8. Summary and conclusion

8.1   The World Health Organisation (WHO) has labeled breast cancer as the most common cancer in women both in developed and developing countries. The global Health Estimates reported that worldwide over half a million women died in 2011 due to breast cancer and a majority of them occur in less developed countries. The World Cancer Research Fund International (WCRFI) projected 1.7 million new cases of breast cancer diagnosed in 2012 that represent 12% of all new cancer cases and 25% of all cancers in women. India is undergoing a period of dramatic social and economic change.

Breast cancer is the most commonly diagnosed cancer in the world and in India it is next to cervical cancer. The peak age of occurrence of breast cancer in developed countries is above 50 years whereas in India it is above 40 years, a decade earlier than West (Population based cancer reports, 1990-1996). The reasons for the recent observed increase in incidence of breast cancer in the Indian population are not clearly understood but thought to be largely explained by 'westernization' of lifestyles and changes in reproductive behavior. Also, with a large population in India, information of risk factors seems to be limited due to different cultures, diets and geographical variations (Badwe et al, 1990, Gajalakshmi et al, 1998, Mathew et al, 2008, Mesh ram et al, 2009, Pakseresht et al, 2009, Gajalakshmi, et al, 2009 and Lodha et al, 2010).Breast cancer is one of the common cancers among women and the incidence is rising in India also. Several risk factors are associated with its occurrence and in the present study it is seen that all the conventional risk factors contribute to occurrence of breast cancer which is seen to occur a decade earlier in the Indian population when compared to the west.

Chapter 1 explains about life style changes and its complications and methods to overcome these complications. Role of reproductive and non-reproductive risk factors, role of estrogen, role of copper and role of nitric oxide in progression and growth of breast cancer are highlighted in this chapter.

8.2   Chapter 2 narrates the specific aims and objectives. The aim of the present study is to evaluate the risk factors for breast cancer and to study the role of copper, estrogen and nitric oxide in the growth and progression of breast cancer.

The Objectives of the present study are

(i) To evaluate the risk factors for breast cancer in the population being studied.

(ii) To estimate the levels of copper in different stages of breast cancer in pre and post menopausal women.

(iii) To estimate the levels of estrogen in different stages of breast cancer in pre and postmenopausal women.

(iv) To estimate the levels of nitric oxide in different stages of breast cancer in pre and postmenopausal women.

(v) To associate the role of copper,estrogen and nitric oxide in growth and progression of breast cancer in different stages in pre and postmenopausal women.

The Hypothesis stated are (i) There will be a significant association between reproductive, non-reproductive risk factors and family history with the development of breast cancer.

(ii)There will be a significant difference in plasma copper concentration in different stages of breast cancer in pre and postmenopausal women.

(iii) There will be a significant difference in plasma estrogen concentration in different stages of breast cancer in pre and postmenopausal women.

(iv)There will be a significant difference in plasma nitric oxide concentration in different stages of breast cancer in pre and postmenopausal women.

(v) Plasma levels of copper, estrogen and nitric oxide are associated with growth and progression of breast cancer.

This chapter also gives information about ethical considerations

8.3 Chapter 3 gives an elaboration of the available literature on the history of breast cancer, prevalence, signs and symptoms of the breast cancer, pathophysiology, staging of breast cancer as per AJCC, diagnosis of breast cancer, prevention strategies. This chapter also covers the reviews on role of estrogen in breast carcinogenesis, duration of estrogen exposure and its adverse effects, relation between estrogen and nitric oxide in growth and progression of breast cancer. This chapter also covers the role of nitric oxide in angiogenesis, immunity, inflammation, vascular permeability, apoptosis, metastasis, glucose transport, platelets and how nitric oxide is derived from tumour cell and its therapy. This chapter also elaborates on role of copper in breast cancer and also its contradictory role.

8.4 Chapter 4 explains the categorisation of the participants in to two groups (control and cases) the detailed classification is given, along with inclusion and exclusion criteria and evaluation of risk factors both the reproductive and non-reproductive factors. Non reproductive factors like BMI, total fat, visceral fat, subcutaneous fat, skeletal mass were assessed using omaran scale. Statistical analysis done, results are presented in fig 4.1, 4.2, 4.3, 4.5, 4.6, 4.7and 4.8.

8.5 Chapter 5 explains the role of copper in breast cancer and estimation of copper by Erbachem 5x, using randox copper assessing kit by calorimetric method. Statistical analysis done, result showed gradual increase of copper levels in postmenopausal group when compared from controls to stage I, stageII, and stage III breast cancer group. Statistically significant difference was seen when postmenopausal control group was compared with stage III breast cancer. In premenopausal group when control were compared with different stages of breast cancer, there was decrease in stage I and stage II cancer but there was increase in stage III breast cancer. Statistically not significant. There is a strong correlation between nitric oxide and copper when compared with control post (p=0.029) and also when compared all (p=0.04). Some of the details are shown in fig 5.1, 5.2 and 5.3.

8.6 Chapter 6 explains the role of estrogen in progression and growth of breast cancer, details of participants, inclusion and exclusion criteria and estimation of estrogen with Beckman coulter unicelDxl 600, using Access unconjugated Estriol Assay kit. It also covers principle of test and sample collection, statistical analysis given. In the premenopausal group compare to control the various stage showed an increase in estrogen level, and increase was depended on stages, as the stage of breast cancer increased the levels are increased. In the postmenopausal group also when compared to control the various stages showed an increase in estrogen level, and increase was depended on stages, as the stage of breast cancer increased the levels are increased. But statistically significant difference was not observed. However a significant difference was observed between premenopausal stage to postmenopausal stage analysed by unpaired t-test.

In premenopausal group compare to control estrogen levels are increased in all stages of breast cancer. But statistically significant difference was not observed. In postmenopausal group compare to control estrogen levels are increased in all stages of breast cancer and statistically significant difference was observed. There was positive correlation between estrogen

levels and copper levels in premenopausal control group and different stages of breast cancer, but statistically not significant, where as strong positive correlation was seen in postmenopausal   control group and different stages of breast cancer with statistically significant value. Some of the details are presented in fig 6.1, 6.2 and 6.3.

8.7 Chapter 7 explains about the role of nitric oxide in breast cancer, selection of participants, inclusion and exclusion criteria.Estimation of total nitric oxide with BIO-RAD PR 4100 instrument, using Arbor assay kit for detection of total nitric oxide kit by calorimetric method, statistical analysis given. In the premenopausal group compare to control the various stage showed a gradual decrease in nitric oxide level, and decrease was depended on stages, as the stage of breast cancer increased the levels are decreased . In the postmenopausal group also when compared to control the various stages showed a gradual decrease in nitric oxide level, and decrease was depended on stages, as the stage of breast cancer increased the levels are decreased. But statistically significant difference was not observed by (one way Analysis of variance). When control premenopausal was compared with stage III of premenopausal breast cancer there was statistically significant value. There is decrease in nitric oxide levels both in premenopausal and postmenopausal group. But statistically significant value was seen in premenopausal control group to all stages of breast cancer in premenopausal group. Negative correlation is seen in both pre and postmenopausal group but not statistically significant difference is seen. Some of the details are presented in fig 7.1, 7.2 and 7.3.

8.8 The whole research work is summarized in chapter 8. In conclusion there is some role of estrogen, copper and nitric oxide in progression and growth of breast cancer. Based on the hypothesis the findings showed that.
   (a) There was significant association of non-reproductive risk factors with the development of breast cancer.
   (b) There was gradual increase in copper levels in postmenopausal group when compared from controls to stage I, stage II and stage III breast cancer group. Statistically significant difference was seen when postmenopausal control group was compared with stage III breast cancer. In premenopausal group when control were compared with different stages of breast cancer, there was decrease in stage I and stage II cancer but there was increase in stage III breast cancer. But statistically not significant.

(c) In the premenopausal group compare to control the various stage showed an increase in estrogen level, and increase was depended on stages, as the stage of breast cancer increased the levels are increased. In the postmenopausal group also when compared to control the various stages showed an increase in estrogen level and increase was depended on stages, as the stage of breast cancer increased the levels are increased. But statistically significant difference was not observed. However a significant difference was observed between premenopausal groups to postmenopausal group analyzed by unpaired t-test.

(d) In the premenopausal group compare to control the various stage showed a gradual decrease in nitric oxide level, and decrease was depended on stages, as the stage of breast cancer increased the levels are decreased. In the postmenopausal group also when compared to control the various stages showed a gradual decrease in nitric oxide level, and decrease was depended on stages, as the stage of breast cancer increased the levels are decreased. But statistically significant difference was not observed by (one way Analysis of variance). When control premenopausal was compared with 3rd stage of premenopausal breast cancer there was statistically significant value. There is decrease in nitric oxide levels both in premenopausal and postmenopausal group. But statistically significant value was seen in premenopausal control group to all stages of breast cancer in premenopausal group.

(e) Negative correlation is seen in both pre and postmenopausal group but not statistically significant difference is seen.

(f) There was positive correlation between estrogen levels and copper levels in premenopausal control group and different stages of breast cancer, but statistically not significant, where as strong positive correlation was seen in postmenopausal control group and different stages of breast cancer with statistically significant value

There is a strong correlation between nitric oxide and copper when compared with control post (p=0.029) and also when compared all (p=0.04).

This study has shown that breast cancer is caused due to certain risk factors prevalent in the community and that it occurs at an earlier age than West. It also explains the role of hormones and copper in the causation of breast cancer which can be considered to be same as that of the population in general as patients visiting this hospital belong nearly to the same geographical region of the country.

Assessing the complete risk factor profile of women is necessary to intervene early and prevent or decrease the incidence of the disease. Also, it would enable to educate and create awareness among women about the disease. It is necessary to teach and motivate women to perform breast self-examination (BSE) regularly so that no lump in the breast goes unnoticed. However, the limitations of the present study are its small sample size taken from a hospital. A larger sample extended to the community might give a better generalized picture of breast cancer in the community.

The role of estrogen may be different in premenopausal and postmenopausal groups. Menopausal status affects the levels of estrogen and hence the disease causation. The copper may play a positive role in the genesis and progression of breast cancer in postmenopausal group.NO may have an inhibitory effect in breast cancer in both pre and post-menopausal groups. In the present study, it was observed that copper levels are elevated with increasing stage of breast cancer both in premenopausal and postmenopausal women. The increase is more significant in postmenopausal women indicating that the pathogenesis maybe different in the two groups. Also, it is seen that the levels of serum copper are highest in stage 3 of breast cancer and is even more in postmenopausal cases than premenopausal cases. This shows that copper plays a more positive role in advanced stages of cancer as more number of tumour cells is available to take up copper. The precise mechanism responsible for the alterations in trace element levels in breast cancer patients is still unclear and need further evaluation. However, serum Cu levels may be used as a biochemical marker in these patients. Copper levels needed for physiologic functions are lower than those favoured by tumour angiogenesis. It is necessary to deplete Copper to a therapeutic level as shown in earlier studies. This study would help in estimating that level.