CHAPTER 7

DISCUSSION
7.1. Discussion study-1

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

The micronucleus (MN) assay is a sensitive tool to assess radiation induced cytogenetic damage, though there is a variation in the base line frequencies from one laboratory to others. After exposure to mutagenic agents, micronuclei in the cells are derived either from acentric fragments or lagging chromosomes. Micronuclei in cytokinesis-blocked peripheral blood lymphocytes (PBLs) are one of the most reliable biomarkers (indicators) in assessing the chromosome damage induced by ionising radiation or exposure to chemicals. Oppitz *et al.* have shown significant correlations between *in vitro* MN frequency and radio-sensitivity.
The MN analysis has been proved to be an effective tool to quantify radiation damage in both exposed population and also the radio sensitivity of various individuals. In a recent work, Mozdarani 2005 et al. demonstrated that there is an elevated spontaneous frequency of MN in breast cancer group compared to the control group. They also showed that Ca-Breast patients were more sensitive (30%) to ionizing radiation than the age- and sex-matched controls. Scott et al. showed that there is indeed a significant correlation between carcinoma of the breast and increased chromosomal radio-sensitivity. Scott et al. proved that in ataxia telangiectasia patients there is an elevated radio-sensitivity observed in lymphocytes. It is observed in our study that the MN frequencies in carcinoma breast patients had a significant correlation with telomeric damage after radiotherapy. Short telomeres or dysfunctional telomeres may contribute to elevated radiation sensitivity or carcinogen sensitivity (Newman, Banerjee, Hande, 2007). The telomeres play crucial role in detection and repair of DNA damage and radiation insult. The presence of telomere signals in micronuclei might have been the result of telomere breakage and/or dysfunctional telomeres in the lymphocytes of breast cancer patients. There was an attempt made by Acar et al, 2001) to find the chromosomal origin in FISH on MN in acute lymphoblastoid leukaemia patients but they did not report telomere damage pattern. In
another work Norppa et al. tried to find the contents of human micronuclei and reported telomeric signals in some MN population. Based on previous reports and our data, we hypothesise that in CA-breast there is a considerable amount of genomic instability in the lymphocytes with short telomeres. It is also possible that there is abnormal telomere maintenance in a sub-population of lymphocytes which makes them more radiosensitive. Desmaze et al (2004) reported that initially telomere dysfunction and genomic instability contribute to radiation susceptibility. Slijepcevic et al (1998) indicated that interstitial breakpoints in chromosome contain telomeric signal. It is also suggested that telomere maintenance play crucial role in radiation susceptibility and radioresistance. Though we have not studied the fate of these micronuclei with telomere damage, it is tempting to speculate that such telomere loss could lead to chromosome end-to-end fusions or chromosome loss ultimately facilitating cells to undergo apoptosis.

MN analysis of 55 breast cancer patients following radiotherapy demonstrates heterogeneity in the response to radiation among these individuals. This indicates varied radio sensitivity within this population. We speculate that individual response to radiation may differ among the breast cancer patients. This observation highlights the fact that it would be
important to know the radio sensitivity of individual patient while administering the radiotherapy to breast cancer patients. Our data also suggest that telomere damage pattern in micronuclei as detected by *FISH* might indicate the individual radio sensitivity and give a brief idea of genome stability status. It might also give an indication of radio-resistance in stage-variant cancer cells. The varied radio sensitivity of the breast cancer patients and the link between telomere damage and radiation sensitivity provides a frame work for further research that may have an impact in radio-therapeutic strategies in cancer.
Discussion study II:-

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

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

The perceived stress also reduced significantly in the intervention group when compared to the control cohort which is also similar to the findings of Spiegel D et al (1995), Casso D (2004) and Carson J (2007). All though there were few patients in the control population who reported
improvement in their sleep quality and anxiety levels but the depression scale showed increase over the period of the study. The radiation induced DNA damage has been widely studied and reported by many including our previous study. In another work Mozdarani et, al (2005) showed that there is an elevated spontaneous frequency of MN in breast cancer group compared to the control group. They also showed that Ca-Breast patients were more sensitive (30%) to ionizing radiation than the control population age and sex matched. Scott et al (1998-1999) reported that breast cancer patients displayed radiation susceptibility when compared to control; we also reported significant genomic instability in Ca breast patients who underwent radiotherapy. In the current study an effort has been made to compare the radiation induced DNA damage as a genotoxic stress and its correlation with the psychological stress levels of the patients. Alkaline gel electrophoresis technique (comet assay) was used as described by Poonepalli and Hande et, el (2005). Comet assay is a very sensitive tool to study DNA damage (2005). In another study Elizabeth Blackburn et al (2004) reported a significant correlation with telomere length in the PBLs and Psychological stress in controlled study. Recently she again reported a significant correlation with the telomere dysfunction and stress in cardiovascular disease. (Blackburn et al (2006). We also reported a significant correlation between radiation induced DNA damage and
telomere dysfunction in Ca-breast patients. Telomere maintenance is strongly associated with DNA damage and repair as reviewed by Hande (2004). Psychological stress is also associated with faulty DNA repair capacity in the lymphocytes as reported by Kiecolt-Glaser et al (1985). Later Cohen et al (2000) showed reduced DNA repair capacity in anxious students. In our study, it may be speculated that in the intervention group the reduced DNA damage as compared to the control group may be linked to higher psychological stress. The background DNA damage levels in both the control and the intervention group may be associated with the varied dose of chemotherapy and increased levels of anxiety. There is a converging link between the psychological (QOL, Anxiety, depression mood disturbances, perceived stress) (Carlson L.E, 2001, Raghavendra Rao et al 2007) and physiological stress at the molecular level such as Cortisol levels, catecholamines, DNA damage, telomere length and DNA repair capacity) (Scott D 1998, Mozdrani, 2005, Kiecolt-Glasser 1985, Glasser R 1985 E Epel 2004, Elizabeth Blackburn, E. Epel 2006). Homes D et al (2006) reported in a large scale survey of more than 2000 patients undergoing various complementary treatments, yoga is the most effective amongst all CAMs in decreasing anxiety, depression and improving the QOL of breast cancer patients. In the current study we tried to investigate the possible link of stress at the molecular level but a lot of studies
remained to be done in future to substantiate the findings of the above mentioned groups including our study. The limitation of our study is a small cohort of population size as faced by other groups (Carlson 2003, Rosenbaum 2004, Casso D 2004, Carson J W 2007), but in the hospital clinical out patients setting it is difficult to conduct large patient trials with physiological parameters such as DNA damage involved. But large and specific trials in future may prove very effective in deciphering the mechanistic link between the emotional trauma, psychological and physiological stress. In summary our study showed preliminary data to support that stress influence the coping rout at the molecular level. The present study like other groups (Henderson J W 2004, Richardson M.A.2000) can influence yoga based program and supportive counselling for cancer in an out patient setting to improve the efficacy of the conventional treatment modality and benefit the cancer patients overall.