8. **Conclusion**

8.1. **Salient findings of the study**

- There was a significant downregulation of E-cadherin in malignant breast tumors in comparison to benign tumors.

- The overall survival was significantly reduced in breast cancer patients lacking E-cadherin expression.

- Among node negative breast cancer patients expressing E-cadherin, there was a significant increase in the overall survival.

- The overall survival was significantly higher in low grade breast cancer patients expressing E-cadherin, supporting its implication as a prognostic marker among the low grade patients.

- There was a decrease in overall survival of younger and older breast cancer patients who do not express E-cadherin.

- Comparison of the prognostic value of E-cadherin with the standard prognostic factors in breast cancer revealed that E-cadherin and NPI are independent prognosticators in breast cancer.

- The expression of P-cadherin was downregulated in node positive breast cancers in comparison with node negative tumors and in invasive tumors in comparison with pre-invasive tumors.

- The overall survival was increased in node negative breast cancer patients expressing P-cadherin.

- The co-expression pattern of E- and P-cadherin is significantly related to overall survival in breast cancer, with the highest survival being shown by patients expressing both the cadherins.

- There was an aberration in beta catenin expression during malignant transformation and nodal metastasis.
Beta catenin could be used as a marker to predict node positivity in breast cancer.

Focal adhesion kinase expression was increased during malignant transformation, supporting its role as an oncogene.

The expression of MMP-9 was increased in cancers which later developed distant metastasis while the expression of TIMP-1 was decreased during malignant transformation and invasion.

The mean MMP-9:TIMP-1 ratio was elevated in invasive breast cancers in comparison with pre-invasive tumors.

The imbalance in co-expression of MMP-9 and TIMP-1 is significantly related to the overall survival of breast cancer patients with the group expressing TIMP-1 but lacking MMP-9 showing the highest survival.

In node negative breast cancer patients, the overall survival was much greater for those expressing uPA than their counterparts.

The expression of VEGF was upregulated in patients who later developed distant metastasis.

P-cadherin, beta catenin, E-selectin and ICAM-1 are significant independent predictors of nodal positivity in breast cancer.

TIMP-1 emerged as the statistically significant predictor of invasiveness of the tumor in breast cancer.

P-cadherin and TIMP-1 could be used as independent prognosticators of recurrence or metastasis in breast cancer.

E-selectin is a predictor of recurrence free survival in node positive breast cancer patients.

8.2. New Hypothetical model for breast cancer metastasis

In the present study, we find that E-cadherin downregulation is a very initial event in the carcinogenesis process and this alteration is more important in oncogenesis than in development of metastasis. Hence loss of cell-cell attachment can be one of the key events in tumor development process. This supports earlier theories that E-cadherin is a tumor suppressor gene. But P-cadherin downregulation occurs significantly during the lymphatic metastasis pathway. The same alteration is not seen during development of local
recurrence/distant metastasis. This supports the theory that the two pathways of metastasis—lymphatic and hematogenous are different.

The beta-catenin downregulation also occurs as an initial event of carcinogenesis and is maintained during lymph node metastasis. Since cadherin-catenin signaling is very important in maintaining cell adhesion, the alteration seen in E-cadherin and beta-catenin during the malignant transformation of breast highlights the hypothesis that this signaling pathway is probably altered in oncogenesis. Impairment of E-Cadherin and beta-Catenin expression is very frequent in early stage cervical cancers, and alterations in the E-Cadherin/beta-Catenin cell adhesion complex are therefore proposed to be involved in the pathogenesis of cervical carcinomas even at their earliest stages (Fadare O, 2005). This is a very interesting point which has to be looked into in detail since reversing the alteration can bring much hope to finding a permanent solution to the dilemma of cancer.

The adhesion signaling molecule FAK is over expressed in malignant cases compared to the benign cases which suggest an early alteration in FAK that can lead to the malignant transformation. Currently, there is no evidence which points that FAK is a classic oncogene and initiates malignant transformation. In the present study, we find that alterations in FAK play a role in the early stages of breast carcinogenesis and hence FAK could be considered as an oncogene in breast cancer. More studies are warranted to look into the definition of FAK as an oncogene not only in breast cancer but also in other cancers.

Proteolytic degradation of extracellular matrix—primarily mediated by MMP-9 is a late event occurring in the development of hematogenous metastasis. Hence MMP-9 mediated lysis of surrounding tissue is important only in the late stages of distant metastasis. Probably other proteinases play a part in the earlier steps of oncogenesis and lymphatic metastasis. On the contrary, TIMP-1, the inhibitor of MMP-9 is downregulated at the early stages of breast cancer and hence the insult in TIMP-1 is probably an early event in breast oncogenesis.

The angiogenic growth factor VEGF is upregulated in patients who later developed distant metastasis. Hence angiogenesis mediated by VEGF is more important in
hematogenous metastasis. Angiogenesis, the establishment of new capillary blood vessels is considered a crucial step in the processes of oncogenesis and lymph node metastasis. In our study, we did not perceive any significant difference in the expression of VEGF during malignant transformation or development of lymph node metastasis. Thus we hypothesise that probably angiogenic factors other than VEGF may play a role in the early steps and the angiogenic switch mediated by VEGF is important in hematogenous metastasis.

There was no significant alteration in the expression of endothelial adhesion molecules studied, viz. VCAM-1, ICAM-1 and E-selectin which are known to be crucial for the intra- and extravasation processes and in overcoming the host anti-tumor defense mechanisms. This implies that once a cell is destined to be malignant/metastatic due to any one of the earlier alterations, the host anti-tumor defense mechanisms mediated by endothelial adhesion molecules do not have much role.

In the light of these findings, we hypothesise a simple model for evolution of breast cancer metastasis. A normal breast cell after undergoing alterations in one or many of the oncogenes or tumor suppressor genes including deregulated expression of E-cadherin and beta catenin, acquires the malignant phenotype. The primary tumor itself contains subpopulations of cells with metastatic ability to lymph node or distant organs. This heterogeneous population of tumor cells differ in their gene expression profile of metastatic proteins. Those cells which have the potential to spread to lymph nodes have downregulated P-cadherin and beta catenin. Those cells which have a genetic alteration resulting in the upregulation of MMP-9 and VEGF are doomed to enter blood circulation and establish metastatic colonies at distant organs.
8.3. Summary & Conclusion

Improvement of current breast cancer diagnostics and therapy, by any means would be invaluable to the suffering of lakhs of patients. With an everrising annual incidence of new cases and deaths each year in the community, breast cancer is the leading malignancy in women and a leading cause of death. The basic understanding of breast cancer initiation and progression is still incomplete. In addition, there is a need to develop improved methods to stratify breast cancer patients into different risk groups more accurately than can be achieved with current
clinicopathologic classification methods. Hence, low-risk patients can be spared unnecessary treatment, avoiding side effects and reducing the cost of treatment. Moreover, high-risk patients could be rapidly identified and offered treatment modalities customized (more aggressive) to individual patients. Newly designed biological therapies aimed at specific tumor cell-associated target molecules could also be devised.

Differences in the size and other characteristics of the studied populations make comparison between different reports difficult. Most of the studied populations are Caucasian (white), but in some American studies, there is also a fraction of black patients [Simpson JF, 2000]. Because populations may differ in prognostic characteristics [Smigal C, 2006], we have decided to stress this point. Thus we present here, for the first time, the prognostic markers that have been newly identified in Indian breast cancer population. Prognosticators evaluating survival in breast cancer vary in significance in respect to lymph node status and hence identifying prognosticators separately in node negative and node positive breast cancer patients is important.

Understanding the biology of cancer metastasis is still unfinished though lot of studies are directed towards the subject. The molecular switches in metastatic progression are also yet to be identified. We could develop a simple model involving the genetic alterations in nodal and distant metastasis after studying the expression of the crucial proteins of the metastatic cascade. We hope that this model could be a humble contribution towards indulgent efforts bound for solving the mystery of the disease.

In the present study, we could identify genes associated with the lymph node and hematogenous metastatic phenotypes of breast cancers. We were also successful in identifying certain new prognostic markers in predicting the overall and recurrence free survival of breast cancer patients. Another important finding was the identification of the prognostic markers in node negative and node positive breast cancers, separately. This is significant since we know that the biology and therapeutic response of the node negative and node positive breast cancers are extremely different. Although our findings need to be confirmed in larger studies with more patients, they provide a useful basis for further investigations to reveal the mechanisms underlying development of these phenotypes.