmer et al 2007(*Nature Neuroscience, 2007*)

It has been known for a long time that humans can remember those things that have an emotional impact, whether positive or negative, far better than we can recall neutral items and events. Drug studies have suggested that the noradrenergic signalling system is essential for this process. The

research team involved in a new work recognized that a gene for one of the receptors in this signalling system of adrenergic receptors of alpha 2 beta G protein receptor family ***(ADRA2B***) exists in two forms. Chemically, the two forms differ by the presence or absence of three amino acids in a section of the protein that has a large negative charge. In the US, about 30 percent of Caucasians and 12 percent of African-Americans have the shorter form of the gene.

The researchers tested a set of over 400 Swiss volunteers for their ability to recall emotional content. The test subjects were genotyped to determine what form of the ***ADRA2B*** gene they carried, and then given sets of photos to examine from a standard set of images with positive, negative, and neutral emotional content.

Their genotype made no difference to their ability to evaluate the emotional content of the image. But it had a huge impact on their ability to recall the image 10 minutes later. Those with the short form of ***ADRA2B*** gene had nearly double the recall ability, with success rates rising from the low 40 percent range up to just short of 80 percent. Recall of neutral images was unaffected.

The authors noted that the ability to recall things associated with feelings of danger or safety could provide a huge selective advantage in evolutionary terms. But they also considered the possibility that there could be too much of a good thing: excessive recall of disturbing events appears to be at the root of post-traumatic stress disorder. They worked with a Ugandan researcher to check how the ***ADRA2B*** variation affected survivors of the Rwandan genocide. Recall of traumatic events was higher in those carrying the short form of ***ADRA2B***.**(Timmer ;et al, 2007)**

2.4. Depression and Mood Regulated Neuronal Signalling

The John Hopkins study on Rats, 2006

In an experiment, Li Jun Zhou and his team at the Johns Hopkins University School of Medicine found that rats after injected with Prozac (anti-depressant) did not merely experience a change in their brain chemistry but also grew new nerve fibres in mood-critical areas of the brain. Through intricate staining techniques, the Hopkins team found that rats treated with Prozac(anti depressants) grew more axons- in the neural branches that send messages- on serotonin-sensitive neurons in cortical and forebrain areas crucial to mood. Lijun Zhou, a researcher in neurosurgery, proposes that this local change is "the key structural effect

of serotonin antidepressants" and may help explain some successful antidepressant therapy. In this study it was proved that prolonged depression causes degeneration of nerve fibres resulting in dead areas in the brain. The neurotrophin levels are directly related to the mood of a person and mood elevators can cause permanent growth signalling in the brain. Therefore, now scientists are proving the rational that mood swings can result in dramatic change in gene expression and neuronal growth profiling.

2.4.1. Stress Signalling and Gene Expression:-

O. Krizanova et al 2002 reported that repeated stress (Immobilisation) reduces the gene expression of the type 1 and 2 IP_3 receptors in satellite ganglia in mice.

Inositol 1, 4, 5-trisphosphate (IP_3) is one of the second messengers produced by phosphoinositid hydrolysis and triggers IP_3 receptor (IP_3R) mediated calcium release from intracellular pools. In another work Bary et al 2002 at Harvard reported that psycho stimulants and prolonged stress influence the gene expression profile of L- type calcium channels and affects neuronal signalling.

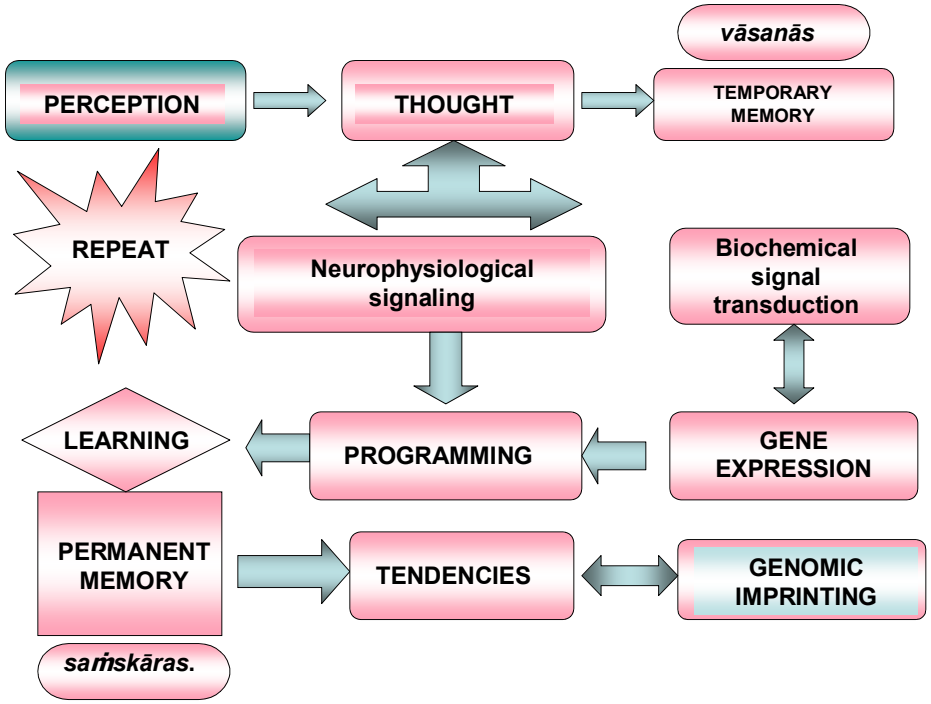


Figure 2.3.The above schematic diagram represents the effect of perception and thought at various levels and how it leads to memory.

Another study by **Stevenson et al 2005** shows that shortages of neurotrophin may contribute to the original structural weakening of neural-network circuit. According to the authors, neurology is deeply affected by perception and our perception deeply affects our neurology. We pause between thought and actions as the segment of time that allows us to *feel* something we call will (that half-second in which we can either stop or allow the action to take place). But what if the time it

takes our thoughts to cascade into action is reduced by other means than what naturally occurs - perhaps by drug therapy that makes thought-into-action far more efficient? Does this mean that the feeling I have of Will becomes only the fleeting illusion of Will? Might conditions like **ADD** (Attention deficit Disorder and **ADHD** Attention deficit hyperactive Disorder is nothing more than too little time available for individuals between thought and action? This is the most important mystery as to how thoughts are converted into action and where are the controlling switches located. Are they in the neurons or in mind which is a non – biological entity? Probably the best solution is from the Mind to the *prana(pancha prana)* level and then to the body level which are neurons.

2.5 Perception and their influence on tendencies according to ancient texts:-

2.5.2. Law of the Last Thought according to Bhagavat Gita:-

आ नो भद्रह् क्रतवो यन्तु विस्वतह्

ā no bhadrah kratavo yantu visvatah
Let noble thoughts come to us from every side

Rig Veda 1-89-I

The theory of last thought.

अन्तकाले च मामेव स्मरन् मुक्त्वा कलेवरम् ।

यः प्रयाति स मद्भावं याति नास्त्यत्र संशयः ॥

Antakāle ca māmeva smaranmuktvā kalevaram
ya□ prayāti sa madbhāvam yāti nāstyatra sam□aya□ **(BG ch-4;5)**

Translation:-
He who departs from the body thinking of me alone even at the time of death attains my state, there is no doubt about it.

यं यं वापि स्मरन् भावं त्यजत्यन्ते कलेवरम् ।

तं तमेवैति कौन्तेय सदा तद्भावभावितः ॥

ya□ ya□ vāpi smaran bhāvam tyjatyante kalevaram
ta□ tamevaiti kaunteya sadā tadbhāvabhāvita□ (BG ch-4;6)

Translation:-
Thinking of what ever entity one leaves the body at the time of death that and that alone attains being ever absorbed in its thought.

We might think that, according to the common belief, a man is reborn according to his thoughts at the time of his death. How, then, can this belief be reconciled to the theory that the rebirth is caused by the unfulfilled desires to be fulfilled in the next life? The ideas and the thoughts which come at the time of the death of a man are responsible for his next life. But, at the same time, the Vedānta asserts that at the time of

death only those thoughts and desires come to mind, which were most dominant during the life of the man. Here we get an explanation of how our life long accumulated thoughts in our permanent memory influence the behaviour and our intense desires. There is a biological programming behind these thought processes. Therefore, only rare few people think of God at the time of their death. The rest of the common people are always engrossed in their thought and *vāsanās* which entangles their *sūkśma śarīra*. Life is a mix of good and bad. We induce good or bad tendencies (vasanas) in our life depending upon our good or bad karmas. Each karma (action), or thought leaves a residual impression or *Vasana* (a Sanskrit word which means the fragrance of food that is left in the cooking vessel even after it is washed) in our mind. Only yogis of high order, who practice *niskāmā Kārmā*, are free from the effects of their actions.

The Vasanas of our actions become tendencies of our personality including us to repeat the same action again and again and, thus, forming habits which become our typical characteristics. Impressions, the root cause of all the desires and resultant actions, accumulated over many birth from stock of our karmas, both good and bad (and mixed) called *Sanchita Karma***.** Vasanas are superficial temporary memory patterns and they become samskaras and they become Samskaras which can be designated a personalitry features by long term memory stores.

vāsanās are like the seeds that have to fructify or manifest (*abhivyakti*) according to the favourable circumstances (*anugunanam*) at appropriate time (*vipaka*). Our psyche is a very fertile land. Nature is very kind to allow each and every seed to sprout.

This *vasana* has to fructify itself according to the environment and fruitful conditions. Swami Sivananda *(chittaśudhhi 1971*) has said if we do any kind of work, it produces an impression in the subconscious mind or *Citta.* This impression is called *Sa□skāra* or tendency. Whatever you see, hear, feel, smell or taste causes *Sa□skāras*. The acts of breathing, thinking, feeling and willing produce impressions. These impressions are indestructible. They can only be fried *in toto* by *Asamprajñāta* Samadhi. Man is a bundle of *Samskaras* which are impressions. It is these *Sa□skāras* that bring a man again and again to this physical plane. They are the cause for rebirths. These *Sa□skāras* assume the form of very big waves through memory, internal or external stimulus. (Swami Śivananda, 1971)

It is these tendencies that a man is born with again. Even the genome it selects is governed by the last thought and the *sa□skāras* or the karmic

signatures of the life lived before the death. Even the Rig-veda asserts that let noble thought come from all sides.

2.5.3 Concept of *Gu□as* and tendencies according to Bhagavat *Gīta*.

सत्वम् रजस् तम इति गुनह् प्रक्रिति-सम्भवाह्

निबध्नन्ति मह बाहो देहे देहिनम् अव्ययम्॥

satvam rajas tama iti gunah prakriti-sambhavāh
nibadhnanti maha bāho dehe dehinam avyayam||(BG Ch 14,5).

The three gunas of Sattva, Rajas and Tamas born out Prakriti bind down the immortal soul to the body it its embodied state.

तमस् तु अज्नन-जम् विद्धि मोहनम् सर्व देहिनाम्

प्रमद् अलस्य निद्रभिस् तन् निबध्नति भारत॥ (BG ch 14,8)

tamas tu ajnana-jam viddhi mohanam sarva dehinām
pramad alasya nidrabhis tan nibadhnati bhārata||

The Tamas Guna is ignorance- born and is the product if delusion in all beings. It is characterises negligence, indolence and sleepiness. It is this Guna which is the cause of all delimas worries tension and violent emotions.

ऊर्ध्वम् गच्चन्ति सत्त्व-स्थ मध्ये तिस्थन्ति रजसह्

जघन्य-गुन व्रित्ति स्था अधो गच्चन्ति तामसाह्॥

ūrdhvam gaccanti sattva-stha madhye tisthanti rajasah
jaghanya-guna vritti sthā adho gaccanti tāmasāh||(BG 14-18)

Those established in sattva evolve to higher goals while those abiding in Rajas remain in the mid- course.Steeped in evil tendencies the Tamas dominated one degenerate.

न तदस्ति पृथिव्यां वा दिवि देवेषु वा पुनः ।

सत्त्वं प्रकृतिजैर्मुक्तं यदेभिः स्यात् त्रिभिर्गुणैः।।

na tadasti p□thivyām vā divi deve□u vā puna□ sattvam p□k□itijaimukta□ yadebhi□ syāttribhirgu□ai□ (B.G. ch, 18. 40).

According to *Bhagavadgīta*, in the eighteenth chapter, *Bhagavān* Sri K□ś□a says that there is nothing, either on earth or in heaven, which is not controlled by the *gu□as*. Right from the bottommost hell to the topmost heaven, we will find that everything is constituted of, controlled and regulated by the *gu□as*.

काम एष क्रोध एष रजोगुणसमुद्भवः ।

महाशनो महापाप्मा विद्ध्येनमिह वैरिणम् ॥

Kāma e□a rajo-guna-samudbhava□

mahāśano mahā-pāpmā viddhyenamiha vairi□am (BG ch, 3:37)

Translation

Bhagavan speaks

Of the three basic attributes (Guṇas)

(Similar to the three basic forces of nature)

Rajo -guna is the one, which imparts motion

And is the origin of *Kaama,* the basic source

For anger and/or other desires,

This karma is the one that

You have to know and master

Because this basic karma

Is the real root cause of all problems.

And can be seen as a foe for all human kind. ||**BG Ch 3:37**|

Even the mind is under subjection to the operation of the *gu□as*. The mind is nothing but the *guṇas* in a subtle form. A rarefied form of the *guṇas* is the substance of the psychological organs - *manas*, *buddhi*, *aha□kāgunas ra*, *citta* - the mind, the intellect, the ego and the subconscious. A gross form of the same *guṇas* appears as the five elements - earth, water, fire, air and ether. Therefore there is a fraternity of feeling between the mind inside and the object outside, since both of these are constituted of the

same *guṇas*, as it has already been. One of three Guṇas is generally predominant in different men. A *Sāttvic* man is virtuous. He leads a pure and pious life. A *Rajasic* man is passionate and active. A *Tamasic* man is dull and inactive.

Sattva makes a man divine and noble, Rajas makes him thoroughly human and selfish, and Tamas makes him bestial and ignorant. There is much *Sattva* in a sage or saint and more *Rajas* in a soldier, politician and businessman.

Limitation of Rajas is excesses. These excessive functioning changes the balance of the three Gunas and leads to stresses at mental levels. Cancer is also the result of excessive functioning of the genes which promotes uncontrolled growth in cancer.

2.5.4. Nature and nurture and the functional duality of environmental Conditions for Karma

प्रकृतेर्गुणसम्मूढाः सज्जन्ते गुणकर्मसु ।

तानकृत्स्नविदो मन्दान् कृत्स्नविन्न विचालयेत् ॥

p□k□tergu□asmmūdha□ sajjate gu□akarmasu
tānak□tsnavido mandān k□tsnavinna vichālayet **(BG-ch3.29)**

Translation:-

They are under the influence of the guṇas of the p□k□ti (They do not know it) they are attached, They should not be disturbed by the ones who know it fully they should not be confused by the ones who have reached the center of the tendencies produced by these three kinds of karma (*icchā, anicchā and parecchā)*, (personally desired, hatred or not desired or due to other desires only those are manifested for which the conditions are favourable. Because of our memory of past tendencies, the chain of cause and effect is not broken by change of species, space, or time Good and bad *sa□skāras* are like seeds of different plants kept in a bottle: some grow in winter, some in the summer, and some in the rainy season. If we throw all the seeds on the earth, the seed which grows in that season will grow and the others will remain dormant waiting for the suitable environmental stimuli is also same in basic genetics (Mendel **1896**). If a pea plant with wrinkled seeds is sown it gives rise to the same morphological seeds. The seeds which nee optimum temperature to grow and Ph shall grow only in those conditions other wise it waits for the optimal conditions to be furnished. It can be also correlated with the daily life styles and stress accumulated during our course of life time. Thos thoughts which are converted into permanent memories s are expressed if give suitable stimuli. (**Goud et; al, 2004**).

2.5.5 Intellect and its controlled function (the power of discrimination)

तत्त्ववित्तु महाबाहो गुणकर्मविभागयोः ।

गुणा गुणेषु वर्तन्त इति मत्वा न सज्जते ॥

tatvavittu mahābāho
gu□a-karma-vibhāgayo□
gu□ā gu□e□u vartanta
iti matvā na sajjate (BG ch 3,28)

But they mahābāho (Arjuna)

Those steady ones thattruly understand

The divisions of Karma and that of Guṇas (attributes)

Guṇas abide in guṇas

Understanding thus, the wise ones

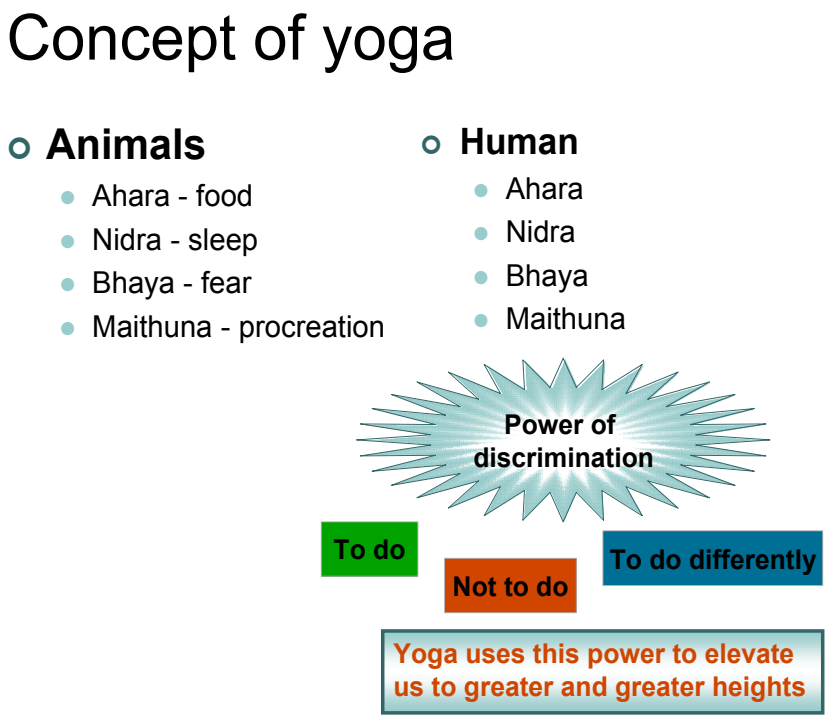
Remain always unattached.

What is happening, is happening, is happening

Let it be, let it be, let it be

The intellect or the *Buddhi* is the most important of all the products of *P□k□iti*. The senses present their objects to the intellect. The intellect exhibits them to the *Puru□a*. The intellect discriminates the difference between *Puru□a* and *P□k□iti*.

Figure 2.4.



The intellect is the instrument or organ which is the medium between the other organs and the Self. All ideas derived from sensation, reflection, or consciousness are deposited in the chief or great instrument, intellect, before they can be made known to the Self for whose use and advantage alone they have assembled. They convey impressions or ideas with the properties or effects of pleasure, pain and indifference, accordingly as they

are influenced by the qualities of *Sattva* (purity), Rajas (passion) or *Tamas* (darkness

2.6 According to Sage Patanjali:-

2.6.1. हेतुफलाश्रयालम्बनैः सङ्गृहीतत्वादेषामभावे तदभावः ॥

hetuphalāśrayāla□banai sag□hītatvāde□āmabhāve tadabhāva□ (YS4.11)

Translations:

Cause, consequence, mental colouring, and object support are interdependent, and when they disappear, desire ceases to manifest.

These tendencies are both maintained and sustained by misapprehensions, by external stimuli, by attachment to the fruits of actions and by the quality of mind that promotes hyperactivity. Reduction of these automatically makes the undesirable impressions ineffective.

2.6.2. PYS4.3

निमित्तमप्रयोजकं प्रकृतीनां वर्णभेदस्तु ततः क्षेत्रिकवत्॥

nimittamaprayojaka□ p□k□tīnām var□abhedastu tata□ kśetrikavat

Translations:

The causes of evolution do not set nature in motion, but withdraw obstacles, like a gardener opening an irrigation canal.

But such intelligence can only remove obstacles that obstruct certain changes. Its role is no more than that of a farmer who cuts a dam to allow water to flow into the field where it is needed.

2.6.3. तत्परं पु ष यातेगु णवैतृ ॥यम

tatpara puruakhyātergu avait ya (PYS: I.16).

In respect of the practice of *vairāgya*, about which we have been studying up to this time, Patanjali says that real *vairāgya* cannot arise unless we gain freedom over the *guṇas*. The spirit of renunciation does not get confirmed and does not become steadfast merely by a readjustment of an outward attitude towards things. What is essential is an adjustment of inward tendencies, and if the tendencies persist, our outward adjustments will not be of much consequence, because what liberate us and what binds us is the tendency inside, and these are the *guṇas*. These *guṇas* are terrific forces, and they cannot be controlled by ordinary effort. They are terrific because they are our masters. We are entirely made up of them, and we are subjected to them in every sense of the term. Every fibre of our being is nothing but the *guṇas*. This is actually the difficulty of self-mastery. The mastery over the *guṇas* is mastery over one's own self

2.6.4PYS4.27

तच्छिद्रेषु प्रत्ययान्तराणि संस्कारेभ्यः ॥

tacchidre□u p□tyayāntarā□i sa□skārebhya□

Any gaps in discriminating awareness allow distracting thoughts to emerge from the store of latent impressions.

In the intervening spaces of that, there are other intentions, due to *sa□skāras*.

2.6.5. Perception of thought in alert mind according to Patanjali. Guru ***Patanjali*** says that on the cultivation, disciplining and perfection of seeded spiritual union, a perfect disciple attains spiritual technology of discrimination. Awareness of this technology about the distinction between the alert mind, which is a product of Nature, and self which is a product of soul starts getting experienced. The perfect disciple starts truly and visibly experiencing the existence of a conjunction between spiritual technology of self and Nature's lack of spiritual technology and self-sense. It is due to this conjunction that the alert mind exists. The existence of the alert mind is due to the presence of this conjunction.

It is on the breaking of this conjunction between the alert mind and the self, that the alert mind gets eliminated. Self, however, is eternal and is a real viewer and a seer. The alert mind is merely a servant. It takes cosmic energy from self in order to function and operate. The alert mind is a cosmic instrument, which by taking energy from self, performs functions in order to serve its sovereign master, the self. The soul's self is a mere viewer and a seer. It does not perform any action. It does not also enjoy any action. It does not harbour any craving or any thought. The flow of lingering past subliminal impressions and the flow of craving for action, takes place in the depth of the alert mind alone. The enjoyment of worldly subjects and objects like before, however, keeps ongoing in the lingering past subliminal impressions due to the seed of the fear of death. It is in this state that on the one hand the alert mind takes the self -alert towards the soul and then takes it to a state of isolation. On the other hand and due to the presence of the seed of fear of death, it fears that by doing so the alert mind itself would die. As such, alert mind runs after the enjoyment of worldly pleasures. It keeps its own self-sense intact under all circumstances.

The Great Sage says that in the state of isolation the alert mind has to perform two functions. On the one hand, remaining lingering past subliminal impressions for the enjoyment of subjects and objects has to be fulfilled and enjoyed. On the other hand, self -alert has to be taken out of the grip of lack of spiritual technology of Nature's essence or reality. Due to these two causes and due to the fear of death lurking in the lingering past subliminal impressions, the state appears as a death like state to the perfect disciple. The fear of death is in the form of a mere seed in the lingering past subliminal impressions. It is in the form of a mere memory.

2.6.6.

ततस्तद्विपाकानुगुणामेवाभिव्यक्तिर्वासनानाम्

Tatastadvipānugu□mevābhivyaktirvāsanānām (PYS4.8)

Translations:
Consequences surely follow these inappropriate tendencies.

Because the tendency of the mind to act on the basis of the five obstacles,[*avidyā* (ignorance), (ii) *asmitā* (egoism), (iii) *rāga*(attachment), (iv) *dwe□a* (hatred) and (v*) abhiniveśa* (fear of death)]. They will surface in the future to produce their unpleasant consequences.

2.6.7. Interaction between the *Guṇas* Leads to Evolution.

YS 3.22

सोपक्रमं निरुपक्रमं च कर्म तत्संयमादपरान्तज्ञानमरिष्टेभ्यो वा ॥

sopakrama□ nirupakrama□ ca karma tatsa□yamāda-parāntajñamari□tebhyo vā

Translations:

Perfect mastery of slow and rapid evolution of actions brings knowledge of the time and circumstances of one's own death. This is also known through premonition.

The results of actions may be immediate or delayed. *Sa□yama (*voluntary control) on this can give one the ability to predict the course of future actions and even his own death.

Karmas are of two kinds; quickly manifesting and slowly manifesting.

The three *Guṇas* are never separate. They support one another. They intermingle with one another. They are intimately related as the flame, the oil and the wick of a lamp. They form the very substance of *P□k□iti.* The *Guṇas* act on one another. Then there is evolution or manifestation. Destruction is only non-manifestation.

2.6.8 PYS4.2

जात्यन्तरपरिणामः प्रकृत्यापूरात् ॥

jātyantarapari□āma□ p□k□typūrāt

Translations:

Positive evolution is the result of one's innermost nature.
Change from one set of characteristics to another is essentially an adjustment of the basic qualities of matter.

2.6.8. Concept of mind according to Sankhya (*kapila muni-oldest of the six systems of Indian philosophy*).

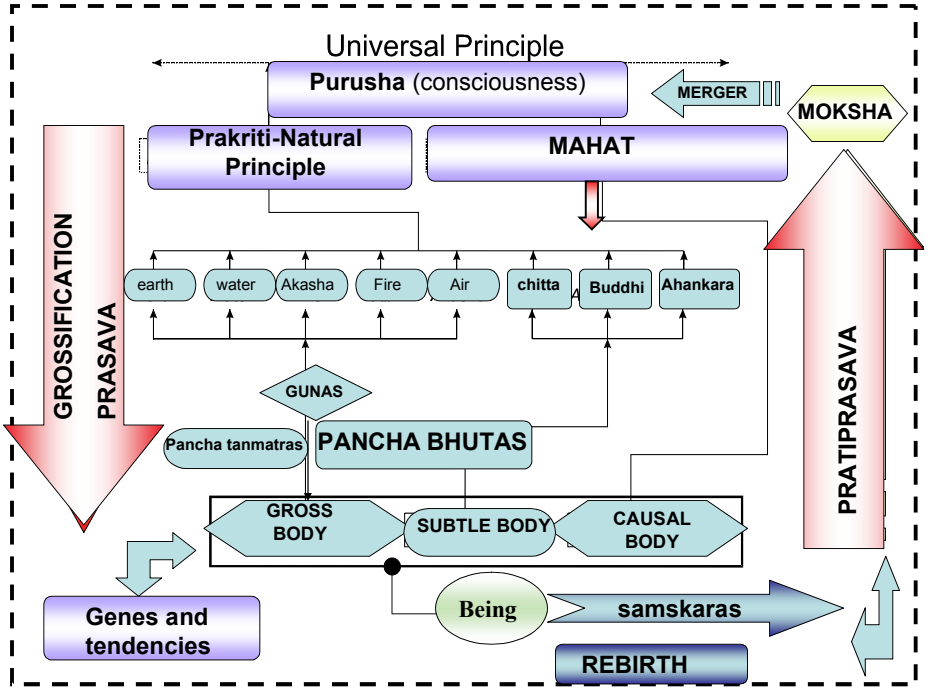
There is no separate *Pra□a Tattva* in the *Sankhya* system. The Vedanta system has a separate *Pra□a Tattva*. In the *Sankhya* system, mind, with the organs, produces the five vital airs. *Praṇa* is a modification of the senses. It does not subsist in their absence.

The *Guṇas* are the objects. *Puru□a* is the witness-subject. *P□k□iti* evolves under the influence of *Puru□a. Mahat* or the Great (Intellect), the Cause of the whole world, is the first product of the evolution of *Pṛkṛiti. Ahankāra* arises after *Buddhi.* Agency belongs to *Ahankāara.* It is the principle that creates individuality. Mind is born of *Ahankāra*. It carries out the orders of the will through the organs of action (*Karma Indriyas*). It reflects and doubts *(Sankalpa-Vikalpa*). It synthesises the sense-data into percepts. The mind takes part in both perception and action.

From this *Pṛkṛiti* emanates the cosmic *Buddhi* or *Mahat.* From *Mahat* proceeds the cosmic *Ahankāra* or the principle of egoism. From this egoism emanate the ten senses and the mind on the subjective side, and the five subtle *Tanmatras* of sound, smell, taste, colour and touch on the objective side. From these *Tanmatras* proceed the five gross elements - earth, water, fire, air and ether.

During dissolution of the world, the products return by a reverse movement into the preceding stages of development, and ultimately into *Pṛkṛiti*. Earth merges in its cause, water, water in fire, fire in air, air in *Akasa*; and *Akasa* in *Ahankāra*, *Ahankāra* in Mahat, and *Mahat* in *Pṛkṛiti*. This is the process of involution. There is no end to Samsara or the play of *Pṛkṛiti*. This cycle of evolution and involution has neither a beginning nor an end.

Figure 2.5.



2.6.9 Memory and its impressions:-

PYS 4.8 अनुभूतविषयासम्प्रमोषः स्मृतिः ॥

anubhūtavi□ayāsapramo□a□ sm□ti□

Translations:

Memory retains living experience. Memory is the mental retention of a conscious experience. Memory is the "non-deprivation" [i.e. retention] of experienced objects.

Recollection is mental modification caused by reproduction of the previous impression of an object without adding anything from other sources. Memory is the not stealing away along with objective mental impression [retained] [i.e. the reproducing of not more than what has been impressed upon the mind. When a mental modification of an object previously experienced and not forgotten comes back to consciousness that is memory. Memory is not allowing an object which has been experienced to escape.

Our memory is intimately linked with emotion and it is very subjective. "Memory is knowledge born out of *samskàra*." Once a memory has been laid down, it can surface at any time when rekindled for some reason. A memory can be of something real or something imagined; the latter is what happens in dreams" All memories arise out of impressions whether of right cognition, misapprehension, vague ideation, deep sleep or of former memory. The foregoing fluctuations are of the nature of pleasure, pain or afflictions.

Every living being, be it a mighty elephant or a tiny insect, runs to save its life when any danger is perceived. Where do this knowledge and the fear of death come from? We generally learn things by experience. But living beings do not have this experience of death. As per Hindu thoughts, this experience of death may not have occurred in this life but is a behaviour learnt in previous lives and inherited as *sa□skāras*

जातिदेशकालव्यवहितानामप्यानन्तर्यं स्मृतिसंस्कारयोरेकरूपात् ॥

Jātideśakālavyavahitānāmapyānantarya sm□tisa□skārayorekarūpāt (PYS4.9)

Translations:

Despite differences in birth, place and era, our behaviour continually perpetuates itself because of the unity of form between memory and mental permeation.

Memory and latent impressions are strongly linked. This link remains even if there is an interval of time, place or context between similar actions

2.6.10. PYS 2.54

स्वविषयासम्प्रयोगे चित्तस्य स्वरूपानुकार इवेन्द्रियाणां प्रत्यहारः ॥

Svavi□ayāsa□prayoge cittasya svarūpānukāra ivendriyā□ā□ pratyāhāra□

Sensory perceptions and attractions from the external world are the basic stimulants to provoke the external reactions. If the sensory perceptions get converted into attractions there should be some screening arrangements in the brain, which will let through only some of the perceptions, which can become attractions. Memories must obviously play an important role in this screening process. *Prātyāhāra* is the process of vitally activating this screening process, and make it so scrupulous that the real core of the mind is left free to take on its fundamental function, which is the realization of *ānanda.*

Here Patanjali explains that there is a programming in the brain after the perception thoughts and memory. But Yoga is the trick to modify the process and remain detached from the impressions. The process has to be voluntary and not forced. The with drawl must come from the innermost efforts of the mind in a gentle manner.

2.7. FROM THE UPANIŚADS:

Beginning our search in the upaniñads, we start of from the most profound and smallest of the upaniñads-

2.7.1. Māndukyopaniñat.

नान्तः प्रज्ञं न बहिष्प्रज्ञं नोभयतःप्रज्ञं न प्रज्ञानघनं न प्रज्ञं नाप्रज्ञम् ।

अदृष्टमव्यवहार्यमग्राह्यमलक्षणमचिन्त्यमव्यपदेश्यमेकात्मप्रत्ययसारं

प्रपञ्चोपशमं शान्तं शिवमद्वैतं चतुर्थं मन्यन्ते स आत्मा स विज्ञेयः ॥

Nānta□prajña□ na bahi□prajña□ nobhayata□prajña□ na prajñānaghana□ na prajña□ nāprajña□ |
Ad□□□amavyavahāryamgrahyamalakśa□amacintyamavyapadeśyamekāt mapratyayasāra□ prapañcopśama□ śānta□ śivamadvaita□ caturartha□ manyante sa ātmā sa vijñeya□ (Mā□□ūkya Up : 7)

Cognitive (wakeful) nor cognitive bothwise (intermediate state in between wakeful and dream states); neither is it an indefinite mass of cognition (deep sleep), nor collective cognition (Īçvara's cognition of whole phenomenal existence in one) nor non- cognition (mere insentiency act).It is unseen, unrelated inconceivable, uninferable, unimaginable, indescribable.

It is the essence of one's Self, a cognition common to all states of consciousness. All phenomena cease in it. It is peace, it is bliss, it is non-duality. This is the Self and it is to be realized.

There is a very subtle hint and advice given here to the humanity or the students of Indian spirituality. The cognition or the thought processes after perception must be let go or sublimally filtered so that it does not leave any lasting impresions by which we are attached. We must therefore, have only the ISvara impressions whcih is the basis of the creation and freedom in the cosmos.

2.7.2.As per Ka□hopaniśat:

In Ka□hopaniśat we read the lines as follows:

यदा सर्वे प्रमुच्यन्ते कामा येऽस्य हृदि श्रिताः ।

अथ मर्त्योऽमृतो भवत्यत्र ब्रह्म समश्नुते ॥

Yadā sarve pramucyante kāmā ye'sya h□di śritā□
Atha martyo'm□to bhavatyatra brahma samaśnute. (Ka□ha Up: 2.3.14)
When all the desires that dwell in the mind are destroyed, then the mortal becomes the Immortal.Right here, he experiences that Brāhmic Consciousness.

There is again a subtle hint here that mind is influenced by desires and those desire form the basis of our karmic signatures. The main basis of Yoga and meditation is to let go these desires. Therefore , we get a direct hint that mind is programmed by our thoughts and action.

2.7.3.In Taittirīya:

यतो वाचो निवर्तन्ते । अप्राप्य मनसा सह । आनन्दं ब्रह्मणो विद्वान् न बिभेति कदाचनेति ॥

Yato vāco nivartante aprāpya manasā saha|
Ānanda□ brahma□o vidvān | na bibheti kadācaneti|| Tai. Upa. 2 .4. 1||

When speech recedes and the mind reaches not there, one realises Ānanda, the Brahman; there he fears not.

तद्वा अस्यैतदाप्तकाममात्मकाममकामं रूपं शोकान्तरम् ॥

Tadvā asyaitadāptakāmamātmakāmamakāma□ rūpa□ □okāntara□ ||Bra. Upa. 4. 3. 21||

Embraced by his beloved wife, he cognizes neither the outer nor the inner worlds.

Båhadāraëyaka, the biggest of the Upaniñads describes the best of the sense pleasures as the state devoid of thoughts outside or inside.They consider all human experiences.

In Bhagavat Gita

मान् अपमान्योस् तुल्यो मित्र् अरिपक्सयोह्

सर्वरम्भ परित्यगि गुन् अतितह् स उच्यते॥

mān apamānyos tulyo mitr aripaksayoh
sarvarambha parityagi gun atitah sa ucyate||(BG-ch 14-25)

A person is said to have transcended all the three Gunas, when he is unaffected by any external imbalances or if he perceives anything he treats everything with equanimity.

पुरुषह् प्रक्रिति- स्थो हि भुम्क्ते सद् अप्रक्रिति जन् गुनान्

कार्नम् गुन- सन्गोस्य सद् असद्- योनि- जन्मसु॥ (BG ch 13-21)

puru□ah prakriti- stho hi bhumkte sad aprakriti jan gunān
kārnam guna- sangosya sad asad- yoni- janmasu||

The *Purusha* or consciousness is the enjoyer of everything and attaches to prakriti to give birth to all the Gunas.

प्रजहाति यदा कामान् सर्वान् पार्थ मनोगतान् ।

आत्मन्येवासना तुष्टः स्थितप्रज्ञस्तदोच्यते ॥

Prajahāti yadā kāmān sarvān pārtha manogatān|
Ātmanyevātmanā tu□□a□ sthitaprajñastadocyate||Gita:2. 5||

When the mind gets relieved of all its desires (thoughts), man is bliss unto himself. He is then the Sthitaprajïa.

All desires sprout in the mind. Hunger and thirst arise in the body no doubt. But these natural urges send up imperative messages or mandates to the relevant portion of the brain. That part of the brain sends up commands to the brain cortex which is the executive commander.

The desire is curbed in a quite a different way, sometimes. The mind is trained to think and believe that overcoming or curbing the desires, is a magnificent achievement. When desires are thus curbed, the mind feels intensely happy

No animal ever tries to curb the desires and feel happy. But the human-animal has learnt this trick. The desire to acquire the power to curb, the natural desires, is also a desire, and is a '*manogatān kāmān'*. Such a desire is glorified, deified and considered noble and so people over the centuries have tried to cultivate, strengthen and eulogize it. Cultivating and developing this may justifiably called one of the main differences between animals and man because; such noble desires lift up man from his animal nature. Succeeding in this attempt, man becomes less of an animal and more of a man. Later on progressing on this path he can become less of a man and more of an idealist, tending towards divinity, divinity as we understand it, however vaguely.

A person that accomplishes this ideal can later on experience *ānanda.* After attaining that stage, he needed no longer exert himself, to maintain the deified desire continues effortlessly. He can thereafter turn on *ānanda* anytime he so desire and gets into *Samādhi.*

Manogatān Kāmān includes the glorified desire also:-

Thus *manogātān kāmān* includes:-

(1) Desires, which relate to the satisfaction of the bodily urges, and these, as everyone is painfully aware, are a legion.

(2) Desires that crop up in the mental plane only, triggered off by sensory stimuli, which drag up the memory storehouse.

(3) Desires conjured up by the mind to curb the animal urges and lift him up to human and later to divine states.

The mind of an experiencer of *ānanda* or of the one in *Samādhi* will be in this state of complete freedom from all desires that may occur in the mind. When all the desires of the mind are *(prajahāti*) cast away, then the person has a firm mind, untrammeled by the happenings all around and within himself. He is said to be in *Samādhi*, then. It certainly is not a void or blank. A part the essence is intensely aware of the whole of cosmos or *brahmānanda.* The awareness is also blissful, and completely satisfying, so satisfying that nothing else remains to be desired. This feeling of *ānanda* identifies one with anything and everything so that there is nothing else at all. In such a state, one feels alone, there is no second or *dvaita.* (picked up terms).When all the desires are gone, whatever is left of the mind is just *ānanda* and awareness. Such awareness is satisfied with itself, by itself and for itself. This must be so. Because the mind has nothing else to mind about.

2.8. Summary of the scientific and ancient concepts of perception and thought and its effect on tendencies and genes.

It is these tendencies that a man is born with again. Even the genome it selects is governed by the last thought and the *samskaras* or the karmic signatures of the life lived before the death. (Bhagavat gita) Even the Rig-veda asserts that let noble thought come from all sides.

The impulse behind most human actions in so far as man is a psychophysical being comes from what are called *samskaras* (subliminal and latent tendencies) and *vasanas* (desires rooted in the psyche at an unconscious level but their force is also consciously felt). Each human being is born with a certain configuration of these *samskaras* and *vasanas* (their precise nature determined by action in a previous life) and these felt as attraction towards some things and aversion towards others, act as driving forces behind our actions, in so far as we act out the dharma of our being as part of nature.

On one hand one can look at identical twins separated at birth who lead very similar lives and share many behaviors -- this seems to support the dominant role of genes. But we can also point to studies of psychiatric

patients with conditions like depression or OCD (obsessive-compulsive disorder ***Benedict et, al 2006***) who improve with talk therapy. That implies that a disorder associated with the brain's "hard wiring" is treatable through changes in "soft" things like behavior, emotions, and thinking.

This seems to leave us with circular reasoning: a machine controlled by genes is locked into pre-determined behavior unless an equally mechanistic influence from the outside changes that behavior. In other words, determinism is used as an explanation both for nature and nurture. If we abandon materialism and allow for the existence of a mind, with its rich panoply of wishes, desires, dreams, and impulses, the picture changes. Genes become the starting point, but free will and environment play an unpredictable role. When genes and uncertainty meet, the mixture is far more creative than neurology presently allows. A gene is inherited from the parents but bears the imprint of memory of function. (**Gould et al 2006**)But memory obviously doesn't rule us completely. *As the Shiva Sutras say, "I use memory, I do not allow memory to use me*." This gives us one of the tenets of enlightenment, that the human mind can free itself from the past by going inward to the source of consciousness. In the Vedic view, the purpose of the past is to provide a vehicle for the present. we were born with a physical body, including the brain, outfitted with enough

past memory (probably imprinted on the genes) to provide a direction for our life, a blueprint of our unique tendencies. As we interact with our surroundings, new material for memories comes in. The brain constantly incarnates its past experience by turning intangible events, emotions, sensations, and drives into cells; there is no need to wait for new genetic mutations when every neuron is capable of expressing itself across a wide range of experience. In other words, the body we have today is metabolized experiencein all its accumulated richness.

However, we do know that accidents, drugs, surgery, shock treatments, strokes and tumours do a very efficient job of disorganizing various aspects of the brain.

The really interesting fact is that none of those qualities that comprise our mental character is immune from such accidents. We can lose our memory, our talents, the normal emotional tone of our personality, and our likes and dislikes because of various cerebral accidents and injuries. And of course, we can lose all our faculties as well, such as the ability to reason, to form speech, to control our bodies, etc.

Now, if the subtle body is sufficient to support the existence of a mental life, including all our faculties and all the unique qualities of our mental character, then why is the brain also necessary, and why cannot these mental qualities continue when the brain is damaged?

One answer we can think of is that, once the subtle body enters a physical body, it somehow becomes limited by that physical body. It is as if we were sitting buckled in at the driver's seat of a car. If the engine breaks down, we can't go anywhere without unbuckling the seatbelt, opening the door, and leaving the car. The engine could correspond to any of our brain functions. So the greatest challenge is to master the meeting point between past, present, and future. In the Indian spiritual tradition, the highest achievement was complete freedom from all three *Guṇas*. The Self or Atman, being pure consciousness, has no predetermined qualities. It exists in the realm of pure potential. Modern society doesn't have a value system that equates freedom with enlightenment, but with new advances in brain research, we are at least seeing the fingerprints left by the mind on the brain, and we can observe how experience gets metabolized into various receptors and neural networks that are unique for each individual. Science cannot yet explain why two people with a similar genetic makeup and experiences can turn out so differently, or so closely, for that matter. but

the potential for recognizing consciousness as the source for both nature and nurture may be the most exciting possibility looming ahead.

In fact our intentional and unintentional visualization inspires DNA 24 hours a day, 365 days a year. We are simply unaware of the process. So we continually affect our genetic code and the nature of the effect is largely down to the nature of our thoughts, feelings, attitudes, beliefs and intentions. Therefore, controlling the mind should lead to the possibilities of controlling or influencing the action of genes to either partially or wholly.This is the basis that Yogic control of the mind is possible to affect the function of the genes and thus facilitate the treatement of cancer.

2.9 Relevance of this literature on perception and mind control in context to cancer.

2.9.1. The modifications of mind and molecular concepts of Cancer:-

Molecular concept of cancer: - According to ***R.A.Weinberg 1998*** Cancer is not one disease but a **disorder**. No matter what cancer takes, it remains a malady of genes, and most, if not all, causes of cancer act by damaging genes directly or indirectly. The genetic paradigm has greatly enhanced

our understanding of cancer and now guides most researches on the disease. Cancer is essentially a rapid and unregulated division of cells. It has been seen that the rate of cell division varies with different cell types. It is possible for perfectly normal cells, e.g., blood forming cells, to have a high rate of division than some cancerous cells.

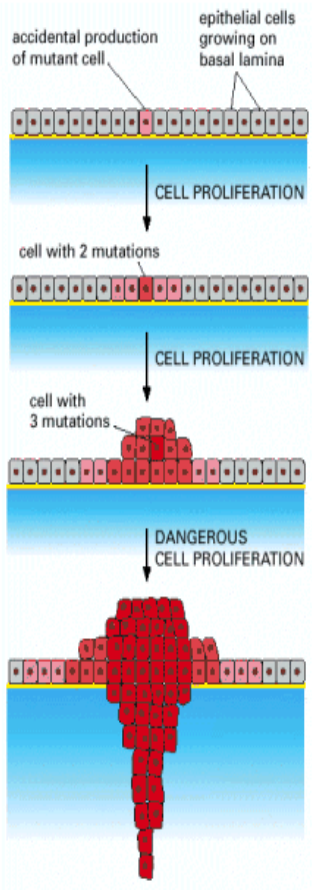
Tim Hunt 1997 reviewed that Cancer results from a breakdown of the regulatory mechanism that governs the division, differentiation and survival of the individual cell. As a result of the loss of regulation, the cancer cells grow and divide in an uncontrolled manner. They ultimately spread throughout the body, interfering with the functions of normal tissue and organs. Each cancer cell has a characteristic life span. Some cells end their life span by death and degradation. Others complete their life span by division. Normally, the overall rate of cell division is high in growing animals, so that cell multiplication is greater than cell death. Once the steady state is reached in others, the origin of new cells and the death of old cells are balanced. Some cells are highly specialized, while others remain relatively undifferentiated.

First, cancer is fundamentally a disease of individual cell (**Kornberg 1979**). The complexity of the cancer can be understood only through

attention of genes, cancer is a perversion of cellular phenotype, and genes are the determinants of that phenotype. DNA and its behaviour is quite sophisticated, and we currently lack an understanding of its exact behaviour or characteristics. DNA is responsible for the creation of perhaps the most sophisticated of creature; the human being. However, nature has also engendered cancer formations, as well as other phenomenon that are capable of destroying us

Hanahan D and Robert Weinberg in their classical review in *Cell, 2000* described cancer as a multifactorial genetic catastrophy. Robert A. Weinberg, PhD, is an internationally recognized authority on the genetic basis of human cancer, having isolated the first human cancer-causing gene and the first known tumour suppressor gene.

Figure 2.6.



Cellular Mechanisms of Tumor Growth and Progression

Cancer results from an accumulation of mutation-induced aberrations in cellular proliferation, survival, migration and invasion

Oncogenes – activating mutation or overexpression promotes tumor growth and progression

Tumor suppressor genes – inactivating mutation or loss promotes tumor growth and progression

2.9.2. Events in Cancer onset (Geoffrey L.Greene, et al 2000)

1. Mutation.(Change in the structure or function of a coding gene)

2. Cell cycle check points are lost.(regulatory mechanism of division)

3. Tumour formation [Benign/Malignant] (accumulation of cells)

4. Benign to malignant transformation.(cancer gains momentum).

5. Increase in tumour size.(more violence in the molecular level)

6. Angiogenesis: formation of arteries and veins [Neo-vascularization*]

7. Metastasis of tumour [delocalization of tumours]

8. Tumour invasion to vital organs.(Gain of function over the normal physiology)

9. Multi-organ malfunctioning.(Homeostasis breaks down completely)

10. Death of the individual who is affected with cancer.(System fails)

Figure 2.7.

Cancer – General

- **Classifications**
  - carcinomas – epithelial cells (most common)
  - sarcomas – connective tissue cells
  - neural tumors
  - non-solid tumors
    - leukemia – blood forming cells
    - lymphoma – immune cells
- **Tumor Types**
  - benign – remains local
  - malignant – invasive
- **Incidence in America**
  - more than 1,000,000 new cases diagnosed each year
  - more than 500,000 deaths each year

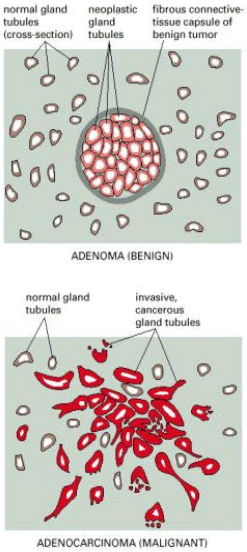
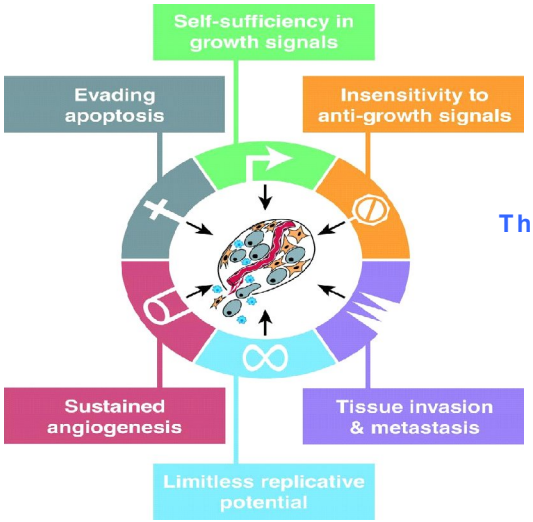


Figure 2.8.



The Cellular Hallmarks of Cancer

Hanahan and Weinberg (2000) *Cell* 100, 57-70.

2.9.3. Pathways of Oncogenesis

The control of the cell proliferation and cell fate is central to the proper differentiation of specific cell types, maintenance of tissue, homeostasis, and the ability of certain cell types to respond to mitogenic signals.(cyclins)(Paul Nurse 1983) Loss of this control underlies many human diseases, including cancer. The gene regulatory pathway governed by the retinoblastoma tumor suppressor protein (Rb) that is responsible for control of expression of genes encoding proteins that effect the replication of DNA and facilitate the orderly passage thro of cell growth. Recent studies have illustrated the fact that various cell fate decisions that trigger apoptotic pathways are tightly linked to the function of the Rb pathway.

The normal cell cycle consists four segments; G1, M, G2, and S. the cell commits to replicating chromosomal DNA and the check-points at the G2 to M transition associated with entry into mitosis.

There are several genes and enzymes which help in controlling cell cycle. The important and master of all is p53 which acts prior to DNA synthesis, by checking DNA damage and delaying entry into S until the damage is

repaired. This gene along with Rb and often several enzymes maintain homeostasis.

The p53 is a protein (phosphor- protein) coded by p53 gene which functions as a transcriptional activator, this property is responsible for its tumour suppressing activity. This p53 induces the transcription of a gene encoding a 21 KD protein that interacts with and controls various cyclin-Cdk complexes. These cyclin-Cdk complexes are responsible for activation and inactivation of Rb gene which gives Rb protein. This activation and inactivation is done by phosphorylation and de-phosphorylation of Rb protein. When the Rb is active (non-phosphorylated form), it goes and bind to the transcriptional factor E2F which is needed for promoting transcription and halts the process of transcription thus, stopping the cell cycle. If the Rb is inactivated (phosphorylated form), then it cannot bind to E2F thus promoting transcription process and helping to move the cell cycle.

The cell cycle works with a great coordination of different enzymes and proteins along with p53. The mutation or change or non-functioning of any of the component of cell cycle leads to tumour or cancer. There are various pathways which leads to cancer, it may be

1. Mutation in p53 (p16, p21).
2. Mutation in Rb.
3. In production of cyclins and kinases.
4. Inactivation of cyclins and kinases.

Improper functioning of many other components of cell cycle also leads to alterations.

2.10 Genetics of Cancer

Cancer originates from changes in DNA that result in unregulated cell division. Most of these changes are mutations that involve changes in the DNA sequence. The mutations may arise as a result of errors in replication or DNA repair, or exposure to carcinogens. Nearly all cancers are genetic in origin, but fortunately most cancers are not inherited. (James P. Allison, 1999)

General classes of cancer genes (Bert Vogelstein et al 1997, 2000, 2001)

There are three general classes of genes, oncogenes, tumor suppressor genes and DNA repair genes.

(i) Oncogenes:

Cells posses a variety of genes called **proto-oncogenes**, which encode proteins that carry out the normal activities of the cell and promote normal

cell growth. Activation of proto-oncogenes by point mutation, converts the proto-oncogenes into oncogenes (tumor-causing genes). Oncogenes encode proteins that promote loss of growth control and transform the cell to a malignant or neoplastic state. Thus oncogenes are genes whose action promotes cell proliferation.

Cellular oncogenes causing human cancer were discovered by Krontiris and Cooper (1981) and Shih et al. (1981), and were thought to be dominantly acting. However, some 20 years before this discovery, cell hybridization experiments had been carried out to make a genetic analysis of malignant. Barski and Cornefert (1962) and Scalettan and Ephrussi (1965) fused some mouse cells of high and malignancy potential and found that the resultant hybrid cells were malignant. This led to the interpretation that malignancy was a dominant trait, supporting the concept of 'dominantly acting' oncogenes.

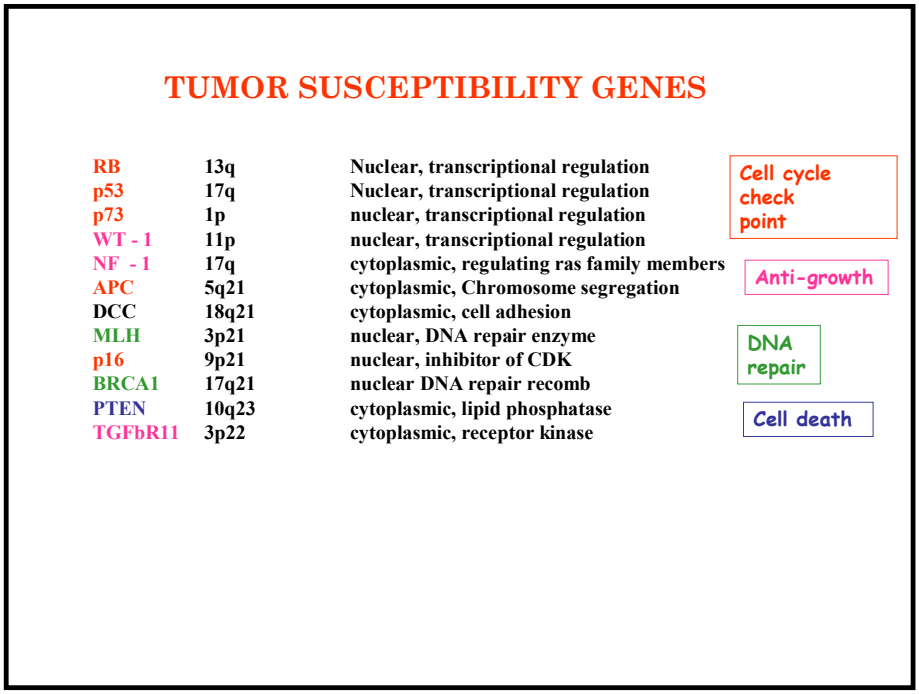
Later, experiments of Harris, Klien and colleagues (1969, 1971) however, yielded results contrary to the concept of dominantly acting oncogenes. Malignancy was shown to be a recessive in these cell fusion experiments.

(i) Malignant cells contain both regulatory genes (TSGs) and oncogenes

Cellular proto-onco-genes are involved in many of the processes regulating cellular growth. The activation of cellular genes that are not expressed in quiescent cells is likely to be involved in the continuation of cellular growth. Several proto-onco-genes encode transcription factors which regulate the transcription of genes in growing cells.

Viral and cellular onco-genes are genes that contribute to the abnormal behaviour of malignant cells. Many proto-onco-genes encode proteins that are involved in the regulation of normal cell proliferation. Their corresponding onco-genes encode onco-genic proteins that result in uncontrolled proliferation of cancer cells. The mechanisms involved in this process include elevated expression (up regulation) of the onco-genes, defective differentiation and failure to undergo apoptosis (programmed cell death). Most onco-protein functions as components of signalling pathways that regulate cell proliferation in response to stimulation by growth factors. These onco-proteins include polypeptide growth factors, receptors for growth factors, components of intracellular signaling pathways and transcription factors.

Figure 2.9.



Tumor suppressor genes or 'anti-oncogenes': (Kenneth Kinzler, 1997 et al)

Normal cells contain genes on their chromosomes that suppress unregulated cell growth. These genes are called tumor-suppressor genes (TSGs) or 'anti-oncogenes'. TSGs encode proteins that restrain cell growth and prevent cells from becoming mutant. Suppression of uncontrolled proliferation, a characteristic of cancer cells, is in effect tumor suppression. The normal products of TSGs have an inhibitory role in cell growth and division. In certain cancers, specific chromosome

regions are deleted from both homologues, resulting in deletion of both alleles of the TSG. Inactivation of the TSG alleles results in the loss of the inhibiting activity. This in turn results in unregulated cell proliferation.

Since mammalian cells are diploid, most genes are represented by pairs of alleles. Somatic mutation of one allele of a TSG results in heterozygosity at that locus. (Knudson 1978) This usually does not have significant consequences, and there is normal growth control. The normal allele codes for sufficient gene products for normal function. In some individuals the cells have inherited loss of function of one allele of the TSG, with the other allele remaining functionally normal. Such individuals do not develop tumours caused by the particular gene. Only when a mutation takes place in the remaining normal allele will there be uncontrolled cell growth. Thus mutation or loss of both alleles of a TSG is necessary for a cell to lose growth control. Such a cell will lack any copy of the wild type (normal) allele, and the protein encoded will therefore be inactive. Thus tumour-suppressor mutations that are found in tumour cells are recessive.

2.11. Effect of Stress and Cancer -Need for mind modifications:-

The cellular stress response is evolutionarily conserved in all living organisms, and a major role is attributed to the induced heat shock proteins (Hsps) and other molecules that confer stress protection. The molecular responses elicited by the cells dictate whether the organism adapts, survives, or, if injured beyond repair, undergoes death. Most of the time these responses are beneficial to the organisms, but sometimes cells like cancer cells mount defensive mechanisms that interfere with therapies. Our detailed understanding of stress responses has paved the way for the development of stress-tolerant crops in several instances (Grover et al 1999).

According Griffith et al 1996, apoptosis is mediated by extrinsic (receptor-mediated) and intrinsic (mitochondria-mediated) signalling pathways that converge in the activation of caspases (which activates the death signal path way).

Apoptosis or programmed cell death is a self-destruct mechanism by which multi-cellular organisms eliminate damaged cells. If DNA repair fails, the p53 protein can trigger damaged cells to undergo apoptosis. Thus the cell is destroyed before its genetic abnormalities can be inherited. In the absence of a functional p53 gene, the p53 protein is not produced. Therefore the p53 apoptosis pathway cannot be activated. The cell cycle thus progresses without DNA damage being repaired. This results in an increase in the overall frequency of gene mutations, chromosomal rearrangements and aneuploidy. Thus the chances of other mutations promoting cell proliferation or blocking apoptosis are increased. The cells become genetically unstable and go on accumulating more and more genetic damage. This may lead to the formation of malignant tumors. The p53 tumor suppressor gene therefore serves as a link between the cell cycle and apoptosis.

Cysteine protease activation is an early hallmark of apoptosis. The activation of the inactive pro-caspases occurs in a hierarchic cascade in which the apoptotic signal activates an initiator caspases, which in turn activates the effectors caspases. These proteases initiate the degradation

phase of programmed cell death, giving rise to the morphological features of apoptosis (cytoplasm and nuclear condensation, cytoplasm shrinkage, cell membrane blebbing, chromatin clumping at the periphery of the nucleus, endonuclease-mediated deoxyribonucleic acid [DNA] cleavage, and formation of apoptotic bodies). Recent evidence suggests that *Hsps* may block the cell death pathways at different levels. Because cellular homeostasis is a balance between survival and death, *Hsps* and other chaperones play a pivotal role to support this sensitive balance. With respect to cancer treatment, chemotherapy is one of the most common therapies. Unfortunately, anti-neo-plastic drug resistance, attributable in part to blocked apoptosis, is an important cause of failure in cancer treatment.

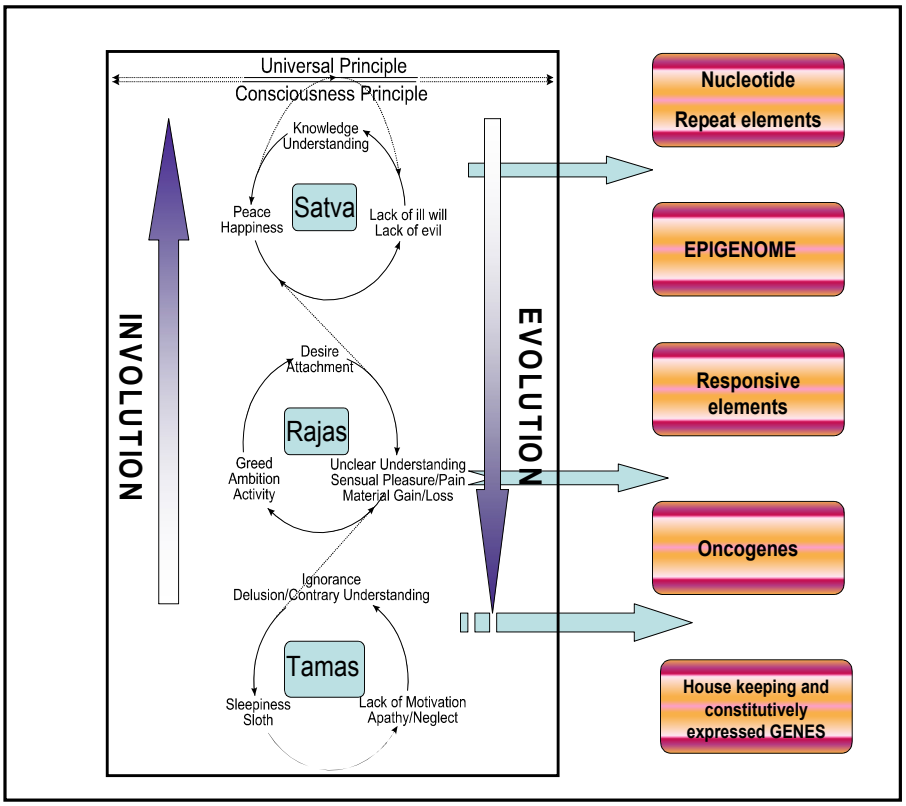
Dr Stuart K. Calder wood explains that under highly concentrated conditions, proteins are at risk of aggregation, which is a cellular catastrophe. Such effects, however, rapidly trigger the activation of the heat shock transcription factor (Hsf) and the expression of Hsp molecular chaperones. This group studied the regulation of Hsf1, Hsf2, and Hsf4 in normal cells and in prostate carcinoma during malignant transformation and under conditions of exposure to cancer therapy. Expression-profiling studies indicated that each of the factors is expressed at equivalent levels

during malignant progression, in line with the high degree of conservation of the response. However, Hsf1 activity and Hsp expression played significant roles in the generation of the malignant phenotype and in the resistance to clinical cancer treatment.

From the above review of literature we find that stress plays a vital role in development of cancer. Dr Stuart K. Calder Wood explains that under highly concentrated conditions, proteins are at risk of aggregation, which is a cellular catastrophe. Such effects, however, rapidly trigger the activation of the ***heat shock transcription factor*** (Hsf) and the expression of Hsp molecular chaperones. This group studied the regulation of Hsf1, Hsf2, and Hsf4 in normal cells and in prostate carcinoma during malignant transformation and under conditions of exposure to cancer therapy. Expression-profiling studies indicated that each of the factors is expressed at equivalent levels during malignant progression, in line with the high degree of conservation of the response. However, Hsf1 activity and Hsp expression played significant roles in the generation of the malignant phenotype and in the resistance to clinical cancer treatment. It is due to chronic stress and production of *Hsps* that tumour display immortality and resistance to various therapeutic treatment modalities. Therefore, stress reduction and a holistic approach to cancer treatment is the current need to

develop an integrated approach to cancer management. As referred earlier that all elements in *Pṛkṛiti* are governed by *Guṇas,* therefore we can draw a rational that even Genes (both functional and non functional) follow the dharma and karma of the three Guṇas. The normal functioning of "House keeping genes" follow the balance of satva, tamas and rajas. The genes which are dormant and do not function on a regular basis can be called as *tamasic*. The genes which expresses on a dynamic and regular scale can be called as *Rajasic* genes. The genes which govern the subtleties of function can be called as *Satvic* genes. The proto-oncogenes which are dormant in most of the normal individuals are influenced by chronic exposure to stress and get transformed into Oncogenes and acquire ***violent emotions***. The signal from the stress forms a vicious cycle and continuously give growth signals which leads to immortality.

Figure 2.10.



Robert W. Woodruff reviews that there is growing attention to the health benefits of mind/body interventions, particularly relaxation and meditation. Biomedical research has provided undeniable evidence of the interconnectedness of the mind and body. The field of *psychoneuroimmunology* has defined the role of stress in reducing effectiveness of the immune system in combating infection and growth of malignant tumours. There are considerable evidences Kiecolt –Glasser et

al 2001 et al (reviewed in the next chapter) that Yoga and meditation practices have been successful in managing various stress related effects due to cancer. In addition, there are encouraging reports of studies citing the influence of melatonin on breast and prostate tumours. A preliminary study finds an association between meditation practice and levels of melatonin produced by the pineal gland.

We know that each parent contributes 50% of the genome of a child and the permutation and combination of each parental genotype gives rise to the genome of the offspring. But the tendencies and characteristic may resemble to some extent to the parents but the ***core tendencies and vasanas or intense desires*** are the grossified form of the prarabdha and sanchita karma. The environment plays an important role in specific gene expression at the both macro and micro level .But these tendencies can be modified or influenced by sadhana and deep meditation. Going into deeper realms of our consciousness we can touch the subtle layers of silence and pure consciousness which may result in modification of the gene functionality. All this above ancient literature and scientific research converges to an idea of using an integrated approach of Yoga and mind modification techniques to bring about a regulatory effect in controlling the catastrophic effects of cancer. There is too much speed in the current

world order which can be compared to the entropy factor in Physics. This excessive speed has lead to the imbalances in the panic channel which has lead to diseases like cancer. These imbalances cannot be treated with only external therapeutic intervention alone. There is a serious need to address the imbalances at all levels of our existence. Therefore, an integrated approach of yoga therapy must be developed each disease and its cure. Stress in any form both psychological and physiological has been known to be associated with cancer from the time of diagnosis. Yoga is an ancient eastern system which is being extensively used in research worldwide to study the effects in reducing the stress at the psychological and physiological levels.

CHAPTER-3

SCIENTIFIC LITERATURE REVIEW OF BREAST CANCER AND CAM STUDIES

3.1 Incidence of Breast Cancer:-

Every three minutes a woman in the United States is diagnosed with breast cancer. In 2007, an estimated 212,920 new cases of invasive breast cancer are expected to be diagnosed, along with 61,980 new cases of non-invasive breast cancer. And 40,970 women are expected to die in 2006 from this disease. (Global breast cancer update.)

Breast cancer is the leading cancer among white and African American women. African American women are more likely to die from this disease.

Breast Cancer is the most common form of cancer (other than skin) and a leading cause of cancer mortality among women in the US. Breast cancer rates in the US are among the highest incidence among 162 areas reporting incidence data to the IARC, with an annual rate of 104.2 per 100,000, adjusted to the world standard population (Pisani P, Parkin DM, Bray F, and Ferlay J, 1999). It is the most common cancer in women, accounting for 16% of cancer-related deaths and ranking second only to lung cancer as a leading cause of cancer-related mortality (Landis S, Murry T, Bolden S, and Wingo PA, 1998).

Incidence rates and mortality rates increase dramatically with age (Garfinkel L, 1995). While the rate of increase in Breast Cancer incidence is greatest in women under age 50, the majority of cases occur after age 50. Incidence rates in women before the age 45 are higher among blacks; after the age of 45, they are higher for whites. Women of higher socioeconomic status, married women, women living in urban versus rural areas have the highest rates. The prevalence is least in African countries. The table below shows a clear trend for increase in prevalence of breast cancer in developed countries. Among the less developed nations India stands first in line, factors may be mainly attributed to life style change, western influence and urbanization.

3.2 Epidemiology of Breast Cancer (Figure 3.1.)

	Prevalence	**Deaths**
World	**1050346**	**372969**
More developed countries	**579285**	**189203**
Less developed countries	**471063**	**183768**
Africa	59167	26616
Central America	18663	5888
South America	69924	22735
Northern America	202044	51184
United States of America	183494	45553
Eastern Asia	142646	38826
China	106014	28787
South-Eastern Asia	55907	24961
South Central Asia	129620	62212
India	**79124**	**40607**
Eastern Europe	110975	43058
Northern Europe	54551	20992
Southern Europe	65284	25205
Western Europe	115308	40443
Australia/New Zealand	12748	3427

GLOBOCAN 2000, Cancer prevalence and mortality worldwide, estimated values, IARC Press, 2001.

3.3. Indian scenario:-

Breast Cancer is rapidly catching up with Cervical Cancer as the most common type of cancer among urban Indian women. According to data compiled by the Delhi-based Indian Council of Medical Research (ICMR), in Delhi and Mumbai Breast cancer is already the No. 1 form of cancer among women. In Bangalore and Chennai, cervical cancer still leads, though the incidence of Breast cancer is on the rise. While increasing hygiene and improved healthcare facilities have helped control the viral infections that lead to cervical cancer, changing urban lifestyles are believed to be behind the rise in the incidence of Breast cancer. The medical community is slowly waking up to this grim fact. According to NCRP data, every year 80,000 new cases of Breast cancer are detected in Indian cities. The disease claims 35,000 lives every year, up by 8% since 1990 today. While statistics like these may sound negligible, the reality is not. In 1970, for instance, the incidence of breast cancer in India was barely 20 per 100,000 urban women. Today, that number has shot up to 28.3 nearly a 50% jump. Among the Parsis in Mumbai, a relatively westernized community in which few women have children and fewer breast-feed them, the incidence rate is higher at 43.8 per 100,000 women. Comparatively, in rural areas the incidence is only 8.5. This is still far less

than the West, where one out of nine women gets the disease. But urban India is not far behind; the incidence of Breast cancer is likely to double in the next 10 years. In fact, it is estimated that one of every 20 women in Mumbai and Delhi is likely to develop Breast Cancer.

3.4 Risk Factors Life Style and Cancer Incidence:-

Unfortunately, there is a new trend, indicating that breast cancer has overtaken cancer of cervix in the urban areas and their surroundings.

Urbanization, early menarche, late menopause, lack of physical exercise, high fat diet and delayed pregnancy have been cited as some of the factors that might have contributed towards higher incidence of breast cancer.

There is a great concern that breast cancer is also surfacing in younger women, between 25 and 30 years. "Breast cancer in early age group is very aggressive and dangerous as the chances of its spreading are faster than in those above 40-45 years," (ICMR survey 2000)

The epidemiological risk factors contributing to breast cancer occurrence are rapidly changing worse; the patients are getting younger. “Unlike a decade ago, women in their 20s and 30s are also developing malignant tumors. But this could also be due to an increased awareness of disease. With more and more women, especially younger, formed individuals

going in for breast examinations, the number of cases detected has gone up while the average age has come down. Interestingly, almost two-thirds of breast cancer patients belong to the upper class.

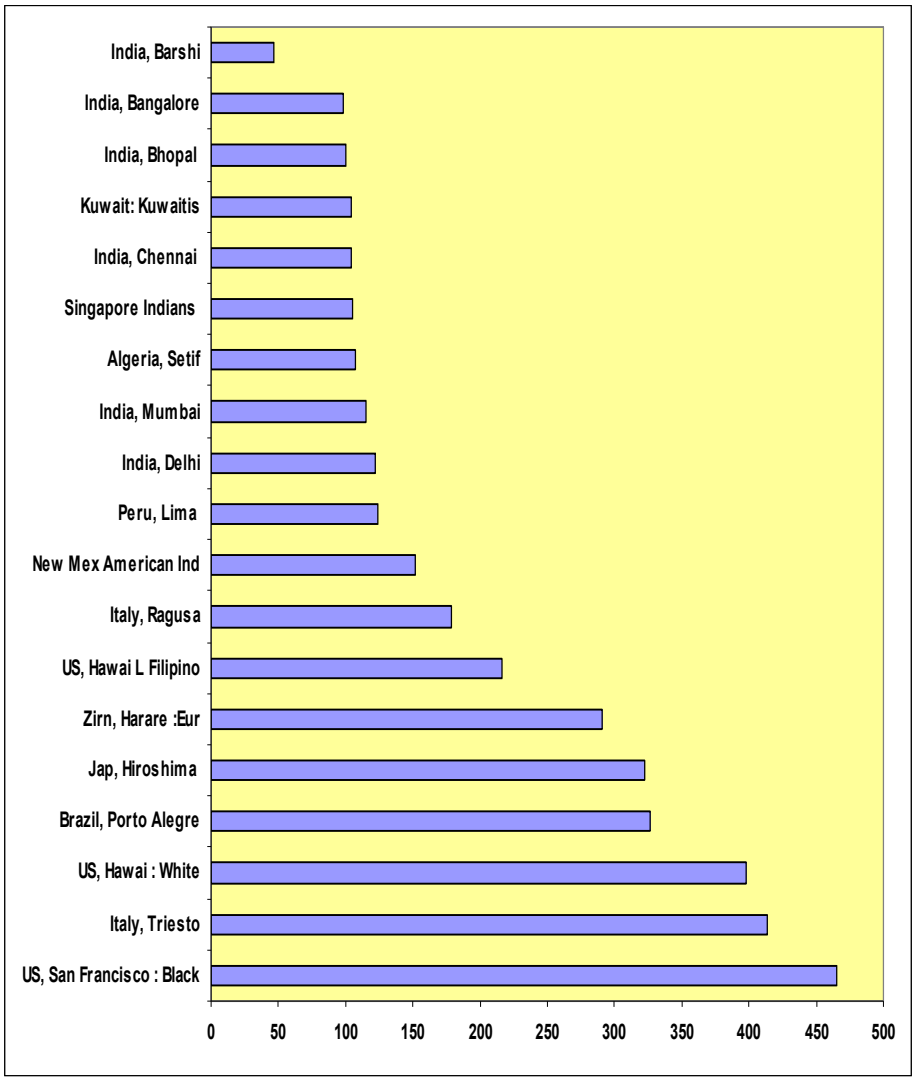
The underlying cause, ironically, is the rapid modernization of Indian society. For instance, sources at clinical oncology dept of the Regional Cancer Center in Thiruvananthapuram, attributes the rise in Breast cancer cases in fully literate Kerela to the high level of education among women which means they marry late, have fewer children and breast feed them for only a couple of months before returning to their jobs. Although urbanization provides the setting, the enemy, as they say, is within; a prolong onslaught of estrogen. During pregnancy, however, estrogen is superseded by another hormone called progesterone, which stops estrogen surges and alters the entire physiology of the breast by providing a long respite from the monthly onslaught of estrogen. Thus women who have two or more kids before 30 and breasted them for several months reduce their risk of getting Breast Cancer by over 50%.Since the number of menstrual cycles seems to have a direct correlation with the incidence of the disease, other factors also come into play. “A generation ago girls got their first period at the age of 14 or 15, today, with better nutrition in the

cities, nine and 10 year olds are beginning to menstruate". The same is true of menopause. While our mothers stopped ovulating in their 40s, today, women reach menopause only in their mid 50s. Coupled with increasing life spans, both early menarche and late menopause greatly prolong the reign of estrogen during a woman's reproductive years, making her more susceptible to Breast Cancer.

3.5 Incidences of Cancer

3.5.1 International burden of cancer in (males)

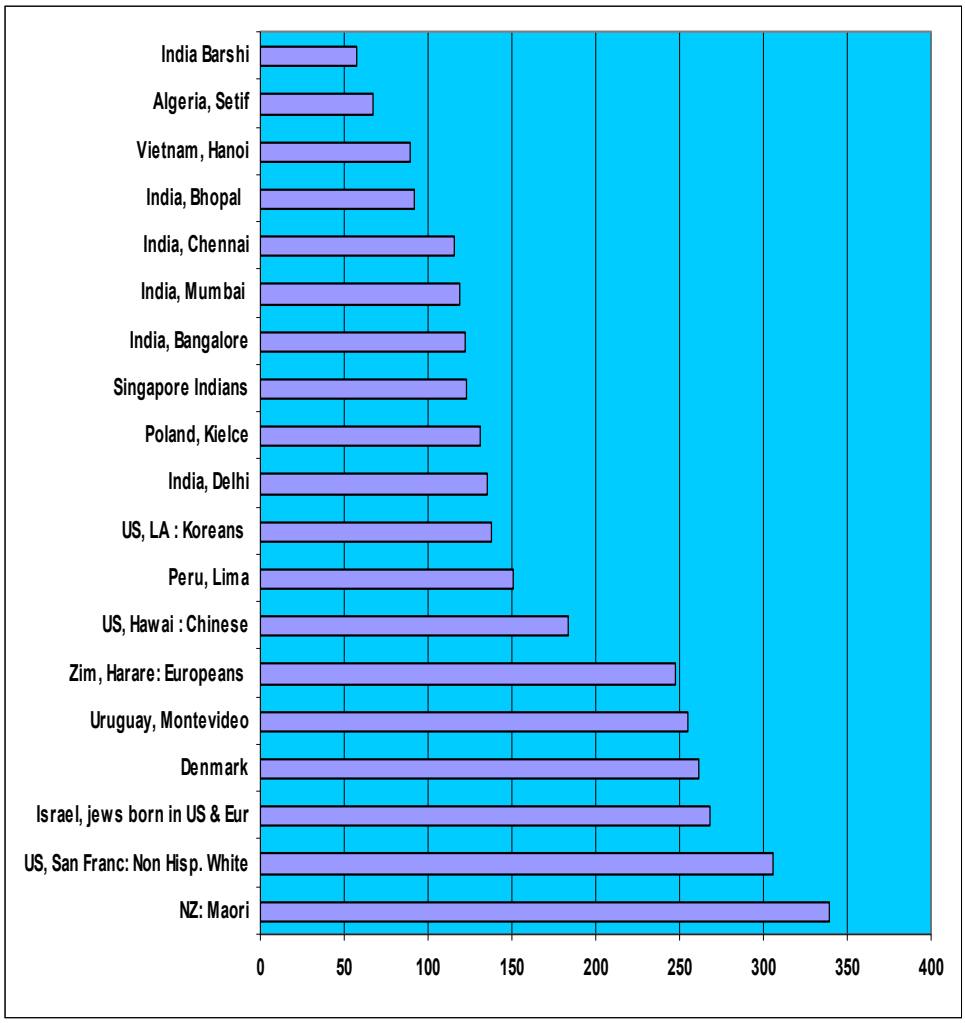
Figure 3.2.



(Adapted from W.H.O Cancer release 2001)

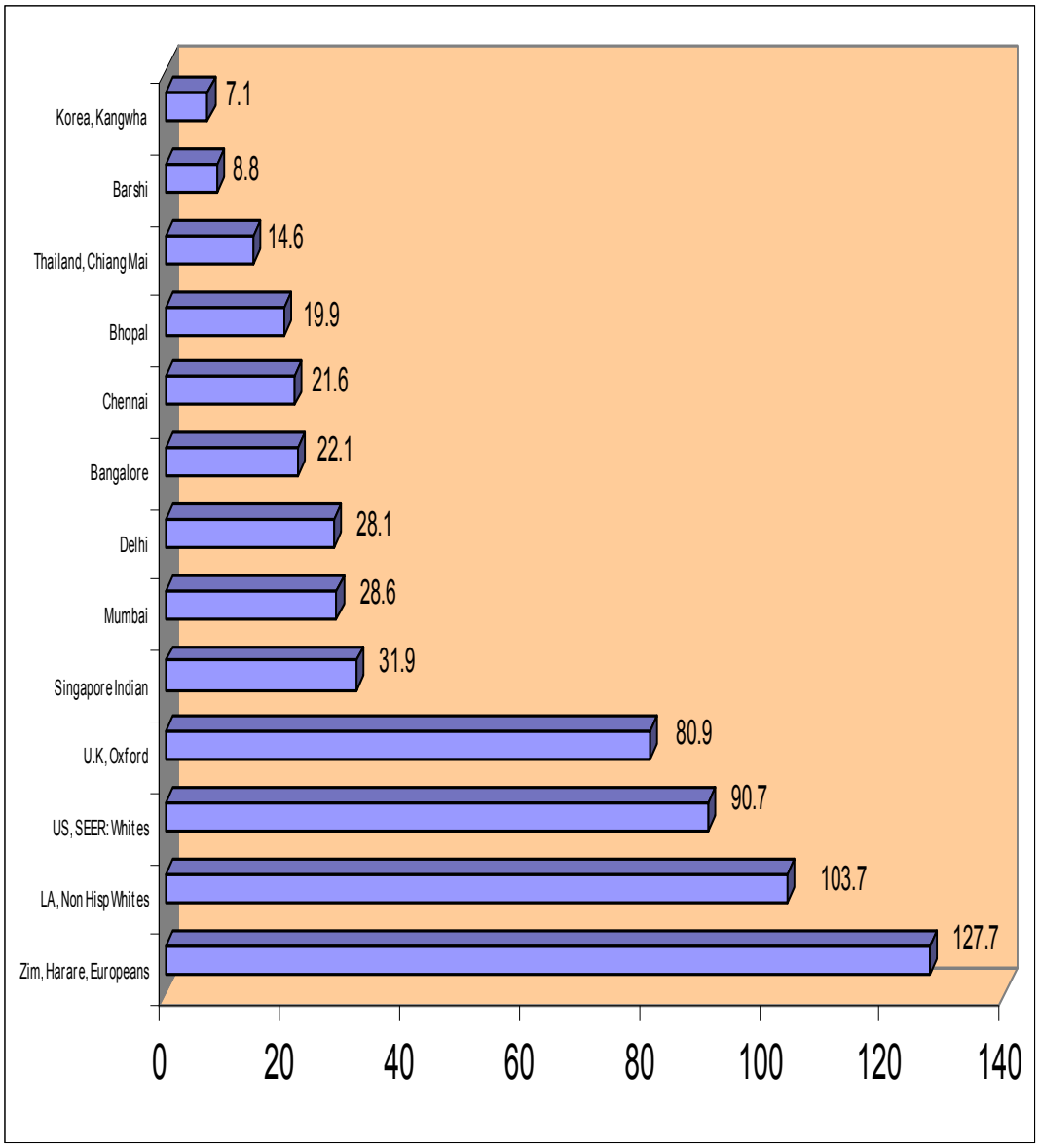
3.5.2. International burden of cancer in (females)

Figure 3.3.



3.5.3. International comparison of female breast cancer (source American association for cancer research)

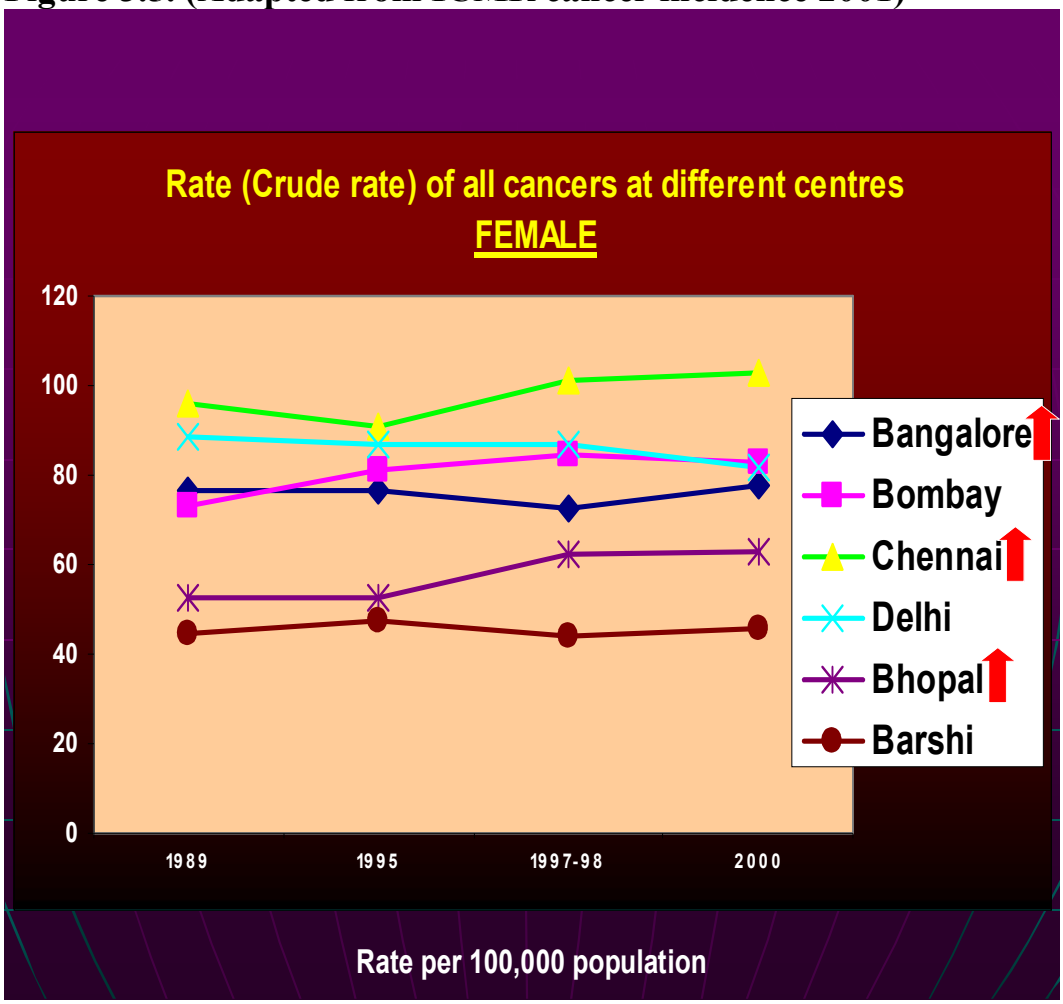
Figure 3.4.



(Adapted from ICMR cancer incidence 2001)

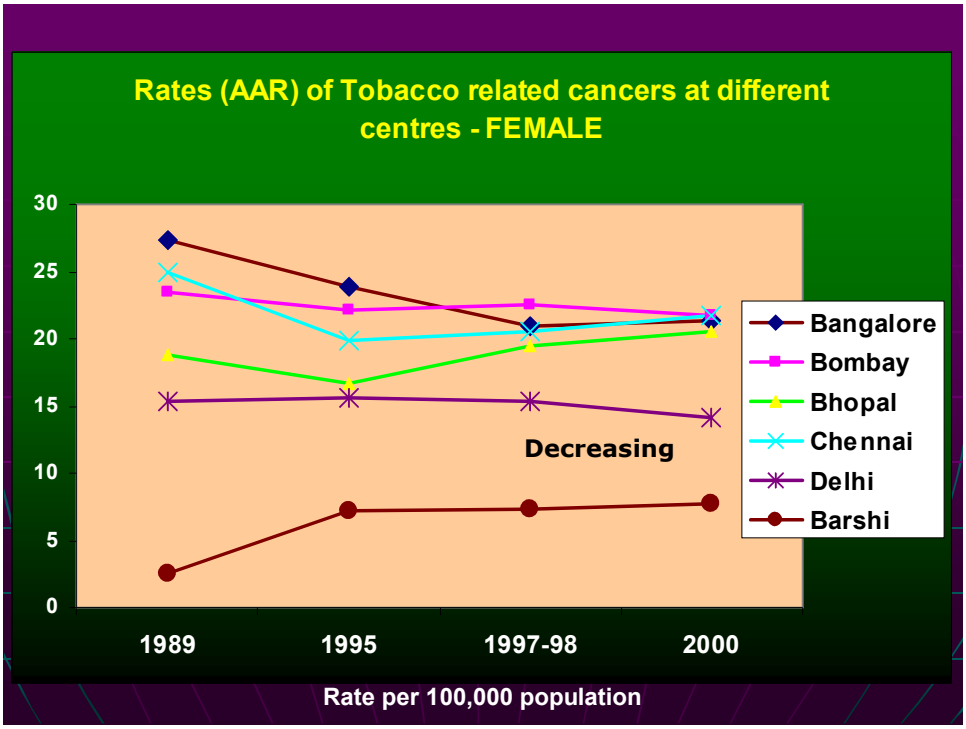
3.5.4. Incidence of cancer amongst females in Indian cities.

Figure 3.5. (Adapted from ICMR cancer incidence 2001)



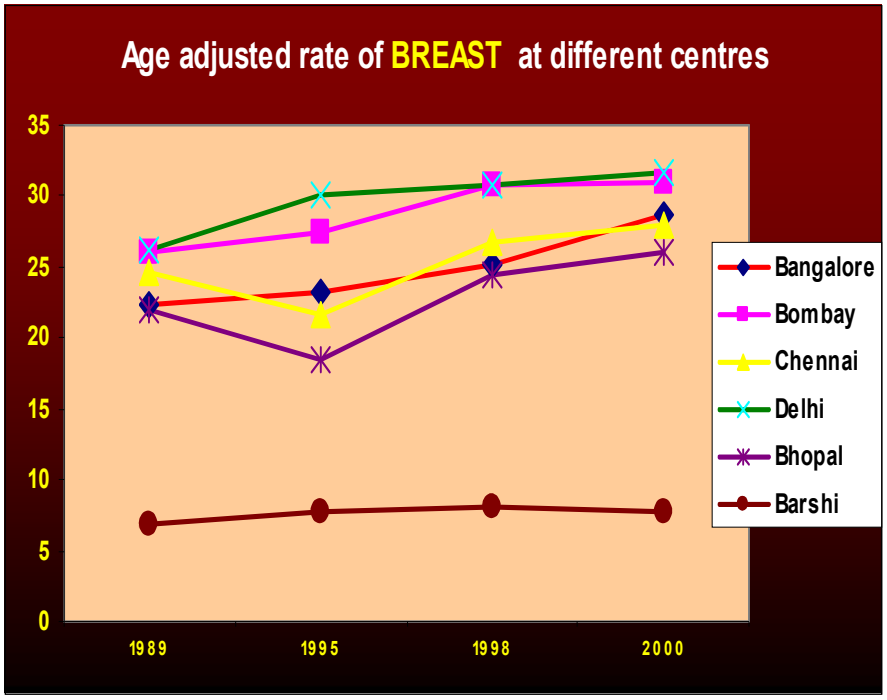
3.5.5. Incidence of tobacco related cancer incidences amongst females.

Figure 3.6. (Adapted from ICMR cancer incidence 2001)



3.5.6. Age Adjusted Rate and Incidence of Breast Cancer.

Figure 3.7.

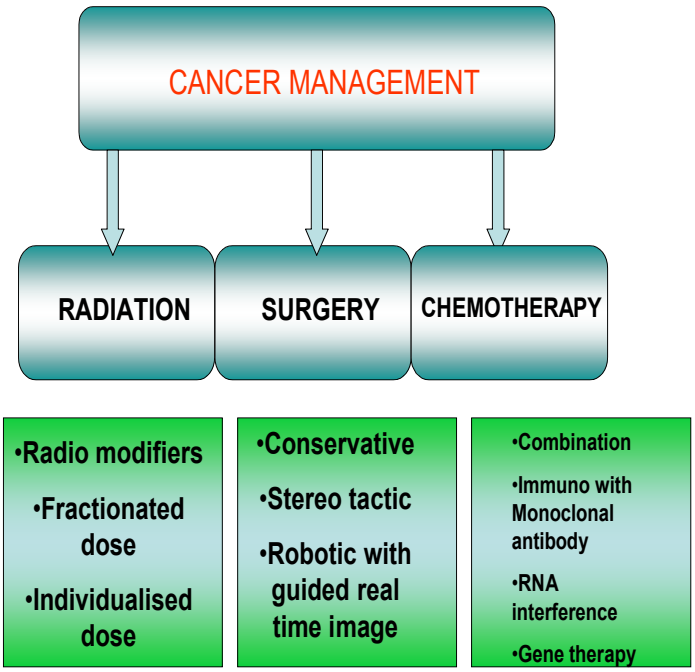


3.6. Conventional Treatment Related Side Effects and Radiation Induced DNA Damage.

In the current scenario the management of cancer is based on three primary therapeutic regimes they are as follows:-

- **SURGERY**
- **RADIATION**
- **CHEMOTHERAPY**

Figure 3.8.



The Current research in cancer is worth billions of dollars worldwide and thousands of scientists working very hard in search of the solutions for the disease.

With the modern advancement of the imaging technology and availability of a library of drugs available for combining the therapeutic regime is changing at a very rapid rate. The world of chemotherapy has been rapidly populated with a number of drugs.

Recent trends suggest that a number of drugs are being combined together to aim various pathways of cancer biology. The combinatorial approach has been approved for a number of clinical trials in various countries. The three major draw back of the cancer management can be described as follows:-

1) **Cytotoxicity of normal cells and side effects**
2) **Radiation and chemo resistance**
3) **Minimal residual disease and recurrence.**

3.7. Genomic Instability and Cancer:

Cancer is a disease of impaired genomic stability. Unstable genome is the hallmark of most cancers and cancer evolves as a consequence of destabilized genome(De Lange, 2005; De Lange, 2005; Desmaze et al., 2003; Hanahan and Weinberg, 2000). Genomic instability is characterized by spontaneous extensive progressive changes in the genome of the cells derived from the same ancestral precursors (Anderson et al., 2001; Raptis and Bapat, 2006b). The molecular mechanisms maintaining the genomic stability are damaged in cancer further resulting in the accumulation of genetic mutations and deficiencies of diverse mechanisms beyond repair (Charames and Bapat, 2003a; Charames and Bapat, 2003b; Raptis and Bapat, 2006a). Majority of the genetic alterations are in the growth regulatory genes, genes involved in cell cycle progression and arrest contributing to the malignant transformation. Genomic instability can be broadly classified into microsatellite instability (MIN) with the mutator phenotype and chromosomal instability (CIN) with gross chromosomal changes(Charames and Bapat, 2003b). MIN results from alterations in the length of short repeat stretches of coding and non-coding DNA, which is largely a consequence of inactivating mutations in DNA damage repair genes. In addition, epigenetic mechanisms resulting in gene silencing

through hyper methylation of promoter regions or increased gene expression through the hypomethylation of such regions also results in instability. Dietary and environmental agents can also further modulate the contribution of genetic instability to tumor genesis (Charames and Bapat, 2003b).

CIN is a defining characteristic of most human cancers and could be numerical and/or structural chromosomal instability. Chromosomal structural aberrations are a hallmark characteristic of human epithelial cancers and the end product of compromised genome stability mechanisms(O'Hagan et al., 2002). Most pathogenetic aberrations are those that result in amplification or deletions of oncogenes or tumor suppressor gene loci with a further imbalance in gene expression and loss of heterozygosity(O'Hagan et al., 2002). The threshold of chromosomal aberrations has been estimated to be higher in epithelial cancers relative to neoplasms originating in mesenchymal or hematopoietic lineages. Recent studies have shown that several factors can result in segregation defects, including abnormal kinetochore–spindle interactions, premature chromatid separation, centrosome amplification, multipolar spindles, and abnormal cytokinesis result in unequal chromosome distribution, defects in the mitotic checkpoint machinery (Elledge, 1996; Nojima, 1997; Wassmann

and Benezra, 2001), increased oxidative stress (Bohr et al., 1998; Olinski et al., 1998),diminished nonhomologous end-joining (Karanjawala et al., 1999; Karanjawala et al., 1999; Sharpless et al., 2001)and chromosomal instability (Pihan and Doxsey, 2003) (Figure 1).

The view that telomere dysfunction can serve as a potent driving force in the production of complex chromosomal rearrangements and aneuploidy was first supported by the study showing tumors arising in mice with telomere dysfunction(Chin et al., 1999; Artandi et al., 2000b). Subsequently, a number of studies have shown that telomeres play a critical role in promoting carcinogenesis by genomic instability (De Lange, 2005; Desmaze et al., 2003; Meeker and Argani, 2004; Meeker et al., 2004; O'Hagan et al., 2002).

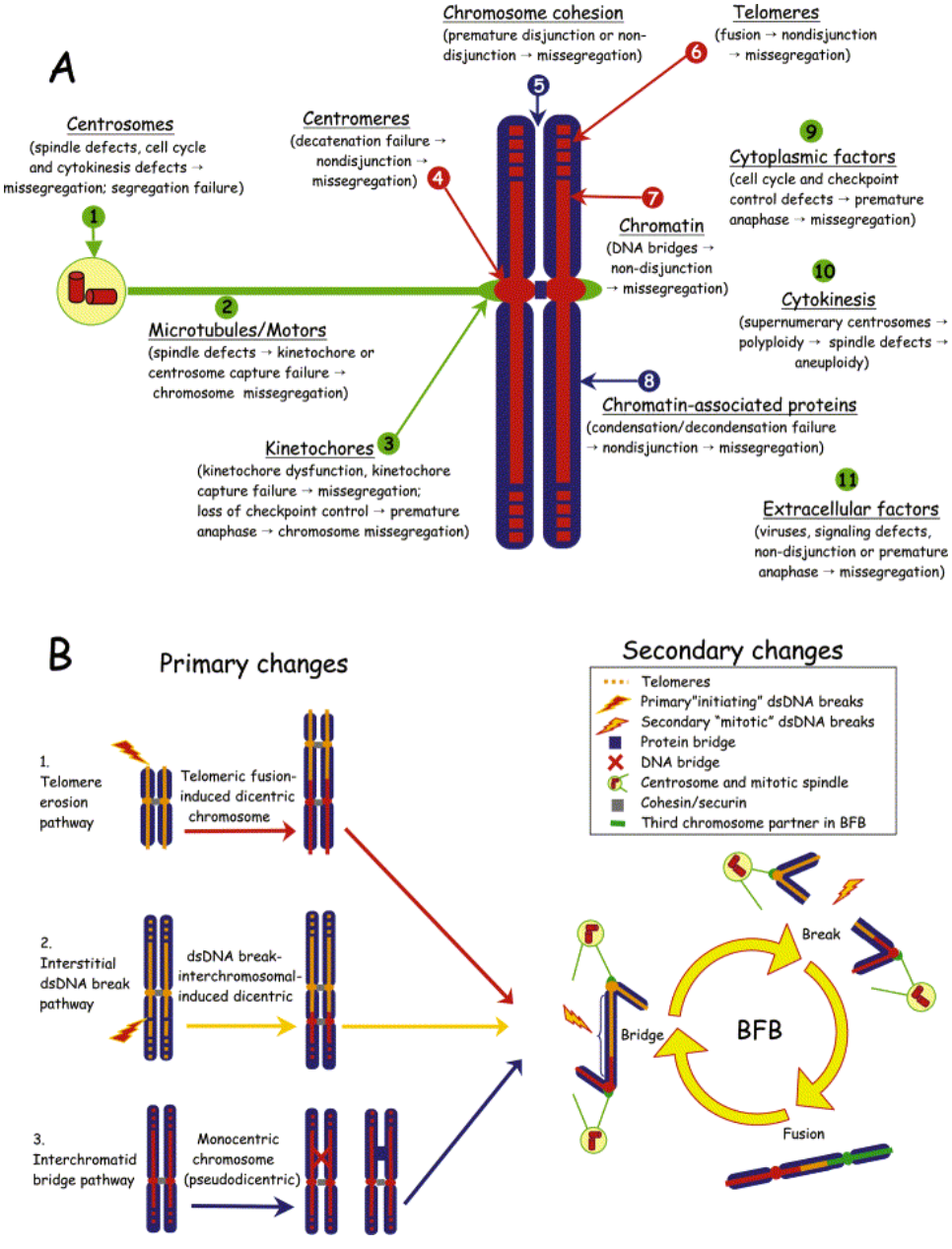


Figure 3.9. : Pathways leading to numerical and structural CIN.A:Numerical CIN;B:Structural CIN (Pihan and Doxsey, 2003; Pihan and Doxsey, 2003).

3.8.TELOMERES:

3.8.1. Telomeric function:

Telomeres are crucial for maintaining genomic integrity (De Lange, 2005; Desmaze et al., 2003; Meeker and Argani, 2004; Meeker et al., 2004; O'Hagan et al., 2002). They are highly regulated specialized nucleoprotein structures that cap the ends of the linear eukaryotic chromosomes. They prevent the chromosomal ends from being recognized as DNA double stranded breaks. They thus prevent the triggering of DNA damage checkpoint or repair machinery that normally acts on an accidental DNA break. They function in protecting the chromosomes from degradation and prevent chromosome fusions(Blackburn, 1991). They are also thought to function in meiotic and mitotic pairing, and chromosome segregation during meiosis and mitosis(Pandita et al., 2007). They prevent the loss of coding and regulatory DNA at the chromosomal ends due to the premature replication termination by the end-replication problem. They also help in nuclear organization and transcriptional silencing(Blackburn, 1991).

3.8.2. Telomeric Structure:

Telomeres are composed of guanine-rich hexameric DNA repeats and specific telomere binding proteins (Blackburn, 1991; Hahn, 2003). The non-coding DNA sequence of the telomeres varies among different

organisms. In mammalian cells it is composed of 5'-TTAGGG-3'(Meyne et al., 1989; Moyzis et al., 1988) ranging from 5 to 20 kbs. In the classical view, the mammalian telomeres were thought to be linear structures (Figure 2A) with the DNA portion starting as double-stranded structures terminating as single-stranded 3'G-rich overhangs of variable length.

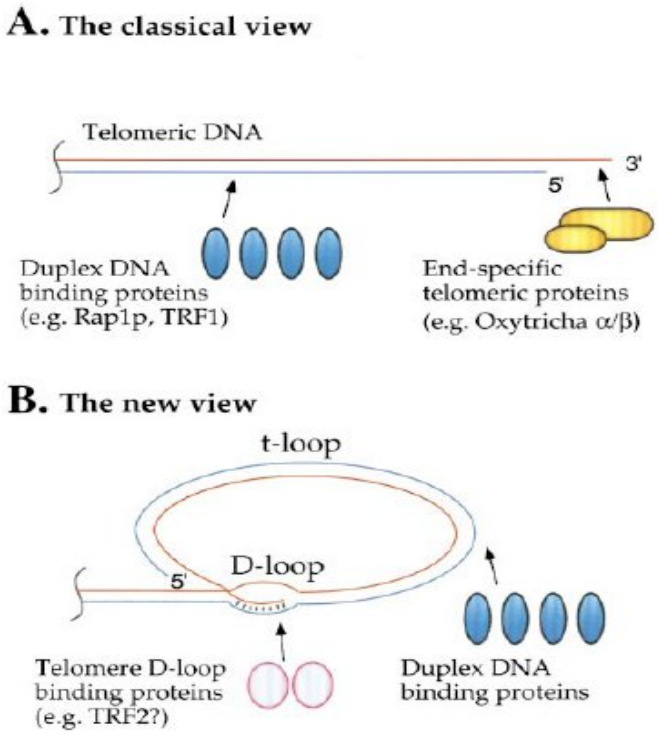


Figure 3.10. : Classical and new views of telomere structure (A). Classical view of telomere structure showing 3'G-rich overhang structure. (B). Modern view showing the D-loop and T-loop formation that is thought to stabilize the chromosomal ends (Greider, 1999).

However, recent electron microscopic studies revealed that the telomere ends can form two loops that contribute to the secondary structure of the telomeres (Henderson and Blackburn, 1989; Makarov et al., 1997; McElligott and Wellinger, 1997). This latter model suggests that the C terminal portion of telomeres folds back on itself to form a large telomere loop (T-loop) and the 3' G-strand binds to the double-stranded telomere repeat sequence of the 5'-end strand, forming a displacement loop (D-loop) (Figure 2B). In this way, the T-loop and D-loop mask the overhang structure and cap the telomeres. They play a protective role by sequestering the overhang terminal inside the double strand(Greider, 1999). In normal human somatic cells telomeres shorten with each cell division due to loss of terminal sequences that accompanies DNA replication due to end-replication problem(Levy et al., 1992).

3.8.3. Telomere end-replication problem:

About 50-150bps from the telomeres are lost with each cell division due to the well known end-replication problem(Levy et al., 1992). DNA replication is bidirectional and starts at one or more concurrent sites. But, DNA polymerase functions unidirectionally which initiates replication from a primer at the 3' end and runs toward the 5' end of the template. The synthesis of the leading strand is towards the replication fork, whereas the

synthesis of the lagging begins at the replication fork (consisting of Okazaki fragments). When the synthesis is complete, the primers are degraded and internal gaps or spaces are formed at each site of replication. The gaps between the newly replicated fragments of the lagging strand are filled by the action of DNA ligase. However, the terminal gap, the space left by the primer at the end of both strands, is not filled (Hug and Lingner, 2006). The terminal gap is further enlarged by the action of putative 5' to 3' exonuclease, which degrades 130-210 nucleotides. Thus, the 5' end of the telomere is shortened with each replication (Figure 3).

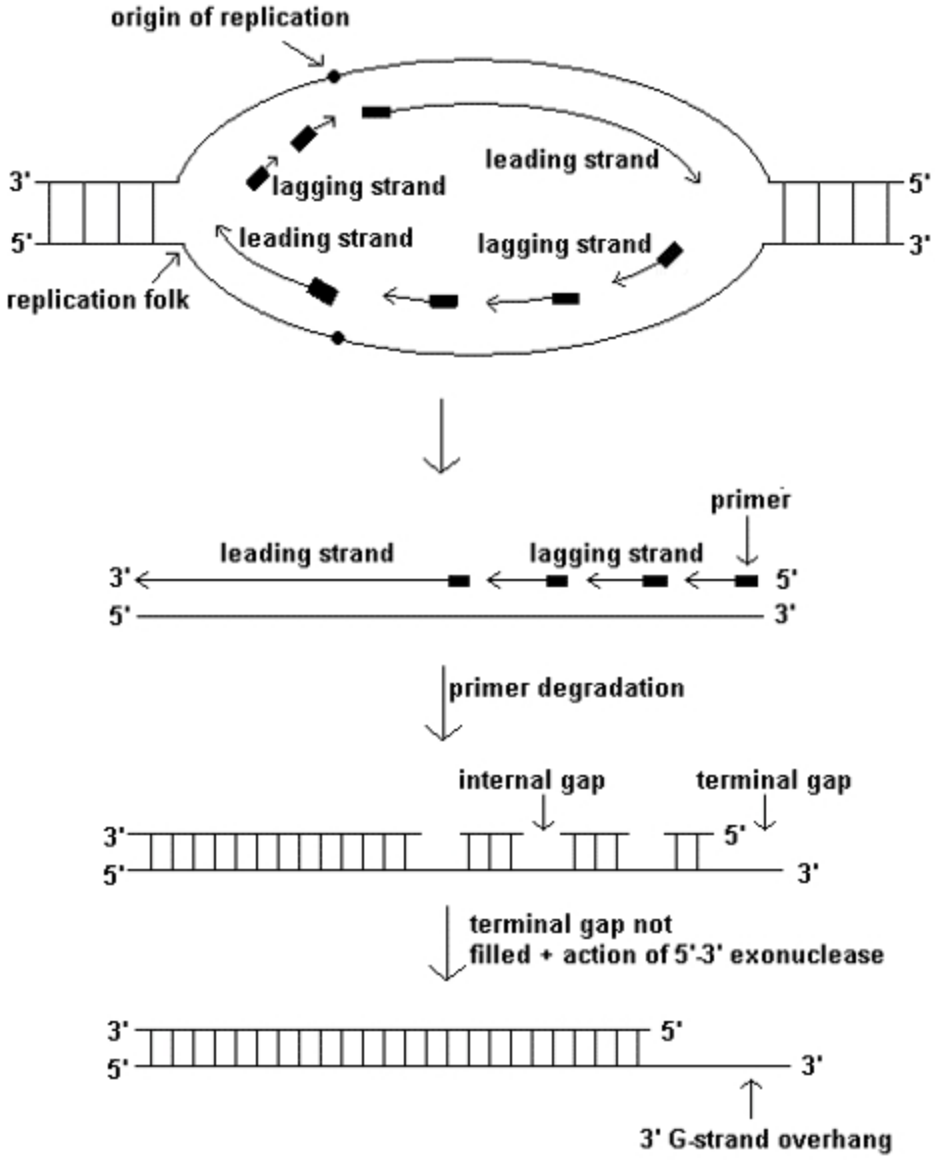


Figure 3.11.: The DNA end replication problem. The replication forks move in opposite directions. DNA polymerases only elongate in the 5′ to 3′ direction, each fork contains a leading (continuous) and a lagging (discontinuous) strand. Lagging strand synthesis cannot be completed because the removal of primers causes net loss of sequence on the lagging strand.(Hug and Lingner, 2006).

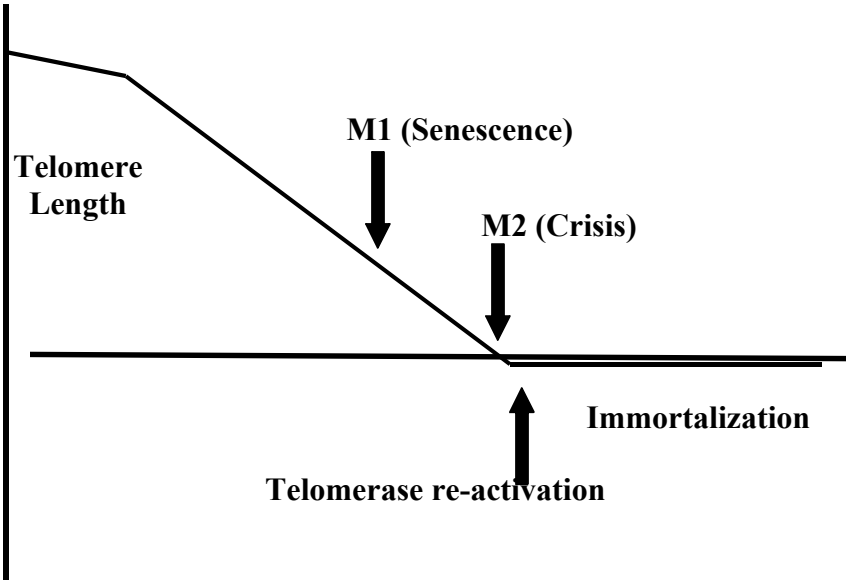
3.8.4. Cellular response to telomere shortening:

The telomeres function as mitotic clocks. The normal cultured cells have a finite replicative potential and enter a stage of replicative senescence when

the telomeres are very short. This stage is called the Hayflick limit (Mortality stage 1 (M1) or replicative senescence), where the cells stop dividing and are arrested at G_0 phase. The senescence can however be bypassed by inactivation of the tumor suppressors, p53 and retinoblastoma (Rb). These transformed cells progress through another 20–30 doublings when the telomeres are critically short resulting in telomere dysfunction and associated genomic instability. The cells then enter into the second stage of massive cell death called cellular crisis or Mortality stage 2 (M2)(Wright and Shay, 1992) (Figure 4).

3.8.5. Telomere-telomerase hypothesis: Telomere length decreases with cell replication and cells with shortened telomeres enter mortality stage 1(M1).After bypassing this stage, the cells enter a stage of crisis (Mortality stage 2 or M2) where apoptosis is triggered. The cells can emerge out of crisis by re-activating telomerase and can become immortal.

Figure 3.12. - Telomere length Homeostasis



The crisis stage is potential barrier for immortal cell growth in culture. Those cells that escape crisis acquire a feature called immortalization which is the ability to divide limitlessly. These rare cells overcome crisis by triggering telomere-maintenance mechanisms, most commonly by re-activating a special reverse transcriptase enzyme called telomerase (Wright and Shay, 1992)

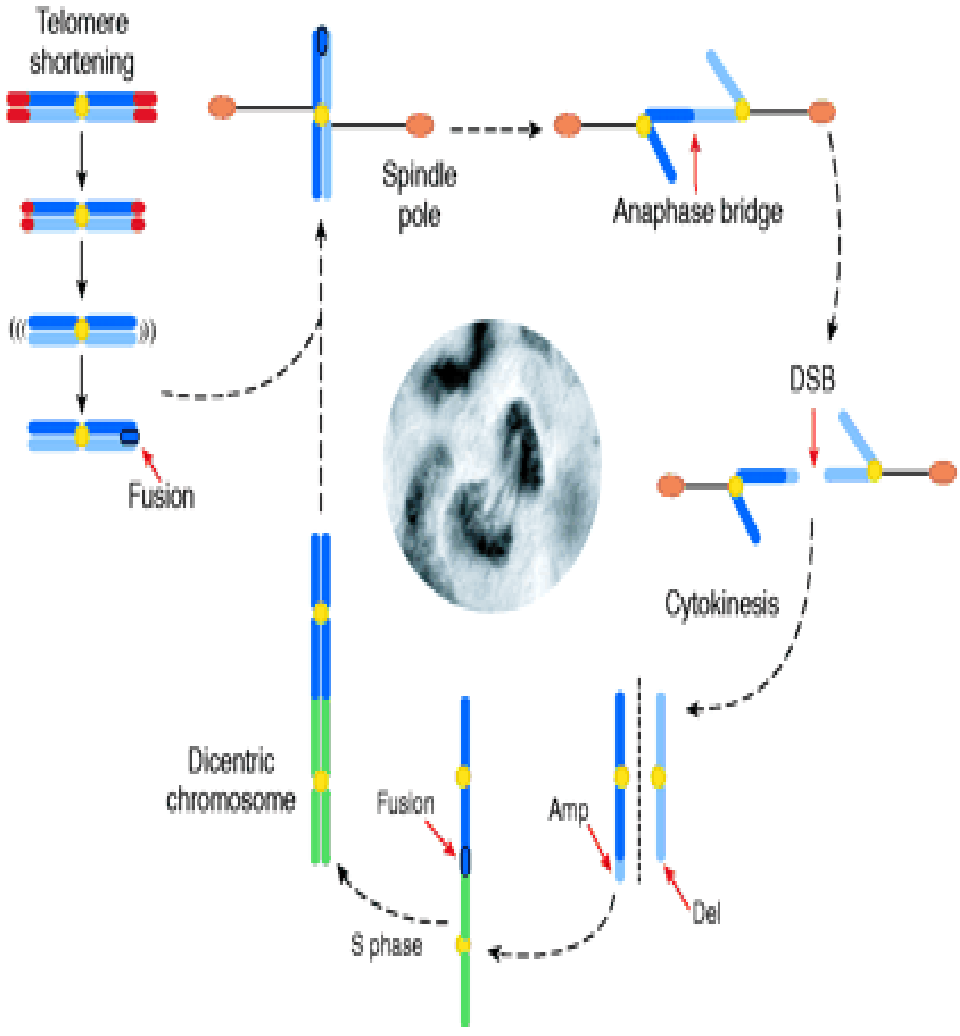
3.8.6. Telomere dysfunction mediated genomic instability in cancer:

Telomere protection and maintenance is necessary to maintain genomic stability and prevent onco-genesis. Telomere dysfunction results in illegitimate chromosomal fusions, inappropriate recombination events and is disastrous for genome integrity. Dysfunctional telomeres could result in genomic imbalances through the mechanism of prolonged breakage-fusion-bridge (BFB) cycles. BFB cycles, first described in maize by geneticist Barbara McClintock in 1938, are frequently the mechanism leading to structural chromosomal instability. As cells divide in the absence of telomerase, telomeres erode, exposing the ends. Fusions form between two sister chromatids or different chromosomes resulting in formation of a dicentric chromosomes, which results in anaphase bridging during segregation in mitosis. The dicentric chromosome is broken when pulled to opposite spindle poles, creating changes in gene dosage [amplifications (Amp) and deletions (Del)] for the resulting daughter cells. The broken chromosome can become fused to another chromosome, generating a second dicentric chromosome and perpetuating the BFB cycle (Figure 6). This leads to a variety of chromosomal rearrangements, non reciprocal translocations, large duplications and double minute chromosomes (Murnane and Sabatier, 2004; Sabatier et al., 2005; Lo et al., 2002) which promote tumorigenesis. Prolonged BFB cycles facilitate

the accumulation of genetic changes that enable cells to emerge from crisis and proceed to malignancy.

3.8.7. Telomere attrtition resulting in prolonged BFB cycles and genomic imbalances.

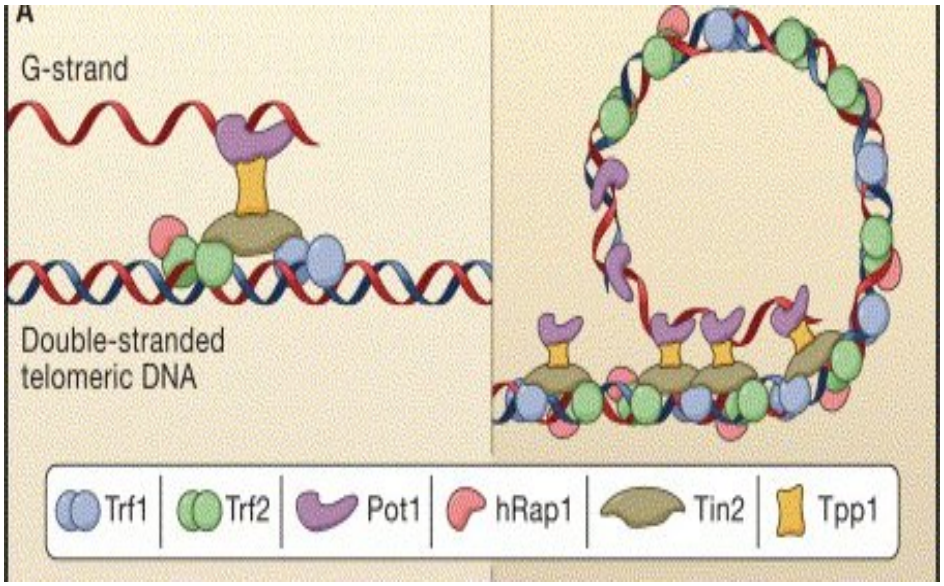
Figure 3.13.



3.8.8. Regulation of telomere function:

Telomeric function is compromised due to either telomere shortening as well as loss of telomeric secondary structure. Telomere length is a balance between the loss of telomeric DNA during cell proliferation and maintenance. Therefore, in addition to the end-replication problem, telomere length and function are determined by telomerase activity and expression of telomere associated proteins like TRF1, TRF2, POT1, and Tankyrase1. hTERT transcription is regulated by a number of negative and positive regulators. Multiple tumor suppressor pathways like Mad1 a repressor of c-Myc, TGFB, acting through SIP1 , Menin, binding directly to the hTERT promoter(Lin and Elledge, 2003), chromosome 3 transfer(Ducrest et al., 2001), pRB and Wilm's tumor 1 suppressor gene(Ducrest et al., 2002)have been shown to negativelyregulate hTERT. The expression of hTERT is also positively regulated by c-MYC, BCL2, E6 human papillomavirus type 16 protein, phosphorylation by PKCa or AKT/PKB(Ducrest et al., 2002). Telomerase access, telomere length and its secondary structure are also regulated by telomere-binding proteins.

Figure 3.14. - Structure of Telomere associated proteins(adapted from Blasco et al 2001)



3.8.9. Telomere binding proteins:

In mammalian cells, these proteins are Telomere repeat binding factor 1(TRF1) and Telomere repeat binding factor 2(TRF2), which bind to double-stranded repeats, along with Protection of telomeres 1(POT1), which binds to G-rich single-stranded telomeric DNA repeats (Figure 8). These three proteins bind to the telomeres by direct interaction and form the part of a protein complex called the telosome (Figure 7) (Liu et al., 2004a). Telosome also includes human repressor activator protein 1 (hRAP1), TRF-interacting protein (TIN2) and TPP1 which are recruited by the directly binding proteins and bind indirectly to the telomeres. This

protein complex helps in capping and protecting the telomeric ends from being recognized as double stranded breaks. They also help in regulating the access of telomerase to the telomeres.

TRF1 plays a primary role in telomere length control and cell cycle. The amino acid terminus contains a homodimerization domain and the carboxy terminus contains a DNA binding domain that directly interacts with TTAGGG repeats (Bilaud et al., 1996; Bilaud et al., 1997; Broccoli et al., 1997). Total number of TRF1 molecules per chromosome end is correlated with the telomere length. TRF1 overexpression causes telomere shortening, whereas overexpression of a DNA-binding-deficient TRF1 variant results in progressive telomere elongation (van and de, 1997b; Smogorzewska et al., 2000). Thus, it acts as a negative regulator of telomere length. TRF1 effects on telomere regulation are independent of any changes in telomerase activity in vitro, suggesting that TRF1 may regulate telomerase access to telomeres (Olaussen et al., 2006a).

TRF2 also binds to the duplex DNA and plays a major role in catalyzing t loop formation. It thus stabilizes the secondary structure of the telomeres. Inhibition of TRF2 has been shown to induce chromosomal end-end fusions and chromosomal instabilities(Ancelin et al., 1998). Recently, TRF2 has also been shown to migrate to the sites of DNA damage(Mao et

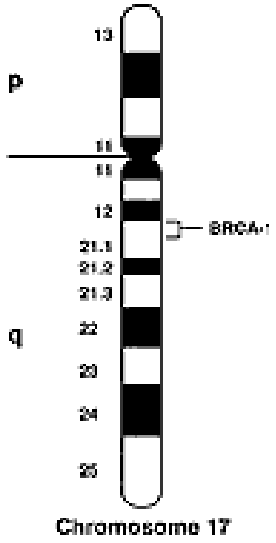
al., 2007).TRF1and TRF2 interact with a number of other proteins, including TIN2 (Kim et al., 2004), TPP1 (Houghtaling et al., 2004; Liu et al., 2004b; Ye et al., 2004b), POT1 (Baumann and Cech, 2001; Loayza and De Lange, 2003a; Loayza and De Lange, 2003a; Loayza and De Lange, 2003a),hRAP1 (Li et al., 2000) and tankyrase 1 and 2 (Cook et al., 2002; Kaminker et al., 2001) to ensure proper telomere maintenance. TIN2 was found to bind TRF1 and TRF2 simultaneously and stabilizes TRF2 on the telomeres(Ye et al., 2004a). hRap1 associates with TRF2 and negatively regulates telomere length(O'Connor et al., 2004)

3.9. Breast Cancer Susceptibility Gene 1 (BRCA1):

BRCA1 is a tumor suppressor gene and has a critical role in major DNA repair pathways. It is involved in genomic surveillance through its interaction with different replication, repair and transcription factors. Germline mutations of *BRCA1* predisposes women to early-onset familial breast, ovarian or other types of cancers (Brody and Biesecker, 1998; Alberg and Helzlsouer, 1997). BRCA1 was mapped in 1990 on to chromosome 17q21 by linkage analysis (Hall et al., 1990) and cloned four years latter in families with autosomal dominant inheritance by positional cloning (Miki et al., 1994). It is a large gene containing 24 exons with a

coding region of 5.5Kb and its mRNA covering 8kb. It encodes a large 220-kDa nuclear phosphoprotein with 1863 amino acids in humans(Miki et al., 1994) and 1812 amino acids in mice(Lane et al., 1995). It has three important structural motifs, including a highly conserved amino-terminal RING (Really Interesting New Gene) finger motif, a nuclear localization motif, and tandem BRCT (*BRCA1* C-Terminal) motifs at its C terminus (Miki et al., 1994; Koonin et al., 1996; Chen et al., 1996; Thakur et al., 1997)(Figure 8).

Figure 3.15. Diagram showing the chromosomal location of BRCA1 gene.



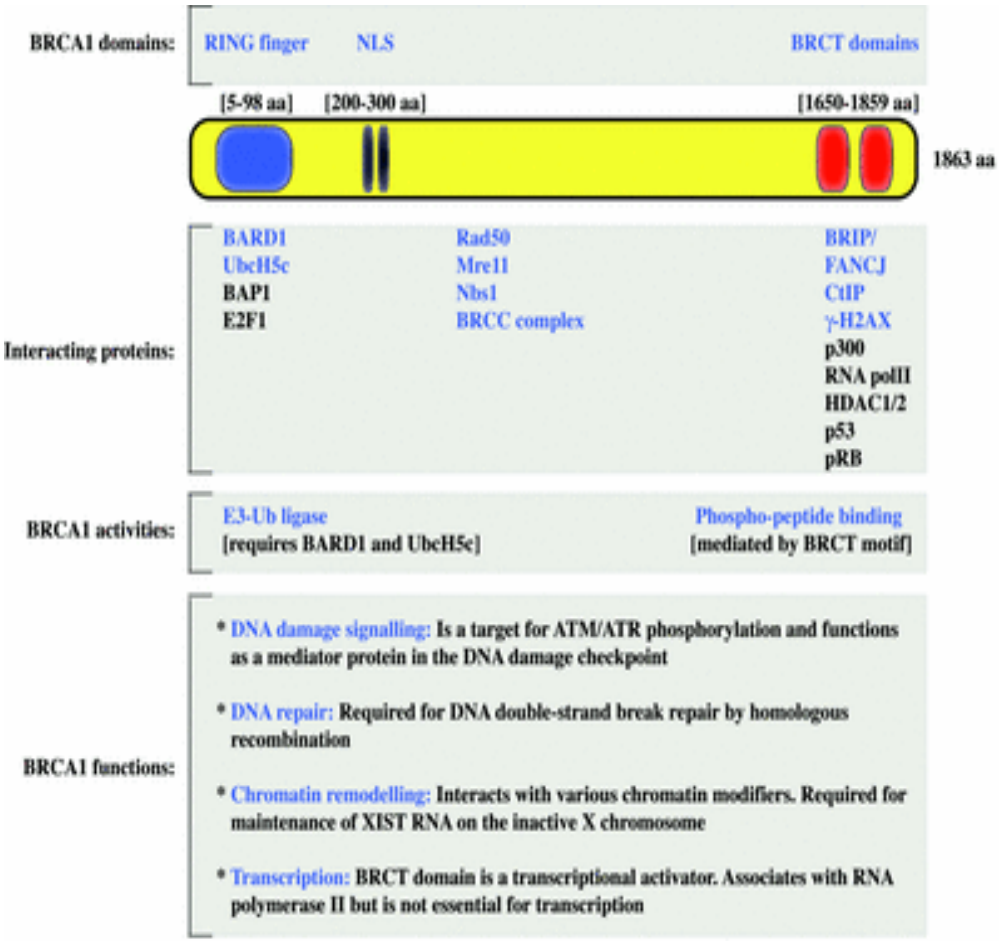


Figure 3.16: BRCA1 (A) Locus of BRCA1 on chromosome 17. (B): Schematic diagram of the BRCA1 protein showing the domains, the interacting partners BRCA1-associated RING domain 1 protein (BARD1), BRCA1-associated protein-1 (BAP1), p53 (TP53), retinoblastoma protein (RB), RAD50, and MYC. A domain within BRCA1 amino acids 758–1064 interacts with RAD51. The BRCA1 C-terminal (BRCT) repeats interact with BRCA2, histone deacetylase (HDAC) 1 and 2, RNA helicase A (RHA), and the CtBp-interacting protein (CtIP) and the functions (Boulton, 2006).

3.9.1. Functions of BRCA1:

BRCA1 is a multifunctional protein that has been implicated in many normal cellular functions such as DNA repair, transcriptional regulation, cell-cycle checkpoint control, and ubiquitination (Boulton, 2006; Starita and Parvin, 2003). Studies have shown that targeted disruption of BRCA1 in mice causes embryonic lethality accompanied by growth retardation, apoptosis, cell cycle defects and genetic instability(Deng and Scott, 2000c). BRCA1 contains several functional domains that interact directly or indirectly with a variety of molecules, including tumor suppressors (p53, RB, BRCA2 and ATM), oncogenes (c-Myc, casein kinase II and E2F), DNA damage repair proteins (RAD50 and RAD51), cell-cycle regulators (cyclins and cyclin-dependent kinases), transcriptional activators and repressors (RNA polymerase II, RHA, histone deacetylase complex and CtIP) and others(Deng and Brodie, 2000a). *BRCA1*-associated mammary gland tumors in humans and murine cells exhibit genomic imbalances and chromosomal aberrations that are hallmarks of genomic instability. These observations, as well as growth abnormalities exhibited by the mutant cells, suggest that BRCA1 acts as a caretaker through its role in maintaining genome integrity, instead of directly inhibiting cell proliferation(Deng, 2001b; Deng, 2001a; Deng and Scott,

2000b). The role of BRCA1 in tumor suppressor function has been linked to its role in genome surveillance attributed to its role in regulating cell-cycle progression, centrosome duplication, DNA damage repair, cell growth and apoptosis, and transcriptional activation and repression(Deng and Brodie, 2000b; Deng and Scott, 2000a).

3.9.2. Role of BRCA1 in DNA repair:

One of the mechanisms of BRCA1 in maintaining genome integrity was thought to be through its roles in DNA damage repair. ncreasing evidence has implicated role of BRCA1 in homologous recombination repair (HRR)(Moynahan et al., 1999), Non homologous end joining (NHEJ)(Zhong et al., 2002) and Nucleotide excision repair (NER)(Gowen et al., 1998). For homologous recombination and DNA repair BRCA1 interacts with RAD51, BRCA2 and the BRCA1-binding protein BARD1, both before and after DNA damage (Scully et al., 1997b) (Scully et al., 1997c). BRCA1 also associates with RAD50, another protein involved in homologous recombination and the DNA damage response. Further studies also showed that BRCA1-deficient cells were highly sensitive to IR and displayed chromosomal instability, with both numerical and structural chromosome aberrations, which may be a direct consequence of unrepaired DNA damage(Shen et al., 1998; Xu et al., 1999). BRCA1

forms a BRCA1-containing complex termed BASC (BRCA1-Associated Genome Surveillance Complex).This complex includes tumour suppressors, DNA damage sensors and signal transducers, including the MRN complex, the mismatch repair proteins MSH2, MSH6 and MLH1, the Bloom syndrome helicase BLM, the ATM kinase, DNA replication factor C (RFC) and PCNA. This also suggests a role for BRCA1 in coordinating various functions of DNA replication that are important for maintaining genomic integrity in the cell(Starita and Parvin, 2003).

3.10. Loss of telomere equilibrium and cancer:

As discussed earlier, telomere dysfunction is one of the factors contributing to genomic instability. The development of genomic instability is an important step in generating multiple genetic changes leading to tumorigenesis. In carcinomas, histomorphologically increasing gradation of dysplasia is associated with the aggressiveness of the tumors. Aggressive tumors are associated with a bad prognosis and genomic instability has been shown to be associated with prognosis of tumors. Hence, it is important and interesting to understand the association of telomere dysfunction with the aggressiveness of the tumors. Since breast

cancer is one of the most common causes of cancer deaths among women, it was chosen as a model for the study.

3.11. Radiation induced DNA damage:-

Radiotherapy is an important therapeutic modality in clinical Cancer management. Lately with advent of better machines and innovative technology individualization of Cancer radiotherapy is gaining greater grounds. There have been number of studies done earlier to prove the radio sensitivity of different individuals undergoing radiotherapy

In a recent work Mozdarani et, al (2005) showed that there is an elevated spontaneous frequency of MN (DNA damage marker) in breast cancer group compared to the control group. They also showed that Ca-Breast patients were more sensitive (30%) to ionizing radiation than the control population age and sex matched .Scott et; al (1995) and (1999) showed that there is indeed a significant correlation between carcinoma breast and increased chromosomal radio sensitivity. Scott et, al (1995) also proved that in ataxia telangiectasia patients there is an elevated radio sensitivity observed in lymphocytes. In our current data we observed that the micro-nuclei frequencies in carcinoma breast patient had significant correlation ship with telomeric damage post radiotherapy. In another study FA

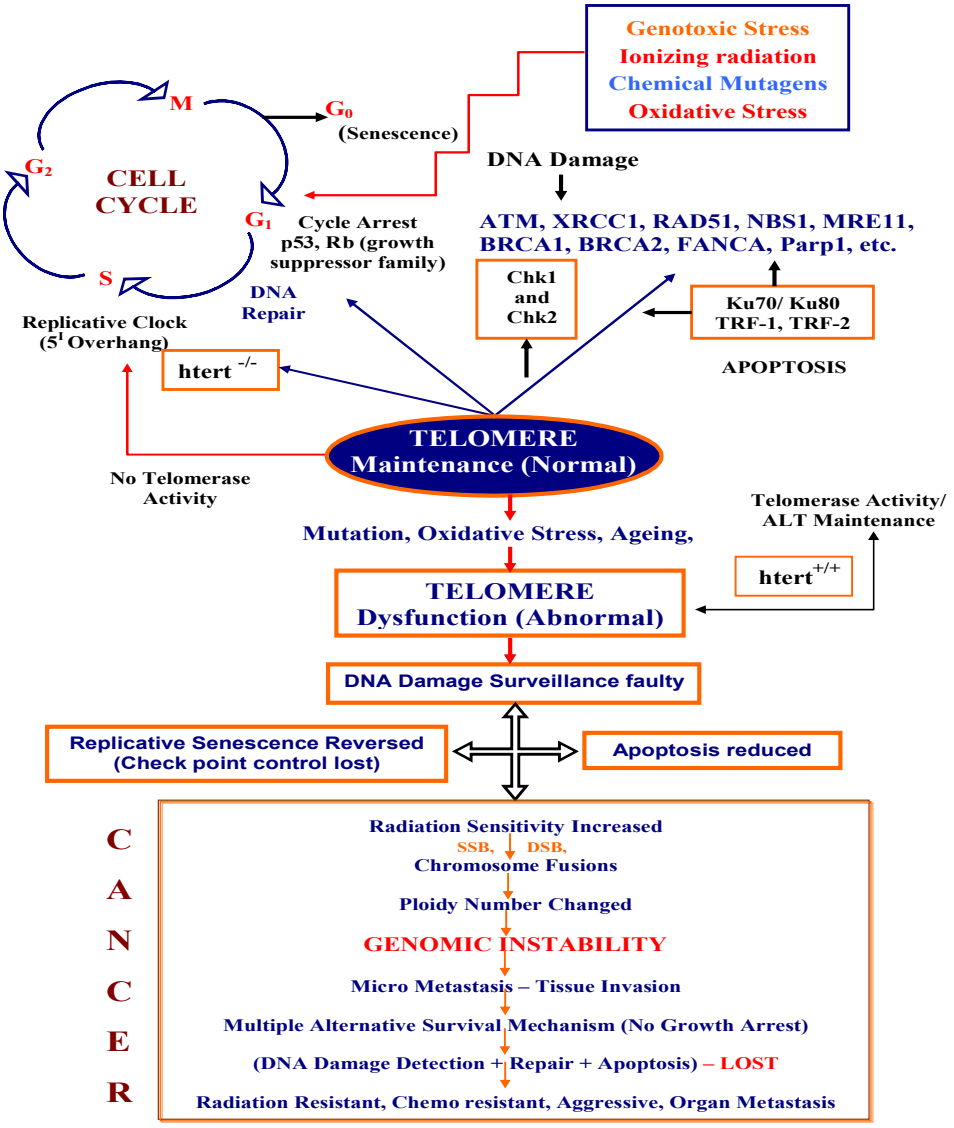
Goytisolo and Blasco (2000) proved that short telomeres or dysfunctional telomeres may contribute to elevated radiation sensitivity. The telomeres (chromosomal ends) play crucial role in detection and repair of DNA damage and radiation insult (Hande 2004).

There is a significant link between Telomere maintenance and radio-sensitivity (Predrag et; al 2000). Telomeres are repetitive non-coding DNA at the ends of the linear chromosomes ranging in size of 5-10kb in human cells (Moyzis et; al 1988). As a consequence of semi conservative modes of DNA replication, the extreme termini of chromosomes are not duplicated completely resulting in successive shortening of telomeres with each cell division. Therefore, they act as a replicative clock and regulate the number of cell divisions and the on set of cellular senescence (Harley et; al 1990). Telomeres also prevent end to end inter chromosomal fusions and take part in efficient DNA repair functions under normal conditions (Blasco et; al 2003).

Hyper radiosensitive cells such as ATM-- shows significant fragmentation and telomere damage establishing the link between the link between telomere maintenance and repair defects (Hande et; al 2001).

3.12. Schematic representation of Telomere and its role in breast cancer-

Figure 3.17. – Telomere and Cancer Pathway



3.13. Impaired DNA repair capacity in Breast Cancer patients:-

According to Parshad R (1996) Women with breast cancer and a family history of breast cancer and some with sporadic breast cancer are deficient in the repair of radiation induced DNA damage compared with normal donors with no family history of breast cancer. DNA repair was measured indirectly by quantifying chromatid breaks in phytohaemagglutinin (PHA)-stimulated blood lymphocytes after either X-irradiation or UV-C exposure, with or without post treatment with the DNA repair inhibitor, 1-beta-D-arabinofuranosylcytosine (ara-C). They have correlated chromatid breaks with un repaired DNA strand breaks using responses to X-irradiation of cells from xeroderma pigmentosum patients with well-characterized DNA repair defects or responses of repair-deficient mutant Chinese hamster ovary (CHO) cells with or without transfected human DNA repair genes. Deficient DNA repair appears to be a predisposing factor in familial breast cancer and in some sporadic breast cancers.

3.14. Stress and its effects on Immunity:-

Stress can be defined as “a state of disharmony or threatened homeostasis provoked by psychological, environmental, and physiological stressors”

3.14.1. STRESSOR: - A stressor is any stimuli that cause a non specific response in an individual, otherwise known as stress (Elliott and Eisdorfer, 1982).

Homeostasis is the term used which means the harmonious equilibrium of many physical and emotional factors that permit the body to maintain a steady state of health (Cannon, 1914). **Stress** is a departure from homeostasis.

The **stress response** is the body's constant effort to right any physical or mental stressor to maintain physiological, mental and emotional harmony or homeostasis. If a person is not able to re-establish homeostasis the typical consequence is disease. Activation of the chemical stress pathway (gluco-corticosteroids) tends to be associated with depression, whereas the activation of the electrical stress pathway (adrenalin) is more frequently correlated with anxiety.

A person's level of stress must reach a certain threshold before the **stress syndrome** develops. The stress syndrome can be produced by physical illness, chronic emotional upset, work problems, status problems, financial worries, divorce and bereavement. Memory plays a significant role in the

perpetuation of stress and people can worry themselves sick and even to death.

3.14.2. The two main categories of stressors: Acute and Chronic.

1) Acute stressors include unpleasant films, under stimulation/work under load, over stimulation/work overload, unexpected or uncontrollable noise, prestige or status loss, electric shock, uncontrollable situations, physical illness, surgery, threats to self-esteem, and traumatic experiences.

2) Chronic stressors include sleep deprivation, daily "hassles", work overload or under load, role strains, or social isolation. There are, of course, many more things that can cause stress, but these are the stressors most commonly used in experimental research and most commonly seen in the general population (Elliott and Eisdorfer, 1982).

3.15 The response to stress

There are changes in both the body's electrical and hormonal pathways underlying the stress syndrome.

Stressful stimuli cause the hypothalamus in the brain to secrete corticotrophin-releasing hormone (CRH) and antidiuretic hormone (ADH). CRH stimulates the release of adrenocorticotrophic hormone

ACTH from the pituitary which then causes the adrenal cortex to release corticosteroids -- primarily cortisol. At the same time the autonomic nervous system initiates the adrenal medulla to release adrenaline -- which increases heart rate, blood pressure and respiratory rates -- resulting in increased arousal and anxiety.

The glucocorticoids, adrenalin and noradrenalin all can inhibit insulin secretion, and this results in the conversion of stored protein and fat to immediately useable energy for exertion. The increased depth of respiration makes more oxygen available and the blood circulation is adjusted to direct more oxygen and glucose (energy) to specific organs and muscles essential for exertion. Hormones related to functions that are not essential for immediate survival, such as reproduction, appetite and immune system function are suppressed. Endorphins, which are strong analgesics, are also released. There are strong connections between the chemical (hormonal) and electrical pathways in response to stress. For example, adrenalin stimulates the hypothalamus to produce CRH which helps to instigate the stress response of the sympathetic nervous system, stimulating the secretion of both adrenalin and nor-adrenalin (Dunn and Berridge 1990, Cuningham et al 1990). Also ADH works synergistically with CRH to stimulate ACTH, which works synergistically with CRH to

stimulate ACTH, also appears to work synergistically to promote behavioural effects -- such as memory enhancement -- of the stress response (Elkaler et al, 1990, Rittmaster et al 1987).

The secretion of CRH usually stops when glucocorticoids reach a certain point by a negative feedback loop. However chronic stress can disrupt the feedback mechanism and cause a prolonged secretion of glucocorticoids, which can be very detrimental to health.

The symptoms that occur with chronic stress correlate to the changes that are induced by acute stress and which support the *'fight or flight response'*. The symptoms such as weight loss, loss of sexual drive, peptic ulcers and immune suppression are an exaggeration of this initial adaptive response.

Allostasis means the body's ability to adjust to various vital functions in order to reset itself to a steady state. It is the ability of the body to achieve stability through changing situations (McEwin 1998) with an Allostatic Load (McEwen and Stellar 1993).

There comes a point when the body can no longer handle all the stress and the person enters into a state of chronic stress accompanied by physiological breakdown -- reduction of the size and functions of the thymus, decreased blood lymphocytes and eosinophils, decrease in lymph

node size, inhibition of cytokine release (essential for T and B cell maturation), suppression of natural killer cells and promotion of programmed cellular death of lymphocytes -- lymphocyte apoptosis (Hetts, 1998, Munck and Guyre 1991). In contrast the acute stress response has been shown to strengthen the immune response and proved an immunological memory (McEwen 1998).

3.15.1. Neurotransmitter and Hormonal Influences on Stress

There are many factors that affect the stress system and the major hormones and neurotransmitters of the system are usually beneficial modulators, but they can also cause malfunction and potential serious illness.

The net effect of glucocorticoids is one of modulation. They prevent immune overactivity and adjust the magnitude and duration of immune reactions (Besedovsky et al 1975, Besedovsky and Sorkin 1977, Munck et al 1984). They suppress the immune system by decreasing the production of many factors that facilitate B- and T- cell proliferation, including cytokines, beta-endorphin, and insulin. Inhibition of these mediators reduces the proper functioning of monocytes and macrophages. In high levels glucocorticoids also reduce natural killer cell activity levels.

However, in low concentrations they have been found to actually enhance the immune system. It has been found that steroids must saturate at least 50% of the glucocorticoid receptors for a minimum of 24 hours before monocyte inhibition occurs (Munch and Guyre 1991).

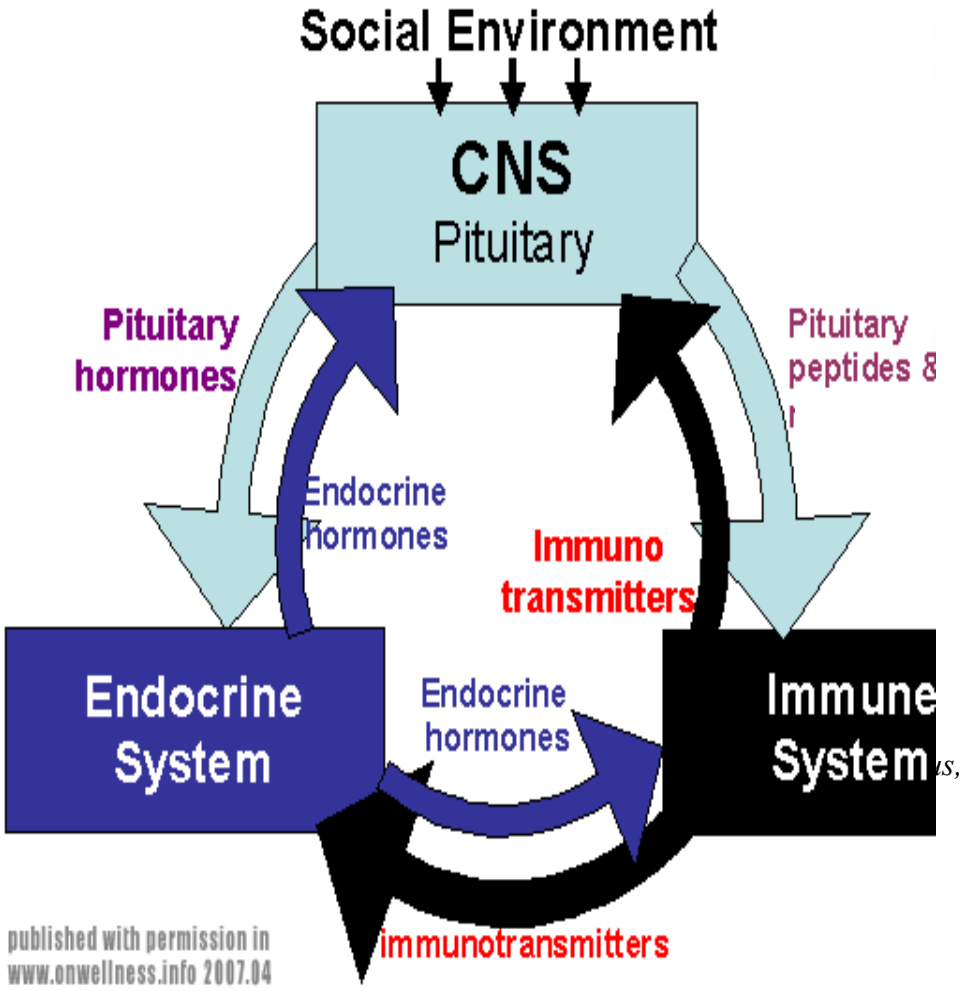
The secretion of ACTH from the pituitary prompted by CRH from the hypothalamus stimulates the adrenals to secrete glucocorticoids. During chronic stress glucocorticoids remain high but ACTH returns to normal or slightly below normal. Chromaffin cells of the adrenal medulla communicate extensively with the steroid producing cells of the adrenal cortex so as to elevate cortisol (Bornstein et all 1997, Haidan et al 1998).

When the pituitary ACTH shuts down during chronic stress, the chromaffin cells of the adrenal medulla become stimulators of adrenocortical production of corticosteroids.

CRH is released from the hypothalamus to stimulate the secretion of ACTH (Saffran and Schaly 1955, Taylor and Fishman 1988). It is a powerful hormone capable of affecting many human functions including mood, growth and reproduction (Pacak et al 1995, Pacak 2000). Nor-adrenalin and CRH are able to stimulate each other and operate differently during acute stress than during chronic stress.

Opioids are also involved in the stress response. They, as endorphins, are secreted by the adrenal medulla during the stress response and are primarily associated with the reduction of pain. Depending on the length and intensity of pain, the body responds with either an opioid or non opioid mediated form of analgesia. The opioid mediated analgesia is associated with depressed natural killer cell activity levels and a decreased tumour median survival time (Shavit et al 1985).

Figure 3.18.- Psychoneuroimmunology Network



3.15.2. How the immune system prepares for action:

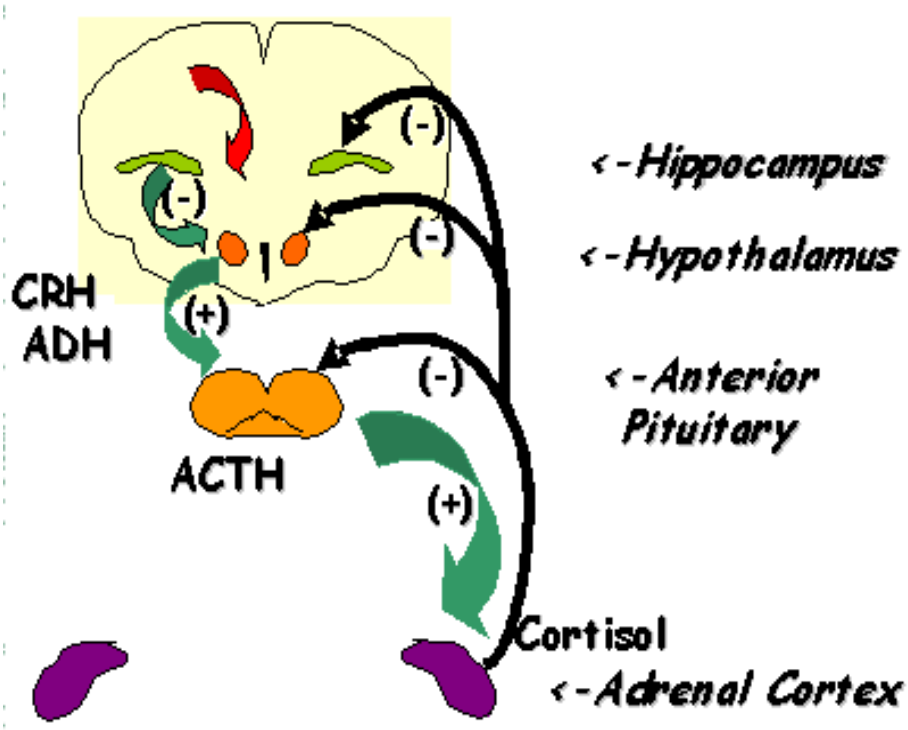
In an acute stress response, the hypothalamic-pituitary-adrenal (HPA) axis stimulates the immune response and arouses immunological memory for the invaders (Dhabhar and McEwen, 1996). The stress stimulus starts a process by which white blood cells -- particularly T cells and monocytes -- move from the blood stream to the walls of blood vessels, lymph nodes or bone marrow, in preparation to mount an immune response (Dhabhar et al, 1996) This results in a reduction of the number of white blood cells in the blood by half and increasing them in other areas, particularly the skin (Dhabhar and McEwen 1996, Dhabhar et al, 1996). After acute stress some of the white blood cells are retained in certain area of the skin and gamma interferon mediates an enhancement of skin immunity and fosters immunological memory (Dhabhar et al 2000). Glucocorticosteroids are the primary mediators of this leukocyte shift. This immune enhancing effect can last for around 3 -- 5 days, after which the allostatic load become too great and features of chronic stress emerge (McEwen, 1998). Chronic stress causes white blood cell function to be inhibited and causes a decrease in the redistribution of white blood cells from the blood to the immune compartments (Dhabhar and McEwen 1997, 1999).

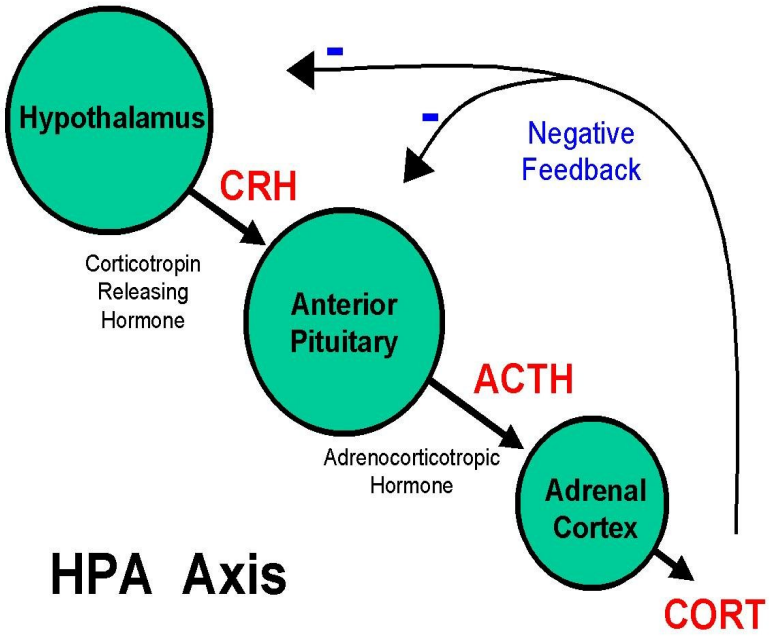
A prolonged stress response depresses the immune system.

Studies on various types of stress, such as bereavement, depression, exams, space flight, sleep deprivation, loneliness, divorce, cancer, helplessness, all show that the body becomes more vulnerable to illness.

3.15.3. The HPA Axis in response to stress/challenge

Figure 3.19.- **HPA Axis**





With malfunctioning of the immune system, the chronically stimulated cytokines can create systemic inflammation which can affect major organs, including those of the cardiovascular system.

Interleukin and tumour necrosis factor levels correlate with the severity of depression and higher levels of these cytokines have been linked to more serious depression.

C-reactive protein is another protein found in inflammation and it is an independent risk marker for coronary heat disease and can also indicate some cancers and some autoimmune diseases.

Neuropeptide Y has been shown to reduce the function of the immune system (Fabiene, Mackay & Herzog, 2005). It is released during periods of stress into the blood stream and inhibits the function of immune cells so that they do not seek out, recognize and destroy pathogens.

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Studies by Manuck, et al in 1991 showed that psychological stressors induced cell division among CD8 cells, thereby increasing the number of CD8 cells and suppressing immune function. However, this response was

only seen in those subjects who also showed high heart rate change and catecholamine change during the stressors. This was consistent with the theory that there are two groups of people - those who are "high reactors", and those who are "low reactors". High reactors are significantly affected by stress, as shown by a significant increase in heart rate, blood pressure, catecholamines, and CD8 cells. Low reactors show little or no change in those areas (Manuck, et al, 1991).

Catecholamines are chemicals produced by the body that work in nerve transmission. The three main catecholamine include dopamine, epinephrine, and nor epinephrine. Dopamine raises the heart rate and blood pressure, epinephrine raises heart rate and opens blood vessels (lowering blood pressure), and nor epinephrine closes blood vessels (raising blood pressure) (Glaser, Anderson & Anderson, 1992). Epinephrine and nor epinephrine are the catecholamine most commonly measured in stress experiments, and both increase under stress. Increases such as these can suppress aspects of immune function, including natural killer cell (cells that attack antigens without having recognized them first) activity. Increases in catecholamine may also rapidly alter cell numbers via redistribution (Naliboff, et al, 1991). In fact, changes in epinephrine

levels are thought to reflect lymphocyte migration from bone marrow, the extremities, and the thymus (Kiecolt-Glaser, et al, 1992) to other areas of the body.

Physical or psychological stressors can alter insulin needs; stressors may often be responsible for episodes of loss of control, especially in diabetic children. Type II diabetes is most often affected by stress, as it tends to occur in overweight adults and is a less severe form of diabetes (Elliot & Eisdorfer, 1982). Additionally, children who had stressful life events stemming from actual or threatened losses within the family and occurring between ages 5 and 9 had a significantly higher risk of Type I diabetes (McEwen & Stellar, 1993).

Psychological stress has also been shown to increase susceptibility to viral infection. Subjects exposed to stress showed increases in infection rates from 74% to 90%, and clinical colds rose from 27% to 47%. Earlier studies have shown that medical students have an increased risk of mononucleosis during examination periods (McEwen & Stellar, 1993). This is not surprising, as stress does suppress the immune system; latent viruses then have an easier time resurging, since the body cannot defend

itself as well. In conclusion, psychological stress does have a significant affect on the immune system. It raises catecholamine and CD8 levels, which suppresses the immune system. This suppression, in turn, raises the risk of viral infection. Stress also leads to the release of histamines, which can trigger severe bronco constriction in asthmatics. Stress increases the risk for diabetes mellitus, especially in overweight individuals, since psychological stress alters insulin needs. Psychological stress also alters the acid concentration in the stomach, which can lead to peptic ulcers, stress ulcers, or ulcerative colitis. Chronic stress can also lead to plaque build up in the arteries, especially if combined with a high-fat diet. This build up is called arteriosclerosis, and is often responsible for angina or heart attacks, which are usually brought on by acute stress. These diseases are by no means the only ones connected with psychological stress, although they are the most common. Further research is needed to clarify exactly how stressors contribute to each of these problems, so that treatment can be given to protect the body from these diseases.

3.16. Neural-Immune Interactions

Two pathways link the brain and the immune system: the autonomic nervous system and neuroendocrine outflow via the pituitary. Both routes

provide biologically active molecules capable of interacting with cells of the immune system,[Ader R, Berczi I et al 1991]

Primary and secondary lymphoid organs are innervated with noradrenergic postganglionic sympathetic nerve fibres.[Felten SY 1991] Peptidergic nerve fibres are also present in bone marrow thymus, spleen, lymph nodes, and mucosal-associated lymphold tissue.[Felten SY, et al 1991]

These nerve fibres form close neuroeffector junctions with lymphocytes and macrophages. Neurotransmitters released from these nerves diffuse to act at distant sites, further extending the potential for neural-immune interactions. Moreover, lymphocytes, Monocytes/macrophages, and granulocytes possess receptors for these neurotransmitters. (Ackerman KD 1991)

The presence of chemically specific nerve fibers associated with primary and secondary lymphoid tissues, the release and availability of neurally derived substances for interaction with immune cells, and identification of immunoregulatory effects are criteria for neurotransmission which have been satisfied for several transmitters, such as noradrenaline and substance P(Felten et al 1991, Bellinger et al1992)] For instance,

noradrenaline interacts with beta-adrenoceptors on thymic lymphocytes to inhibit thymocyte mitogenesis and enhance expression of cell-surface differentiation antigens.(Singh U ,1985) In secondary lymphoid organs, noradrenaline,at physiological concentrations, potentiates primary in-vitro IgM antibody responses that can be prevented by beta blockersSanders (V M et al 1992).Also, noradrenaline is reported to inhibit complement activation and macrophage-mediated lysis of tumour or herpes simplex virus infected cells(Koff WC, et al 1985)In rodents, chemical sympathectomy attenuates primary splenic antibody responses to systemic immunisation and lymph-node antibody responses to footpad challenge, suppresses cytotoxic T-cell responses to allogeneic cells, and reduces delayed-type hypersensitivity reactions; it is also associated with an enhancement of in-vivo lymphoproliferation in some lymph nodes and an increase in natural killer (NK) cell activity.(Madden et al1991 and Madden et al 1994).

Chemical sympathectomy also increases the severity of experimental allergic encephalomyelitis [Chelmicka et al1998] and adjuvant-induced arthritis in susceptible Strains of rats. [Felten et al 1992]

Lymphocytes and macrophages bear receptors for substance P, somatostatin, and vasoactive intestinal peptide. Substance P facilitates lymphocyte migration to inflammatory sites, enhances lympho-proliferative responses tomitogenic stimulation and lymphocyte production of IgA, and promotes phagocytosis and chemo taxis.(Payan DG et al 1992)

The denervation of substance P nerve fibres reduces the inflammation associated with herpes zoster infection (shingles) and rheumstold arthritis.(Levin J 1984) In arthritis, the greater involvement of distal joints is correlated with the density of substance P-containing afferent nerve fibres to these areas. Focal neural lesions alter the bilateral symmetry of rheumatoid arthritis in humans and in rats. Patients with paralysing central or peripheral lesions, who later develop arthritis, do not develop joint inflammation in the paralysed limb. (Levin J 1984) Thus, neurotransmitter release in joints may be an additional pathway, besides the secondary lymphoid organs, through which the nervous system contributes to the pathogenesis and severity of inflammatory diseases.

Other neurotransmitters inhibit or counteract the effects of substance P (Felten et al, 1991). Even before sympathetic innervations of lymphoid

tissues were recognized, it was known that lesions of the brain, especially the hypothalamus and limbic system, had immunological consequences (Felten D L Ader R 1991).

Preoptic/anterior hypothalamic lesions suppress splenocyte and thymocyte numbers, proliferate responses to T-cell mitogens, NK-cell cytotoxicity, antibody production, and lethal anaphylactic responses. Many of these effects are mediated by neuro endocrine changes since hypo-physectomy of lesioned animals obviates these immune changes. Medial or posterior hypothalamic lesions are associated with reduced numbers of T and B cells and enhanced allograft rejection. There is also laterality in the immuno modulatory effects of the cerebral cortex.[Rennoux G Bigerre K 1991)]

3.17. Psycho-social factors and immune function:-

In view of the central role of the neocortex in the perception and interpretation of environmental circumstances, including stressful life experiences, the immuno modulatory effects of the cerebral cortex could

be an important link between psychosocial factors and alterations in immuno-competence.

Pathways between the brain and the immune system are bidirectional. For example, Besedovsky and colleagues[Besedovosky HO 1991)] observed that activation of the immune system is accompanied by changes in hypothalamic, autonomic, and endocrine processes.

Immune system activation increases the firing rate of neurons in the ventromedial nucleus of the hypothalamus at the time of peak antibody production; sympathetic activity, indexed by nor adrenaline turnover, is increased in the spleen and the hypothalamus; and some immune responses, including those initiated by vital infections, are associated with dramatic increases in blood levels of adrenocorticotropic hormone (ACTH) and corticosterone. Such data indicate that signals generated by an activated immune system are being received and acted upon by the CNS.

Cytokines released by activated immune cells, in addition to their role in regulating cellular interactions, are one means by which the immune system communicates with the CNS and thereby influences behavior.

Interleukin (IL-1, IL-2, IL-6, interferon-gamma, and tumor necrosis factor influence activation of the hypothalamic-pituitary-adrenal (HPA) axis and are, in turn, influenced by glucocorticoid secretion (Berkenbosch J,1991)

The precise sites at which cytokines act within the brain has not been fully worked out. It is known that cytokines are endocrinologically, electrophysiologically, and behaviourally active. Central and peripheral administration of cytokines influence fever, sleep and eating behaviours, locomotor and exploratory behaviour, and mood states; the recent therapeutic use of interferons in human disease has been associated with neurological and psychiatric side effects.(Dantzer R, Kelley KW et al.1989)

3.18. Endocrine-immune interactions

In addition to autonomic nervous system activity, the immune system is influenced by neuroendocrine outflow from the pituitary. All immuno-regulatory processes take place within a neuroendocrine environment that is sensitive to the influence of the individual's perception of and response

to events in the external world. Because lymphocytes bear receptors for various hormones and neuropeptides, the cellular interactions that mediate humoral and cellular immune responses can be modulated by the neuroendocrine environment in which these immune responses occur.

In rodents, deficiencies of growth hormone are associated with abnormal cellularity of the bone marrow and thymus, together with diminished antibody production, T-cell function, and NK-cell activity. These effects are, to a large extent, overcome by administration of exogenous growth hormone (Kelley KW 1991).

Prolactin exerts a stimulatory effect on immune functions? Inhibition of pituitary prolactin secretion suppresses antibody and cell mediated immune functions and increases susceptibility to infections such as Listeria monocytogenes. These defects in immune function can be reversed by exogenous treatment with prolactin or dopamine antagonists given to stimulate endogenous release of prolactin. Prolactin released in response to stressful experiences counters many of the immunosuppressive effects of corticosteroids.

Lymphocytes bear receptors for corticotropin-releasing factor (CRF), ACTH, and endogenous opioids. Endorphins (and enkephalins) directly influence antigen-specific and non-specific in-vivo and in-vitro responses, the direction and magnitude of the effects being determined by several factors including the nature and quality of the peptides, their binding sites, and the timing of administration in relation to dose and route of antigenic stimulation.(Heijnen CJ, Kavelaars A, Ballieux 1991).

Although there are direct immunomodulatory effects of CRF and ACTH, their major in-vivo effects are exerted through interactions with other hormones and immune system products.[Ballieux 1991]

The most conspicuous hormonal influences on immune function are achieved through ACTH-induced release of adrenocortical steroids. The administration of glucocorticoids to reduce inflammatory responses and to prevent rejection of transplanted tissues is based on their immunosuppressive effects. However, many immunosuppressive properties of Corticosteroids were observed after pharmacological rather than physiological doses of the hormone. In physiological doses, glucocorticoids are essential for normal immune function (compromised adrenal function increases susceptibility to infections) and, in some

circumstances, corticosteroids can be immuno enhancing.[Jeffries WM et al 1991] The generally immunosuppressive effects of glucocorticoid release may protect the organism against an overreaction of the immune system that could lead to autoimmune disease.[Munck A, Guyre 1984] In the case of experimental allergic encephalomyelitis, a central demyelinating autoimmune disease, the anti-inflammatory effects of corticosterone attenuate the time-limited course of the paralysis.[Levine S, 1962]

However, adrenalectomised animals do not recover from this condition unless treated with glucocorticoids. An apparent defect in the release of CRF and the diminished adrenocortical activity in Lewis compared with Fisher strain rats makes the former more susceptible to the induction of rheumatoid arthritis. [Sternberg EM, 1989 these findings show the patho-physiological consequences of neuroendocrine-immune system interactions.

Pathways between the endocrine system and the immune system are also bidirectional.

Neural or lymphocyte-derived cytokines contribute to the interacting feedback mechanisms regulating the HPA axis and its target organs by

triggering CRF release or stimulating (eg, growth hormone) and inhibiting (eg, prolactin) production of pituitary hormones.[Rettori V, Jurcovicova J, 1987 Sapolsky R, Rivier C 1988] .

The potential interaction between neuroendocrine and immune processes is further shown by observations that immune cells activated by immunogenic stimuli are capable of producing neuro-peptides. (Weigent DA, 1994)

3.19. Behavioural-immune interactions

Changes in behavioural and emotional states that accompany the perception of, and the effort to adapt to, environmental circumstances are accompanied by complex patterns of neuroendocrine changes. Animal and human studies implicate psychosocial factors in the

predisposition to and initiation and progression of various pathophysiological processes, including infectious, bacterial, allergic, autoimmune, and neoplastic diseases that involve alterations in immunological defence mechanisms.[Falden 1991] The chain of psycho-physiological events has not yet been firmly established, but changes in several components of antibody and cell mediated immunity have been

associated with naturally occurring and experimentally induced behavioral and emotional states.

3.20. Depression stress and Immunity:-

The death of a family member is an especially stressful experience and can be associated with depression and an increased morbidity and mortality.[Weiner H. 1987).

Several reports describe immune alterations in the setting of bereavement and depression, especially in severe depressive states and in older men.

A detailed analysis (up to 1991 Herbert TB, Cohen S.) in meta analytic review revealed reliable effects for both enumerative and functional measures of immunity.

Clinical depression is associated with an increased number of circulating neutrophils and a decreased number of NK cells, T and B lymphocytes, and helper and suppressor/cytotoxic T cells. Depression is also associated with a reduction in NK cell activity and lympho-proliferative responses to mitogen stimulation. Three Mile Island nuclear accident (Sheridan

JF1994) suggesting that cell-mediated immunity may mediate between stressful experiences and reactivation of latent viruses.

Stressful life events also increase the rate of infectivity in response to experimental inoculation with rhinoviruses, although there is no increase in the incidence of common colds (Cohen S. 1991 and 1993)

In laboratory animals and in human beings, various stressful behavioral manipulations influence immune responses. Depending on the environmental demands and the nature of the pathophysiological process, stress can also alter host defence mechanisms, thereby altering susceptibility to bacterial and viral infections, modifying the neuroinvasiveness of normally non-neurovirulent strains of virus, or allowing an otherwise inconsequential exposure to a pathogen to develop into clinical disease.[Cohen s 1994)] Stress activates the HPA axis, increases circulating glucocorticoids, and is associated with alterations of immune function and susceptibility to infection and neoplastic disease.

However, it is not possible to attribute all immunological can sequences of altered behavioural states to increased adrenocortical steroids. There are numerous examples ofstress-induced, adrenocortically mediated changes in immunity, but there are many other observations of behavioural and stress-induced changes in immunity that are independent of adrenocortical

activation.(Adar R 1987) A striking example of CNS involvement in the modulation of immunity is the classical (Pavlovian) conditioning of antibody and cell mediated immune responses.(Felten DL 1991.)

When a distinctively flavoured drinking solution, the conditioned stimulus, is paired with the injection of an immunosuppressive drug, eg, cyclophosphamide, the unconditioned stimulus, the subsequent antibody response to sheep red blood cells is attenuated in conditioned animals re-exposed to the conditioned stimulus. Similarly, the immunological effects of stress have been conditioned. (Lysle DT, 1988).Other studies Ader R, Kelly K 1993) have shown conditioning effects using antigen as the unconditioned stimulus. Mediation of conditioned immunopharmacological effects, stress effects, and of the direct conditioning of immune responses are not yet known but probably involve sympathetic and/or neuroendocrine mechanisms, including feedback regulation by the immune system. The hypothesis that conditioned alterations of immunity are merely a reflection of stress responses, notably adrenocortical secretions, is not supported by the evidence. (Ader R 1987)

3.21. Summary of Immune-modulation and stress (Psychoneuroimmunology)

There is a new appreciation of the interactions between behavioral, neural, endocrine, and immune processes. Indeed, there has been a paradigm shift in the attempt to understand immunoregulatory function.

The innervations of lymphoid organs and the availability of neurotransmitters for interactions with cells of the immune system add a new dimension to our understanding of the microenvironment in which immune responses occur. Similarly, the interaction between pituitary, endocrine, and lymphocyte derived hormones, which define the neuroendocrine environment in which immune responses take place, adds another level of complexity to the analysis of cellular interactions that drive immune responses.

Collectively, these observations provide the basis for behaviorally induced alterations in immune function and immunologically based changes in behavior. They may also provide the means by which psychosocial factors and emotional states influence development and progression of infectious autoimmune and neo-plastic disease.

Neurotransmitters and cytokines, the signal molecules of the nervous and immune systems, are expressed and perceived by both systems and, as such, are misnomers. What have been considered separate "systems" can be considered components of a single, integrated defence mechanism in which the interaction between systems is as important to an understanding of adaptation as the interactions within a system.

In summary the association between stressful life experiences and changes in immune function do not establish a causal link between stress, immune function, and disease. This chain of events has not yet been definitively established. However, major links between these “systems" have been described and a new understanding of interactive biological signaling has begun. Psychoneuroimmunology is developing the means to explore these relations and their clinical and therapeutic implications.

3.22. Stress DNA damage and cancer:-

According to Kang DH 2002 Oxidative stress is a disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant defenses. It occurs when excessive production of ROS

overwhelms the antioxidant defense system or when there is a significant decrease or lack of antioxidant defenses. Oxidative stress, in turn, is known to cause DNA damage and mutations of tumor suppressor genes that are critical initial events in carcinogenesis. Interestingly, early findings of the studies suggest that environmental factors, such as high psychological stress and poor nutritional profile (eg, low antioxidant and high fat intake), increase ROS production. Given that breast cancer is a complex disorder in which gene-environment interactions play a significant role in the development of cancer, oxidative stress may be an excellent model for exploring mechanisms mediating gene-environment interactions for nurse scientists and advanced practice nurses. Such investigations may help to suggest future strategies for non pharmacological interventions for decreasing cancer risk.

In a land mark study in 2004 Cohen L et al says that there has been extensive research into the effects of stress on immune function but little on the effects of stress on DNA repair capacity (DRC), a process central to maintaining a normal cell cycle. Defective DRC is one of the factors responsible for carcinogenesis. In a study Lorenzo and group assessed DRC in healthy medical students during times of high and low stress. Sixteen medical students were evaluated during the third day of a 5-day

exam period and then again 3 weeks later, after vacation. At both time points, participants underwent a brief physical examination, had venous blood drawn, and completed questionnaires to identify subjective stress levels. The DRC was assessed by the host-cell reaction assay, which measures nucleotide excision repair capacity. Participants reported significantly higher levels of subjective stress during the exam period than after vacation. DRC was also significantly higher during the exam period than after vacation, suggesting a positive association between subject stress levels and DRC.

3.23. Breast cancer as Psychosocial Disease:-

Most women with breast cancer usually undergo surgery as a primary treatment followed by 4-6 months of chemotherapy and 6 weeks of radiotherapy as adjuvant treatments. Psychosocial morbidity is common in breast cancer patients after mastectomy and increased during radiotherapy and chemotherapy, wherein the majority of patients reported some degree of depression, anxiety, social dysfunction and inability to work (De Boer-Dennert M, de Wit R, & Schmitz PI, 1997; Gelber RD, Goldhirsch A, & Cavalli F, 1991; Hughson AV, Cooper AF, McArdle CS, & Smith DC, 1987)

3.24. Stress, Glucocorticoids, Immune Response and Cancer

Even though stress induced changes in immune system have shown to facilitate tumor development (Riley V, 1981) and progression in animal models (Giraldi T, Perissin L, Zorzet S, Piccini P, & Rapozzi V, 1989), the data on psychological factors and cancer in human studies are controversial, although several authors cautiously agree that to some extent, they may contribute to disease manifestation and progression (Cooper CC & Watson M, 1991; Levenson JL et al., 1994).

Stress also influences NK cell counts and function which are involved in antitumor immune responses. In general, research findings suggest that "chronic stressors are associated with continued down-regulations of immune function rather than adaptation" (Kiecolt-Glaser J.K & Glaser R, 1992).

Studies have shown that the biological sequel of depression is known to affect HPA Axes dysregulation in metastatic cancer patients (Sephton S.E, Sapolsky R.M, Kraemer H, & Spiegel D, 2000) and also immune function (NK Cell numbers and activity) (Brittenden J, Heys S.D, Ross J, & Eremin O, 1996). This is well corroborated by studies which show

immunosuppressive effects of these gluco corticoids on NK cell function (Garland M.R et al., 2004; Mikosz C.A, Brickley DR, Sharkey M.S, Moran TW, & Conzen SD, 2001). Studies on depressed subjects (Zorrilla EP et al., 2001) and those with breast cancer show an impairment in NK activity and independent association between this, psychological states, nodal status and stage of disease progression(Levy S.M, Herberman R.B, Maluish A.M, Schlien B, & Lippman M, 1985; Levy SM, Herberman RB, Lippman M, & d'Angelo T, 1987; Levy SM et al., 1990). However other studies have failed to show a relationship between in vivo cortisol measures and reported decreases in either circulating NK Cell numbers or NK cell activity (Irwin M, 1999; Maes M et al., 1992; Miller AH, Asnis GM, Lackner C, Halbreich U, & Norin AJ, 1991). Similar studies in cancer populations have also been inconclusive (Garland M.R et al., 2004; Lechin F et al., 1990; Sephton S.E et al., 2000).These inconclusive findings could be related to the fact that first two studies had several methodological issues as they focused on metastatic disease where in multiplicity of confounding factors could have affected the cortisol- NK relationships, the last study compared the preoperative cortisol rhythmicity with post operative psychological morbidity.

3.25. Complimentary and alternative medicine (CAM) studies:-

Breast cancer survivors commonly use CAM to improve quality of life (QoL) [Henderson JW 2004, Richardson MA, 2000, Boon H 2000) yet few studies have evaluated QoL correlates of CAM therapies in these women. Burstein et al., prospectively examining newly diagnosed early-stage breast cancer patients, found worse mental health 3 months after diagnosis in women who began using CAM than in nonusers but no significant difference after one year. Alferi et al. also examined CAM use in recently diagnosed early-stage breast cancer patients, but found no differences in QoL between CAM users and nonusers. Despite the evidence that breast cancer survivors have lower QoL years after diagnosis [Casso D, 2004], they found no study examining the relationship between use of CAM and QoL in breast cancer survivors of more than 1 year. However, among general cancer patients diagnosed between 2 months to more than 5 years previously, Paltiel et al. found lower QoL scores among CAM users than nonusers [Paltiel O 2001]. A limiting problem with many studies is different definitions of CAM and the grouping of different CAM therapies under one or a few broad categories. CAM is defined as ‘‘a group of diverse medical and health care systems, practices, and products

that are not presently considered to be part of conventional medicine.’’[Barnes PM, 2004] Thus, this definition is subject to change as some therapies become accepted into mainstream medicine and others fade from use.

Comparison between studies is challenging, and assessment of factors associated with overall CAM use is of limited utility, since factors such as race, religious affiliation, and breast cancer treatment (e.g., chemotherapy) may be associated with the use of some CAM therapies but not others (Lee MM, Barnes PM 2004, Alferi SM, 2001).

Burstein et al set out to identify factors (including demographic, health, breast cancer clinical factors) associated with overall CAM use and, more importantly, with different types of CAM therapies [Burstein HJ, 1999, Alferi SM, 2001]. Holmes D et al also sought to assess whether different aspects of QoL are related to use of individual therapies. Because some [. Edgar L, 1999, Swisher EM, Wyatt GK 2001], Davidson R, studies have suggested that CAM users are more optimistic than nonusers; they also examined levels of optimism

3.26. The use of various CAMs in last two years

(Harvard 2006)

Figure 3.21.

Complementary and alternative medical therapies used for any reason by respondents during the past 2 years

Therapy	*N*	Number using therapy	Percent of total respondents
Relaxation/imagery	1942	616	32%
Spiritual healing	1909	257	13%
Yoga	1929	228	12%
Energy healing	1929	147	8%
Acupuncture	1923	81	4%
Massage	1932	440	23%
Chiropractic	1928	225	12%
High-dose vitamins	1918	391	20%
Herbs	1895	361	19%
Homeopathy	1901	70	4%
Other CAM therapies[a]	1932	85	4%
Any CAM used[b] (Total respondents using at least 1 CAM therapy)	2022	1249	62%

[a] Includes infrequently used therapies, such as biofeedback (used by 24), hypnosis (used by 24), naturopathy (used by 20), osteopathy (used by 16), folk remedies (used by 16), chelation (used by 4)

[b] Because some women used more than one therapy, the total of individual therapies is greater than the total of ''Any CAM Used''

CAM use in breast cancer survivors is high, with 62% of study participants reporting use of at least one CAM therapy in the last 2 years,

most commonly ‘‘for general Wellness.’’ Among the 27% of CAM users for cancer or its symptoms, the majority found the therapies helpful.

A key finding of a study lead by (Holmes D et al 2006) is that different factors are associated with the use of different types of CAM therapies, which likely explains why some factors, such as chemotherapy and radiation, are inconsistently identified as significant correlates of CAM use when CAM is modelled as a combined outcome [Ashikaga T, 1999 Richardson MA, Boon H, 2000]. These findings suggest that grouping all CAM use under one heading is inappropriate. The association of the use of relaxation/imagery and spiritual healing with having received chemotherapy is of interest, particularly because nearly one-third of women who used CAM reported using relaxation/imagery and spiritual healing to help treat cancer or its symptoms, and the overwhelming majority rated these therapies as helpful. In addition, the notion that either relaxation/imagery or spiritual healing might be used concurrently with chemotherapy causes little alarm for the clinician concerned about possible interactions between conventional and complementary/alternative practices.

Knowledge of an association between radiation therapy and use of high-dose vitamins could be of concern, if the therapies were used

simultaneously and women were unaware of potential interactions between these therapies [Bairati I, 2005]. The finding that women who use homeopathy are less likely to use tamoxifen or anastrozole warrants further investigation, given that the evidence supporting use of adjunctive hormonal therapy for prevention of breast cancer recurrence is strong.

In general, several types of CAM were statistically significantly associated with worse QoL subscales, but most were not considered to represent clinically meaningful differences.

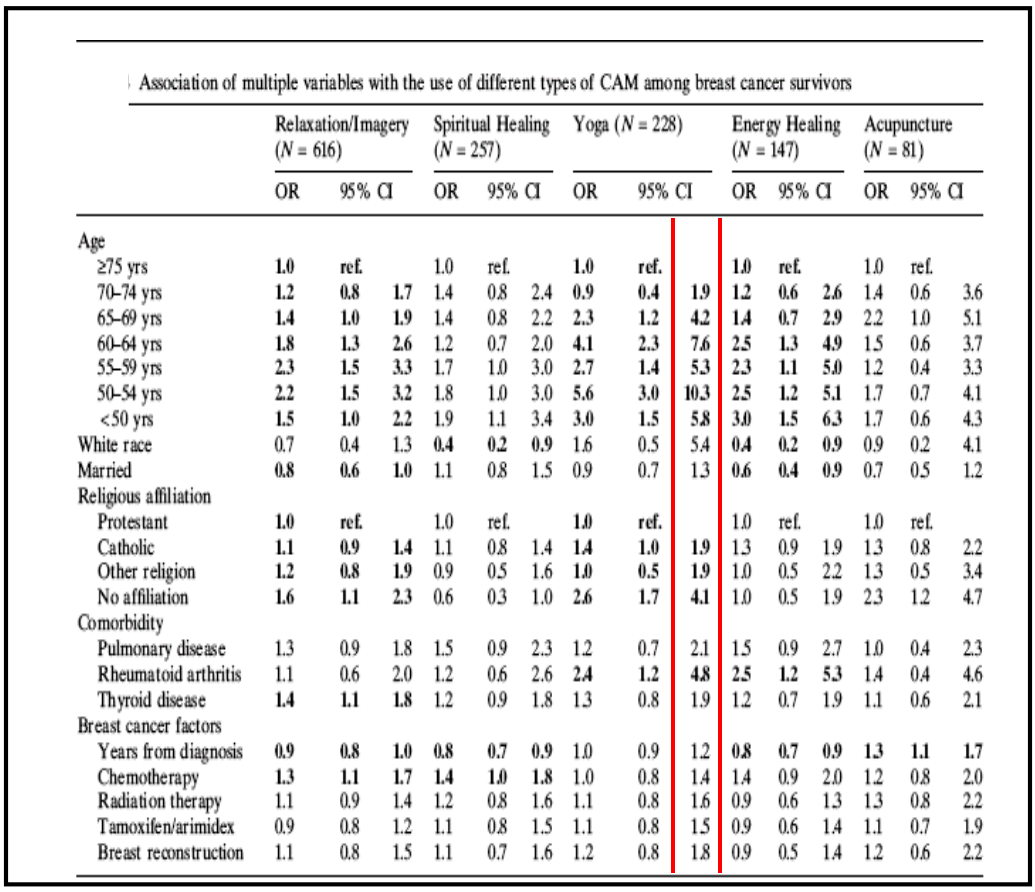
In the **Holmes survey 2006**, at Harvard

1) A striking exception was found among women who used energy healing, who demonstrated statistically and clinically significantly lower scores on almost every QoL subscale.

2) In addition, although the difference in many QoL scores associated with the use of yoga was modest, unlike use of all other types of CAM, yoga was reported to produce better QoL. It is not known whether use of CAM therapies affects QoL, or vice versa, and future research should evaluate whether these therapies changes QoL over time.

3) Although women who used relaxation/imagery demonstrated significantly more optimism, the difference in optimism scores was small, and CAM users or nonusers demonstrated similarly high levels of optimism. Advantages of this study were the inclusion of breast cancer survivors from multiple US states, women diagnosed with all stages of breast cancer, and a large sample size with a wide selection of CAM therapies.

Figure 3.22.



Association of multiple variables with the use of different types of CAM among breast cancer survivors

	Relaxation/Imagery (N = 616)			Spiritual Healing (N = 257)			Yoga (N = 228)			Energy Healing (N = 147)			Acupuncture (N = 81)		
	OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI	
Age															
≥75 yrs	1.0	ref.		1.0	ref.		1.0	ref.		1.0	ref.		1.0	ref.	
70–74 yrs	1.2	0.8	1.7	1.4	0.8	2.4	0.9	0.4	1.9	1.2	0.6	2.6	1.4	0.6	3.6
65–69 yrs	1.4	1.0	1.9	1.4	0.8	2.2	2.3	1.2	4.2	1.4	0.7	2.9	2.2	1.0	5.1
60–64 yrs	1.8	1.3	2.6	1.2	0.7	2.0	4.1	2.3	7.6	2.5	1.3	4.9	1.5	0.6	3.7
55–59 yrs	2.3	1.5	3.3	1.7	1.0	3.0	2.7	1.4	5.3	2.3	1.1	5.0	1.2	0.4	3.3
50–54 yrs	2.2	1.5	3.2	1.8	1.0	3.0	5.6	3.0	10.3	2.5	1.2	5.1	1.7	0.7	4.1
<50 yrs	1.5	1.0	2.2	1.9	1.1	3.4	3.0	1.5	5.8	3.0	1.5	6.3	1.7	0.6	4.3
White race	0.7	0.4	1.3	0.4	0.2	0.9	1.6	0.5	5.4	0.4	0.2	0.9	0.9	0.2	4.1
Married	0.8	0.6	1.0	1.1	0.8	1.5	0.9	0.7	1.3	0.6	0.4	0.9	0.7	0.5	1.2
Religious affiliation															
Protestant	1.0	ref.		1.0	ref.		1.0	ref.		1.0	ref.		1.0	ref.	
Catholic	1.1	0.9	1.4	1.1	0.8	1.4	1.4	1.0	1.9	1.3	0.9	1.9	1.3	0.8	2.2
Other religion	1.2	0.8	1.9	0.9	0.5	1.6	1.0	0.5	1.9	1.0	0.5	2.2	1.3	0.5	3.4
No affiliation	1.6	1.1	2.3	0.6	0.3	1.0	2.6	1.7	4.1	1.0	0.5	1.9	2.3	1.2	4.7
Comorbidity															
Pulmonary disease	1.3	0.9	1.8	1.5	0.9	2.3	1.2	0.7	2.1	1.5	0.9	2.7	1.0	0.4	2.3
Rheumatoid arthritis	1.1	0.6	2.0	1.2	0.6	2.6	2.4	1.2	4.8	2.5	1.2	5.3	1.4	0.4	4.6
Thyroid disease	1.4	1.1	1.8	1.2	0.9	1.8	1.3	0.8	1.9	1.2	0.7	1.9	1.1	0.6	2.1
Breast cancer factors															
Years from diagnosis	0.9	0.8	1.0	0.8	0.7	0.9	1.0	0.9	1.2	0.8	0.7	0.9	1.3	1.1	1.7
Chemotherapy	1.3	1.1	1.7	1.4	1.0	1.8	1.0	0.8	1.4	1.4	0.9	2.0	1.2	0.8	2.0
Radiation therapy	1.1	0.9	1.4	1.2	0.8	1.6	1.1	0.8	1.6	0.9	0.6	1.3	1.3	0.8	2.2
Tamoxifen/arimidex	0.9	0.8	1.2	1.1	0.8	1.5	1.1	0.8	1.5	0.9	0.6	1.4	1.1	0.7	1.9
Breast reconstruction	1.1	0.8	1.5	1.1	0.7	1.6	1.2	0.8	1.8	0.9	0.5	1.4	1.2	0.6	2.2

(Adapted from Homes D, 2006 Breast Cancer Research and treatment)

Figure showing that YOGA had a significantly high correlation with multiple parameters.

Figure 3.23.

Association of CAM with health-related quality of life factors

	Relaxation/ Imagery			Spiritual healing			Yoga			Energy healing			Acupuncture		
	Yes	No	*P*	Yes	No	P	Yes	No	P	Yes	No	P	Yes	No	P
Physical function	80.1	80.1	1.0	77.5	80.5	*	**84.8**	**79.4**	**	**72.2**	**80.7**	**	77.1	80.3	0.2
Diff (95% CI)	0.0	-2.1	2.1	-3.0	-5.9	-0.1	**5.4**	**2.4**	**8.5**	**-8.5**	**-12.2**	**-4.8**	-3.2	-8.0	1.7
Bodily pain	71.6	72.6	0.4	71.1	72.4	0.4	75.3	71.8	*	**62.6**	**73.0**	**	68.6	72.4	0.1
Diff (95% CI)	-1.0	-3.1	1.2	-1.3	-4.2	1.6	3.5	0.4	6.7	**-10.4**	**-14.2**	**-6.6**	-3.9	-8.8	1.1
Role-physical	66.6	71.0	*	65.4	70.1	0.1	71.9	69.2	0.3	**54.8**	**70.7**	**	66.9	69.7	0.5
Diff (95% CI)	-4.5	-8.2	-0.8	-4.6	-9.7	0.4	2.7	-2.8	8.1	**-15.9**	**-22.4**	**-9.4**	-2.8	-11.4	5.8
Vitality	63.6	63.4	0.8	62.3	63.6	0.3	66.5	63.0	*	**57.6**	**63.9**	**	62.0	63.5	0.5
Diff (95% CI)	0.3	-1.7	2.2	-1.3	-4.0	1.4	3.6	0.7	6.4	**-6.3**	**-9.7**	**-2.8**	-1.6	-6.1	3.0
Social function	84.7	88.2	**	83.8	87.5	*	88.3	86.8	0.3	**78.8**	**87.7**	**	82.9	87.2	0.1
Diff (95% CI)	-3.5	-5.4	-1.5	-3.7	-6.3	-1.0	1.4	-1.4	4.2	**-8.8**	**-12.2**	**-5.5**	-4.4	-8.8	0.1
Role-emotional	77.4	81.3	*	76.4	80.5	0.1	79.3	80.1	0.7	**71.9**	**80.6**	**	73.2	80.3	*
Diff (95% CI)	-4.0	-7.0	-0.9	-4.1	-8.2	0.0	-0.9	-5.3	3.5	**-8.8**	**-14.1**	**-3.5**	-7.1	-14.1	-0.1
Mental health	78.2	80.3	**	78.9	79.7	0.4	79.5	79.6	0.9	76.5	79.8	*	76.8	79.7	0.1
Diff (95% CI)	-2.0	-3.4	-0.7	-0.8	-2.6	1.1	-0.1	-2.1	1.9	-3.3	-5.7	-0.9	-2.9	-6.1	0.3

(Adapted from Catherine Buettner breast cancer research and treatment 2006)

The above figure shows a very high Quality of Life (QOL) amongst Yoga practitioners

Figure 3.24.

Selected reasons[a] given for why CAM therapies were used among those using CAM (N = 1249)

	"To treat cancer or its symptoms" (N = 338)	% of CAM used for cancer or its symptoms (%)	"To treat other illnesses" (N = 396)	% of CAM used for other illnesses (%)	"For general wellness" (N = 889)	% of CAM used for general wellness (%)	Reason not specified[b] (N = 254)
Relaxation/imagery	130	38	57	14	430	48	140
Spiritual healing by others	113	33	45	11	108	12	58
Yoga	16	5	12	3	184	21	41
Energy healing	39	12	52	13	58	7	22
Acupuncture	17	5	50	13	30	3	2
Massage	63	19	83	21	298	34	62
Chiropractic	11	3	152	38	74	8	14
High-dose vitamins	72	21	52	13	271	30	54
Herbal therapies	99	29	99	25	222	25	27
Homeopathy	25	7	29	7	33	4	4
Other therapies	20	6	26	7	44	5	12

(Adapted from Homes D, 2006 Breast Cancer Research and treatment)

Figure: - The above figure suggests that among all CAM studies Yoga has the highest effect in increasing wellness.

In another study Osoba D, 2005 although the list of CAM therapies was not comprehensive, it allowed for several CAM therapies to be modeled as individual outcomes. Their decision to group relaxation and imagery together was justified by the high correlation of these two therapies. As with any observational study, their findings may be subject to unmeasured confounding. By including only registered nurses, their study indirectly controls for education and occupation. However, both higher education and higher income have been linked to greater use of CAM. Thus, the prevalence of CAM use estimated by their study may be higher than in the general population of breast cancer survivors. Nonetheless, the finding that 62% of breast cancer survivors (averaging 3.2 years since diagnosis) had used CAM is similar to two other recent surveys of breast cancer survivors (averaging 3–3.5 years since diagnosis), Boon H, Stewart M, 2004 and Henderson JW 2001 which both found approximately two-thirds of survivors had used CAM. Although they had information to confirm breast cancer stage for 84% of participants, they did not assess current disease status.

Given the associations identified between use of different types of CAM and QoL, current disease status and information on a broader selection of

co-morbidities associated with physical pain and disability would be useful

3.27 Summary of CAM studies:-

Breast cancer survivors commonly use CAM. Because correlates of CAM use vary according to type used, one should not assume general factors associated with its use extend to use of all types of CAM. Breast cancer factors associated with the use of individual CAM therapies included the use of relaxation/imagery and spiritual healing with receiving chemotherapy, use of high-dose vitamins with receiving radiation or breast reconstructive surgery, and the use of homeopathy, which was inversely associated with the use of tamoxifen or anastrozole. The use of energy healing was associated with statistically and clinically significantly worse scores for several aspects of QoL, while use of **YOGA** was associated with better QoL. These findings more clearly describe patterns of use of CAM by breast cancer survivors and highlight the need for longitudinal studies of specific CAM therapies to evaluate their efficacy for alleviating cancer-related symptoms.

The mood benefits of Hatha yoga and swimming, two activities that differ greatly in aerobic training benefits, were examined (Berger BG & Owen

DR, 1992) . The consistent mood benefits of yoga supported our earlier observation that the exercise need not be aerobic to be associated with mood enhancement. The immediate effects of relaxation therapy were assessed in 40 hospitalized children and adolescents with diagnoses of adjustment disorder and depression. These effects were assessed using a within subjects pre-test/post-test design and by comparison with a control group of 20 depressed and adjustment disorder patients who watched a 1-hr relaxing videotape Adjustment disorder patients and a third of the depressed patients showed decreases in cortisol levels following Relaxation therapy (Platania-Solazzo A et al., 1992).

3.2.8.Summary of CAM research:-

- Throughout past millennia, human beings have shared the common goal of improving health for longevity. However, different cultures around the world have developed their own approaches to achieve this goal. Various traditions have emerged, rendering distinct medical systems such as Ayurveda, Yoga, Chinese-Japanese medicine, shamanism, and Native American healing. Traditional medicine involves a holistic approach to the human body to integrate healing with culture, environment, and tradition. Modern

allopathic medicine originated from Greco-Roman Medicine and Northern European traditions and is built on the science of anatomy, physiology, and biochemistry and the structure-function relationship between cells, tissues, and organs. This foundation focuses on diagnosis, treatment, and cure for acute illnesses via potent pharmaceutical drugs, surgery, radiation, and other treatment modalities. Within this past century, we have doubled the life-span of human beings. Genomic medicine, including stem cell research, cloning, and gene therapy, will increase our capability to treat even more diseases. In the new millennium, we face more chronic illnesses related to aging, environment, and lifestyle, such as cancer, diabetes. Osteoporosis and cardiovascular diseases. Thus, health care providers face the challenge of prospecting for health and disease prevention. Modern science and medical advancements provide the rationale for the integration of various traditional healing techniques, which have been termed Alternative and Complementary Medicine, to promote healing, health, and longevity. Advances in medicine must include the holistic approach of traditional medicine to face the current challenges in health care. Therefore, the New World of Medicine must fuse the antiquity of ancient healing with the innovations of

modern medicine to increase life-expectancy and improve quality of life throughout the world.

- In 1987 in Dubrovnik, Yugoslavia, N.H. Spector named a new discipline: Neuroimmunomodulation. R. Ader called this new discipline psychoneuroimmunomodulation when the major emphysis was on its behavioral aspects. Neuroimmunomodulation (NIM) is devoted to the study of the interactions at different morphologic and functional levels among the immune, nervous, and endocrine systems. In fact, this science is the modern manifestation of an old science: in the words of B.D. Jankovic (1987), "Neuroimmunomodulation is a modern reflection in neurosciences and immunosciences of the ideas and experience of philosophers and ingenious observers of ancient Egypt, Greece, China, India, and other civilizations that the mind is involved in the defense against diseases." Twelve years ago NIM was regarded by many conventional scientists almost as a form of witchcraft. Today it may be the fastest growing area of biomedical science research in the world. Important clinical applications will not be far behind. NIM research has also progressed in the field of oncology research. Topics such as treatment of hormone-dependent cancer with analogues of hypothalamic hormones, the

role of opioids and T cells in cancer, stress-cancer-immune connections, the anticancer role of melatonin and cytokines, immunotherapy of cancer, and the role of psychotherapy in cancer patients represent some lines of research that have been or are being investigated by scientists. Some areas remain to be thoroughly investigated such as the influence of physical exercise (sports), music (classical or modern), and/or relaxation techniques (e.g. yoga) on the development of human cancer. This paper reviews the role of NIM in oncology and provides some perspectives for further research and development of clinical applications.

- Robert W. Woodruff reviews that there is growing attention to the health benefits of mind/body interventions, particularly relaxation and meditation. Biomedical research has provided undeniable evidence of the interconnectedness of the mind and body. The field of psychoneuroimmunology has defined the role of stress in reducing effectiveness of the immune system in combating infection and growth of malignant tumors. There is growing consensus in the development of meditation practice and explores the indications that the practice of meditation is effective reducing the harmful effects of stress. In addition, there are encouraging

reports of studies citing the influence of melatonin on breast and prostate tumors. A preliminary study finds an association between meditation practice and levels of melatonin produced by the pineal gland.

Chineese Qigong and gene expression studies:-

- The great similarity of the genomes of humans and other species stimulated us to search for genes regulated by elements associated with human uniqueness, such as the mind-body interaction. DNA microarray technology offers the advantage of analyzing thousands of genes simultaneously, with the potential to determine healthy phenotypic changes in gene expression. The aim of this study was to determine the genomic profile and function of neutrophils in Falun Gong (FLG, an ancient Chinese Qigong) practitioners, with healthy subjects as controls. SUBJECTS AND DESIGN: Six (6) Asian FLG practitioners and 6 Asian normal healthy controls were recruited for our study. The practitioners have practiced FLG for at least 1 year (range, 1-5 years). The practice includes daily reading of FLG books and daily practice of exercises lasting 1-2 hours. Selected normal healthy controls did not perform Qigong, yoga, t'ai chi, or any other type of mind-body practice, and had not

followed any conventional physical exercise program for at least 1 year. Neutrophils were isolated from fresh blood and assayed for gene expression, using microarrays and RNase protection assay (RPA), as well as for function (phagocytosis) and survival (apoptosis). RESULTS: The changes in gene expression of FLG practitioners in contrast to normal healthy controls were characterized by enhanced immunity, downregulation of cellular metabolism, and alteration of apoptotic genes in favor of a rapid resolution of inflammation. The lifespan of normal neutrophils was prolonged, while the inflammatory neutrophils displayed accelerated cell death in FLG practitioners as determined by enzyme-linked immunosorbent assay. Correlating with enhanced immunity reflected by microarray data, neutrophil phagocytosis was significantly increased in Qigong practitioners. Some of the altered genes observed by microarray were confirmed by RPA.

The study concluded that Qigong practice may regulate immunity, metabolic rate, and cell death, possibly at the transcriptional level. The pilot study provides the first evidence that Qigong practice may exert transcriptional regulation at a genomic level. New approaches are needed to study how genes are regulated by

elements associated with human uniqueness, such as consciousness, cognition, and spirituality.

3.28. Summary of Effects of Stress on Immune Response.

Figure 3.25.

Authors	Study sample	Stressors	Immune measures	Outcome
Biondi and Pancheri	25 female inpatients	Awaiting breast surgery	E-rosetttes,PHA,skin test	Subjects with reduced PHA-lymphoproliferative responses,reduced skin test reactivity and E rosettes formation had higher scores om depression, social introversion and repression denial coping styles than those awaiting surgey with low scores.
Castle S,Cousions net et al 1990	Elderly care giver wives of demented patients	Stress of care-giving	Immune cell phenotype and cell proliferative capacity.	Depression was associated with increase in CD8 cells and reduction in Nk cells. Explains higher risk of mortality in care givers.
Zorilla EP et al 2001	Meta analysis on 180 studies	Relation between various forms of depression and immune system.	Fixed effects model showed depression to be associated with Leucocytosis, increased CD4, CD8 ration IL-6 levels, rediuced Nk cell cytotoxicity and lymphoproliferative responses to mitogens.	
VedhraK,cox NK Met et al 1999	50 spouses of dementia patients	Care giving of family member with severe illness.	Ig G Ab titers flu vaccine cortisol	Increased cortisol and poor antibody response to influenza vaccine,care givers may be more vulnerable to infectious disease.
Fawzy F Cousins et al 1990	61 cancer care givers and 35 psychiatric patients 26 controls	Cancer diagnosis	T helper T cells,T suppressors and large granular lymphocytes number NK cell activity	Intervention group had higher anger than control, anxiety and depression are related to LGLs and Nk cells %age and activity. At six months the difference between the groups persists.
Aragona M, Muscatello MRetal et al 1996	106 breast cancer patienst and 37 patients with benign breast cancer (controls)	Stressful life events, hospital admissions uncertain diagnosis and awaiting surgery	Catecholamines excretion and blood cortisol levels. CD3+ CD4+ CD8+ CD 16+ Lymphocytes %age.	Breast cancer patients show increased catecholamanine excretion and positive correlation between blood cortisol and lymphocyte percentage.
Pettingale et al 1977	160 women admitted for breast tumor biopsy.	Awaiting surgery	Serum immunoglobulins	Serum IgA levels are higher in subjects who suppress anger than those who express it.
Pettingale K W Greer S et al 1977	57 breast cancer patients	Life threatening illness	Ig G, Ig M, and IgA	Increase of IgA is associated with emotional repression, increase of IgM with suppressed reactivity to Con A PHA etc

3.29. Summary of Yoga Interventions in Cancer Patients Figure 3.26.

Authors, Year	Subjects and Settings	Design	Outcomes
Joseph CD,1983	50 cancer patients undergoing radiotherapy	Uncontrolled pre post design	Improved self reported symptoms of appetite, well being, sleep
Coker KH,1999	Prostate cancer patients	Uncontrolled preliminary study MBSR intervention	Reduction in stress enhancing general health and wellness, increased production in melatonin
Speca Met.al.2000,Carlson LE	Heterogeneous cancer population,(n=61 in yoga and n=48 in control)	Randomized wait list control design;7 week MBSR intervention	Better compliance to intervention and improved mood states
Shapiro SL, Bootzin RR et.al 2003	Breast cancer patients	RCT – MBSR vs free choice as controls	Improvement in daily sleep quality in both groups
Moadel A, Shah Cet.al 2004	Undeserved Breast cancer patients ,(n=59 in yoga and n=29 in control)	Randomized wait list control design;12 weekly sessions of Hatha Yoga	Poor adherence, improvements in emotional well being in yoga group decline in social well being in controls and increase in stress symptoms
Cohen L,Warneke Cet.al 2004	39 stage I-IV lymphoma subjects at different stages of treatment	Randomized wait list control design; 7 weekly sessions of Breast cancer patients Tibetan Yoga	Improved sleep quality
Carlson LE,Speca Met.al 2004	Breast and prostate cancer patients (n=59)	Uncontrolled study 8 weeks MBSR	Improvement in Qol, stress & health behaviors, no changes in mood, cancer related symptoms, salivary cortisol, DHEA and melatonin levels, change in intracellular cytikines.
Culos-Reed S,Carlson LEet.al,2004	38 mixed cancer patients	Randomized wait list control design; 7 weekly sessions of Hatha Yoga	Reduced mood disturbance,stress and improved Q0l,Cardiopulmonary functions and physical
Cohen L, Thornton Bet.al,2005	58 stage I-III Breast cancer patients	Randomized wait list control design; 7 weekly sessions of Breast cancer patients Tibetan Yoga	Fever cancer related symptoms no improvements in mood, Qol and sleep
Carlson LE, Culos-Reed Net.al 2005	20 mixed cancer survivors	Randomized wait list control design;7 weekly sessions of Hatha Yoga	Reduced mood disturbance and improved Qol, No change in cortisol slope or diurnal rhythms
Culos-Reed SN, Carlson LEet.al,2006	Breast cancer survivors (n=20 in yoga and n=18 in control)	Randomized wait list control design; 7 week Yoga program	Improvement in psychosocial (ie global quality of life, emotional function and diarrhea variables at post assessment.

3.30. Yoga studies in Breast cancer

Earlier studies validating the effects of a psychosocial intervention in cancer patients are met with methodological problems with cortisol measure where only one measure of cortisol was used (Schedlowski M, Jung C, Schimanski G, & Tewes U, 1994), plasma samples were used instead of saliva samples and time of day wasn't specified for collection of sample (Van der Pompe G, Antoni M.H, & Heijnen C.J, 1996). Meditation has been shown to decrease cortisol levels in populations of healthy volunteers (MacLean CR, Walton KG, & Wenneberg SR, 1994; Sudsuang R, Chentanez V, & Veluvan K, 1991). Recent studies using a eight-week mindfulness based stress reduction program for early stage breast and prostate cancer patients have shown to decrease levels of salivary cortisol in those with initially high values and also change the abnormal pattern of cortisol secretion. Improvements were also seen in quality of life, moods, and decrease in stressful symptoms (Carlson LE, Culos-Reed N, & Daroux LM, 2005; Carlson LE, Speca M, & Patel DK, 2004)

3.31. Prelude to the Current study:-

Breast cancer is a profoundly stressful disease posing both physical and psychological threats to the patient. Moreover, patients with breast cancer normally receive multimodal treatment over a long period of time .Psychological distress and trauma is often associated with the diagnosis of cancer and is common (Derogatis L R et al 1983, Stefanek M et al 1987, Farber JM et al 1983). There is an uncertainty about the prognosis of cancer, and social isolation along with physical symptoms or functional losses resulting from the disease or its treatment are the most important factors. Due to these various difficulties (Spiegel D et al, 1995, Fox B .H et al 1995) many patients believe that stress, including that which is caused by their cancer experience, may contribute to poor coping as well as recurrence or progression of their disease. In the last decade there is a growing interest amongst the cancer survivors to use various complementary therapies adjuvant to the conventional treatment in the anticipation of reducing the burden of stress and better coping to the treatment. (Holmes M.D et al 2006, Cassileth B.R et al 1998) There is a considerable use of these therapies in recent times in approach to cancer treatment; therefore there is a need to understand the links between social, psychological, and physiological determinants of health (Brawley L .R et

al 2002). Yoga is an ancient eastern practice which has been used for therapeutic benefits world wide and is being scientifically studied by many clinicians (Gimbel M .A et al 1998) It has been suggested that 'gentler' physical activities, such as yoga or tai chi, may help to promote regular participation, especially in chronic disease populations who face additional barriers to engaging in an active lifestyle (Johnson NA et al 1998, Brawley L .R et al 2002). There have been a number of studies including randomized trials which reported positive therapeutic outcomes following Yoga program including our group (S Telles and Nagendra et al et al 1998). There was also a wide range of benefits reported earlier such as in asthma(Nagarathna and Nagendra et al 1985), increase in immune function(Henderson L.E et al 1989,Solberg E .E et al 1995,Sainani G.S et al 2003),hypertension (Walton K.G et al 1995-Schneider R.H. et al 1995)improvement in cardiovascular effects(Johnson NA et al 1998,Raub J A et al 2002,Jayasinghe S.R et al 2004) ,decrease in blood pressure (Wenneberg S R et al 1997,Sudsuang R et al 1991),diabetes(Sahay B.K, et al 2002),and serum Cortisol levels(Sudsuang R et al 1991)The use of CAM as an adjuvant therapy in breast cancer patients have attracted the attention of many researchers world wide (Holmes M.D et al 2006). Burstein et al. (1999), reported that newly diagnosed early-stage breast cancer patients had stressful mental health 3 months after diagnosis in

women who began using CAM. Meditation was basically used as a religious or spiritual practices, now it has been accepted world wide as a very effective tool to calm down the mind and harmonize the physiological and psychological parameters to have a balanced effect(2). Meditation based relaxation program have been implemented in a number of randomized and pilot studies particularly by Carlson L. E, et al(2001,2004,2003) and they reported to have stress reduction effectively, reduced total mood disturbance and specific symptoms of Anxiety, Depression, Anger, and Confusion. In all these studies mentioned above the main aim was to improve the quality of life of either the breast cancer survivors or those who were undergoing treatment. There have been reports of improvement of quality of life (QOL) in breast cancer patients who under went Yoga based programs or supportive counselling along with relaxation and imageries. (Rosenbaum E, et al 2004, Casso D, et al 2004). Inspired by favourable out comes of these interventional studies Carson J W,et al 2007 recently reported significant improvement in pain as well as psychological parameters of metastatic breast cancer patients. Recently there is a report where there is no physical improvement of breast cancer survivors over control patients after yoga intervention but there was a significant improvement in the global quality of life scores and mood disturbance scores (Culos-Reed S.N et al 2006). In our recent study

Raghavendra et al 2007, reported that yoga program has significant improvement in the chemotherapy induced nausea and emesis and the breast cancer survivors had significant improvement in the quality of life. The current study aims to study the effect of an intensive and integrated yoga program which is customized for the breast cancer patients in modulating the psychological and physiological stress. It is known that radiation causes DNA damage to the peripheral blood lymphocytes (PBLs) of the patients undergoing radiotherapy treatment (Scott D et al 1998, Hossein Mozdarani et al 2005). We also reported, Banerjee et al (2007)**,** significant radiation induced DNA damage in breast cancer patients undergoing radiotherapy**.** There was also a study in which DNA damage in the form of telomere shortening was linked to increased stress in the population of care givers by Blackburn et al, 2004**).** Our group (Banerjee et al 2007) also reported a significant increased in telomere associated DNA damage in breast cancer patients after radiotherapy. DNA repair capacity is also associated with psychological and physiological stress (Kiecolt-Glaser et al 1985 Cohen L et al 2000, Glaser R et al 1985). Therefore the fact that breast cancer patients are under stress and they also undergo considerable radiation induced DNA damage, we set to investigate in the present study the effect of an intensive yoga program on the Psychological parameters (HADs and PSS) as well as the radiation

induced DNA damage in the PBLs derived from the breast cancer patients pre and post radiotherapy in both the intervention and supportive counselling group.

CHAPTER 4

OBJECTIVE AIMS AND SCOPE OF STUDY

4.0. Objective, Aims and the Scope of study:-

4.1. OBJECTIVE: - The study is designed to evaluate the effect of Yoga intervention over a certain period of time in modifying the genotoxic stress induced by radiation treatment and whether there is a significant link between Yoga intervention and psychological stress when compared to the control subjects.

4.2. AIMS

Study-I: - The first aim of the study is to find out the background radiation induced DNA damage and genomic instability in the breast cancer patients undergoing radiotherapy using molecular cytogenetic techniques.

Study-II: The second aim of the study is to conduct a randomized control trial to study the effect of yoga intervention in modulating the genotoxic and psychological stress levels in breast cancer patients undergoing radiotherapy.

4.2.1. HYPOTHESIS: - Integrated Yoga Intervention can modulate anxiety, depression, perceived stress and radiation induced DNA damage in Breast cancer patients undergoing radiotherapy.

4.2.2. Null Hypothesis: - Integrated Yoga program do not influence the out come measures of anxiety, depression and perceived stress in Breast cancer patients undergoing radiotherapy.

4.3. Scope of the study: - The current research study does not aim to find the molecular genetic basis of stress reduction and modulation of DNA damage. The molecular genetic interaction is beyond the scope of the study. The goal is to find out whether Stress and response to DNA damage are associated with yoga.

CHAPTER 5

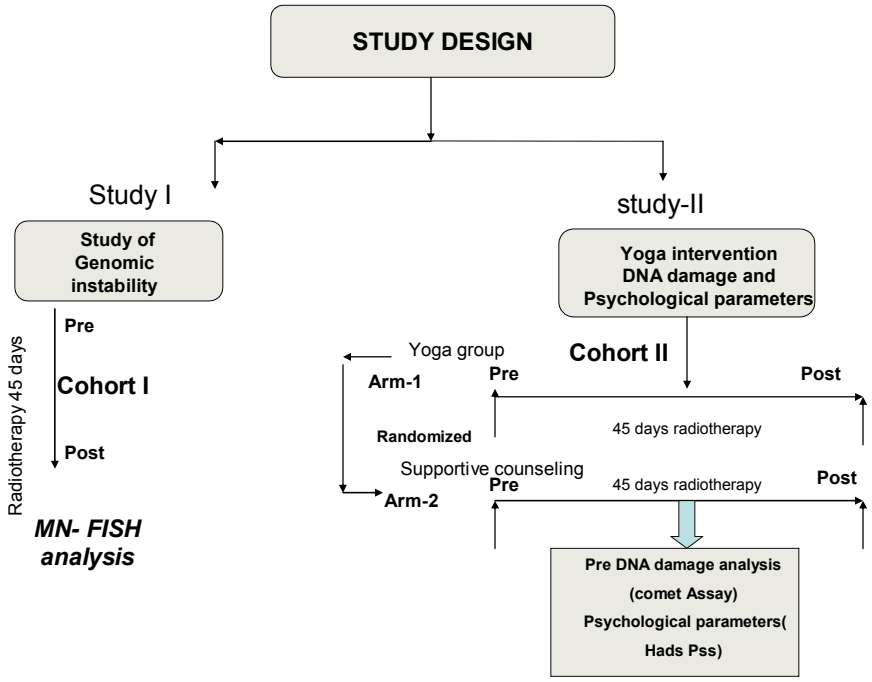
METHODS

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 *Insitu* 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.

Figure 5.1. The overall research design of the whole study:-



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.
2. Metastatic Cancers.
3. Those in Zubrod's performance status of 4 or severely ill.
   [3 = Symptomatic, ambulatory < 50% of the time. 4 = Bed ridden]
4. Major psychiatric/ neurological and autoimmune diseases.

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 CO_2 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 NucleicAcid 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 Axoiplan 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.

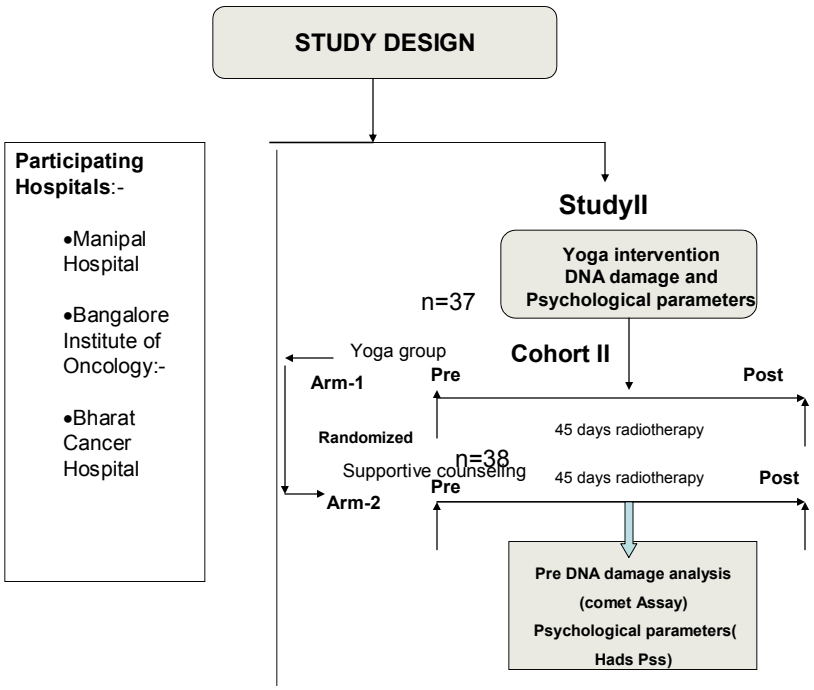
5.10. Overview of the Study -II:-

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.
6. Metastatic Cancers.
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 al 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> (Yujyate anena iti yogaù). Yoga is that

which joins. In the traditional terminology it is joining of jIvaTma (jévätmä) with prmaTma (paramätmä) 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 ***REALITY.***

Patanjali Yoga is one among the six systems of Indian philosophy known as 'Ñat darçanas'. One of the great Åñis [Seer], Patanjali compiled the essential features and principles of Yoga in the form of *'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 buiÏ [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 yaeg> icÄ **v &**iÄ inraex> 'Yogaù Citta Våtti Nirodhah' (Yoga Süträ: 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 Drañtuù 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> [Upäyaù], 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 mid. 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' (Géta 2.48). Instruments of action and understanding (karmendriyäs and jnanendriyäs) 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 **(Räja Yoga)** gives a practical and easy approach to reach higher states of consciousness. It is based on the Añtänga Yoga of Patanjali's Yoga system.

<u>Añtänga Yoga</u>

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

1. ym - Yama (the disciplines, 'DONT'S': Niçedhas)
2. inym - Niyama (the injunctions, 'DO'S': Vidhis)
3. Aasn - Äsana (the posture of the body)
4. P+aa[ayam - Präëäyäma (the control of Präëa, the life force)
5. P+aTyahar - Pratyähära (restraint of senses from their objects of enjoyment)
6. Xar[a - Dhäraëa (focusing of mind)
7. Xyan - Dhyäna (deconcentration)
8. Samaix - Samädhi (super consciousness)

The first five limbs come under Bahiranga Yoga. In this the Bahirindriyäs are used for indirect control of mind. It includes;

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

b) **Jnänendriyäs:** 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 yogäsanas 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, Kapälabhäti, Agnisära, Vamana Dhouti (Kunjal kriya), etc are used extensively.

Physical exercises and Movements - Çithilékaraëa Vyäyäma: 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.

Yogäsanas – Postures: Yogäsanas 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 Pränamaya Koça (The layer of Präëa)

Präëa is the basic life principle. **Präëäyäma** is a process for gaining control over Präëa. The five manifestations of Präëa and the corresponding most comprehensive definition of Präëäyäma in the human system are

described in praçnopaniñat. Also the conventional Präëäyäma through regulation of breath is described therein.

Through the practice of proper breathing, kriyäs and präëäyäma, we start operating on the Pränamaya Koça. Suitable types of präëäyäma and breathing help to remove the random agitations in pränic flows in the Pränamaya Koça. Thus, the ailments are handled at this Pränamaya Koça level.

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

Dhäraëa and Dhayäna: A direct operation on this level is made possible by the last three limbs of Añtänga Yoga of Patanjali – Dhäraëa, Dhayäna, and Samädhi. The culturing of mind is accomplished by focussing of the mind (Dhäraëa) initially, followed by relaxed dwelling of the mind in a single thought (Dhayäna) for longer and longer durations leading ultimately to superconsciousness (Samädhi). A progressive habituation allows the mind to remain relaxed during the period of meditation (Dhayäna). 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, Nämavalis, 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 Vijnänamaya Koça (The layer of wisdom)

A basic understanding is the key to operate from vijnänamaya koça. **Upaniñads** are the treasury of such knowledge which is the redeemer of all miseries and obsessions. It is the lack of that inner jnäna which is responsible for many wrong habits, agitations, etc. The Happiness Analysis - Änanda Mimämsa 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 präëa and mind emerge – the Änandamaya 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 *Ś*avāsana with legs aparts, hands away from the body, head and neck in a very6 convenient position. The whole body is completely collapsed on the ground.
- Let us start the session with the prayer 'Mrtyunjaya Mantra.

Om Trayambakam yajamahe

Sugandhim Pustivaradhanam

Urvarukakamivabndhanat

Mrtyormuksiya 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 vibrationsand 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 sesitivity. 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 quieten 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 Koça (The layer of bliss)

To bring the bliss of our causal body (Käraëa Çaréra) called Änandamaya Koça 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 *Praṇayama (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 propiduim 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. PerceivedStress 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.

CHAPTER 6

RESULTS

6.1. Results of Aim I:-Radiotherapy induced telomere damage patterns:-

6.1.1. Pre Radiotherapy MN

We measured the MN frequency in 55 breast cancer patients undergoing partial-body irradiation. We mainly evaluated the relationship between total MN yield and the percentage of MN with telomere damage before and after radiation therapy in the patients. The MN baseline yield for the 55 patients before radiotherapy. The mean MN frequency in the PBLs from breast cancer patients was 22.6 ± 3.21 (Mean ± S.D) and ranged from 12.5 - 30.2. The post-radiotherapy MN frequency was analysed after a cumulative dose of 50.4 Gy which was administered at a fractionated dose of 1.8 Gy per day of external beam radiotherapy from a ^{60}Co γ radiation source. There is a high degree of damage *in vivo* in the lymphocytes of breast carcinoma patients post-irradiation. The post-radiotherapy MN frequency increased to 283.1 ± 23 (Mean ± SD; Fig 2).and ranged from 230-350. Though the distribution of micronuclei produced after radiotherapy was heterogeneous, the data clearly indicate the higher damage produced by fractionated irradiation.

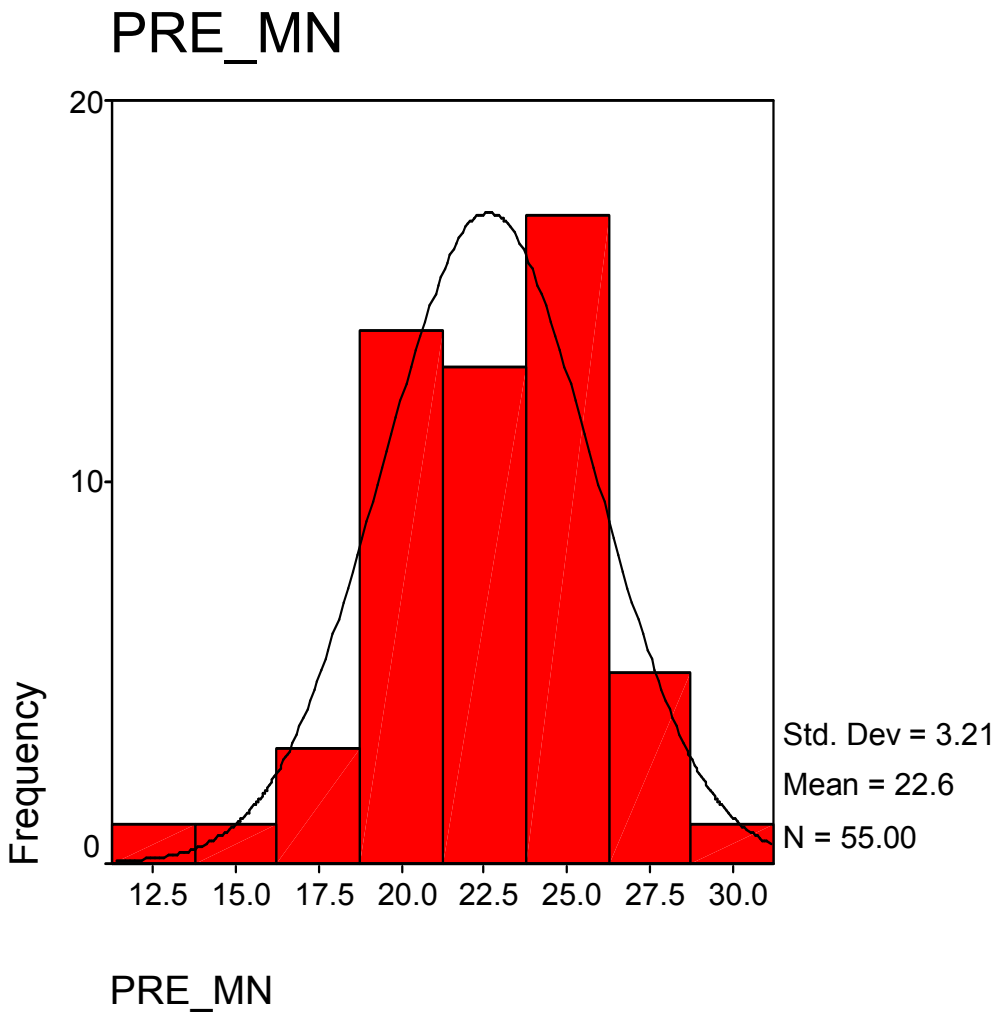


Figure. 6.1 showing the distribution of Micronuclei frequency in the Ca –Breast patients pre-Radiation therapy .The MN frequency shows the baseline damage of the Go lymphocyte DNA Pre -radiation treatment.

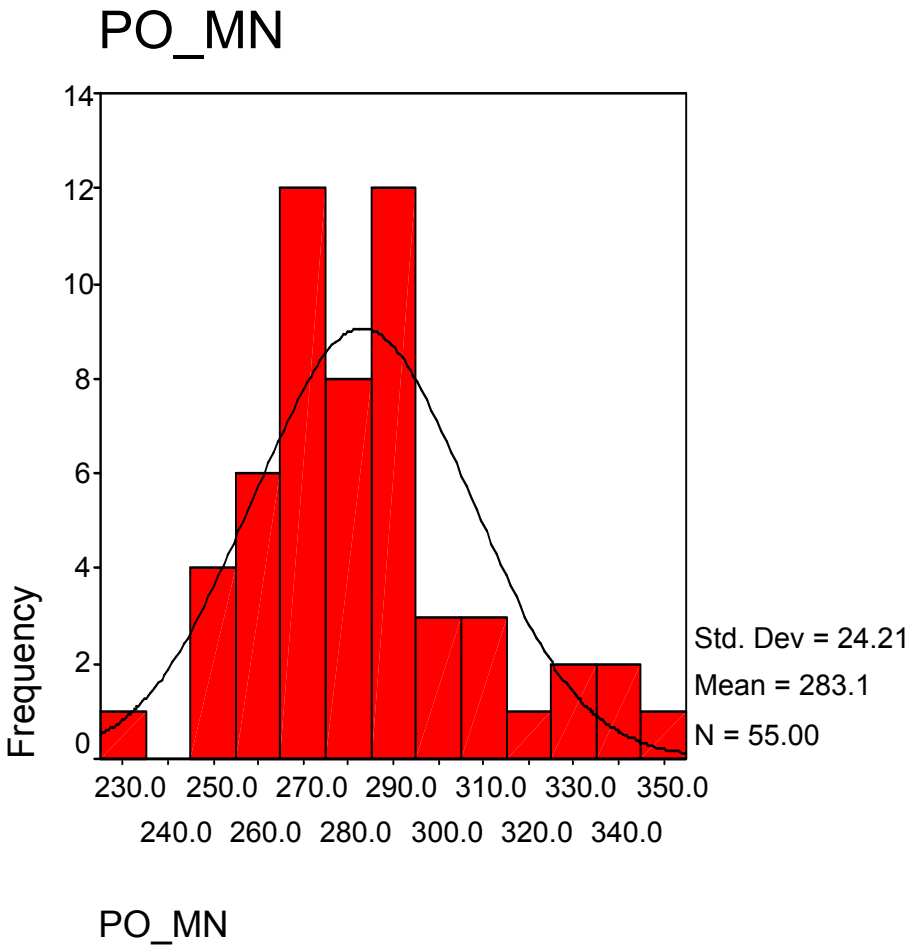


Figure 6.2: Frequency distribution showing the micronuclei in the Ca –Breast patients post radiation therapy. There is very high degree of damage in the G_0 lymphocytes of Carcinoma Breast patients *in vivo* after a cumulative dose of 50.4 Gy at the rate of 1.8 Gy per day of external beam radiotherapy from a ^{60}Co source. (Mean 253.1 SD 24.21)

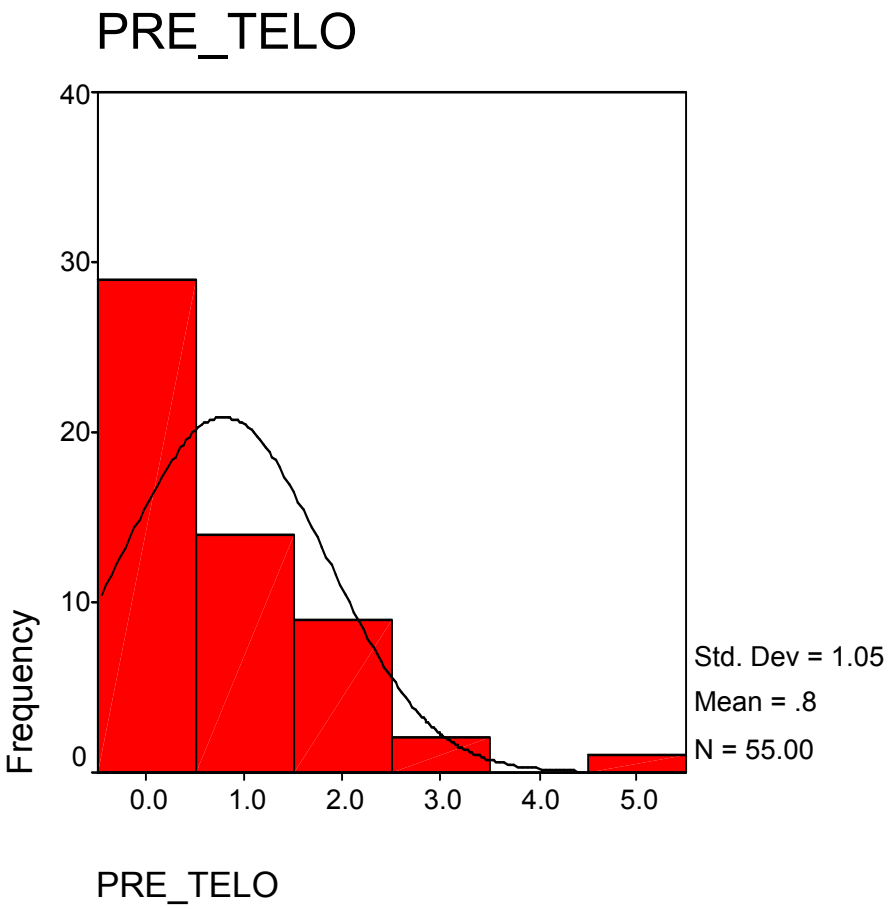
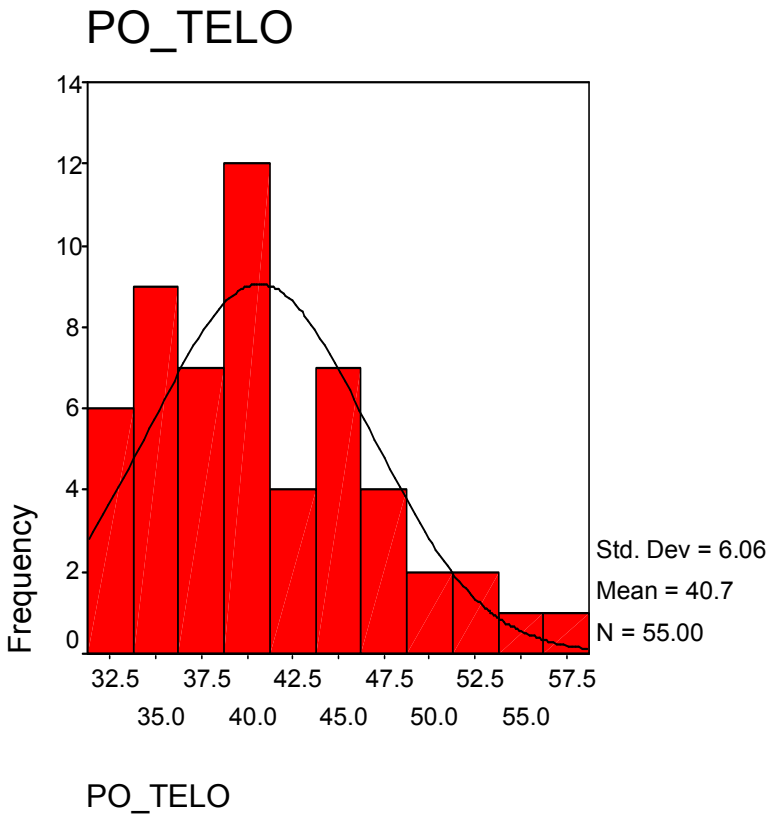


Figure 6.3 Distribution showing the telomere damage frequency in the micronuclei of the patients before radiotherapy. There is not much of telomeric damage in the Micronuclei population of maximum patients Most of them had zero Telomere signals in the MN.

Figure 6.4. Distribution showing the Telomere damage frequency in the Ca –Breast patients post radiation therapy There is a significant (p>.001) telomere damage frequency in the micronuclei population and the damage pattern is normally distributed with a mean of 40.7.



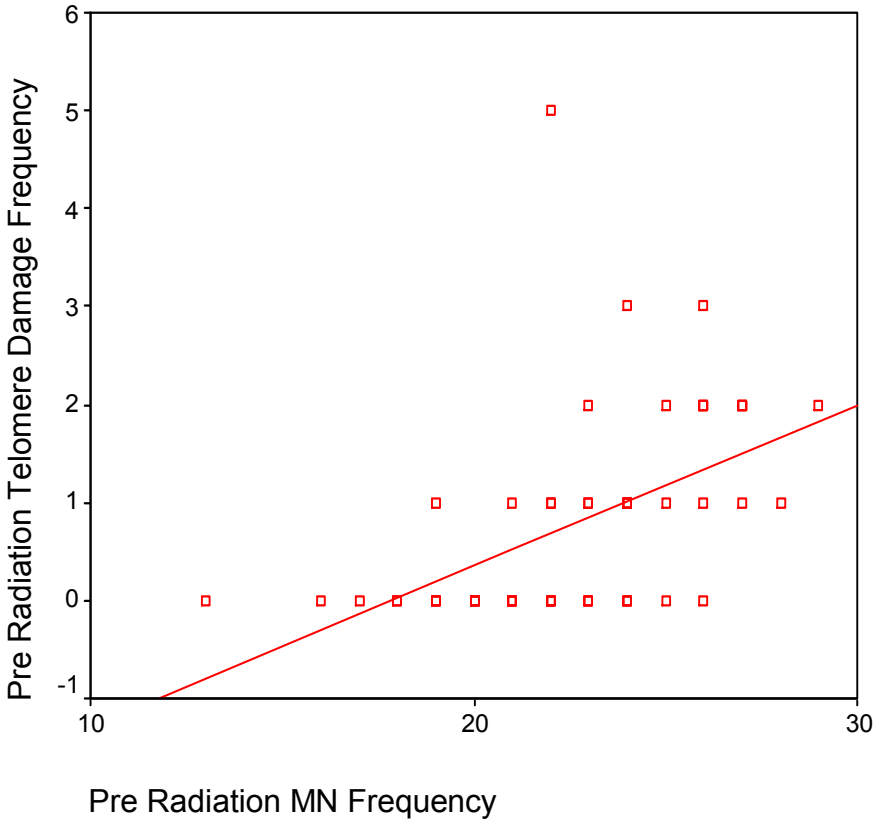


Figure 6.5. The correlation showing the mean frequency of Micronuclei in pre-radiotherapy and the Telomere damage frequency. There is no considerable co relation ship of Telomere damage frequency and the Micronuclei

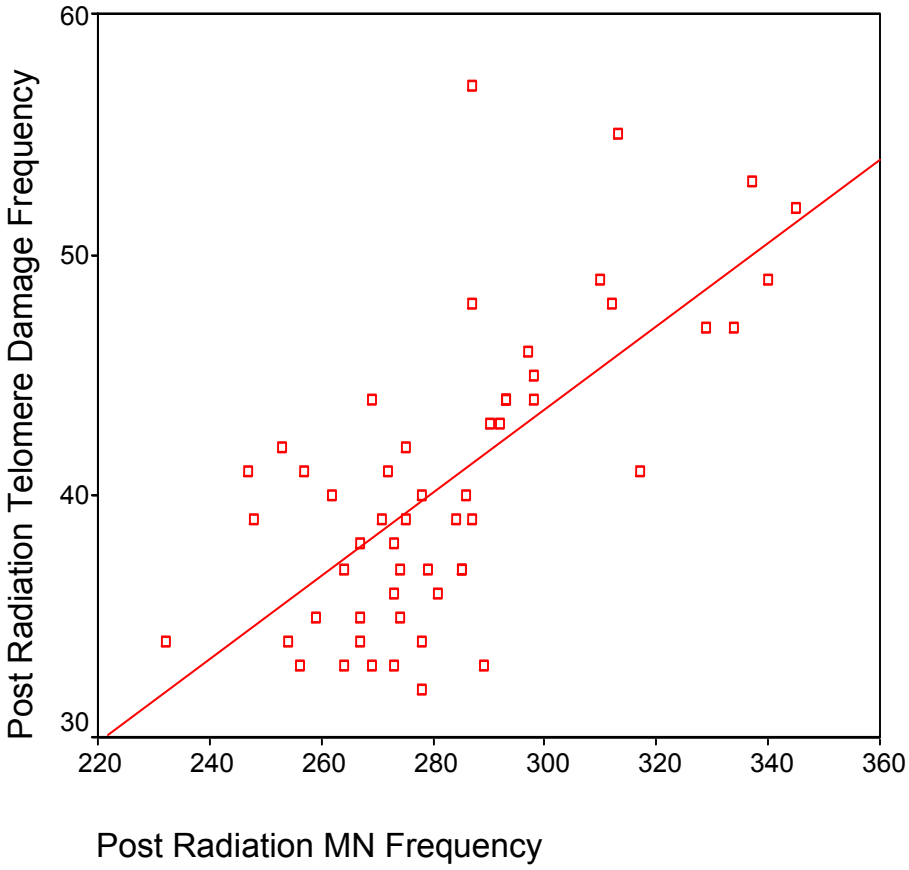


Figure 6.6 The correlation showing the mean frequency of Micronuclei post radiotherapy and the Telomere damage frequency .The telomere damage association with the micronuclei frequency is highly significant(p>.001).

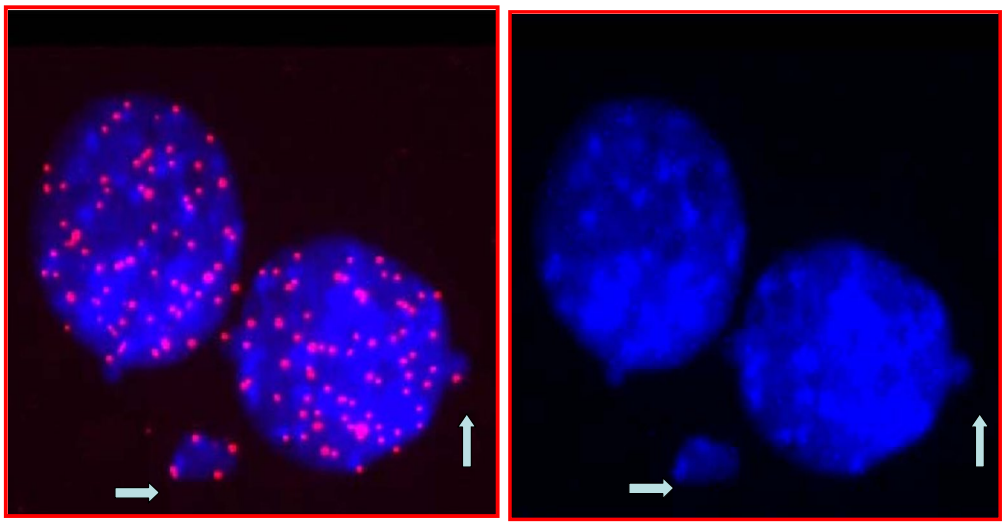


Figure 3, Banerjee et al.

Figure 6.7. Cytokinesis-blocked binucleated lymphocyte of breast cancer patients after radiotherapy. FISH with telomere specific peptide nucleic acid (PNA) probes were used to determine the presence of telomere signals in the micronuclei as pointed by arrow.

Slides with CB-MN were subjected to *FISH* using telomere specific PNA probes. A representative image is displayed in Fig. 3 showing a typical binucleated cell with a micronucleus. Telomere signals could be visible in the micronucleus (Fig. 3). Telomere distribution patterns for patients before and after radiotherapy in Figs.6. 4 and 6.5 respectively. FISH analysis revealed that there is not much telomere damage in most of the patients before radiotherapy (Fig. 6.4). This was evident by the presence of very low number of telomere signals in the MN. However, the data obtained from the patients after radiotherapy showed significant telomere damage (Fig 6.5). Many of the MN displayed telomeres signals (Mean 40.71 ± 6.06). The difference is statistically significant ($p<0.001$).

We then compared the mean frequency of micronuclei with the micronuclei with telomere damage to determine whether or not there is a correlation between micronuclei production and telomere dysfunction. The data for patients before radiation therapy is presented in Fig.6.5. As is displayed, there is not much correlation ($r^2 = 0.45$) between telomere damage and total MN production in pre-radiotherapy patients. However, in post-radiotherapy patients, the relationship between MN frequency and MN with telomere damage is significantly higher ($r^2 = 0.68$; >95 % confidence limit) indicating the fact that patients following radiotherapy suffered significant telomere damage.

Figure 6.8. - Trial Profile

Results of Study II (Primary and secondary outcome measures:-

Trial Profile:-

Cohort-II

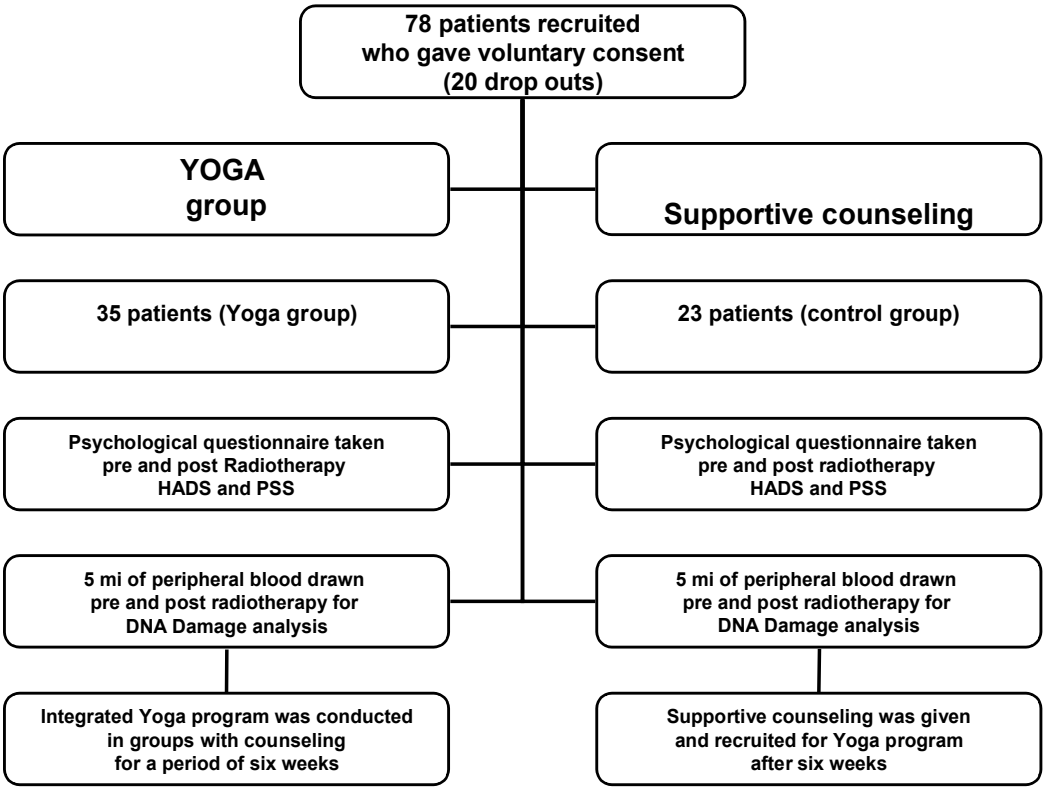
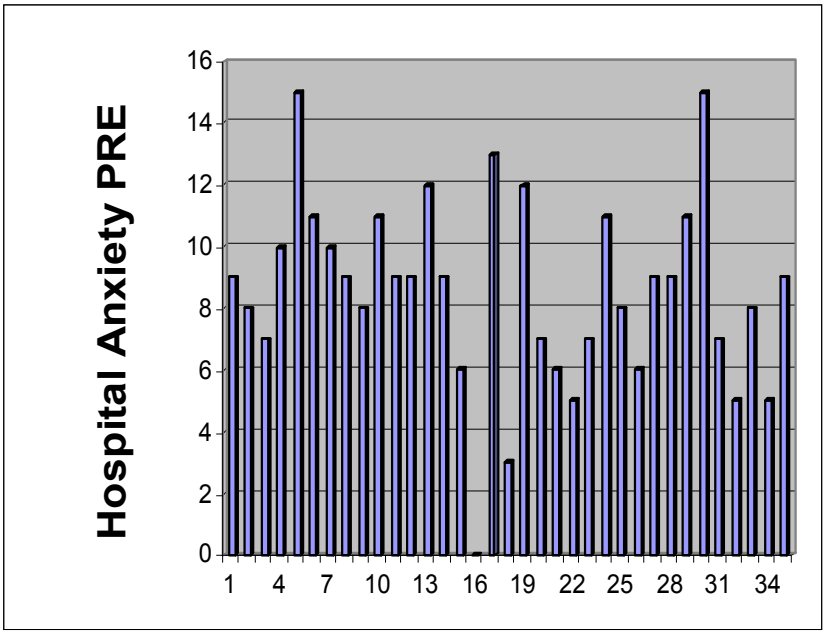


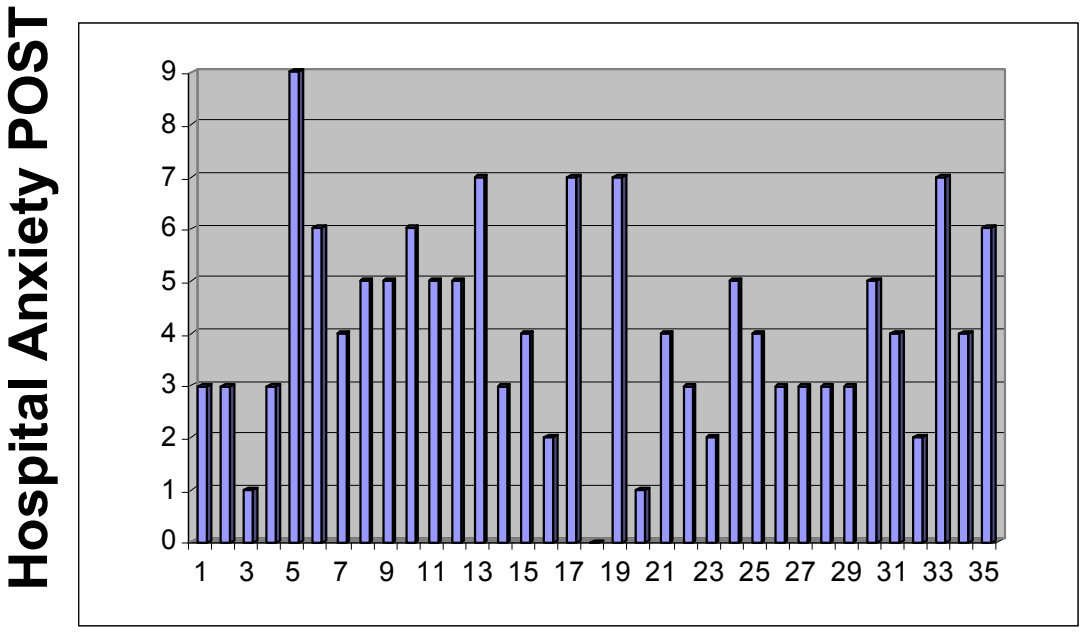
Figure 6.9. Demographic characteristics of the Cohort-II

Demographic Particulars		All Subjects Age Mean=44yrs (SD=1.3)		Yoga group Age Mean=47yrs (SD=1.1)		Control Group Age Mean=43yrs (SD=1.5)	
Radiation dose 50.4 Gy(28 cycles)		N=58	(%)	N=35	(%)	N=23	(%)
Cycles of Chemotherapy	Nil	30	52	14	40	16	70
	I	5	9	3	9	2	9
	II	16	28	12	34	4	17
	>II	7	11	6	17	1	4
Stage of Ca-Breast	II	26	45	16	46	10	43
	III	32	55	19	54	13	57
Grade of Ca-Breast	II	27	46	17	48	10	43
	III	31	54	18	52	12	57
Menopausal status	Pre	34	59	18	51	16	69
	Post	24	41	17	49	7	31
Stressful events in life	Yes	14	24	10	28	4	17
	No	44	76	25	72	19	83
Post Hysterectomy		5	9	3	8	2	9

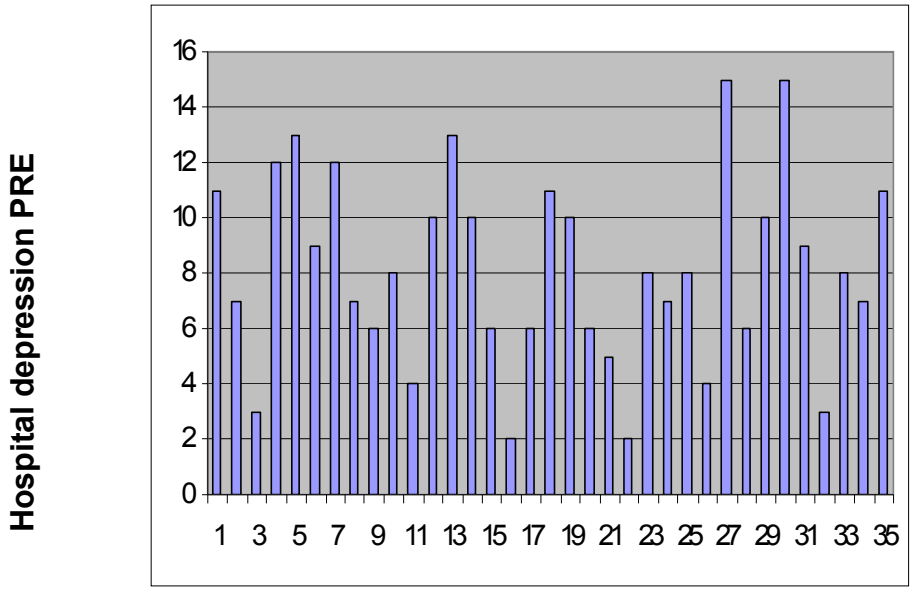
Graphs: 6.10 - Hospital anxiety Pre- Intervention in Yoga group (Raw- data)



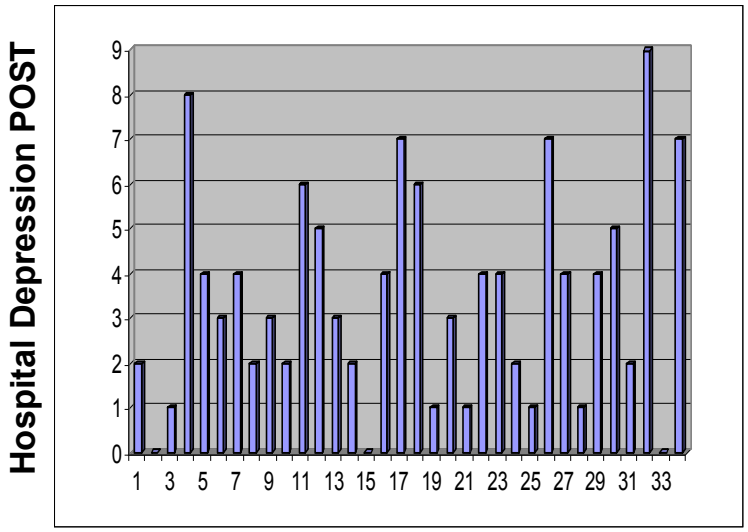
Graph -6.11. Hospital-anxiety Post- Intervention in Yoga group (Raw- data)



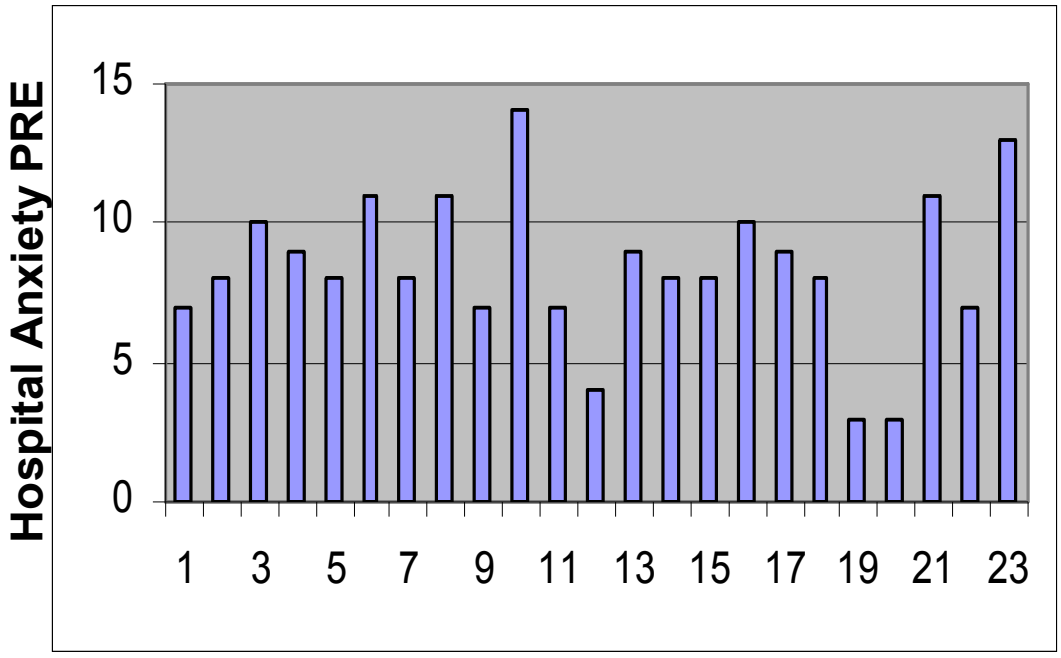
Graph -6.12. Hospital-Depression PRE- Intervention in Yoga group (Raw- data)



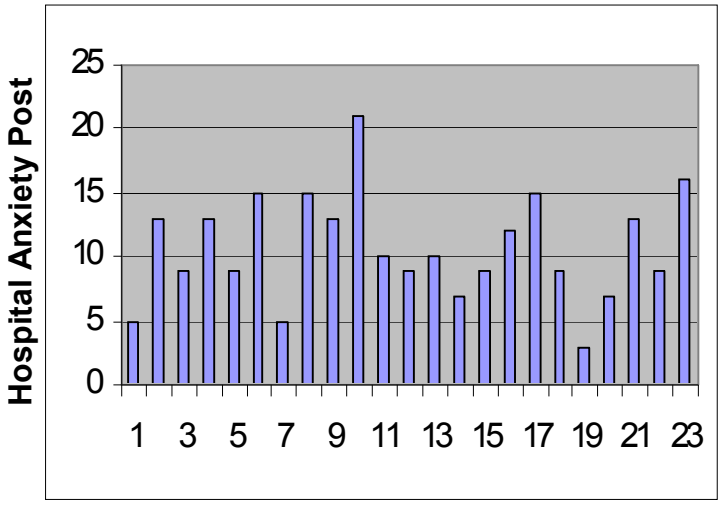
Graph -6.13. Hospital-Depression POST- Intervention in Yoga group (Raw- data)



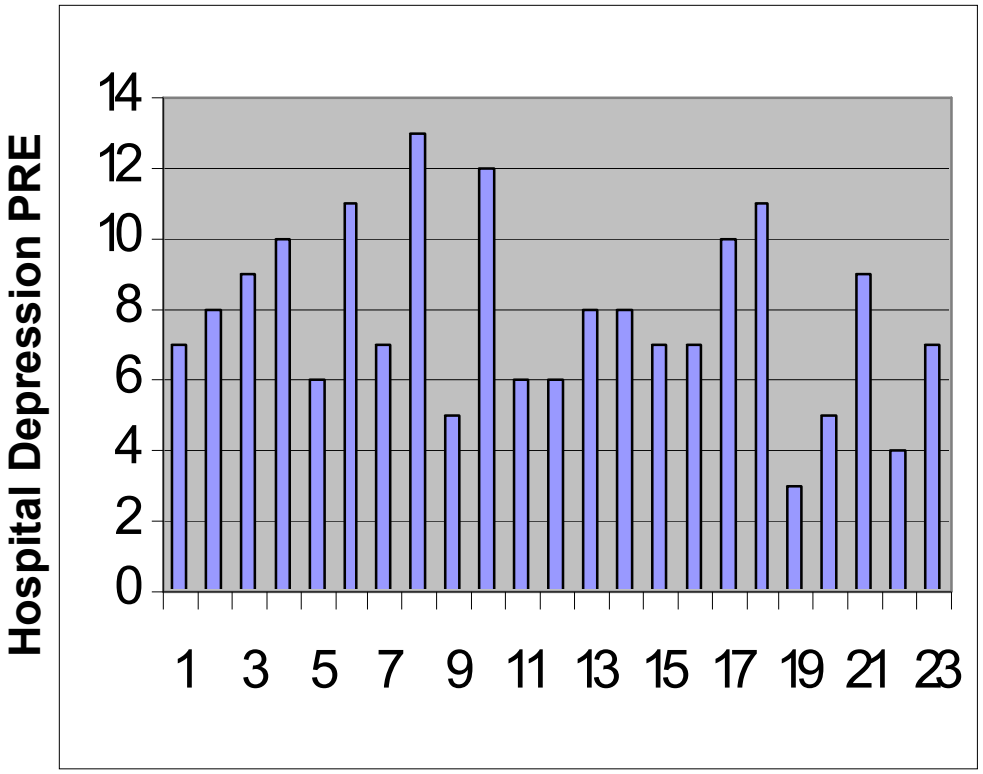
Graph -6.14. Hospital Anxiety Pre radiotherapy in Supportive counselling group. Raw data)



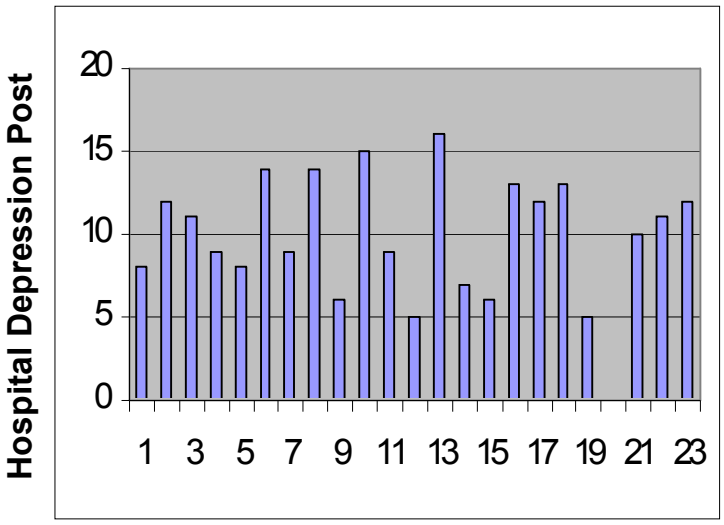
Graph -6.15. Hospital Anxiety Post radiotherapy in Supportive counseling group.(Raw data)



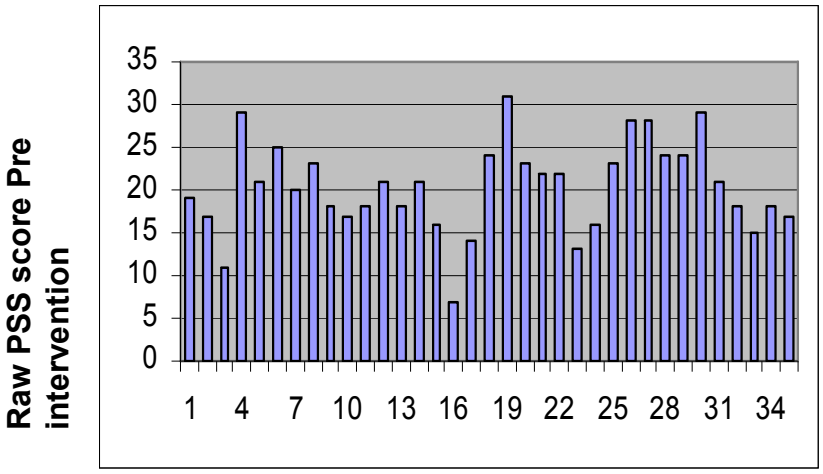
Graph 6.16. Hospital Depression pre- radiotherapy in Supportive counseling group.(Raw data)



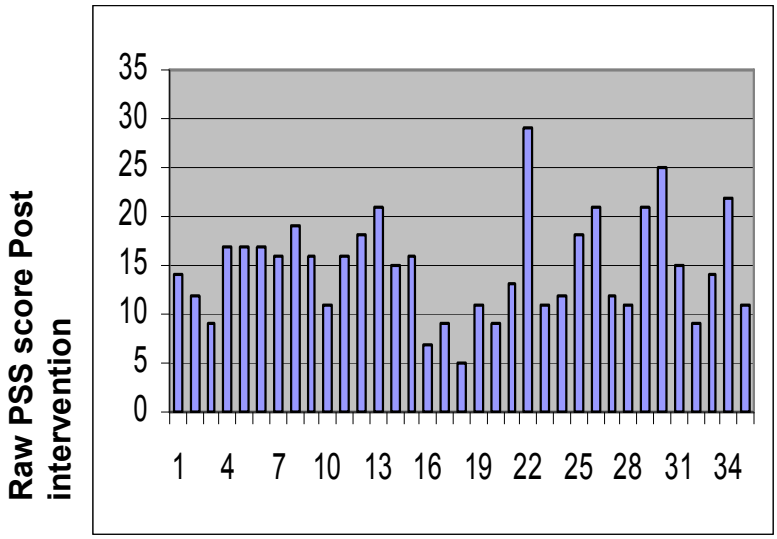
Graph 6.17. Hospital Depression pre- radiotherapy in Supportive counseling group.



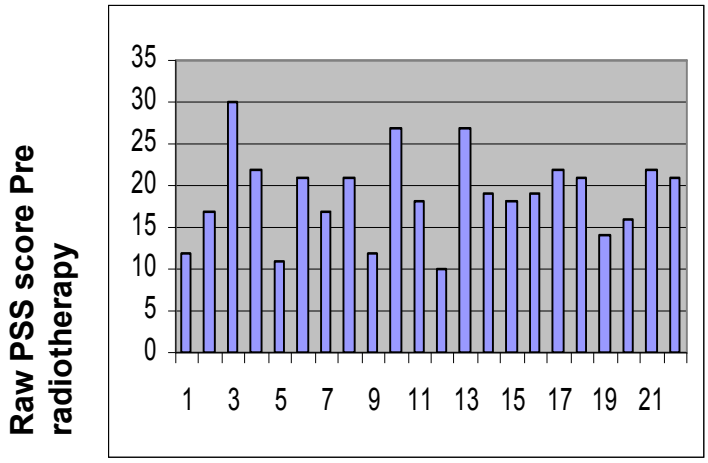
Graph 6.18.Perceived stress scale raw scores Pre-intervention (Yoga group)



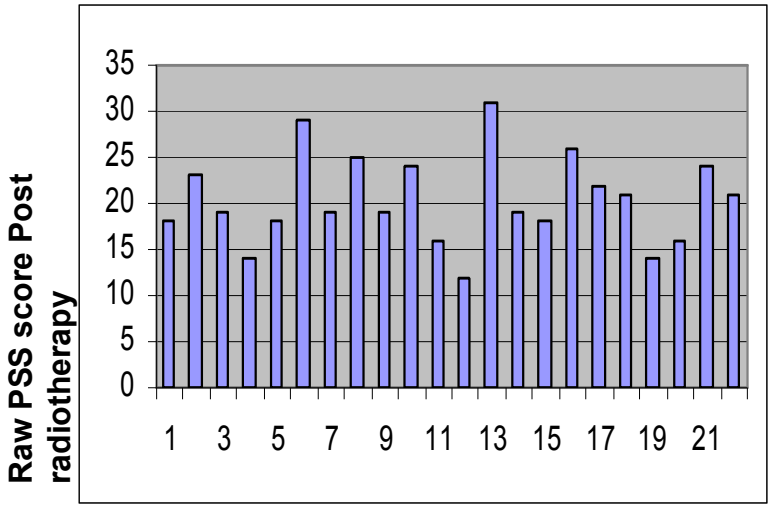
Graph 6.19.Perceived stress scale raw scores Post-intervention (Yoga group)



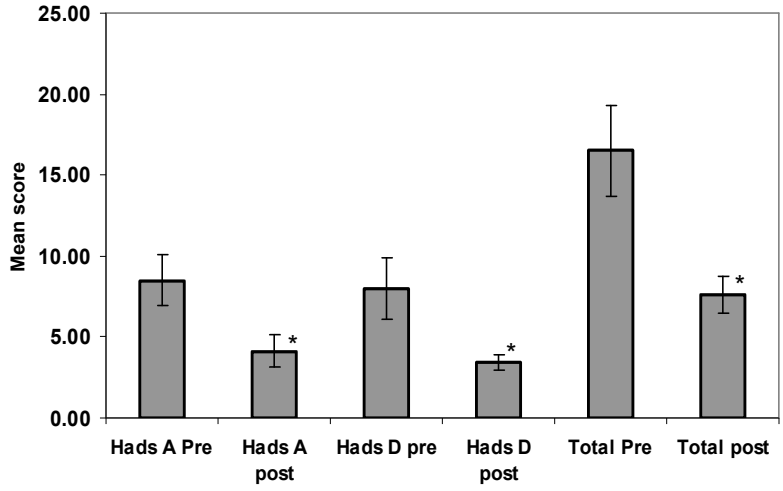
Graph 6.20.Perceived stress scale raw scores Pre radiotherapy (Control group).



Graph 6.21.Perceived stress scale raw scores Post radiotherapy (Control group).



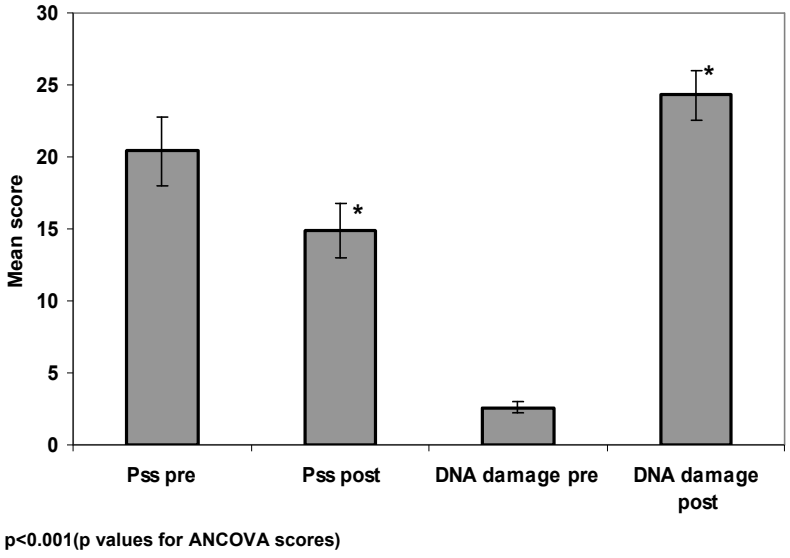
Graph 6.2.2



* p<0.001(p values for ANCOVA scores)

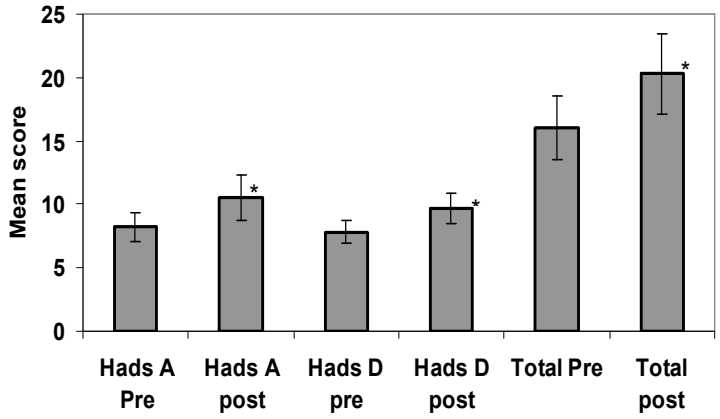
The graph shows the Mean Hospital anxiety and depression (HADs) scores of the yoga intervention group. The pre (baseline) data showed significant decrease after the intervention for a period of six weeks. The total HADS score also showed decrease from the baseline. ($p<0.001$ repeated measure ANCOVAs were performed, controlling for baseline values of each dependent variable

Graph 6.2.3.



The graph shows the Mean Perceived stress scores and Mean DNA damage frequency of the yoga intervention group. The pre (baseline) data of PSS showed significant decrease after the intervention for a period of six weeks. The DNA damage showed significant increase after the radiation treatment. ($p<0.001$ repeated measure ANCOVAs were performed, controlling for baseline values of each dependent variable.

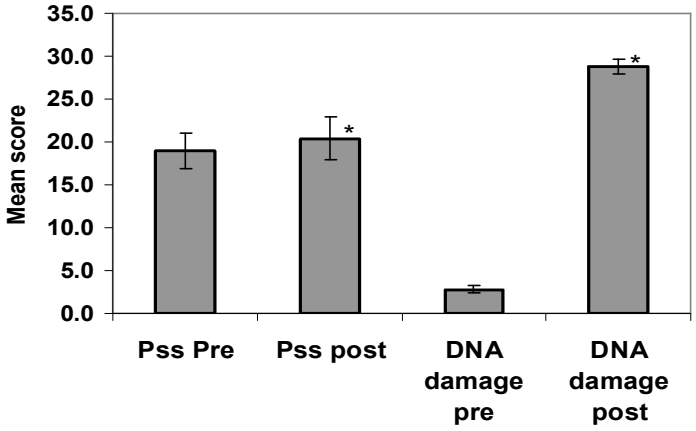
Graph 6.2.4



* p<0.001(p values for ANCOVA scores)

The graph shows the Mean Hospital anxiety and depression (HADs) scores of the **Control group**. The pre (baseline) data showed significant increase after the intervention for a period of six weeks. The total HADS score also showed increase from the baseline. ($p<0.001$ repeated measure ANCOVAs were performed, controlling for baseline values of each dependent

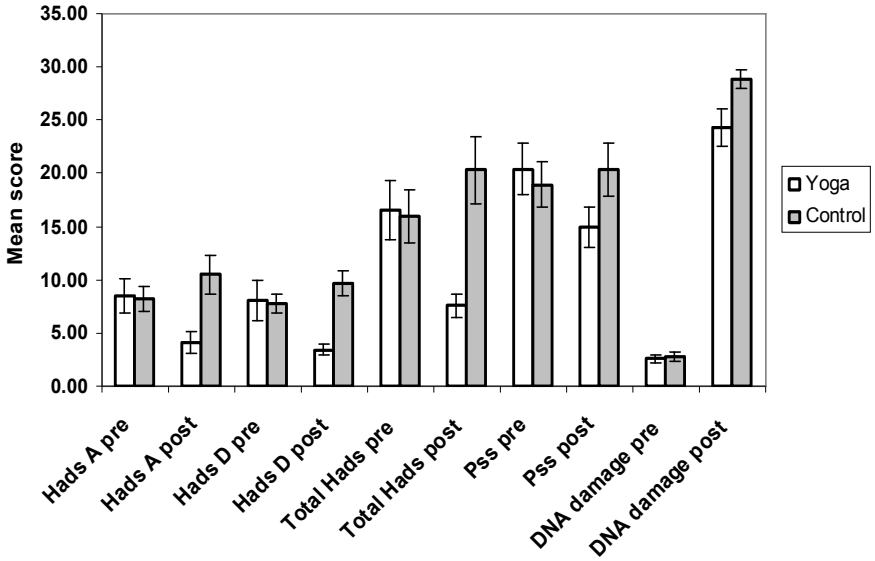
Graph 6.2.5



* p<0.001(p values for ANCOVA scores)

The graph shows the Mean Perceived stress scores and Mean DNA damage frequency of the **control group**. The pre (baseline) data of PSS showed significant increase after the intervention for a period of six weeks. The DNA damage showed significant increase after the radiation treatment. ($p<0.001$ repeated measure ANCOVAs were performed, controlling for baseline values of each dependent variable

Graph 6.2.6



The graph shows the comparative HADS, PSS and DNA damage frequency scores of the **Yoga intervention and the control group.** In comparison to the control group the yoga group showed significant decrease in the anxiety, depression and the Perceived stress sores when compared to the base line before treatment. Although both the groups showed increased DNA damage, but the yoga group showed significant lesser damage compared to the control.

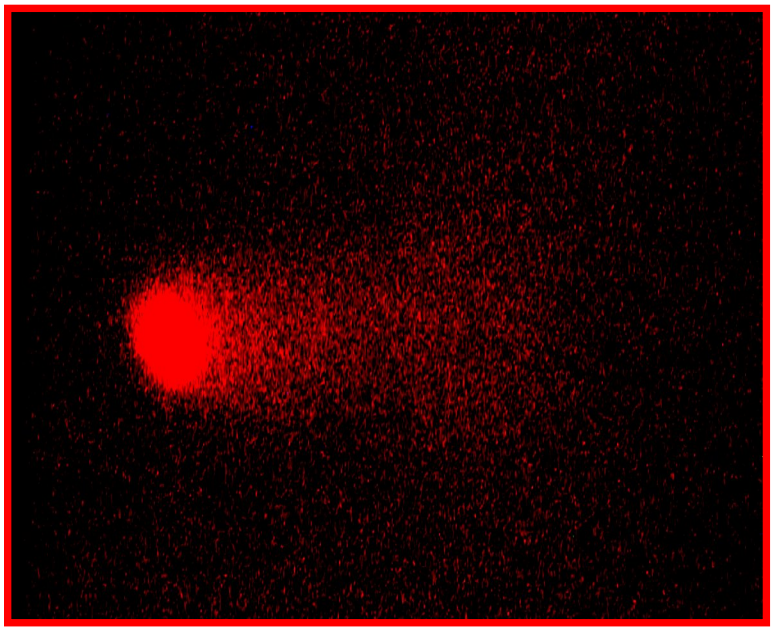
Table 6.2.7

Groups	HADS-A		HADS-D		PSS		DNA Damage	
	Pre	post	Pre	post	Pre	post	Pre	post
Yoga, n= 35 Mean	8.5	4.1*	8.0	3.4*	20.4	14.9*	2.6	24.3*
SD	1.6	1.0	1.9	0.5	2.8	2.4	0.4	1.70
Control, n=23 Mean	8.2	10.5*	7.8	9.7*	19.0	20.4*	2.8	28.8*
SD	1.1	1.8	0.9	1.2	2.1	2.5	0.4	0.9

*** p<0.001(p values for ANCOVA scores)**

The table shows the comparative mean scores (HADs, PSS, DNA damage) of the yoga and the control groups with their respective standard deviation.

Figure 6.2.8



It is a representative image of a comet from a post radiotherapy treated leukocyte nuclei of a patient stained with propidium iodide dye and it shows considerable tailing

6.3 Data Analysis

6.3.1. DNA Damage

The DNA damage due to radiation was significantly high in both the Yoga and control group after radiotherapy But the post radiotherapy DNA damage was slightly lesser 14.5% Mean=24.3(SD =1.7) when compared to the control group Mean=28.8 (SD =0.9).$p<0.001$ repeated measure ANCOVAs were performed, controlling for baseline values of each dependent variable

The baseline DNA damage being Mean=2.6 (SD =0.4) and Mean=2.8 (SD =0.4) respectively and they have significant correlation (Pearson's correlation coefficient=0.97)

6.3.2. Hospital anxiety and Depression scale

The was a significant decrease in the anxiety levels in Yoga intervention group from Mean=8.5 (SD =1.6) (baseline) to Mean=4.1 (SD =1.0) (48.2%) (Figure 2, Table 2) after the 6 weeks of Yoga program, where as in the control group the Mean anxiety score increased from Mean= 8.2 (SD =1.1) to 10.5 (SD =1.8) (28%) (Figure 4) $p<0.001$ repeated measure ANCOVAs were performed, controlling for baseline values of each dependent variable

The post depression score for the intervention group decreased from Mean= 8.0(SD =1.9) (baseline) to Mean= 3.4(SD =0.5) (57.5%) (Figure 2, Table 2) where as the in the control group the score increased from 7.8(SD =0.9) (baseline) to 9.7(SD =1.2) (24%). (Figure 4, Table 2) $p<0.001$ repeated measure ANCOVAs were performed, controlling for baseline values of each dependent variable

6.3.3. Perceived Stress Scale

In the Yoga group (Figure 3, Table 2) the mean perceived stress score (PSS) decreased from Mean= 20.4(SD =2.8) (baseline) to14.9 (SD =2.4) (26.9%) where as the control group(Figure 5, Table 2) showed no change in pre and post radiotherapy Mean= 19.0(SD =2.1) (baseline) and Mean=20.4(SD =2.5).

CHAPTER 7

DISCUSSION

7.1. Discussion study-1

Radiotherapy is an important therapeutic modality in clinical cancer management. Lately with advent of better machines and innovative technology, individualisation of cancer radiotherapy is gaining greater grounds. There have been number of studies done earlier to prove the radio sensitivity of different individuals undergoing radiotherapy. Fibroblasts are the most commonly used *in vitro* experimental model for studying the radio sensitivity of normal tissue. Johansen *et al* observed a significant correlation between the surviving fractions of fibroblasts after 3.5 Gy and subcutaneous fibrosis in breast cancer patients.

The micronucleus (MN) assay is a sensitive tool to assess radiation induced cytogenetic damage, though there is a variation in the base line frequencies from one laboratory to others. After exposure to mutagenic agents, micronuclei in the cells are derived either from acentric fragments or lagging chromosomes. Micronuclei in cytokinesis-blocked peripheral blood lymphocytes (PBLs) are one of the most reliable biomarkers (indicators) in assessing the chromosome damage induced by ionising radiation or exposure to chemicals. Oppitz *et al.* have shown significant correlations between *in vitro* MN frequency and radio-sensitivity.

The MN analysis has been proved to be an effective tool to quantify radiation damage in both exposed population and also the radio sensitivity of various individuals. In a recent work, Mozdarani 2005 et al. demonstrated that there is an elevated spontaneous frequency of MN in breast cancer group compared to the control group. They also showed that Ca-Breast patients were more sensitive (30%) to ionizing radiation than the age- and sex-matched controls. Scott et al. showed that there is indeed a significant correlation between carcinoma of the breast and increased chromosomal radio-sensitivity. Scott et al. proved that in ataxia telangiectasia patients there is an elevated radio-sensitivity observed in lymphocytes. It is observed in our study that the MN frequencies in carcinoma breast patients had a significant correlation with telomeric damage after radiotherapy. Short telomeres or dysfunctional telomeres may contribute to elevated radiation sensitivity or carcinogen sensitivity (*Newman, Banerjee, Hande, 2007*). The telomeres play crucial role in detection and repair of DNA damage and radiation insult. The presence of telomere signals in micronuclei might have been the result of telomere breakage and/or dysfunctional telomeres in the lymphocytes of breast cancer patients. There was an attempt made by Acar et al, 2001) to find the chromosomal origin in FISH on MN in acute lymphoblastoid leukaemia patients but they did not report telomere damage pattern. In

another work Norppa et al. tried to find the contents of human micronuclei and reported telomeric signals in some MN population. Based on previous reports and our data, we hypothesise that in CA-breast there is a considerable amount of genomic instability in the lymphocytes with short telomeres. It is also possible that there is abnormal telomere maintenance in a sub- population of lymphocytes which makes them more radiosensitive. Desmaze et al 2004) reported that initially telomere dysfunction and genomic instability contribute to radiation susceptibility. Slijepcevic et al 1998) indicated that interstitial breakpoints in chromosome contain telomeric signal. It is also suggested that telomere maintenance play crucial role in radiation susceptibility and radio-resistance. Though we have not studied the fate of these micronuclei with telomere damage, it is tempting to speculate that such telomere loss could lead to chromosome end-to-end fusions or chromosome loss ultimately facilitating cells to undergo apoptosis.

MN analysis of 55 breast cancer patients following radiotherapy demonstrates heterogeneity in the response to radiation among these individuals. This indicates varied radio sensitivity within this population. We speculate that individual response to radiation may differ among the breast cancer patients. This observation highlights the fact that it would be

important to know the radio sensitivity of individual patient while administering the radiotherapy to breast cancer patients. Our data also suggest that telomere damage pattern in micronuclei as detected by *FISH* might indicate the individual radio sensitivity and give a brief idea of genome stability status. It might also give an indication of radio-resistance in stage-variant cancer cells. The varied radio sensitivity of the breast cancer patients and the link between telomere damage and radiation sensitivity provides a frame work for further research that may have an impact in radio-therapeutic strategies in cancer.

Discussion study II:-

Background genomic instability and damage due to radiotherapy: - Based on previous reports and our data, we hypothesise that in CA-breast there is a considerable amount of genomic instability in the lymphocytes with short telomeres. It is also possible that there is abnormal telomere maintenance in a sub- population of lymphocytes which makes them more radiosensitive. Desmaze et al 2003) reported that initially telomere dysfunction and genomic instability contribute to radiation susceptibility. Slijepcevic et al 1998) indicated that interstitial breakpoints in chromosome contain telomeric signal.

MN analysis of 55 breast cancer patients following radiotherapy demonstrates heterogeneity in the response to radiation among these individuals. This indicates a varied radio-sensitivity within this population. We speculate that individual response radiation may differ among the breast cancer patients. This observation highlights the fact that it would be important to know the radio-sensitivity of individual patient while administering the radiotherapy to breast cancer patients

The results in our study suggest that the patients, who were recruited in the Yoga and supportive counselling group (control), both had significant degree of background stress and anxiety in the beginning (Figure 6). The recruitment and randomization processes resulted in two groups whose equivalence was confirmed by analysis of demographic factors and pre intervention test scores. This data correlates with the previous reports by other groups such as Carlson et al 2001, 2004, and 2003) and Carson et al (2007). The back ground anxiety and depression levels can be attributed to severe traumatic experience of the cancer as a disease as well as the anticipation of end of life as a crisis situation. (Farber et al 1983, Spiegel D et al 1995, Fox B .H, et al 1995). The decrease in the anxiety as well as depression levels can be attributed to the relaxation response gained from the integrated yoga approach which had a reduction effect of the stress induced arousal in traumatized patients S Nicole et al (30) where they reported an improved QOL post yoga module.

The perceived stress also reduced significantly in the intervention group when compared to the control cohort which is also similar to the findings of Spiegel D et al (1995), Casso D (2004) and Carson J (2007). All though there were few patients in the control population who reported

improvement in their sleep quality and anxiety levels but the depression scale showed increase over the period of the study. The radiation induced DNA damage has been widely studied and reported by many including our previous study. In another work Mozdarani et, al (2005) showed that there is an elevated spontaneous frequency of MN in breast cancer group compared to the control group. They also showed that Ca-Breast patients were more sensitive (30%) to ionizing radiation than the control population age and sex matched. Scott et al (1998-1999) reported that breast cancer patients displayed radiation susceptibility when compared to control; we also reported significant genomic instability in Ca breast patients who underwent radiotherapy. In the current study an effort has been made to compare the radiation induced DNA damage as a genotoxic stress and its correlation with the psychological stress levels of the patients Alkaline gel electrophoresis technique(comet assay) was used as described by Poonepalli and Hande et, el(2005).Comet assay is a very sensitive tool to study DNA damage(2005) In another study Elizabeth Blackburn et al (2004) reported a significant correlation with telomere length in the PBLs and Psychological stress in controlled study. Recently she again reported a significant correlation with the telomere dysfunction and stress in cardiovascular disease. (Blackburn et al (2006). We also reported a significant correlation between radiation induced DNA damage and

telomere dysfunction in Ca- breast patients. Telomere maintenance is strongly associated with DNA damage and repair as reviewed by Hande (2004). Psychological stress is also associated with faulty DNA repair capacity in the lymphocytes as reported by Kiecolt-Glaser et al (1985). Later Cohen et al (2000) showed reduced DNA repair capacity in anxious students. In our study, it may be speculated that in the intervention group the reduced DNA damage as compared to the control group may be linked to higher psychological stress. The background DNA damage levels in both the control and the intervention group may be associated with the varied dose of chemotherapy and increased levels of anxiety. There is a converging link between the psychological (QOL, Anxiety, depression mood disturbances , perceived stress) (Carlson L.E, 2001, Raghavendra Rao et al 2007) and physiological stress at the molecular level such as Cortisol levels, catecholamines, DNA damage, telomere length and DNA repair capacity) (Scott D 1998, Mozdrani,2005,Kiecolt-Glasser 1985, Glasser R 1985 E Epel 2004. Elizabeth Blackburn, E. Epel 2006). Homes D et al (2006) reported in a large scale survey of more than 2000 patients undergoing various complementary treatments, yoga is the most effective amongst all CAMs in decreasing anxiety, depression and improving the QOL of breast cancer patients .In the current study we tried to investigate the possible link of stress at the molecular level but a lot of studies

remained to be done in future to substantiate the findings of the above mentioned groups including our study. The limitation of our study is a small cohort of population size as faced by other groups (Carlson 2003, Rosenbaum 2004 , Casso D 2004, Carson J W 2007), but in the hospital clinical out patients setting it is difficult to conduct large patient trials with physiological parameters such as DNA damage involved. But large and specific trials in future may prove very effective in deciphering the mechanistic link between the emotional trauma, psychological and physiological stress. In summary our study showed preliminary data to support that stress influence the coping rout at the molecular level. The present study like other groups(Henderson J W 2004, Richardson M.A.2000) can influence yoga based program and supportive counselling for cancer in an out patient setting to improve the efficacy of the conventional treatment modality and benefit the cancer patients overall.

CHAPTER 8

SUMMARY AND APPRAISAL

8.1 Summary:-

To summarize we found that the current research has tried to focus on the psychological and physiological impact of the cancer patients. The background stress level of the patients was higher due to the trauma of the disease itself. The yoga intervention benefited the patients psychologically which was due to physiological modifications at the molecular level. The current therapeutic modality of cancer management adds to lot of stress and therefore an integrated approach of yoga intervention along with the conventional treatment regime will immensely benefit the patients.

8.2. High points of the study:-

- That breast cancer patients undergoing radiotherapy are under added stress and tension due to the burden of the disease itself.
- Radio-therapeutic intervention causes DNA damage in the normal peripheral lymphocytes of the breast cancer patients which has differential variability on a case to case basis but a definite amount of DNA

damage is incurred on the lymphocytes over a period of six weeks of treatment.

- The varied radio sensitivity of the breast cancer patients and the link between telomere damage and radiation sensitivity provides a frame work for further research that may have an impact in radio-therapeutic strategies in cancer.

- An integrated approach to yoga therapy is effective to a significant extent in negotiating the background stress levels at the psychological as well as physiological levels.
- There are no side effects detected due to Yoga intervention during the period of study.
- The integrated approach of Yoga intervention can be used as an adjuvant to the conventional regime of cancer management for an effective and beneficial outcome.

8.3. Limitations of the study:-

The major limitation of the study is the heterogeneity of the human behaviour. A human trial is never a perfectly controlled trial as human emotions vary immensely under various stimuli and environmental modification.

The second limitation of the study is the population size. The current study is a pilot research programme which can be extended to a large trial to increase the efficacy and effectiveness of the intervention.

Such interventional trial must be conducted in large population over a period of time for effective conclusive results. But to do such large trials in an out patient set up in a hospital is always a limitation as faced by other groups such as Carlson et al, Carson et al and Gasser et al.

The third limitation is the monitoring and follow -up of the patient at their domestic set up. Each patient must be monitored on a long term basis and their effective parametric changes must be carefully studied.

The fourth limitation is the molecular study in details at the gene expression level. The cohort of the stress genes must be studied which

may high light the individual response to the relaxation and yoga based intervention at the molecular level on a single cell basis.

8.4. FUTURE DIRECTIONS: -

Study of stress and relaxation response at the molecular level (differential gene expression)

The emergence of new tools enables investigators to address previously intractable problems and to reveal novel potential targets for therapies. Researchers are using microarray technology to try to identify fundamental aspects of growth and development as well as to explore the underlying genetic causes of many human diseases including stress related ones. (Debouck C, 1999) Microarray based researches have enormous potential in the exploration of disease processes such as cancer, and in drug design and development Ultimately, these studies are certain to expand the size of existing gene families, discover new patterns of coordinated gene expression across gene families, and disclose entirely new classes of genes. (Chuaqui RF 1999) The application of functional genomics approaches are beginning to have an impact, enhancing our understanding of stress related diseases. Genomics or high-throughput

genome-wide technologies, promises insights and answers on genes that are sensitive to various kinds of stress. **Figure8.1**

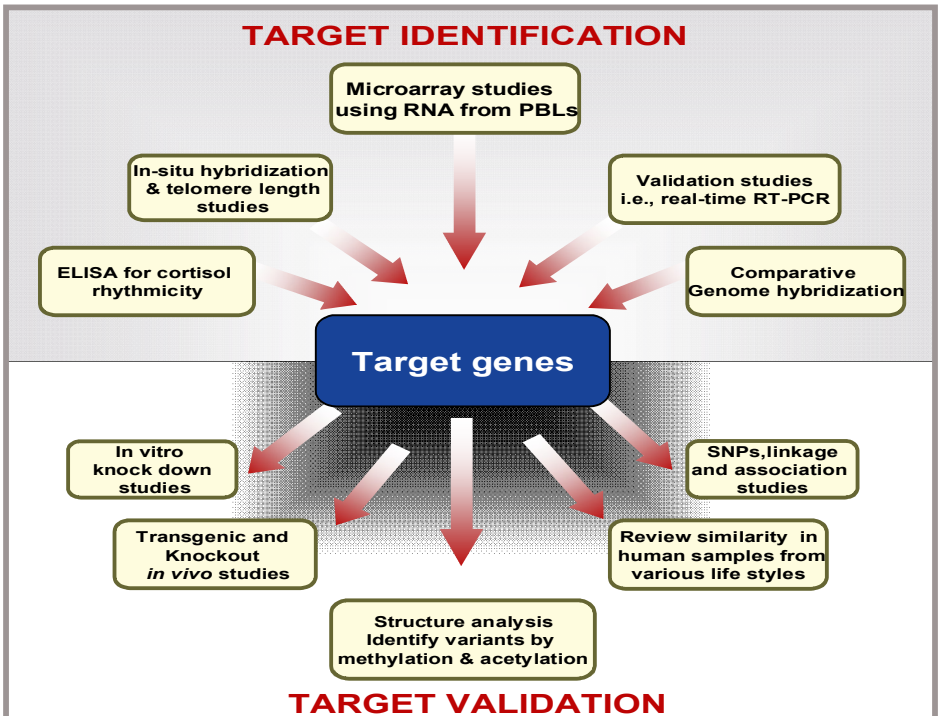


Figure 8.1: Stress Genomics: Strategies applied in target identification and validation**.** Summarizes a strategy applied for identifications of target genes and biological validation of potentially identified target by various schemes.

Learning the pattern of gene expression in samples and identifying the target gene is the first and critical step. Once a gene is identified as a potential candidate, investigations move to the subsequent stage of

biological validation. Figure 8.1 summarizes a functional genomics approached used for discovering target genes involved in stress related conditions and validation of the identified targets. (Gerhold DL, Jensen RV, 2002) The functional role of a target gene is determined by strategies like tissue culture, transgenic or knockout animal models, SNPs and association studies. Biomarkers are important in assessing the exposure and for predicting future adverse health outcomes. The development of cellular and molecular biomarkers for stress related conditions are important goal of modern research. Once biomarkers are identified by functional genomics approaches, eventually, microarrays could be used as a routine diagnostic tool called "microarray readers," with which treatments could be tailored for an individual patient suffering from stress. (Julian White.2004) The relaxation response and the differential expression of several stress related genes can be studied by the above approach and the effect of an integrated approach of yoga can be studied in details.

Pressed by patients and advancing technology, health care will soon change its focus **from treatment to enhancement,** from **repair to improvemen**t, from **diminished sickness to increased performance**. Further research is needed to clarify exactly how stressors contribute to each of these problems, so that treatment can be given to protect the body

from these diseases. Mind Body Medicine (**MBM**) will become a vitally important clinical field-perhaps the most important field in the 21st century."

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APPENDIX I

Weekly Yoga Modules

Program Content

1st week

Concepts

1. Philosophy and didactic and interactive lecture of yoga
2. Rules and regulations of yoga based stress reduction program
3. Stretching and loosening exercises and body awareness
4. Full relaxed breathing and breath awareness
5. Breathing exercises
6. Guided awareness of body sensation in supine posture

Practices

1. Hands stretch breathing, hands in and out, ankle stretch breathing, sectional breathing and full yogic breathing
2. Loosening exercises
3. Quick Relaxation Technique, guided awareness of body sensations and proprioception

4. Ardhakati chakrasana
5. Padahastasana
6. Ardhachakrasana
7. Instant Relaxation Technique (IRT)
8. Nadi Shuddhi Praṇayama

2nd week

Concepts

1. Stress re response and physiological and behavioral adaptation to stressor
2. Identification of inner self to these responses
3. Awareness of nature of inner self and its association with cognition, emotion and sensory perceptions through past memories, reactions to situations.
4. The process of building awareness of self

Practices

1. Loosening exercises
2. Ardhakati chakrasana
3. Padahastasana
4. Ardhachakrasana
5. Instant Relaxation Technique (IRT)

6. Supine - uttana padasana
7. Pavanamuktasana
8. Quick Relaxation Technique (QRT)
9. Meditation - breath awareness
10. Nadanusandhana - Sound awareness

3rd week

Practices

1. loosening exercises
2. Ardhakati chakrasana
3. Padahastasana
4. Ardhachakrasana
5. Instant Relaxation Technique (IRT)
6. Makarasana breathing with "A" Kar, "U" Kar, "M" Kar Chanting.
7. Bhujangasana breathing.
8. Shashankasana breathing.
9. Praṇayama - Alternate Nostril, Uni-nostril breathing.
10. Deep Relaxation Technique with imagery.
11. Meditation - Breath awareness,
12. Nadanusandhana - Sound awareness.
13. Instant Relaxation Technique (IRT)

4th week

Practices

1. Loosening exercises - 5 Min
2. Ardhakati chakrasana.
3. Padahastasana.
4. Ardhachakrasana.
5. Quick Relaxation Technique(QRT)
6. Makarasana breathing with "A" Kar, "U" Kar, "M" Kar Chanting.
7. Bhujangasana breathing.
8. Praṇayama - Alt Nostril, Uni-nostril breathing.
9. Deep Relaxation Technique with imagery.
10. Meditation - Breath awareness,
11. Mind Sound Resonance Technique.

5th week

Practices

1.Loosening exercises - 5 Min

2. Cyclic meditation.

3. Praṇayama - Alternate Nostril , Uni-nostril breathing

4. Meditation _ Breath awareness,

6th week

Practices

1. Loosening exercises - 5 Min.
2. Cyclic Meditation With imagery.
3. Review of all practices, developing his/her own module of the practices, which suits them best.

YOGA PRACTICES USED IN THE INTERVENTION

NAME OF PRACTICES:

Breathing Exercises

1. Hands in and out breathing
2. Hands stretch breathing
3. Ankle stretch breathing
4. Straight leg raising (Alternate legs)

Sūkṣma Vyāyāma (Strenghtening Exercises)

5. Maṇibandha Śakti Vikāsaka (Wrists)
6. Karapriṣṭha Śakti Vikāsaka (Back of hand)
7. Kaphoni Śakti Vikāsaka (Elbows)
8. Grīva Śakti Vikāsaka -I (Neck)
9. Grīva Śakti Vikāsaka -II (Neck)
10. Kaṭi Śakti Vikāsaka -I (Back)
11. Kaṭi Śakti Vikāsaka -II (Back)
12. Netra Śakti Vikāsaka (Eyes)

Yogāsana

13. Uṣṭrāsana
14. Bhujangāsana
15. Viparīta Karaṇi with wall support
16. Deep Relaxation echnique

Prāṇāyāma

17. Kapālabhāti
18. Vibhāga Prāṇāyāma (Sectional Breathing)

6. Sūrya Anuloma Prāṇāyāma
7. Candra Anuloma Prāṇāyāma

4. Nādi Śuddhi

22. Śītalī / Sītkārī /Sadanta Prāṇāyāma

23. Bhrāmarī

Meditation (Dharana, Dhyana)

24. OM Meditation

25. Cyclic Meditation

26. Mind Sound Resonance Technique(M S R T)

27. Prānic Energisation Technique (P E T)

Kriyas

28. Jala Neti

29. Sūtra Neti

30. Vaman Dhouti

BREATHING PRACTICES

1. HANDS IN AND OUT BREATHING

a) STARTING POSITION:

- **Sthiti:** Tādāsana

b) PRACTICE:

- Stretch out your arms in front, in level with your shoulders & bring the palms together.
- While inhaling, spread your arms sideways in the horizontal plane.
- While exhaling bring the arms forward with palms touching each other.
- Repeat five rounds, making your arm movements continuous, breath flowing in and out rhythmically.
- Relax in Tādāsana. Feel the changes in the breath and the body, especially the arms, shoulders and the back of the neck.

2. HANDS STRETCH BREATHING

a) STARTING POSITION:

- Stand erect with feet together (heels together and toes four to five inches apart) relaxed by the side of the body.
- Gently bring your hands in front of the chest.
- Interlock the fingers and place the palms on the chest
- Collapse and relax your shoulders
- Close your eyes.

b) PRACTICE:

- **STAGE-I** (Horizontal)
- While inhaling, stretch the arms straight out in front of your body so that the arms are at shoulder level.
- At the same time twist the hands so that the palms face outwards.
- Fully stretch the arms, but do not strain.
- Now, while exhaling reverse the process and bring the palms back on to the chest.
- Collapse the shoulders again.
- This is one round. Repeat five rounds.

STAGE-II (At 45^0)

- Repeat the same movements now stretching the arms above the forehead at an angle of 45^0.
- Repeat the entire thing five rounds.

STAGE-III (Vertical)

- Again repeat the same movements, this time stretching the arms vertically above the head.
- While moving up and down the hands may gently touch the nose tip.
- Repeat five rounds.

c) NOTE:

- Collapse the shoulders at the beginning and end of each cycle.
- Maintain perfect awareness of the breathing.
- Exhalation should be longer than the inhalation.
- If required, it can be practiced sitting on a chair too.
- Properly synchronize the breathing with hand movements.

3. ANKLE STRETCH BREATHING

a) STARTING POSITION:

- **Sthiti:**Tādāsana

b) PRACTICE:

- Open the eyes and fix your gaze on a point on the wall ahead. Keep the palms on front of your thighs.
- While inhaling, raise your hands and stretch the ankles. Feel yourself growing taller and firm
- As you exhale, bring your hands and heels down.
- Repeat five rounds keeping the movement of hands and ankles continuous breathing in synchronization. Feel the stretch from your ankles to your fingers as you reach upwards.
- Relax in standing position, hands by the side of the thighs. Observe your breath and enjoy the stability for a few seconds.

Breathing exercises in sitting position:

- The initial stance for all these practices is the Legs-stretched relaxed posture i.e., sitting with legs spread out in front. Palms behind the buttocks, fingers facing away from the body, neck hanging loosely backwards.
- Feel the weight of the body on the buttocks and the palms.

4. STRAIGHT LEG RAISING (Alternate legs)

a) STARTING POSITION:

- Sthiti: Supine.

b) PRACTICE:

- While inhaling slowly raise the right leg without bending at the knee, as far as comfortable (up to 90^0, if possible).
- While exhaling return the leg to the floor as slowly as possible.
- Repeat the practice with the left leg.
- This is one round. Perform ten rounds.

c) NOTE:

- If you need, you can keep the arms by the side of your body with the palms facing the floor at any convenient position or at shoulder level even.
- Do not bend the knee at any cost.
- Do not disturb the leg lying straight on the ground in order to be able to raise the other leg further.
- Even if you can, do not raise the leg beyond 90^0 because that will create an undesirable pressure on the abdomen.
- Perfectly synchronize breathing with the leg movements.
- Maintain perfect breath awareness during the practice

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SUKSMA VYAYAMA(Strengthening Exercises)

5. MAṆI BANDHA ŚAKTI VIKĀSAKA (Wrists)

a) STARTING POSITION:

- **Sthiti:**Tādāsana

b) PRACTICE:

STAGE-I (Arms stretched in front)

- Stretch your arms straight in front of the chest at shoulder level, keeping them parallel to the ground.
- Make loose fists of your hands (palms facing down).
- Now, move the fists up and down from the wrists with force.
- Repeat ten rounds.

STAGE-II (Arms bent)

- Stretch your arms sideways at shoulder level, keeping them parallel to the ground.
- Now, bend them at the elbows and bring the hands near the chest, palms facing downward.

- Make loose fists of your hands and move them up and down from the wrist with force.

c) NOTE: (For both Stages)

- The movement should be from the wrists and forceful and vigorous.
- While bringing your fists up and down, try to touch the forearm.
- Keep the arms as stiff as possible.

d) BREATHING: Normal breathing

6. KARA - PṚṢṬHA ŚAKTI VIKĀSAKA (Back of hand)

a) STARTING POSITION:

- **Sthiti:** Tādāsana

b) PRACTICE:

STAGE-I (Arms stretched in front).

- Stretch the arms in front of the chest at shoulder level.
- Palms open and facing downwards, fingers close together.
- Now, move the palms up and down forcefully from the wrist.

- Repeat ten rounds.

STAGE-II (Arms bent).

- Bring the palms near the chest as in Stage-II of I.
- Here, keep the palms open and palms facing downward, and all the fingers together.
- Now, move the palms up and down forcefully from the wrist.
- Repeat ten rounds.

c) NOTE:

- The movement should be from the wrists and forceful and vigorous.
- While bringing the palms up and down try to touch the forearm (without bending the fingers)
- Keep the arms as stiff as possible.

d) BREATHING: Normal

7. KAPHONI ŚAKTI VIKĀSAKA (Elbows)

a) STARTING POSITION:

- **Sthiti:** Tādāsana

b) PRACTICE:

STAGE-I (with fists)

- Stretch the arms straight downwards beside the body and make fists.
- Bend the arms at the elbows and raise your clenched fists forward to the level of the shoulder with a jerk.
- Then stretch them downwards again with a jerk.
- Repeat twenty rounds.

STAGE-II (with open palms)

- Here, keep your palms open (facing forward) and with the fingers close together.
- Repeat jerking your arms up and down from the elbows as before.
- Repeat twenty rounds.

c) NOTE: (For both Stages)

- The elbows should remain stationary.
- The fists / palms must come up to the level of the shoulders and then down straight.
- The fists / palms must not touch the shoulders when going up, nor touch the thighs when coming down.

8. GRĪVA ŚAKTI VIKĀSAKA - I (Neck)

a) STARTING POSITION:

- **Sthiti:** Tādāsana
- Relax your neck completely, keep your eyes wide open.

b) PRACTICE:

STAGE-I (Turning or Twisting).

- Relaxing your neck, turn your head with a jerk first towards your right shoulder, then towards your left shoulder.
- Repeat this ten rounds.

STAGE-II (Forward and Backward)

- Relaxing your neck, jerk your head, first forward and then backward.
- When it is forward, the chin should touch the sternal notch (chest).
- When it goes back, it should touch the nape of your neck.
- Repeat this ten rounds.

c) NOTE: (For both Stages)

- Movements are done with a jerk to have appropriate effect.
- Neck must be completely relaxed.
- Keep the mouth closed and eyes wide open throughout the practice.

d) BREATHING: Normal.

9. GRĪVA ŚAKTI VIKĀSAKA - II (Neck)

a) STARTING POSITION:

- **Sthiti:** Tādāsana
- Relax your neck completely, keep your eyes wide open.

b) PRACTICE:

- Keep your chin in and rotate the head from left to right and then right to left alternately.
- Repeat this ten rounds.

c) NOTE:

- Try to make your ear touch the shoulder.
- Take particular care to avoid raising the shoulder.
- Keep the chin in throughout the practice to have good benefit.

d) BREATHING: Normal

9. KAṬI ŚAKTI VIKĀSAKA -I (Back)

(Forward & backward bending)

STAGE-I

a) STARTING POSITION:

- **Sthiti:** Tādāsana
- Clench your right hand to form a fist with the thumb tucked in and take it behind the back.
- Now, hold the right wrist with the left hand.
- Both hands remain in contact with the back.

b) PRACTICE:

- While inhaling deeply bend backwards as far as you can by keeping the hands in contact with the back.
- Maintain this posture for a few moments.
- Then, while exhaling, bend forward trying to touch the knees with your head.
- Repeat ten rounds.

STAGE-II

a) STARTING POSITION:

- As above except that the left hand should be formal into a fist and right hand holding the left wrist.

b) PRACTICE:

- Same as in Stage-I.

c) NOTE: (For both Stages)

- The hands at the back must always be in contact with the body.
- Hold the positions (forward & backward) for a moment.

10. KAṬI ŚAKTI VIKĀSAKA - II (back)

(Forward & backward bending)

a) STARTING POSITION:

- Stand with your legs separated as far as possible.
- Place your hands on the hips with the thumbs pointing forward and the fingers pointing backward.

b) PRACTICE:

- While inhaling bend backward from the waist as far as you can go. Maintain this posture for sometime.

- Then while exhaling bend forward trying to touch the ground with the head (without bending the knees).
- Repeat this ten rounds.

c) NOTE:

- Hands continue to be on the hips all through.
- Do not bend the knees at any time during the practice.
- Make the movements within your capacity.

12. NETRA ŚAKTI VIKĀSAKA (Improving the Eye Sight)

a) STARTING POSITION:

- **Sthiti:** Tādāsana.

b) PRACTICE:

- Tilt your head backwards as far as it will go.
- Look at the spot between the two eyebrows without blinking and with full concentration.
- The eyes must squint in doing so.
- When the eyes feel tired or start watering, stop it and do palming.
- You can repeat it a few times but with rest in between.

c) NOTE:

- You must relax the eye muscles between two consecutive practices.
- You can practice this with Nāsāgra Dṛṣṭi also (i.e. gazing at the tip of the nose).
- If it is difficult, you can keep your head in normal position and practice it.
- You can do it even sitting in Vajrāsana etc.

d) BREATHING: Normal

BENEFITS

- The entire neuro-muscular apparatus of the eye-ball is toned up for better performance and endurance.
- It also has an important effect on improving concentration of the mind.

YOGĀSANA

13. UṢṬRĀSANA

a) STARTING POSITION:

- **Sthiti:** Dandāsana.

b) PRACTICE:

- Sit in Vajrāsana.
- Stand on your knees.
- Place the palms on the waist and fingers pointing forwards.
- Inhale and bend the body backwards and place the palms on the heels.
- Exhale while coming back to Sthiti.

14. BHUJANGĀSANA

a) STARTING POSITION:

- **Sthiti:** Prone

b) PRACTICE:

- Bring the palms to the level of the last rib bone and place them on the ground. Keep the hands bent at elbows; least pressure to be exerted on the hands. Maintain the elbows touching the body; let it not spread out.
- Raise the head first and then the upper portion of the trunk slowly, till the navel portion, just as the cobra raises its hood. Arch the dorsal spine well. Keep the body below the navel straight and in touch with the ground. Maintain this position for a minute.
- Come back to Sthiti position & relax in Makarāsana.

15. VIPARĪTA KARAṆI

a) STARTING POSITION:

- Lie flat on your back with the legs and feet together in a straight line. Place the hands and arms close to the body with the palms facing down.
- Raise the legs to 90^0, keeping the knees straight, less stretching inwards slightly.

- Raise the buttocks and the trunk off the floor by supporting the body with the hands at the waist to transfer the weights to the arms and elbows. Keep the elbows as close to each other as possible.
- Slowly move into the final position of Viparītakaraṇi by raising the straight legs to the vertical position and keeping the trunk at an angle of 45^0 to the ground.
- In the final position the weight of the body rests on the shoulders, neck and the elbows the trunk is at 45^0 angle to the floor, the legs straight and vertical to the floor & note that the chin should not press against the chest.
- Close the eyes and feel comfortable.
- Focus the awareness on the perineum i.e., the area between the anus and the genitals.

b) PRACTICE:

- Now slowly in a rolling motion, pull the buttocks inwards.
- Then contract the anal sphincter muscles and pull the anus inwards and upward.
- Hold it as long as possible with normal breathing.

- Release the bandha and then retrace the steps to come down to the supine sthiti and rest in Śavāsana for a while.

c) NOTE:

- Apart from Mūlabandha the following practices can also be done in the final position of Viparītakaraṇi.
- Deep abdominal breathing.
- Kapalābhāti
- Aśvini Mudra
- Mūlabandha can be performed also with antarkumbhaka (holding the breath after inhalation).
- It can be practiced for a few rounds.
- Aśvini Mudra, in fact, is a good preparatory practice for Mulabandha & can be practiced in the following way:
- "Contract the anal sphincter muscles and pull the anus inward and upward. Hold for 2 to 3 seconds. Then relax. Repeat this as many times as you can with normal breathing. Continues to briefly contract and relax thus as rhythmically and evenly as possible. Once this is mastered, Mūlabandha can be performed quite effectively."

16. DEEP RELAXATION TECHNIQUE

a) STARTING POSITION:

- **Sthiti:** Śavasana.
- Gently move your whole body, make yourself comfortable and relax completely.

b) PRACTICE:

Phase-I

Phase-I

- Bring your awareness to the tip of the toes, gently move your toes and relax. Sensitize the soles of your feet, loosen the ankle joints, relax the calf muscles, gently pull up the knee caps release and relax, relax your thigh muscles, buttock muscles, loosen hip the joints, relax the pelvic region and the waist region. Totally relax your lower part of the body. R**..e..l..a..x……** Chant A-kāra and feel the vibration in your lower parts of the body.

Phase-II

- Gently bring your awareness to the abdominal region and observe the abdominal movement for a while, relax your abdominal muscles and relax the chest muscles. Gently bring your awareness to your lower back, relax your lower back, loosen all the vertebral joints one by one. Relax the muscles and nerves around the back bones. Relax your middle back, shoulder blades and upper back muscles, totally relax. Shift our awareness to the tip of the fingers, gently move them a little and sensitize. Relax your fingers one by one. Relax your palms, loosen the wrist joints, relax the forearms, loosen the elbow joints, relax the hind arms-triceps, biceps and relax your shoulders. Shift your awareness to your neck, slowly turn your head to the right and left, again bring back to the center. Relax the muscles and nerves of the neck. Relax your middle part of the body, totally relax. **R..e..l..a..x…….**Chant U-kāra and feel the vibration in the middle part of your body.

Phase-III

- Gently bring your awareness to your head region. Relax your chin, lower jaw and upper jaw, lower and upper gums, lower and upper teeth and relax your tongue. Relax your palates-hard and soft, relax your

throat and vocal chords. Gently shift your awareness to your lips, relax your lower and upper lips. Shift your awareness to your nose, observe your nostrils, and feel the warm air touching the walls of the nostrils as you exhale and feel the cool air touching the walls of the nostrils as you inhale. Observe for a few seconds and relax your nostrils. Relax your cheek muscles, feel the heaviness of the cheeks and have a beautiful smile on your cheeks. Relax your eye balls muscles, feel the heaviness of eye balls, relax your eye lids, eye brows and in between the eye brows. Relax your forehead, temple muscles, ears, the sides of the head, back of the head and crown of the head. Relax your head region, totally relax. **R..e..l..a..x…….**and chant M-kāra feel the vibration in your head region.

Phase-IV

- Observe your whole body from toes to head and relax, chant an Om-kāra. Feel the resonance throughout the body.

Phase-V

- Slowly come out of the body consciousness and visualize your body lying on the ground completely collapsed.

Phase-VI

- Imagine the vast beautiful blue sky. The limitless blue sky. Expand your awareness as vast as the blue sky. Merge yourself into the blue sky. You are becoming the blue sky. You are the blue sky. Enjoy the infinite bliss. **E..N..J..O..Y…** the blissful state of silence and all pervasive awareness.

Phase-VII

- Slowly come back to body consciousness. Inhale deeply. Chant an "Om-kāra". Feel the resonance throughout the body. The soothing and massaging effect from toes to head.

Phase-VIII

- Gently move your whole body a little. Feel the lightness, alertness and movement of energy throughout the body. Slowly bring your legs together and the hands by the side of the body. Turn over to the left or the right side and come up when your are ready.

PRĀṆĀYĀMA

17. KAPĀLA BHĀTI(Preparatory Exercise for Prānāyāma)

a) STARTING POSITION:

- Sit in any meditative posture.
- Keep your spine, neck erect and perfectly vertical to the ground.
- Eyes closed, shoulders collapsed and the whole body completely relaxed.

b)PRACTICE:

- In this practice, exhalations will be very active and forceful whereas the inhalations will be totally passive and happening on its own.
- In fact, it is done by blasting out the air and is accomplished by vigorous flapping movement of the abdomen in quick succession.
- Inhale passively by relaxing the abdominal muscles at the end of each expulsion.
- Repeat at the expulsion as quickly as possible starting with 60 strokes or expulsions per minute and increasing with practice up to 120 expulsions per minute.

- At the end of one minute, stop the practice.
- Now you will observe an automatic suspension of breath. In fact, there will be no urge for breathing.
- Simultaneously the mind achieves a deep silence. Enjoy this state of silence.
- Then gradually, breathing resumes when you start breathing in and out slowly and then after few rounds it becomes normal.

NOTE:

- Through out the entire practice the spine must be erect. Otherwise, there is a possibility of injuring the spine because of the vigorous flapping of the abdomen.
- In the beginning it may not be possible for one to do the practice continuously for one minute and for so many expulsions or strokes. Therefore, one can start with 10 to 20 strokes or expulsions in one round without bothering for the time it takes and do it for 2 to 3 rounds. Once he gets the technique of doing properly, he can do it rapidly coming to the level of required speed emphasizing on these points.

- Emphasis on the limitation aspect. People with High BP, IHD problems, vertigo, epilepsy, Hernia, Gastric Ulcer, Slipped disc, Spondylosis should not do. Women during menses and advance pregnancy should also avoid.
- In fact, you need not bother about the inhalations. It will happen automatically when the abdominal muscles are relaxed at the end of each expulsion.
- You finally concentrate on the expulsions and do it properly.
- Kapālabhāti can be practiced through alternative nostrils also.

18. VIBHĀGA PRĀNĀYĀMA (SECTIONAL BREATHING)

- This is a preparatory breathing practice for Prānāyāma. It corrects the breathing pattern and increases the vital capacity of the lungs. It has three sections:

ABDOMINAL BREATHING OR DIAPHRAGMATIC BREATHING (ADHAMA)

a) STARTING POSITION:

- Sit in any meditative posture.

b) PRACTICE:

- Inhale deeply, slowly and continuously. This is called pūraka, the abdomen is made to bulge continuously with the air entering specially in the lower section of the lungs.
- Before exhaling stop the breath (antaryā kumbhaka) for a second.
- While exhaling (recaka) the abdomen is drawn inwards continuously and slowly.
- Before the breath is reversed, stop the breath (bāhya kumbhaka) for a second and then inhale.
- Repeat the breathing cycle. There should be no jerks in the whole process. It should be smooth, continuous and relaxing.
- The diaphragm separating the thorax from the abdomen descends during inhalation with the bulging of the abdomen. This increases

the airflow into the lower sections of the lungs. The rhythmic movement of the diaphragm massages the contents of the abdomen gently, and helps the organs to function normally. It promotes the general circulation also.

THORACIC (CHEST) BREATHING OR INTERCOSTAL BREATHING (MADHYAMA)

a) STARTING POSITION:

- Sit in any meditative posture.

b) PRACTICE:

- In this practice expanding and contracting the chest only performs inhalation and exhalation. Air flows through both nostrils, slowly and continuously. The abdomen is controlled to avoid its bulging.
- The middle lobes are opened up fully by this type of breathing.

UPPER LOBAR BREATHING OR CLAVICULAR BREATHING (ĀDYA)

a) STARTING POSITION:

- Sit in any meditative posture.

- **PRACTICE:**
- Raise the collarbones while inhaling.
- Keep the abdominal muscles contracted.
- The air is forced into the upper most region of the lungs thus ventilating the upper lobes. The sparingly used upper lobes of the lungs will be properly aerated by this breathing.

FULL YOGIC BREATHING

a) STARTING POSITION:

- Sit in any meditative posture.

b) PRACTICE:

- *In full yogic breathing technique all the other three types will be combined.*
- *During inhalation, the adhama, madhyama and ādhya occur sequentially and during exhalation the same sequence namely abdominal, chest and clavicular breathing occur.*
- *The whole process should be relaxing and comfortable, without any tension in the face.*

Four Mudrās are generally associated with these sectional breathing practices. They are:

- Cin mudrā – abdominal breathing or adhama
- Cinmaya mudrā – thoracic or chest or madhyama
- Ādi mudrā– clavicular or ādya
- Brahma mudrā – compelete yogic breathing

19. SŪRYA ANULOMA PRĀNĀYĀMA

a) STARTING POSITION:

- Sit in any meditative posture.
- Adopt Nāsika mudrā with your right hand (folding index & middle fingers towards the palm).

b) PRACTICE:

- Inhalation & exhalation are carried out through the right nostril (sūrya nadi) only.
- Keep the left nostril closed all the time during the practice.
- Practice nine rounds.

20. CANDRANULOMA PRĀṆĀYĀMA

a) STARTING POSITION:

- Sit in any meditative posture.
- Adopt Nāsika mudra with your right hand (folding index & middle fingers towards the palm).

b) PRACTICE:

- Inhalation & exhalation are carried out through the left nostril (candra nādi) only.
- Keep the right nostril closed all the time during the practice.
- Practice nine rounds.

21. NĀDI ŚUDDHI PRĀNĀYĀMA

a) STARTING POSITION:

- Sit in any meditative posture.

b) PRACTICE:

- Close the right nostril with the right thumb by adopting Nāsika mudrā and exhale completely through the left nostril, then inhale deeply through the same left nostril.
- Close the left nostril with your ring & small fingers of the right hand, then open the right nostril and exhale through the right nostril, again inhale through the same right nostril.
- Then close the right nostril and exhale through the left nostril. This is one round of Nādisuddhi prānāyāma.

- This practice also helps to maintain balance between nādis.
- Repeat nine rounds.

22. ŚĪTKĀRĪ/ŚĪTALĪ/SADANTA

ŚĪTALĪ

a) STARTING POSITION:

- Sit in any meditative posture.

a) PRACTICE:

- Stretch the tongue forward out of the mouth and fold it so as to resemble the back of a crow.
- Slowly suck the air through the beak and feel the jet of cool air passing down the trachea into the lungs.
- Enjoy the turnover and Kevala- Kumbhaka(automatic cessation of breath).
- Slowly exhale through the nostrils, carefully feeling the movement of warm air all the way up from the lungs through the trachea and the nasal passage.

- Enjoy the stoppage of breath and promote this blissful kevala kumbhaka before the breath starts moving in again through the beak of the tongue.
- This completes one round of Śītalī Prānāyāma. Repeat 9 rounds.

ŚĪTKĀRĪ

a) STARTING POSITION:

- Sit in any meditative posture.

b) PRACTICE

- Fold the tip of the tongue inwards and press the root of the upper palate with the tip of the tongue. The folded tongue slightly comes out between the two rows of teeth and provides a narrow opening on both the sides.
- Slowly suck the air, which enters in through the two sides of the tongue, diffuse throughout the mouth and move down the trachea into the lungs.
- Promote kevala kumbhaka and feel its effects.
- The warm air is exhaled out slowly through the trachea, and the nostrils and the breath stops automatically.

- The deep relaxation obtained due to cooling, extends the kevala kumbhaka.
- This completes one round of Śītkārī. Repeat 9 rounds.

ŚĪTALĪ

a) STARTING POSITION:

- Sit in any meditative posture.

b) PRACTICE:

- Stretch the tongue forward out of the mouth and fold it so as to resemble the back of a crow.
- Slowly suck the air through the beak and feel the jet of cool air passing down the trachea into the lungs.
- Enjoy the turnover and Kevala- Kumbhaka (automatic cessation of breath).
- Slowly exhale through the nostrils, carefully feeling the movement of warm air all the way up from the lungs through the trachea and the nasal passage.

- Enjoy the stoppage of breath and promote this blissful kevala kumbhaka before the breath starts moving in again through the beak of the tongue.
- This completes one round of Śītalī Prānāyāma. Repeat 9 rounds.

SADANTA

a) STARTING POSITION

- Sit in any comfortable posture.

b) PRACTICE

- Let the upper teeth touch the lower teeth.
- The tip of the tongue kept behind the teeth and air is sucked in.
- Inhale through the crevices of the teeth and the air moves over the gums slowly and continuously into the mouth and passes down the trachea into the lungs.
- The warm air is exhaled out slowly through the trachea, and the nostrils and the breath stops automatically.
- The deep relaxation obtained due to cooling, extends the kevala kumbhaka(automatic cessation of breath).
- This completes one round of Sadanta. Repeat 9 rounds.

23. BHRĀMARĪ PRĀNĀYĀMA

UNDERSTANDING M-kāra, N-kāra & BHRĀMARĪ

(M-kāra)

- Chant 'Mā' a few times and you will see that while chanting it
    - Your lips are closed
    - Rows of teeth are separated &
    - The tongue is just behind the lower row of teeth in normal position

- So, in order to chant M-kār, you can chant any word ending with 'M' such as 'Om', 'Mum', 'Swim', 'Phālam' etc. but stretch the 'M' part only. This will result in 'M-kār' chanting.
- While chanting M-kār, you will notice that the sound is nasal and is produced near the upper (soft) palette.

(N-kāra)

- Chant 'Nā' a few times and you will notice that while chanting it
    - Your lips are separated
    - Rows of teeth are separated also
    - Tongue moves upward and touches the upper (hard) palette &
    - Most importantly, there is a partial closure of the epiglottis because of the lifting up of the soft palette.
- While doing Brahmari, in fact, the sound of 'N' occurs as it happens in the pronunciation of words such as 'King', 'Ring', 'Sing' etc.

BHRĀMARĪ

Vegad Ghośam Pūrakam Bhringanādam

Bhringanādam Recakam Mandammandam

Yogīndrānam Evamābhyāsayogat

Citte Jāta Kacidānandalīla

[Meaning: By quick forced inspiration one should produce a high humming sound like that of a male bee and by very slow expiration a low sound should be produced resembling that of a female bee**]**

By a continuous practice of this type there easily supervene a condition of bliss in the minds of Yogin that defies all description.

- So, in order to produce the sound of a female bee (i.e., Bhrāmarī) utter any word ending with 'ng' such as 'Sing', 'Ring' etc. and stretch the 'n' part which will give rise to Bhrāmarī.

NOTE:

- While doing Bhrāmarī, if you observe, you will notice that the sound vibration is more at the throat region.
- During practice of Bhrāmarī, touch the tongue to upper (hard) palette
- While practicing Bhrāmarī, feel the strong vibrations particularly in the head region. Also feel its resonating effect. Before going for the next round Bhrāmarī, allow the vibrations to settle down thoroughly

MEDITATION (DHĀRAṆA, DHYĀNA)

24. OM MEDITATION

a) STARTING POSITION:

- Sit in any comfortable meditative posture feeling completely relaxed.

b) PRACTICE:

PHASE-I

- *Close your eyes and start chanting OM mentally. Allow the mind to repeat OM continuously without break. If there are distractions, you chant OM faster, not giving a chance to distractions. After a while the chanting slows down. Consciously slow it down further. If the mind jumps to distractions, again increase the speed of japa of OM kara. Thus, by increasing and allowing the speed to slow down, you should be able to have an unbroken stream of the japa in your mind.*

PHASE-II

- *Make the chanting softer and softer and gentler and gentler, and more and more effortless. As you progress on the path of meditation, you will reach the second phase of japa in which you start feeling the vibration of the japa in the particular part of the body and later throughout the body.*

PHASE-III

- As you slow down the japa of OM kāra observe the gap between OM . Further you slow down the gap widen and widen to diffuse into silence.

PHASE-IV

- The very deep experience of silence helps to expand from the 3dimensional awareness of the body to all pervasive awareness. The bed of silence becomes deeper and more expansive – an ocean of silence with waves on it – merge into complete silence – AJAPA. This silence is the source of Creativity, Power, Knowledge and Bliss.

25. CYCLIC MEDITATION (C M)

STEP-I: STARTING PRAYER

- Lie on your back. Relax and collapse the whole body on the ground legs apart, hands apart, palms facing the roof, smiling face, let go all parts of the body. As you repeat the prayer feel the resonance throughout the body.

Laye Sambodhayet Cittam Vikśiptam Śamayet Punah/

Sa Kaşāyam Vijānīyāt Samaprāptam Na Cālayet//

Meaning: In the state of oblivion awaken the mind, when agitated pacify it, in between the mind is full of desires. If the mind has reached the state of perfect equilibrium, then do not disturb it again.

STEP–II(A): INSTANT RELAXATION TECHNIQUE (I R T)

- Bring your legs together, join the heels, toes together, palms by the side of the thighs. Keep your face smiling till the end. Gently bring your awareness to the tip of the toes. Stretch the toes, tighten the ankle joints, tighten the calf muscles. Pull up the kneecaps. Tighten the thigh muscles. Compress and squeeze the

buttocks. Exhale and suck in the abdomen. Make the fists of the palms and tighten the arms. Inhale and expand the chest. Tighten the shoulders, neck muscles and compress the face. Tighten the whole body from the toes to the head. Tighten……tighten……tighten…… Release and relax. Legs go apart, arms go apart, palms facing the roof. Assume the most comfortable position, let the whole body sink down. Let all the groups of muscles beautifully relax. Collapse the whole body. Enjoy the relaxation.

STEP-II(B): LINEAR AWARENESS

- Now slowly bring the left hand over head along the ground. Slowly turn over the left side. Place the head on the left biceps. The right leg on the left leg. Right palm on the right thigh. Let the whole body relax. The entire weight of the body coming down to the ground through the left side. Fine linear awareness. Slowly start coming up to **Tādāsana**. Let all the movements slow down. Let the breathing be deep, slow, and continuous. Eyes are kept closed. Carefully feel the changes in your body as you reach the vertical position. Feel the flow of blood down the heart. Feel the

heartbeat and the pulse. Let us chant Bhramari to generate 3D awareness. MMM…….. Feel the whole body resonating. Feel the fine massaging effect.

STEP-III: CENTERING

- Now centering. Slowly lean forward. Feel the weight of the entire body on the toes. Pointed awareness. Slowly lean backwards. Feel the weight on the heels. Surface awareness. Come to the center. Lean to the right. The weight of the entire body is on the right edge of the right foot. Linear awareness. Lean to the left. Come to the center. Fine surface awareness. Now the whole body is centered, the weight of the body is equally distributed throughout the soles of the feet. Collapse the shoulders, arms hanging freely down. Smiling face. Feel all the changes taking place throughout the body.

STEP-IV: STANDING ĀSANA

ARDHA KATI CAKRĀSANA (AKC)

- Now we pass on to the first set of stimulation and relaxation.
- Ardha Kati Cakrāsana the half wheel posture.
- Slowly start raising the right arm sideways upwards, 45º raise the arm further slowly and continuously to horizontal position, enjoy the movement. As the right arm reaches the 90º position twist the palms at the wrist. Pointed awareness and glide the right arm up to 135º position. Beautiful pointed awareness on the deltoid muscles on the right arm. As the right arm reaches up the vertical position feel the nice stimulation in the shoulder muscles. The right biceps touching the right ear, feel the beautiful surface awareness. Feel the blood gushing down the arm. Smiling face. Stretch the right arm from the tip of the fingers of the right palm. The entire right portion of the body gets stretched, but not the face. Face always smiling and relaxed. Slowly start bending down to the left. Left palm sliding down along the left thigh. Fine movement of surface awareness. Enjoy the fine stretch of the waist muscles on the right side and compression on the left side. Observe all the changes

taking place in your body. Slowly start coming back to vertical position. Feel the blood flowing down, the nerve impulses throughout the body. Again stretch and pull up the right arm and the entire right portion of the body stretched from the toes to the tip of the fingers. Slowly start bringing the right arm down to 135º gliding down smoothly. Feel the pointed awareness at the shoulder as you reach horizontal position and at the wrist as you slowly turn the palm downwards. Further bring down the right arm to 45º. Feel the tingling sensation at the tips of the fingers. Continuously glide down the hand by the side of the thigh and hang it freely. Have a glance of the whole body again from toes to head. Entire right portion of the body is beautifully charged with nerve impulses, light and energized.

- Now let us perform AKC from the left side. Slowly start raising the left arm sideways upwards. 45º. Gliding smoothly upwards to horizontal position, palm twisted upwards. Beautiful pointed awareness at the wrist. Left arm beautifully moving up to 135º. Then to vertical position. Left biceps touching the left ear. Now stretch up the left arm from the tip of the left fingers. Entire left portion of the body gets stretched up but not the face, face smiling

and relaxed. Slowly start bending to the right. Right palm sliding down the right thigh. Movement of surface awareness, beautiful stretch of the left waist muscles. Enjoy the changes going on. Feel the heart beat, the nerve impulses spreading throughout the body. Slowly start coming up to the vertical position. Feel the nerve impulses from the tips of the fingers of the left palm. Pull up the left palm. Entire left portion of the body gets stretched up. Slowly bring the left arm down to 135º, then further down to horizontal position. Twist the wrist downwards and enjoy the pointed awareness. Glide your arm down further to 45º. Continuously glide down the hand by the side of the thigh and hang it freely. Collapse the shoulders. Have a glance of the whole body again from toes to head. Entire left portion of the body is charged with nerve impulses, energized and light. Enjoy the sense of well being. Check the centrifugal. Both the sides of the body are equally energized.

STEP-V: QUICK RELAXATION TECHNIQUE (Q R T)

- Now slowly sit down and then lie down to Śavāsana from the right side. Let all the movements be slow and continuous. The entire right arm stretched, head on the right biceps, left leg on the

right leg, left palm on the left thigh, the weight getting transferred to the ground from the right side, beautiful sharp linear awareness. Slowly turn over, the muscles of the back collapsing on the ground, bring down the right arm along the ground. Legs apart, arms apart, palms facing the roof. Assume the most comfortable position.

Phase I – Observing the abdominal movements

- Bring your awareness to the movements of the abdominal muscles moving up and down as you breathe in and out. Recognize the haphazardness and jerky movement of the abdominal muscles. Do not manipulate the breathing, let it be natural, simply observe the abdominal movement. Count yourself five rounds mentally, one inhalation and one exhalation forming one round.

Phase II – Associate with breathing

- Synchronize the abdominal movements with the breathing. While inhaling the abdomen bulging up and while exhaling the abdomen-sinking down. Inhale……. deeply and exhale….. completely. Continue upto five rounds.

Phase III –Breathing with feeling

- As you inhale, the abdominal muscles are coming up feel the whole body getting energized and feel the lightness. As you exhale, feel the whole body collapses and sinks down nicely, releasing all the stresses and tensions completely. Inhale……. deeply and exhale…… completely. Continue upto five rounds.
- Bring your legs together and hands by the side of the body. Come up straight with the support of the elbows to the sitting legs stretched relaxation position *Ś*ithili Dandāsana. Let all the movements be slow and continuous without jerk. Legs apart take the support of the palms backwards. Relax the neck muscles. The head hanging freely down backwards or resting either one of the shoulders. Feel the changes throughout the body.

STEP–VI: SITTING ĀSANA

- Now we pass on to the next set of stimulation and relaxation. Vajrāsana, Śaśānkāsana and Ardhauştrāsana/Uştrāsana combination.

A. VAJRĀSANA

- Slowly fold the right leg backward and then the left leg, sitting on the heels, coming to the Vajrāsana position. Palms on the thighs and keep the spine erect. Enjoy the effect of harmonizing, the beautiful balance. Recognize all the changes in the body.

B. ŚAŚĀNKĀSANA

- Now slowly start taking the arms behind. Hold the right wrist with the left palm. Start feeling the pulse at the right wrist, feel the heart beat. Now slowly start bending down forward for Śaśānkāsana**.** The abdominal and chest muscles pressing on the thigh, beautiful surface awareness. Now collapse the forehead on the ground. Fine surface awareness. Collapse the shoulders. Observe all the changes going on, the increased flow of blood into the head and feel the heaviness in the head region. Inhale and chant M-kara, MMM…… Feel the resonance throughout the head, 3D awareness. Slowly come up to Vajrāsana. Carefully follow all the changes in the head region. Feel the lightness in the head. Feel the heart beat, fine 3d awareness throughout the body. Slowly release the arms, place them on the thighs near the knees.

C. UŞTRĀSANA

- Slowly rise up to stand on the knees for Ardhauştrāsana, the back bending camel posture. Standing on the knees, observe all the changes in the head region. Slowly slide the palms up along the thighs, fingers together and support the waist with the palms, fingers pointing forwards. Slowly start bending backwards from the waist. Relax the neck muscles; head hanging freely down. Beautiful stretching of the abdominal and thoracic muscles. This is Ardhauştrāsana. Those who can, go further down to Uştrāsana by placing both the palms on the soles of the feet. Have a beautiful smile on the face. Inhale and chant an A-kāra, AAA…….. Slowly return by releasing the arms and placing them on the waist. Feel the avalanche of nerve impulses throughout the body. Feel the heartbeat. Slowly come back to Vajrāsana and place the palms on the thighs. Feel all the changes and let the changes continue. Fine 3 dimensional awareness throughout the body. Unfold the right leg and the left leg. Assume the leg stretched position. Head hanging freely backward or resting on either of the shoulders.

STEP–VII: DEEP RELAXATION TECHNIQUE (DRT)

- Slowly slide down to Śavāsana with the support of the elbows. Legs apart, hands apart, palms facing the roof. Let the whole body collapse on the ground. Let us make ourselves comfortable and relax completely. We will now go for DRT:

Phase-I

- Bring your awareness to the tip of the toes, gently move your toes and relax. Sensitize the soles and relax, relax your feet, loosen the ankle joints, relax the calf muscles, pull up the knee caps, release and relax, relax your thigh muscles, buttock muscles, loosen the hip joints, relax the pelvic region and the waist region. Totally relax your lower part of the body. **R..e..l..a..x……** Chant A-kāra, AAA……. Feel the vibration in your lower parts of the body.

Phase-II

- Gently bring your awareness to the abdominal region and observe the abdominal movements for a while, relax your abdominal muscles, relax the chest muscles. Gently bring your awareness on your lower back, relax your lower back and loosen all the

vertebral joints one by one. Relax the muscles and nerves around the backbones. Relax your middle back, shoulder blades and upper back muscles, totally relax. Shift your awareness to the tip of the fingers, gently move them a little and sensitize. Relax your fingers one by one. Relax your palms, loosen the wrist joints, relax the forearms, loosen the elbow joints, relax the hind arms-triceps, biceps and relax your shoulders. Shift your awareness to your neck, slowly turn your head to the right and left, again bring back to the center. Relax the muscles and nerves of the neck. Relax your middle part of the body, totally relax. **R..e..l..a..x…….**Chant U-kāra, UUU……. Feel the vibration in the middle part of your body.

Phase-III

- Gently bring your awareness to your head region. Relax your chin, loosen your lower jaw and upper jaw, relax your lower and upper gums, lower and upper teeth and relax your tongue. Relax your palates-hard and soft, relax your throat and vocal chords. Gently shift your awareness to your lips, relax your lower and upper lips. Shift your awareness to your nose, observe your nostrils, and feel the warm air touching the walls of the nostrils as you exhale and feel the cool air touching the walls of the nostrils

as you inhale. Observe for a few seconds and relax your nostrils. Relax your cheek muscles, feel the heaviness of the cheeks and have a beautiful smile on your cheeks. Relax your eye balls muscles, feel the heaviness of eye balls, relax your eye lids, eye brows and the space between the eye brows. Relax your forehead, temple muscles, ears, the sides of the head, back of the head and crown of the head. Relax your head region totally relax. **R..e..l..a..x……** and chant M-kāra, MMM……. Feel the vibration in your head region.

Phase-IV

- Observe your whole body from toes to head and relax, chant AUM in a single breath A……U……M……. Feel the resonance throughout the body.

Phase-V

- Slowly come out of the body consciousness and visualize your body lying on the ground completely collapsed.

Phase-VI

- Imagine the vast beautiful blue sky. The limitless blue sky. Expand your awareness as vast as the blue sky. Merge yourself into the blue sky. You are becoming the blue sky. You are the

blue sky. Enjoy the infinite bliss. **E..N..J..O..Y…** the blissful state of silence and all pervasive awareness.

Phase-VII

- Slowly come back to body consciousness. Inhale deeply. Chant an Om-kāra. Feel the resonance throughout the body. The soothing and massaging effect from toes to head.

Phase-VIII

- Gently move your whole body a little. Feel the lightness, alertness and energy throughout the body. Slowly bring your legs together and the hands by the side of the body. Turn over to the left or the right side and come up when your are ready.

STEP–VIII: CLOSING PRAYER

Sarve bhavantu sukhinah Sarve santu nirāmayāh

Sarve bhadrāni paśyantu Mā kaścit duhkha bhāgbhavet

Om Śāntih Śāntih Śāntih.

Meaning: May all be happy. May all be free from disease. May all see only things auspicious. May none be subject to misery.

26. MIND SOUND RESONANCE TECHNIQUE (M S R T)

STARTING POSITION:

- Sit in any meditative comfortable position or lie down in *Śavāsana* with legs aparts, hands away from the body, head and neck in a very6 convenient position. The whole body is completely collapsed on the ground.
- Let us start the session with the prayer 'Mrtyunjaya Mantra.

Om Trayambakam yajamahe

Sugandhim Pustivaradhanam

Urvarukakamivabndhanat

Mrtyormuksiya 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, 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 vibrationsand 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 sesitivity. 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 quieten 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.

27. PRANIC ENRGISATION TECHNIQUE (P E T)

Please refer to the book "Pranic Energisation Technique", Published by SVYASA

KRIYĀS

NETI (Clearing the nasal passage)

28. JALA NETI

a) STARTING POSITION

- Stand in Tādāsana

b) PRACTICE

- Spread the legs apart.
- Hold the neti pot in your right hand.
- Insert the nozzle of the Neti pot into the right nostril.
- Keep the mouth open to allow free breathing through the mouth.
- Tilt the head first slightly backward, then forwards and sidewards to the left so that the water from the pot enters the right nostril and comes out through the left by gravity. Allow the flow till the pot is empty.
- Repeat the same on the left side.
- Blow out the water accumulated in both the nostrils by active exhalation through alternate nostrils as in Kapālabhāti to clean the nasal passage of the remaining water.

C) NOTE:

- Add about half a teaspoon of salt to a neti pot full of lukewarm water

29. SŪTRA NETI OR RUBBER CATHETER NETI

- Insert the blunt end of a thin soft rubber catheter from the front horizontally in the right nostril.
- Push it along the floor of the nose until the tip is felt in the back of the throat.
- Insert the right index and the middle fingers through the mouth and catch the tip of the catheter at the back of the throat.
- Pull it out through the mouth and gently massage the nasal passage by catching the two ends of the tube.
- Remove the catheter through the nose
- Repeat on the left side.

30. VAMAN DHOUTI

- Sit on heels, and drink luke-warm saline water till you can take no more, or till you feel like vomiting it out.
- Churn the stomach by twisting exercises.

- Stand with feet together and bend the trunk forward forming an angle of about 90 and vomit.
- Now with the help of the middle three fingers tickle the back of the throat to vomit out (vaman) all the water.
- Repeat the process of tickling, till no more water is forth coming, which means that all water has been vomited.
- With continued practice one can stimulate the vomiting sensation and vomit out the water without using the fingers at the throat. Further practice can lead to a continuous vomiting of all the water through mouth as if it is coming in a jet.

c) NOTE:

- This is to be done early morning on an empty stomach.
- Relax completely in Śavāsana for about half an hour(D R T)
- Eat kichadi (rice and dhal boiled together preferably without salt) with about four to six spoonful of pure ghee for breakfast. Lunch can be a normal diet.

APPENDIX II

PSYCHOLOGICAL QUESTIONNNAIRES AND CONSENT FORMS SWAMI VIVEKANANDA YOGA ANUSANDHANA SAMSTHANA

Perceived Stress Scale

Name: ……. Date: …….

Patient No: …… Visit No: ……

Instructions: The questions in this scale ask you about your feelings and thoughts during the last month. In each case, please indicate with a check how often you felt or thought a certain way.

1. In the last month, how often have you been upset because of something that happened unexpectedly?

0	1	2	3	4
Never	Almost	Sometimes	Fairly Often	Very Often

2. In the last month, how often have you felt that you were unable to control the important things in your life?

0	1	2	3	4
Never	Almost	Sometimes	Fairly Often	Very Often

3. In the last month, how often have you felt nervous and "stressed"?

0	1	2	3	4
Never	Almost	Sometimes	Fairly Often	Very Often

4. In the last month, how often have you felt confident about your ability to handle your personal problems?

0	1	2	3	4
Never	Almost	Sometimes	Fairly Often	Very Often

5. In the last month, how often have you felt that things were going your way?

0	1	2	3	4
Never	Almost	Sometimes	Fairly Often	Very Often

6. In the last month, how often have you found that you could not cope with all the things that you had to do?

0	1	2	3	4
Never	Almost	Sometimes	Fairly Often	Very Often

7. In the last month, how often have you been able to control irritations in your life?

0	1	2	3	4
Never	Almost	Sometimes	Fairly Often	Very Often

8. In the last month, how often have you felt that you were on top of things?

0	1	2	3	4
Never	Almost	Sometimes	Fairly Often	Very Often

9. In the last month, how often have you been angered because of things that were outside of your control?

0	1	2	3	4
Never	Almost	Sometimes	Fairly Often	Very Often

10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

0	1	2	3	4
Never	Almost	Sometimes	Fairly Often	Very Often

Psychometric Properties Of Questionnaires

Perceived stress will be measured with the10-item version of the *Perceived Stress Scale* (Cohen, 1988). The Perceived Stress Scale, which was designed for use with community samples, is now the most widely used self-report measure of psychological stress. Participants respond how often during the past month they experienced thoughts and feelings such as "felt that you were unable to control the important things in your life," "felt that things were going your way," "been unable to control irritations in your life."

HOSPITAL ANXIETY AND DEPRESSION SCALE

Name: date:

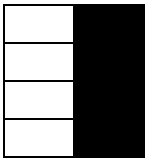
PID No:

Doctors are aware that emotions play an important part in most illnesses if your doctor knows about this feelings he will be able to help you more. This questionnaire is designed to help your doctor to know how you feel. Read each item and place a tick in the box opposite the reply which comes closest to how you have been feeling in the past week.

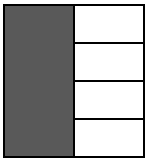
Don’t take too long over your replies. Your immediate reaction to each item will probably be more accurate than a long thought of response.

Tick only one box in each section.

1. I feel tensed or wound up:
   Most of the time.
   A Lot of the time.
   Time to time, Occasionally
   Not at all.

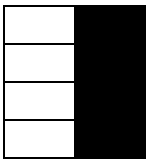


2. I still enjoy the things I used to enjoy:
   Definitely as much.
   Not quite so much.
   Only a little.
   Hardly at all.

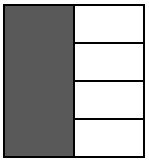


3. I get a sort of frightened feelings as if some thing awful is about to happen:

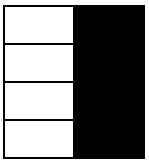
   Very Definitely and quite badly.
   Yes but not too badly.
   A little but it does not worry me.
   Not at all.



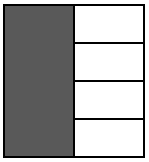
4. I can laugh and see funny side of things:
   As much as I always could.
   Not quite so much now.
   Definitely not so much now.
   Not at all.



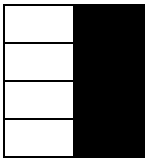
5. Worrying thoughts go through my mind:
   A great deal of the time.
   A lot of the time.
   From time to time but not too often.
   Only occasionally.



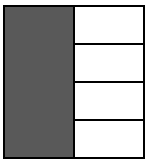
6. I feel cheerful:
   Not at all.
   Not often.
   Sometimes.
   Most of the time.



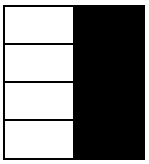
7. I can sit at ease and feel relaxed:
   Definitely.
   Usually.
   Not often.
   Not at all.



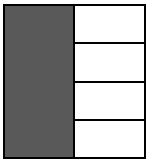
8. I feel as if I am slowed down.
   Nearly all the time.
   Very often.
   Sometimes.
   Not at all.



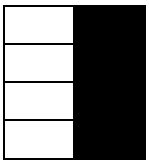
9. I get a sort of frightened feelings like 'butterflies' in the stomach.
   Not at all.
   Occasionally.
   Quite often.
   Very often.



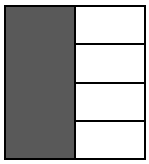
10. I have lost interest in my appearance:
    Definitely.
    Quite a lot.
    I may not take quite as much care.
    I take just as much care as ever.



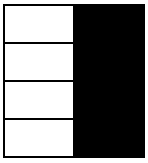
11. I feel restless as if I have to be on the move:
    Very much indeed.
    Quite a lot.
    Not very much.
    Not at all.



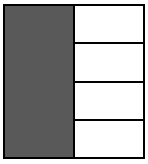
12. I look forward with enjoyment to things:
    As much as ever I did.
    Rather less than I used to.
    Definitely less than I used to.
    Hardly at all.



13. I get sudden feelings of panic:
    Very often indeed.
    Quite often.
    Not very often.
    Not at all.



14. I can enjoy a good book or radio or TV Programme.
    Often.
    Sometimes.
    Not often.
    Very seldom.



Keys: Questions for Anxiety score: 1, 4, and 6,7,8,11,13
Responses graded from 0 to 3.
MAX- 21
MIN- 0 for anxiety subscale.

Questions for depression Score: 2,3,5,9,10,12,14.
Responses graded from 0 to 3.
MAX- 21.
MIN- 0 for depression subscale.

Psychometric Properties Of Questionnaires

Hospital Anxiety and Depression Scale: 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.

INFORMED CONSENT FORM

Project

Evaluation of yoga intervention in modulating genotoxic stress in patients with carcinoma of Breast undergoing radiation and chemotherapy.

IRB Approval: **Date:**

Contact Information:

Drs GopinathK.S/ B.S Ramesh
Bangalore Institute of Oncology
Sampangiramnagar
Bangalore.
Phone- 22225644 / 98.

Dr Vadiraja H.S
Dr Jayashree
Yoga Wellness Center
No-41/14, 2nd cross
Rajarammohanroy extn
Bangalore.Ph: 55313368.

Description of Study: In this research study, we will be looking at effects of radiation induced alterations in the immune cells and also study the relationship between stress and these changes. We also plan to study the effect of yoga on stress and radiation induced alterations in the immune cells. The objective of this study is to assess whether stress such as depression enhances these alterations and if therapies like yoga can reduce them. A better understanding of the relationship between stress, radiation induced alterations in immune cells and yoga will help to improve quality of treatment, quality of life and may improve patient responses to radiation.

You have been selected as a participant based on your cancer diagnosis and because you are receiving radiation and chemotherapy. The total duration of study is eight weeks where you will be asked to attend yoga classes five days a week. If you agree to participate, you will be interviewed at our clinic, and then

asked to complete six surveys to determine your mood, symptoms, level of depression and quality of life.

You will have a 50/50 chance (like flipping a coin) of being place in one of two groups. Neither your doctor nor you will make the choice, so that bias in the study is reduced. The two groups are (a) the Intervention Group or (b) the Control Group. The Intervention group will get the yoga classes at the beginning of the study. The second group will also get the yoga classes, but after 3 months in the study

You will also be asked to give a 8ml blood sample at the start of the study and after eight weeks of practicing yoga/ receiving radiation to assess your genotoxic damage and repair. The blood samples will be collected by a trained laboratory technician after breakfast between 8.00am to 10.00 am by using a sterile needle. You will also be asked to provide saliva samples 3 times/day on 3 consecutive days before and after the study. The samples can be stored in the vials provided and you will have to give the stored vials after 3 days of collection. Collecting the blood sample will take about 10 minutes and filling out the surveys will take about 40 minutes. Therefore, participation in this research study will require approximately 5 hours of your time every week for 3 months. At the end of this research study, you can request a copy of the findings by contacting Dr Vadiraja H.S.

Risks/Benefits to the Participant: There are no significant risks involved with participation in this study. The information you will be asked to discuss will be personal and will require you to reveal information about your mental well being, moods, and how you are coping with cancer. However, you will only be asked to respond with a number or a single word, and will not be asked to elaborate upon responses. You will also be asked to provide a blood sample, which will be collected by trained personnel. The risks of drawing blood include temporary discomfort from the needle stick, bruising, and, rarely, infection. The investigations pertaining to the study and yoga therapy charges will be free during this study. Since these blood tests are expensive, you may benefit financially from participation in this study. You will also have the opportunity to gain knowledge that might improve treatment options available to patients with cancer.

Yoga practices will be introduced in a gentle and slow pace to help you relax. These practices will have no religious connotation nor affect the religious sentimentality of any participant If you have any concerns about the risks or benefits of participating in this study, you can contact Dr Ramesh/Gopinath, Dr Vadiraja H.S or Dr Jayashree at the numbers listed on the previous page.
The place of intervention will be in the premises of BIO.

Incase of Injury to the subject:
If you are injured as a result of being in this study due blood draws or yoga postures, you will be provided all the necessary treatment for injuries arising due to these circumstances. You should contact the study staff or report to the clinical supervisor and in the event of such claim.

Cost and Payments to the Participant: There is no cost for participation in this study. Participation is completely voluntary and no payment will be provided. Also any patient requiring any other form of therapy will be informed of the same and no cost will be born for these from our end. However such participants will be permitted to continue with the treatment program already commenced unless they wish otherwise.

Confidentiality: Information obtained in this study is strictly confidential unless disclosure is required by law. You will be assigned a research number, rather than your name, which will be recorded on the assessments you receive. All data will be secured in a locked filing cabinet. Your name will not be used in the reporting of information in publications or presentations.

Participants Right to withdraw from the Study: You have the right to refuse to participate in this study, the right to withdraw from the study and the right to have your data destroyed at any point during or after the study, without penalty, except in situations that violate state and/or federal law and regulations.

Termination of Participation: My participation in the study may be terminated by the Investigator under such circumstances wherein:

i) The subject fails to adhere to the requirement and regulations put forth in the study.
ii) The subjects default on the treatment or intervention or investigations frequently.

VOLUNTARY CONSENT BY THE PARTICIPANT

Participation in this research project is completely voluntary, and your consent is required before you can participate in this research. If significant new information related to this study becomes available and this information may affect your willingness to participate in this study, Dr Gopinath/Dr Ramesh or Dr Vadiraja H.S will alert you immediately.

I have read this consent form (or it has been read to me) and I fully understand the contents of this document and voluntarily consent to participate or consent to have my child participate. All of my questions concerning this research have been answered. If I have any questions in the future about this study, they will be answered by the investigator listed above or his/her staff. I understand that this consent ends at the conclusion of this study. A copy of this form has been given to me.

Participant's Signature _____ Date: _____

Witnesses Signature _____Date: _____

Counter signed by;

Signature of the staff Clinical Supervisor

APPENDIX III

EXPERIMENTAL PHOTOGRAPHS

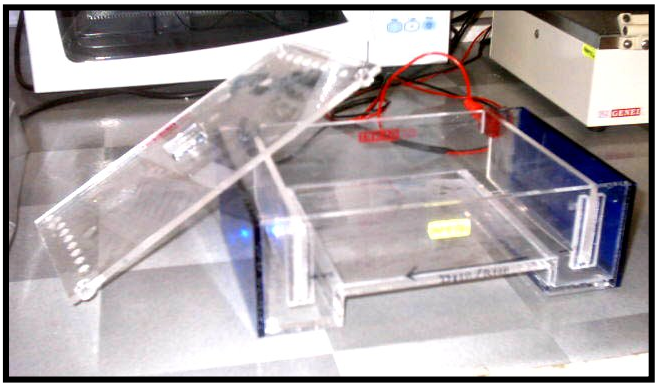


Figure 1: Electrophoresis tank used for single cell gel electrophoresis

Figure 2: Micro centrifuge used for separation of lymphocytes

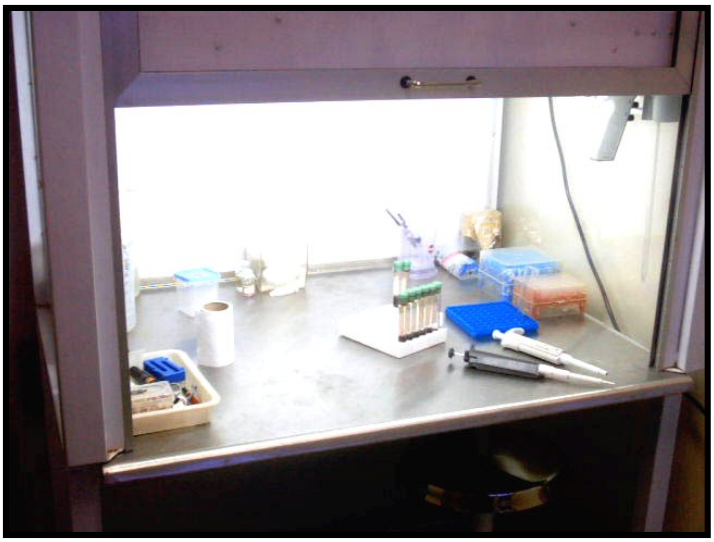


Figure 3: Laminar Air Flow ensures a sterile environment and indirect incandescent lighting conditions

Figure 4: Trinocular Microscope with fluorescent filters used for counting cells stained with Propidium Iodide

Figure 5: Minus twenty refrigerator for micro gel preparation

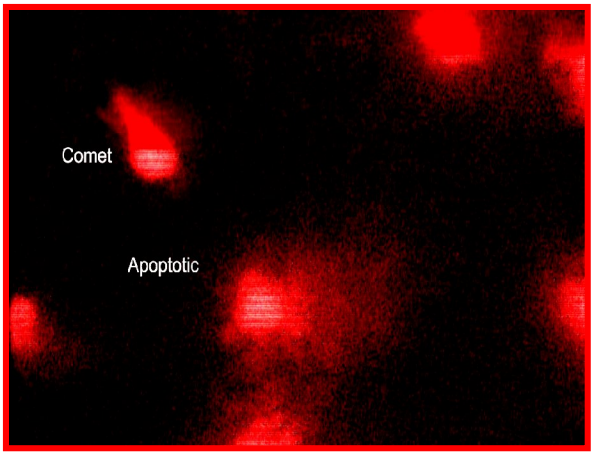


Figure 6: results of comet assay showing all two types of cells Apoptotic cells Comet cell

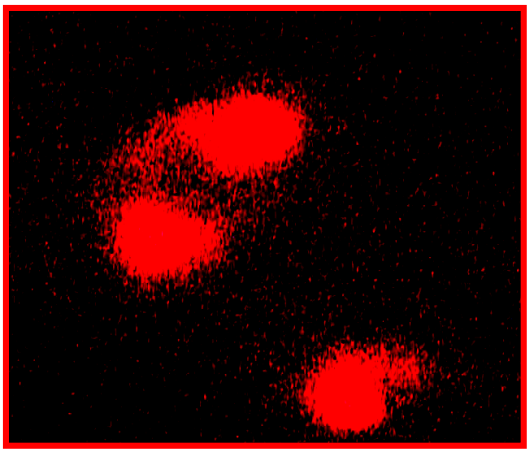


Figure 7: Results of Comet assay showing two normal cells and one comet

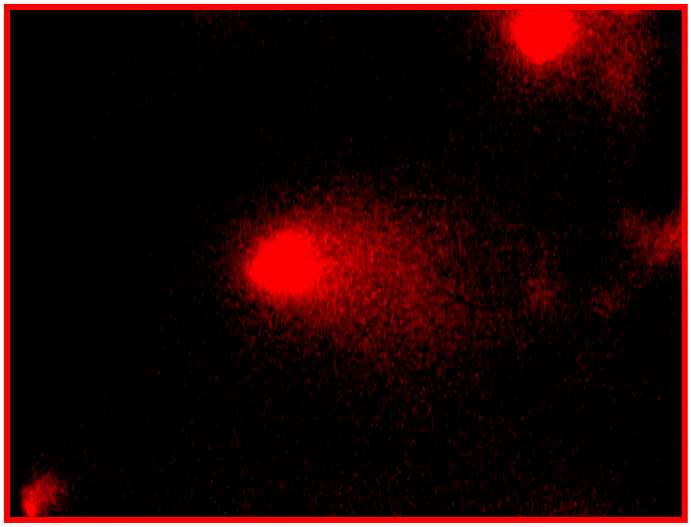


Figure 8: results of Comet Assay showing comet cell with a high DNA damage

APPENDIX IV

PROTOCOLS

COMET ASSAY PROTOCOL

The use of alkaline comet assay with lymphocytes in human bio-monitoring studies Floriane faust et al, mutation research 566,(2004) 209-229

1. Lymphocytes isolated by ficol method
2. Lymphocyte mixed with 1% low melting agar (3:1 mixture)
3. Layered on pre-agar-coated slides
4. Slides are immersed in SDS lysing buffer for two hours
5. Slides are electrophoresed for 45 minutes at 20V
6. Neutralized in neutralizing buffer for 20 minutes
7. Stained with propidium iodide and covered with a cover slip
8. Analyzed under fluorescent microscope
9. The tail of the comets measured using KOMET software
10. The measure of the tail length is directly co-related with the DNA damage

TELOMERIC FISH

Fluorescent in situ hybridization with comets, Santos et al, experimental cell research 232 (1997),(407-411)

1. Lymphocytes isolated by ficol method
2. Lymphocyte mixed with 1% low melting agar (3:1 mixture)
3. Layered on pre-agar-coated slides
4. Slides are immersed in SDS lysing buffer for two hours
5. Slides are electrophoresed for 45 minutes at 20V
6. Neutralized in neutralizing buffer for 20 minutes
7. The telomeric probes for all chromosomes with fluorescent dyes are added to the micro-gels
8. Specific telomeric damages are looked for under the microscope and the image is captured using a FISH image processor.

Neutral Comet Assay

	- [PH = 8]
Lysis Buffer	
1. H_2O	- 150ml
2. EDTA	- 1.674g
3. SDS	- 750mg
4. Proteinase K	- 250µl 1mg/ml
	- [PH = 8]
TBE Buffer	
	- 350ml
1. H_2O	
2. EDTA	- 260.4mg
3. Tris HCl	- 4.956g
4. Boric Acid	- 2.082g
Neutralisation Buffer	
1. Tris HCl	- 1.21g in 25ml of H_2O

Alkaline Comet Assay

Lysis Buffer	
	- 100ml
1. H2O	
2. NaCl	- 14.6g
3. Na2EDTA	- 3.6g
4. Tris	- 0.12g
5. NaOH	- 0.12g

6. Triton X	- 2ml	
Running Buffer		
1. H2O	- 350ml	
2. NaOH	- 4.2g	- 300mM
3. EDTA	- 130.2mg	- 1mM
Neutralisation Buffer		
1. Tris HCl	- 1.21g in 25ml of H2O	

APPENDIX -V RAW DATA TABLES

YOGA GROUP HOSPITAL ANXIETY AND DEPRESSION

Sample	Hads A pre	Hads A post	Hads D Pre	Hads D Post	Total Pre	Total Post
1	9	3	11	5	20	8
2	8	3	7	2	15	5
3	7	1	3	0	8	3
4	10	3	12	1	22	4
5	15	9	13	8	28	17
6	11	6	9	4	20	10
7	10	4	12	3	22	7
8	9	5	7	4	16	9
9	8	5	6	2	13	8
10	11	6	8	3	19	9
11	9	5	4	2	13	7
12	9	5	10	6	19	11
13	12	7	13	5	25	12
14	9	3	10	3	19	6
15	6	4	6	2	12	6
16	0	2	2	0	2	2
17	13	7	6	4	19	11
18	3	0	11	7	14	7
19	12	7	10	6	22	13
20	7	1	6	1	13	2
21	6	4	5	3	11	9
22	5	3	2	1	7	3
23	7	2	8	4	15	6
24	11	5	7	4	18	9
25	8	4	8	2	16	6
26	6	3	4	1	10	4
27	9	3	15	7	24	10
28	9	3	6	4	15	7
29	11	3	10	1	21	4
30	15	5	15	4	30	9
31	7	4	9	5	16	9
32	5	2	3	2	8	4
33	8	7	8	9	16	16
34	5	4	7	0	12	4
35	9	6	11	7	20	13

CONTROL GROUP HOSPITAL ANXIETY AND DEPRESSION

RAW DATA

Sample	Hads A pre	Hads A post	Hads D Pre	Hads D Post	Total Pre	Total Post
1	7	5	7	8	12	15
2	8	13	8	12	16	25
3	10	9	9	11	19	20
4	9	13	10	9	19	22
5	8	9	6	8	14	17
6	11	15	11	14	22	29
7	8	5	7	9	15	14
8	11	15	13	14	24	29
9	7	13	5	6	12	19
10	14	21	12	15	26	36
11	7	10	6	9	13	19
12	4	9	6	5	10	14
13	9	10	8	16	17	26
14	8	7	8	7	15	15
15	8	9	7	6	15	15
16	10	12	7	13	17	25
17	9	15	10	12	19	27
18	8	9	11	13	19	22
19	3	3	3	5	6	8
20	3	7	5	0	12	7
21	11	13	9	10	20	23
22	7	9	4	11	11	20
23	9	12	14	10	23	22

YOGA GROUP PSS SCORE:

Sample Y	PSS PRE	PSS POST
1	19	14
2	17	12
3	11	9
4	29	17
5	21	17
6	25	17
7	20	16
8	23	19
9	18	16
10	17	11
11	18	16
12	21	18
13	18	21
14	21	15
15	16	16
16	7	7
17	14	9
18	24	5
19	31	11
20	23	9
21	22	13
22	22	29
23	13	11
24	16	12
25	23	18
26	28	21
27	28	12
28	24	11
29	24	21
30	29	25
31	21	15
32	18	9
33	15	14
34	18	22
35	17	13

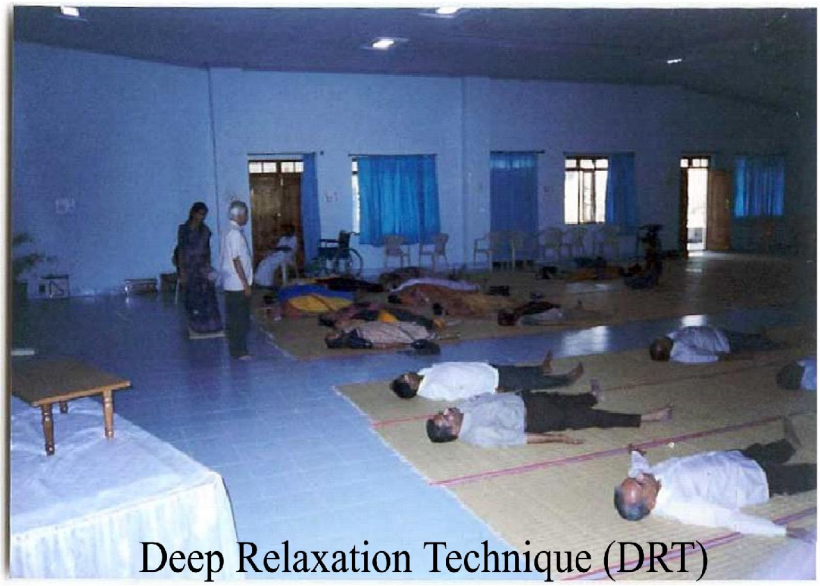
Sample C	PSS Pre	PSS Post
1	12	18
2	17	23
3	30	19
4	22	14
5	11	18
6	21	29
7	17	19
8	21	25
9	12	19
10	27	24
12	18	16
13	10	12
14	27	31
15	19	19
16	18	18
17	19	26
18	22	22
19	21	21
20	14	14
21	16	16
22	22	24
23	21	21

DNA DAMAGE RAW DATA (YOGA GROUP)				
Sample	Number of cells analysed	Comet% Pre	Number of cells analysed	Comet% Post
1	400	4.00	360	23.00
2	450	3.50	450	21.50
3	355	2.20	430	26.00
4	440	3.60	400	24.30
5	400	2.50	330	23.90
6	430	3.20	350	23.00
7	450	2.60	340	24.70
8	450	2.80	400	21.60
9	400	3.40	430	24.30
10	390	2.10	410	24.30
11	450	3.40	445	22.30
12	430	3.10	460	20.40
13	400	2.20	415	23.70
14	400	2.80	400	25.70
15	450	2.40	440	27.00
16	430	1.80	350	24.70
17	435	2.60	355	27.40
18	450	2.20	360	23.50
19	440	2.10	450	26.00
20	350	2.40	430	22.70
21	445	3.40	400	25.70
22	430	2.40	330	23.20
23	400	2.30	350	22.30
24	430	1.85	340	24.50
25	350	2.30	400	25.20
26	375	2.46	430	24.20
27	450	3.30	410	26.50
28	430	2.30	445	26.70
29	340	2.30	460	26.40
30	365	2.20	415	23.40
31	430	2.30	400	24.50
32	400	3.20	440	25.00
33	350	2.10	350	23.60
34	360	2.60	355	23.90
35	410	2.15	390	24.60

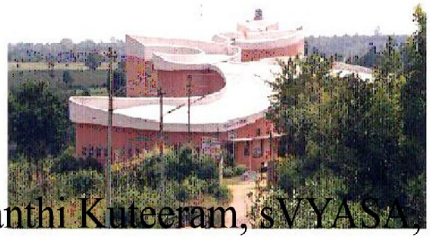
DNA DAMAGE RAW DATA

(YOGA GROUP)

Sample	Number of cells analysed	Comet% Pre	Number of cells analysed	Comet% Post
1	440	3.4	450	29.50
2	350	2.7	430	28.60
3	355	3.5	400	30.20
4	430	2.85	400	30.40
5	350	3.2	450	28.60
6	360	2.9	430	27.70
7	410	2.7	435	30.30
8	330	3.3	450	28.50
9	350	2.75	440	27.80
10	430	3.1	350	29.50
12	350	3.4	445	27.60
13	400	2.85	430	29.40
14	350	2.3	400	27.40
15	340	2.56	430	29.30
16	375	2.74	350	28.50
17	430	2.45	375	29.40
18	350	2.65	450	28.50
19	430	3.2	430	27.90
20	400	2.3	340	28.90
21	350	2.4	365	29.50
22	300	2.15	430	28.75
23	430	2.14	400	27.23



Deep Relaxation Technique (DRT)



Prashanthi Kuteeram, sVYASA, Bangalore

Cancer Patients at Prashanthi

YOGA SESSION IN PROGRESS

Patient at ease with Module

NEVER FORCED

OR

OVER DO

VYASA, Bangalore, India

Collaborating Organizations

Division of Life Sciences
Swami Vivekananda
Yoga Anusandhana
Samsthana, Bangalore,
India

Department of Medical Genetics
Manipal Hospital Bangalore,
India

Department of Surgical
& Radiation Oncology,
Bangalore Institute of Oncology,
Bangalore, India.

Bharath Cancer Hospital
Mysore

VYASA, Bangalore, India