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.)

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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.

![Chart showing age adjusted rate of breast cancer at different centres from 1989 to 2000 for Bangalore, Bombay, Chennai, Delhi, Bhopal, and Barshi.](chart.png)
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.

A. The classical view

![Classical view of telomere structure showing 3’G-rich overhang structure.](image)

B. The new view

![Modern view showing the D-loop and T-loop formation that is thought to stabilize the chromosomal ends.](image)

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).
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 G₀ 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.
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 attrition 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 negatively regulate 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.
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
TRF1 and 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
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. Increasing 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 radiosensitivity (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 onset 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 adrenocorticotropic 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 al 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). Noradrenalin 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 nonopioid 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 heart 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 resurfing, 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 lymphoid 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 al 1992)] 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 blockers (Sanders VM 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 al, 1991 and Madden et al, 1994).

Chemical sympathectomy also increases the severity of experimental allergic encephalomyelitis [Chelmicka et al, 1998] 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 to mitogenic stimulation and lymphocyte production of IgA, and promotes phagocytosis and chemotaxis. (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 neuroendocrine changes since hypophysectomy 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 [Besedovsky 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 immunoregulatory 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 pathophysiological 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 psychophysiological 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 of stress-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.

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

\(^a\) 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.

![Table showing association of multiple variables with the use of different types of CAM among breast cancer survivors](image)

(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.

<table>
<thead>
<tr>
<th></th>
<th>Relaxation Imagery</th>
<th>Spiritual Healing</th>
<th>Yoga</th>
<th>Energy Healing</th>
<th>Acupuncture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>P</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Physical function</td>
<td>80.1</td>
<td>80.1</td>
<td>1.0</td>
<td>77.5</td>
<td>80.5</td>
</tr>
<tr>
<td>Diff (95% CI)</td>
<td>0.0</td>
<td>-2.1</td>
<td>2.1</td>
<td>-3.0</td>
<td>-5.9</td>
</tr>
<tr>
<td>Bodily pain</td>
<td>71.6</td>
<td>72.6</td>
<td>0.4</td>
<td>71.1</td>
<td>72.4</td>
</tr>
<tr>
<td>Diff (95% CI)</td>
<td>-1.0</td>
<td>-3.1</td>
<td>1.2</td>
<td>-1.3</td>
<td>-4.2</td>
</tr>
<tr>
<td>Role-physical</td>
<td>66.6</td>
<td>71.0</td>
<td>*</td>
<td>65.4</td>
<td>70.1</td>
</tr>
<tr>
<td>Diff (95% CI)</td>
<td>-4.5</td>
<td>-8.2</td>
<td>-0.8</td>
<td>-4.6</td>
<td>-9.7</td>
</tr>
<tr>
<td>Vitality</td>
<td>63.6</td>
<td>63.4</td>
<td>0.8</td>
<td>62.3</td>
<td>63.6</td>
</tr>
<tr>
<td>Diff (95% CI)</td>
<td>0.3</td>
<td>-1.7</td>
<td>2.2</td>
<td>-1.3</td>
<td>-4.0</td>
</tr>
<tr>
<td>Social function</td>
<td>84.7</td>
<td>88.2</td>
<td>**</td>
<td>83.8</td>
<td>87.5</td>
</tr>
<tr>
<td>Diff (95% CI)</td>
<td>-3.5</td>
<td>-5.4</td>
<td>-1.5</td>
<td>-3.7</td>
<td>-6.3</td>
</tr>
<tr>
<td>Role-emotional</td>
<td>77.4</td>
<td>81.3</td>
<td>*</td>
<td>76.4</td>
<td>80.5</td>
</tr>
<tr>
<td>Diff (95% CI)</td>
<td>-4.0</td>
<td>-7.0</td>
<td>-0.9</td>
<td>-4.1</td>
<td>-8.2</td>
</tr>
<tr>
<td>Mental health</td>
<td>78.2</td>
<td>80.3</td>
<td>**</td>
<td>78.9</td>
<td>79.7</td>
</tr>
<tr>
<td>Diff (95% CI)</td>
<td>-2.0</td>
<td>-3.4</td>
<td>-0.7</td>
<td>-0.8</td>
<td>-2.6</td>
</tr>
</tbody>
</table>

(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.

<table>
<thead>
<tr>
<th>Selected reasons given for why CAM therapies were used among those using CAM (N = 1249)</th>
</tr>
</thead>
<tbody>
<tr>
<td>“To treat cancer or its symptoms” (N = 338) % of CAM used for cancer or its symptoms (%)</td>
</tr>
<tr>
<td>Relaxation/imagery</td>
</tr>
<tr>
<td>Spiritual healing by others</td>
</tr>
<tr>
<td>Yoga</td>
</tr>
<tr>
<td>Energy healing</td>
</tr>
<tr>
<td>Acupuncture</td>
</tr>
<tr>
<td>Massage</td>
</tr>
<tr>
<td>Chiropractic</td>
</tr>
<tr>
<td>High-dose vitamins</td>
</tr>
<tr>
<td>Herbal therapies</td>
</tr>
<tr>
<td>Homeopathy</td>
</tr>
<tr>
<td>Other therapies</td>
</tr>
</tbody>
</table>

(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
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.

Table 3.25.

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

#### Figure 3.26.

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