DISCUSSION
Discussion

The IGF axis is comprised of IGF ligands, IGF binding proteins and IGF receptors. The whole axis is well studied and is ascribed with a number of important biological actions. IGF signaling is known to play important role in cancers of breast, colon, liver, prostate, lung, pancreas, kidney, thyroid, brain, ovary and uterus and hence; is an important drug target. Currently, several epidemiological, clinical and laboratory research methods are being used to investigate novel cancer prevention and treatment strategies related to IGF signaling. IGF signaling may be related to dietary and lifestyle factors that influence cancer risk and cancer prognosis. IGFs differ from the other regulatory peptides that are relevant to cancer because they regulate physiology at the level of entire organism as well as at cellular level.

IGF signaling can be targeted through pharmacological strategies that include the use of novel receptor-specific antibodies and receptor tyrosine kinase inhibitors. Phase III clinical trials have been conducted so far only with IGF-1 receptor-specific antibodies with disappointing results.

Even after an extensive research in the area of growth factors, there are many unclear issues regarding involvement of IGFs in cancer. The studies to date have found inconsistent and inconclusive results on IGF’s effect on cancer as they are derived both from systemic regulation as well as local expression. It is unclear which source of IGFs is more important to tumor growth.

The present prospective, comprehensive study is designed to assess the utility of IGFs in breast cancer prognosis, prediction and therapeutics. Both the ligands (IGFs) and four IGFBPs were studied at the circulatory levels. Moreover, both the ligands, receptors and IGFBP-3 were studied at the transcript level. Several studies incorporated either one or two molecules (IGFs / IGFBP / IGF receptors) singly or in combination.

The rationale behind the inclusion of many molecules in the current study was to assess the entire IGF axis; role of each molecule in breast cancer patients from western India along with the matched controls was evaluated. Circulatory levels were compared with the clinico-pathologic variables of breast cancer with parametric statistics to find out prognostic utility of the growth factors and their binding proteins. Non parametric analysis was also performed for each molecules based on the cut off levels derived from ROC curve analysis.

Moreover, the impact of IGFs in tumor formation and / or promotion and / or in metastatic spread; an unsolved question so far was attempted. The follow-up blood samples were collected at regular intervals and estimation of the molecules was attempted to determine their predictive utility including overall- and relapse- free survival with Kaplan Meier
survival analysis. Efficacy of treatment modality (especially Tamoxifen) as well as interaction of IGFs with ER, PR, and HER2 and their combinations were also studied. Therapeutic utility of these likely targets and their crosstalk with hormone receptors (ER, PR) and Human Epidermal Receptor 2 (HER2) singly as well as in combination were also studied in the current thesis. At the translational level, IGF-1, IGF-2, IGFBP-2, IGFBP-3, IGFBP-4 and IGFBP-6 were studied by immunoassays (RIA/IRMA/ELISA), a fast, easy and convenient method of circulatory biomarker estimation. At the transcript levels, IGF-1, IGF-2, IGFBP-3, IGFR1, IGFR2, ERα, ERβ, PR, HER2 and GAPDH (as housekeeping gene) were estimated from tumors and corresponding adjacent normal tissues (ANT) with absolute quantitation with real time PCR. Copy numbers of each target per μg total RNA were then correlated with the known clinico-pathological parameters as well as positive/negative groups (defined as per ROC cutoffs) and up-/down- regulation (using the expression of tumors vs corresponding ANTs). Further, the expression of all these molecules was attempted from reduction mammoplasty tissue (RMT) and compared with tumors and ANT. Macromastia of the breast is a benign overgrowth of breast tissue and hence, was used to compare expression of the molecules in the tissues obtained at reduction mammoplasty.

Several studies incorporated the circulatory and/or transcript levels of the IGFs but none of them have combined circulatory levels with absolute quantitation of gene expression till date. In addition, circulating levels of IGF signaling molecules have never been evaluated from women of Indian origin. They have been reported in women of diverse origin like Hispanic\textsuperscript{209} and non-hispanic\textsuperscript{421}, Norwegian\textsuperscript{422,423} and Caucasian\textsuperscript{423,424}.

A case control study by Allen et al\textsuperscript{84} found relatively high circulating concentration of IGF-1 and low IGFBP-3 to be connected to increased risk of developing breast cancer. No association of IGF-2 in pre- or post- menopausal breast cancer risk was observed in their study. IGF-1 and age was inversely correlated\textsuperscript{24,84,176}. IGF-1 was positively correlated with IGF-2 and IGFBP-3. IGF-2 was also linearly associated with IGFBP-3 within the control group. They also demonstrated that a relatively higher IGFBP-3 concentration was associated with a reduced risk in pre-menopausal patients but not in post-menopausal women and suggested a protective role of IGFBP-3. In the present study, it was observed that IGF-2 and IGFBP-3 were significantly higher in controls as compared to patients, whereas IGF-1 was non significantly higher in the patients. An inverse correlation was found between IGF-1 and age. Jones et al\textsuperscript{8} suggested that higher IGFBP-3 in controls may offer protection against tumor formation since higher IGFBP-3 is likely to result into reduced bioavailability of IGFs to initiate IGF signaling. Moreover, a similar association was also found in breast cancer patients by them. In addition, IGFBP-3 was shown to inhibit cell growth and induce apoptosis.
of breast cancer cells directly, independent of its effect on IGF-1. Similar finding was evident in a study by Chen et al. in other malignancies. They conducted a systematic meta-analysis of 96 studies in 2009 and inferred that higher IGF-1 levels were associated with an increased risk of pre-menopausal breast cancer, colorectal- and prostate- cancer. They also suggested that estrogen could interact with IGFs and hence IGF-1 might have different roles in pre- and post- menopausal breast cancer. A higher concentration of IGFBP-3 significantly decreases the risk of advanced prostate cancer. Contrary to these findings, no such association of IGF-1 and increased risk of pre-menopausal breast cancer was reported in two studies by Toniolo and Kaaks. Several other studies have also failed to demonstrate an increased breast cancer risk with increasing IGFBP-3 in pre-menopausal women.

Gronbaek et al. in 2004, reported different results. This group studied IGFs in post-menopausal women and the association of IGFs with the ER system. They found no association between increased breast cancer risk and IGF-1 or IGFBP-2 but a positive association with IGFBP-3. They found a positive association of IGFBP-3 and IGF-2 with breast cancer risk on dividing the patients in ER positive and negative subgroups. In the current study, significantly higher IGFBP-2 in breast cancer patients than controls was suggestive of its role in tumor formation. This was contrary to other authors who demonstrated an inverse relation between IGFBP-2 and breast cancer risk in post-menopausal women. Moreover, higher IGF-2 in controls as compared to the patients did not indicate its significant role in breast cancer in the current study. On dividing the patients according to ER status, IGF-1 and IGFBP-3 expressions were higher in ER positive tumors while IGF-2 expression was lower. Moreover, IGF-1 was higher in PR and HER2 positive tumors whereas IGF-2 was higher in ER, PR and HER2 negative groups which suggest that both these growth factors act differently in presence of ER and there might be an important link between IGFs and ER. The method of estimation (IRMA) of IGFBP-3 was similar to the method used by Gronbaek et al. It was suggested that this is an only correct estimate of IGFBP-3 levels in absence of IGFBP-3 proteolysis since immunoradiometric assay measures both intact and fragmented IGFBP-3. IGFBP-3 fragmentation was reduced in patients responding to Tamoxifen treatment, whereas non-responders exhibited increased IGFBP-3 fragmentation. Majority of our patients received tamoxifen in the adjuvant setup with relatively lesser recurrence. IGFBP fragmentation studies therefore are warranted in such patients.

Rollison et al. in 2010 demonstrated that IGF-1 and breast cancer association differed by ethnicity. They found that IGF-1 and IGFBP-3 levels significantly decreased with age in cases and controls and were lower among post-menopausal compared to pre-menopausal
women. This result is similar to the current study. IGF-1 was inversely correlated with age and menopausal status whereas IGFBP-3 was inversely correlated only with age. Further, they reported that higher levels of IGFBP-3 in pre- and post-menopausal women increased the risk of breast cancer where IGF-1 and post-menopausal breast cancer had a significant association. This is somewhat contradictory to other studies that showed an association of IGF-1 with pre-menopausal breast cancer. The possible reasons for such inconsistencies across published studies have been explained by researchers as variations in laboratory assays, environmental and dietary exposures and differences in study populations including the difference in stage at diagnosis and hormonal profiles. In the present study, young age patients had higher levels of IGF-1 as compared to middle age group suggesting a higher risk in them.

Vatten et al reported the breast cancer risk in young women (<50 years) in 2008 and found a modest association between IGF-1 and breast cancer risk. They demonstrated a possibility of use of oral contraceptive that could link IGF-1 levels. Younger age group and pre-menopausal patients lie within the same younger age group and in these groups, IGF-1 as well as IGF-2 showed a higher levels. These patients may have higher risk of developing breast cancer because of a link between higher sex hormone activity (especially estrogens) and IGFs. IGFs can directly interact with estrogens to increase the risk. Moreover, Estrogens can up-regulate IGF-1 receptor; thereby IGF signaling.

Baglietto et al reported that IGF-1, IGFBP-3 and breast cancer risk association varies according to menopausal status and the age dependence of this association. We did not find any association in pre-menopausal patients while a positive association of IGF-1 and IGFBP-3 was seen in post-menopausal (>50 yrs) patients that were similar to the Nurses’ Health Study II. Varying results from positive to negative associations have been available in the literature; positive relation of IGF-1 and IGFBP-3 in pre-menopausal breast cancer risk to no association in post-menopausal patients. Variations in circulating IGF-1 and IGFBP-3 concentrations during menstrual cycle have been suggested while the others do not agree with such results. These authors analyzed the data according to ‘attained age’ at follow-up and found that age at which high hormone concentrations start to get associated with increased breast cancer risk was close to age of menopausal transition for both IGF-1 and IGFBP-3. EPIC (European Prospective Investigation into Cancer and nutrition) study postulated that highest circulating levels of IGF-1 or IGFBP-3 had 40% increased risk of breast cancer after the age of 50 years (post-menopausal status).

Eppler et al measured IGF-1 (using radioimmunoassay) and found that IGF-1 expression was significantly lower in grade III tumors compared to grade I and II tumors. In all
histopathological grades, IGF-1 immunoreactivity increased along with ER and PR level but was inversely related to S phase fraction. In low grade tumors, tumor IGF-1 level was associated with longer survival time. The highest levels of IGF-1 at the transcript and at circulation were seen for well differentiated tumors in the present study. Though other studies did not report significant differences in serum IGF-1 or IGFBP between breast cancer patients and controls matched for age and menopausal status\textsuperscript{441,442}, the current study found differences amongst them. Thus, the role of circulating IGF-1 in breast cancer development continues to remain debatable.

Toropainen et al\textsuperscript{443} obtained contradictory finding; they showed that IGF-1 may have independent prognostic role in the early phase of disease. They found no correlation between ER PR content and IGF-1 immunoreactivity. These authors\textsuperscript{230} also studied IGF-2 expression and showed that high expression was significantly associated with less advanced stage, lower tumor grade, diploid and lower S-phase fraction; further, its expression in the tumor or stromal cells was not related to survival. In the present study, a higher IGF-2 expression was observed in moderately differentiated tumors where as the peptide levels were highest in well differentiated tumors.

Schemhammer et al\textsuperscript{175} (2006) estimated IGFs before diagnosis of breast cancer; evaluated them as the predictors of the disease (not as tumor markers) and found that IGF-1 and IGFBP-3 were positively correlated with each other and IGF-1 was inversely correlated with age. A similar correlation was also observed in the present study. It was demonstrated that IGF-1 or IGFBP-3 did not vary between cases and controls in pre- or post- menopausal women and was inferred that the relation between IGF-1 and breast cancer risk does not differ by the menopausal status. We have also obtained a decreasing trend both for IGF-1 and IGFBP-3 with age. In pre- menopausal women, IGF-1 was not associated with ER negativity/positivity. Seven prospective analyses have evaluated associations between IGFs and pre-menopausal breast cancer risk\textsuperscript{83,84,176,220,427,428,444,445}. Three nested case control studies among pre-menopausal and post-menopausal women\textsuperscript{83,84,427,428} reported a positive association among pre-menopausal women. The discrepancies between these studies can be explained by the lifestyle and nutritional factors, methodological issues, secular changes over time, hormonal status of the woman\textsuperscript{446,447}. Another mechanistic interpretation\textsuperscript{448} of previous studies showing association of breast cancer risk with higher IGF-1 levels lies in higher levels of IGF-1 receptor activation in at-risk mammary epithelial cells. This was consistent with the hypothesis of increased survival of cells with accumulating DNA damage would facilitate stepwise carcinogenesis and lead to a higher proliferation rate of early cancers\textsuperscript{449}. 

300
Schemhammer et al (2005) measured circulatory IGF-1, FIGF-1, IGFBP-1 and IGFBP-3 and showed that IGF-1, IGFBP-3 as well as IGF-1 and Free IGF-1 were strongly and positively correlated with each other. IGF-1 levels and age were inversely correlated. Among pre-menopausal women (<50 years), a significant difference was seen in plasma IGF-1 levels between cases and controls. Higher circulating IGF-1 levels were associated with a higher breast cancer risk. Neither free IGF nor two binding proteins, IGFBP-3 and IGFBP-1, seemed to be associated with breast cancer risk in this cohort. Moreover, no association between IGF-1 and breast cancer risk among post-menopausal women was seen. IGF-1 and breast cancer risk may differed by menopausal status. Similar inter-molecular correlations and correlation with age were observed in the present study. In our study cohort, IGF-1 was higher in patients than controls. However, FIGF-1 was inversely associated with relapse and was significantly higher in the expired patients compared to patients who were alive. Thus, it is suggested that in our cohort, the patients who had higher FIGF-1 were at higher risk of aggressive tumors and higher chances of recurrence and death due to a higher IGF signaling. We did not dichotomize the data according to the menopausal status but the majority of our patients were post-menopausal.

Yu et al in 1998 performed the study from tissue extracts and found that age and ER was inversely correlated with IGFBP-3; showed that it was not associated with the recurrence. An elevated death risk with higher IGFBP-3 levels was seen. This result was different than the present study. Yu et al (1996) described that ER and PR were inversely associated with cytosolic IGF-2, IGFBP-3 which was opposite to the results of the current study. no significant association was seen in HER2 and any IGF molecules except IGFR2. IGFBP-3 was positively correlated to EGFR. The authors also showed that high levels of IGF-2 and IGFBP-3 were related to the unfavorable prognosis in breast cancer. Response of breast cancer cells to tamoxifen treatment was found to be associated with changes in IGFs and IGFBPs; it was shown that IGF-1 levels decreased and IGFBP-3 levels increased in the serum of breast cancer patients after administration of tamoxifen. In animal experiments, tamoxifen was shown to suppress the expression of IGF-1 gene. The association between tamoxifen treatment and IGFs or IGFBPs suggests that the production of IGFs or IGFBPs may be regulated by steroid hormones. The presence of estrogen receptor (ER) or the progesterone receptor (PR) was also found to be associated with levels of both IGFs and IGFBPs in breast cancer cell lines and tissues. An inverse relationship between ER and IGFBP-3 has been reported in a number of studies. In the present study, IGF-1 and IGFBP-3 were highly expressed in ER positive group as compared to ER negative group; showed synergy amongst the hormone receptors and other receptor. Moreover, PR was linearly associated with all the molecules of IGFs. IGF-1 and both receptors were
significantly higher in PR positive tumors than PR negative tumors. Cell culture studies demonstrated that IGFs as mitogens could facilitate the growth of tumor cells and increase the resistance of cells to apoptosis. IGF levels were shown to be reduced in patients who responded well to tamoxifen treatment. It was found that patients with low levels of IGFBP-4 in their tumor had longer disease-free survival, and this association was seen only in patients with large tumors. Other IGFBPs did not show any relationship with survival.

The present study demonstrated significantly higher IGFBP-4 in patients as compared to controls. Moreover, it was significantly higher in all age groups and in all menopausal groups as compared to the controls, in stage II and III disease and in poorly differentiated tumors. However, it was lower in relapsed patients as compared to not relapsed patients suggest that though IGFBP-4 was associated with the poor prognostic features of the breast cancer, the lower levels in stage IV disease and in relapsed patients indicate no participation of these molecule in the disease progression that needs to be elaborated.

Rocha et al (1997) studied IGFBP-3 and IRS-1 level using ELISA and immunoblot respectively. They estimated these proteins from cytosols. They also compared ligand blotting, immunoblotting and IRMA. They found IRMA as the most practical and the most sensitive test to detect variations in IGFBP-3 levels. ELISA kits can be used to measure IGFBP-3 levels in breast tumors as reliably as IRMA kits. IGFBP-3 was negatively associated with age, ER and PR and positively associated with tumor size. These authors also analyzed the survival and found that IGFBP-3 was not associated with poor survival. In the present study, an opposite scene was inferred; IGFBP-3 protein was linearly correlated with ER, PR and inversely associated with tumor size. The inverse correlation with age and no significant association of IGFBP-3 related survival was observed which was similar to the present study.

Pazaitou-Panayiotou et al (2007) measured IGF-1, IGF-2, IGFBP-1, -3, -4, and -6, insulin, leptin and Growth hormone binding protein. They showed a protective role of IGFBP-3 in the post-menopausal women and suggested the beneficial actions of IGFBP-3 which may include anti-proliferative effects and induction of apoptosis, both through sequestration of IGF-1 and IGF-independent effects. It is also possible that cleaved IGFBP-3 fragments may either promote or inhibit tumor growth. They also demonstrated that IGFBP-1, -4, -6 were not significantly associated with the breast cancer risk. Serum IGF-1 appeared to be associated with risk of breast cancer among pre-menopausal women, but no similar association was found amongst post-menopausal women. This can be explained by higher estrogen levels that induce IGF-1 production that causes an increased risk in pre-menopausal women. Moreover, they did not obtain any association between IGF-2 and
breast cancer risk. Similarly, in the current study we have also obtained higher levels of IGF-2 in the controls. IGF-2 was also not associated with relapse. However, evidence from animal and cell line experiments examining the role of IGF-2 in carcinogenesis indicates increased rates of malignancies, including breast tumors\textsuperscript{227,454,456}. Results from a case-control study found a positive association between IGF-2 and ER positive but not ER negative breast cancer among post-menopausal women\textsuperscript{429a}. In the present study, ER\textsubscript{B} was linearly associated with IGF-2 and not associated with ER\textsubscript{A} but the effect was not examined in pre- or post-menopausal women separately due to less numbers. While comparing ER positive and negative groups, the IGF-2 transcript was higher in the negative group. These authors also showed that IGFBP-4 and -6 were directly related to the age and have a significant positive association with each other and inverse association of IGF-1 with age. In the current study, IGFBP-4 and IGFBP-6 were not found to be associated with age or with each other. IGF-1 and age were inversely correlated. Thus, it may be hypothesized here that inverse correlation corresponds to higher IGF-1 levels in younger women; hence they have more chances of breast cancer / recurrence.

Diorio et al\textsuperscript{457} studied different forms of IGFBP-3 in breast cancer patients and demonstrated that all forms of IGFBP-3 were positively correlated with age. They also showed that different forms of IGFBP3 may bear different relations to pre-menopausal breast cancer risk. Goodwin et al\textsuperscript{351} reported that IGFBP-3 was modestly associated with IGF-1 and IGF-2; was also associated with a higher risk of distant metastasis which differs according to the menopausal status. A significant effect was seen in ER positive post-menopausal patients and not in pre-menopausal patients. Higher IGFBP-3 was strongly associated with the tumor grade, with ER and PR negativity and weakly associated with advanced tumor stage and was not associated with survival. Neither IGF-1 nor IGF-2 was associated with recurrence or death. In the present study, IGFBP-3 was found to be inversely correlated with tumor size, linearly associated with histologic type and was not associated with relapse or survival. IGF-1 and FIGF-1 were inversely associated with relapse whereas IGF-1 showed significant linear association with IGF-2 and IGFBP-3. IGFBP-3 mRNA expression in primary breast cancer tissue has been associated with poor prognostic features like ER and PR negativity, aneuploidy, high S-phase fraction\textsuperscript{304,319}. In the present study, IGFBP-3 mRNA was higher in peri-menopausal patients, small tumors, ILC, well differentiated tumors, tumors without vascular invasion and lymphatic infiltration, in tumors with presence of stromal reaction, in not relapsed patients and in alive patients; suggesting a protective role of transcript levels. Circulating IGFBP-3 rises on administration of tamoxifen that enhance the binding of IGFs (with elevated IGFBP-3); this is the mechanism by which tamoxifen exert its beneficial
effects in breast cancer patients. No consistent association of circulating or tumor IGF-1 levels with prognostic factors have been reported\textsuperscript{25,209,458}. Circulating IGF-1 also was not found to influence survival\textsuperscript{225}. Similar results were obtained in the present study that none of the molecules showed significant association with relapse-free or overall survival of patients even when the different combination was applied along with ER, PR, HER2 and combinations of all the three molecules.

Mu et al\textsuperscript{459} performed experiments on breast tumor tissues and measured the levels by real-time RT PCR and ELISA. They suggested that peptide levels of IGF-1, IGFBP-3 and IGF-2 were inversely associated with age at both the levels (peptide and transcript). Total IGF-1 was positively correlated with free IGF-1 and was much higher than free IGF-1. IGFBP-3 protein was lower in receptor positive than negative tumors. IGF-1 peptide either total- or free- was not related to disease progression. Higher IGF-2 protein was associated with relapse of the disease and not with death. Hormone receptors were positively correlated with IGF-1 and IGF-2 expression. Small tumors, early TNM stage or low grades were associated with high mRNA expression of IGFs and IGFBP-3. In the current study, inverse association of age with IGF-1 and IGFBP-3 were observed and IGF-1 levels were much higher than free IGF-1; both were linearly correlated with each other. At the transcript level, copy numbers were compared between the hormone receptor positive and negative subgroups. It was observed that IGFBP-3 was significantly higher in ER positive subgroup as compared to ER negative. Both, FIGF-1 and IGF-1 were inversely correlated with relapse. FIGF-1 was significantly higher in relapsed and expired patients; suggesting a significant role of FIGF-1 in progression and relapse since the free form of IGF-1 is likely to readily bind to the receptors and initiate the signaling. We have also observed a positive correlation of ER with IGF-1 and IGFBP-3 transcript; PR with IGF-1, IGF-2 and IGFBP-3. However, we did not find any difference in IGF-2, disease relapse and death as reported by Mu et al\textsuperscript{459}. It was observed in the current study that early tumors, ILC histologic type and the tumors with lymphatic permeation had higher levels of growth factors. The highest IGF-1 level was seen in well differentiated tumors while IGF-2 was the highest in moderately differentiated tumors. IGFBP-3 was higher in tumors of small size, early stage, ILC histologic type, well differentiated, without vascular permeation, lymphatic infiltration and necrotic tumors. Though these results were statistically non-significant (mostly due to small numbers in each subgroup), it gives the idea on the trend that both the growth factors and IGFBP-3 are likely to be associated with less aggressive features as observed by these authors in their study. These authors concluded that mRNA and protein levels of IGFs may be regulated by different mechanisms; similar to the current study. However, these authors measured the peptide levels from tissues and it was measured from the circulation in the current study. Even though, when the molecules were correlated
between transcript and circulatory levels, they were inversely correlated and confer strength to the hypothesis.

Overall, the role of IGFBP-3 remains inconclusive as reviewed in the literature. Two studies reported higher IGFBP-3 in tumor cells than stromal cells or adjacent normal tissues\textsuperscript{460,461}; two studies indicated association of high IGFBP-3 to poor prognosis\textsuperscript{288,304}. However, one study reported higher IGFBP-3 protein in DCIS than in invasive tumor\textsuperscript{460}; three other studies found no association between IGFBP-3 and breast cancer prognosis\textsuperscript{287,291,462}. Few studies measured IGFBP-3 mRNA in breast tumors and assessed its association with disease characteristics and survival outcomes. Circulating IGFBP-3 is the most studied binding protein in epidemiologic studies with highly different and controversial results.

Vadgama et al\textsuperscript{209} suggested an inverse correlation of age with IGF-1. However, they did not yield any correlation of age with IGFBP-3. Higher IGF-1 in breast cancer patients as compared to controls, no significant difference in IGF-1 levels between node positive and negative patients was found in their study. IGF-1 was significantly higher in pre-menopausal patients as compared to post-menopausal patients. Moreover, it was higher in relapsed patients than not relapsed patients. The IGFBP-3 increased with tumor size and was higher in pre-menopausal patients. They suggested that lower IGF-1 reduces the development of breast cancer and progression. IGF-1 and IGFBP-3 were positively correlated. Tamoxifen treatment decreases IGF-1 levels and reduces the recurrence and increase the survival.

In the present study, it was seen that IGF-1 and IGFBP-3 were inversely correlated with age. IGF-1 was inversely correlated with menopausal status and relapse. It was significantly higher in pre-menopausal patients as compared to post-menopausal patients whereas IGFBP-3 was inversely correlated with tumor size and was non-significantly higher in pre-menopausal patients. Both these molecules were inversely correlated with each other. In addition, we found higher levels of IGF-1 in node positive patients as compared to node negative ones which was opposite to that reported by Vadagama et al\textsuperscript{209}.

Vestey et al\textsuperscript{460} studied IGFBP-3 in breast cancer patients with IHC and found an inverse association with age. They also suggested that IGFBP-3 negativity confer favorable outcome. There were no significant associations between IGFBP-3 and established prognostic indicators in invasive disease (lymph node involvement, increasing tumor size, increasing tumor histological grade, ER negativity [quick-score; 0-8], lymphovascular invasion and NPI). IGFBP-3 was associated with cell proliferation, ER negativity and HER2 over expression. These authors have also showed that IGFBP-3 positive breast cancer patients had lower DFS and OS that did not reach statistical significance. Moreover, it was suggested that IGFBP-3 regulates breast cancer epithelial growth through IGF-dependent and IGF-
independent pathways that involve both growth inhibition and enhanced apoptosis with a potential to switch to growth stimulatory pathways interacting with EGFR, HER2 and fibronectin. Defining these mechanisms demands further 'in vitro' and 'in vivo' studies. In the current study, IGFBP-3 protein and the transcript levels were not found to be significantly associated with poor prognostic features. These authors found a linear correlation of IGFBP-3 with ER and higher levels of IGFBP-3 in ER and HER2 positive subgroup and suggested a synergistic action between them which was similar to the current study. Association of IGFBP-3 with DFS and OS however, was not evident in the present study. Increasing levels of IGFBP-3 in breast cancer tissues correlate with poor prognostic features, as demonstrated in four studies performed using ELISA and IRMA assay. IGFBP-3 is a potential mitogen that interacts with EGFR and HER2 signaling pathways.

Kahan et al proposed circulating IGF-1 levels as a surrogate biomarker for breast cancer risk in premenopausal women. ER status of breast tumors is independent of the concentrations of endogenous estrogens. These authors reported significantly higher plasma IGF-1 in breast cancer cases than controls in the study population. IGFBP-3 levels did not differ. A significant correlation was found between IGF-1 and IGFBP-3. No significant correlations were found between the plasma IGF-1 and sex hormone levels. Positive associations were revealed between IGF-1 concentrations and overall risk of breast cancer. Highest IGF-1 was associated with the risk of ER+PR+ breast cancer and lowest risk was connected to ER+PR− breast tumors. IGFBP-3 remained significant for the ER+PR− subtype, suggesting an independent effect for IGFBP-3. No correlation between IGF-1 levels and any of the sex hormones was seen. In the present study, higher IGF-1 in patients was seen as compared to controls. However, the difference was not significant. There was a linear correlation of IGF-1 and IGFBP-3. IGF-1 was linearly correlated with ER and PR where as the IGFBP-3 was linearly correlated with PR. The highest level of IGF-1 and IGFBP-3 was seen in ER+PR+ breast tumors as compared to ER+PR−.

A high IGF-1 concentration was a marker for breast cancer risk irrespective of menopausal status; one investigation revealed a high breast cancer risk parallel to an elevated IGF-1 concentration in post-menopausal Afro-American women. Since levels of circulating IGF-1 and IGFBP-3 strongly depend on race, lifestyle and diet, the possibility of finding that racial or nutritional factors also play a role in breast tumorigenesis can not be excluded. Results on IGFBP-3 in various studies are in agreement; some showed an increased risk, others showed a decreased risk, still some did not observe any difference in post-menopausal women. Endogeneous estrogens have been linked as significant breast
cancer risk factors in prospective studies\textsuperscript{469,470} but case-control studies displayed a significant heterogeneity\textsuperscript{470}.

The Endogeneous Hormones and Breast Cancer Collaborative \textsuperscript{421} group concluded that the post-menopausal risk of breast cancer was related to the levels of sex hormones\textsuperscript{469}. The same group analyzed the data on pre-diagnostic IGF-1 and IGFBP-3 from 17 prospective studies in 12 countries in 2010 and showed an association of circulating IGF-1 levels to breast cancer risk. Further, this association was not substantially modified by IGFBP-3 and does not differ markedly by menopausal status but seems to be confined to ER positive tumors.

Probst-Hensch\textsuperscript{471} studied IGF-1, IGFR1, IGFBP-2 and IGFBP-3 expression in 855 primary breast carcinomas as measured by immunohistochemistry using tissue microarrays. Majority of patients in this study cohort were post-menopausal and major histologic type was ductal carcinoma which was similar to our study. They showed that tumor IGF-1 expression was positively correlated with IGFR1 and IGFBP-3 expression. In addition, IGFBP-2 and IGFBP-3 expressions were positively correlated with each other and with IGFR1 expression. IGFBP-3 expression was associated with high-grade tumors. ER-positive tumors were more likely to express IGFBP-2, but less likely to express IGFR1 and IGFBP-3. Expression of IGFR 1, IGFBP-2 and IGFBP-3, but not IGF-1 protein, were correlated with ER status. In addition, IGFBP-3 expression was positively correlated with grade, BMI and premenopausal status. Only IGFBP-2 expression was positively associated with overall survival, and only upon adjustment for other prognostic factors including BMI.

In the present study, circulatory IGF-1 was linearly correlated with IGFBP-3 and inversely with IGFBP-2. At the transcript level, IGF-1 mRNA was linearly associated with IGFBP-3. IGFR1 was linearly associated with IGFBP-3. IGFBP-3 mRNA was the highest in peri-menopausal and in well differentiated tumors. ER was linearly associated with IGF-1, IGFR1 and IGFBP-3 transcripts. No association of survival was observed with IGFBP-2 levels in the current study. Some results were similar and some were dissimilar. The interactions observed between IGF and estrogen signaling pathways seem to relate to mitogenic and growth stimulatory effects. In addition, IGF signaling pathways may exhibit estrogen-independent non-mitogenic effects. IGFBP-3, due to its expression in endothelial cells, is increased in highly vascular tumors\textsuperscript{471}. In Shanghai Breast Cancer Study, IGFBP-3 mRNA expression in breast cancer tissue was higher in pre-menopausal as opposed to post-menopausal women\textsuperscript{462}.

The correlations observed in this study between IGF protein expression and sex steroid hormone-related factors (ER status, menopausal status, and obesity) match the experimental data on an intense cross-talk between the IGF system and sex steroids in breast cancer cell
lines and tissues. The relative importance of locally produced versus systemically induced IGF-related proteins remains incompletely understood.\(^{440,472}\)

High IGFBP-2 concentrations in blood or malignant cells and tissues were predictive of poor prognosis in many malignancies including leukemia, brain tumors, as well as cancers of the colon, ovaries, lung, and prostate. Mixed opinions in the literature are available related to IGFBP-2 protein.\(^{473}\) Wang et al\(^{474}\) observed an inverse correlation between IGFBP-2 and ER. They reported tumor IGFBP-2 expression to predict metastases in early stage breast cancer. The largest study on the prognostic role of IGFBP-2 included tumor specimens from 4,186 breast cancer patients showed that IGFBP-2 expression was higher in ER positive tumors, but not associated with overall survival.\(^{473}\) Zhang et al\(^{475}\) recently identified IGFBP-2 as a potent stimulant of hematopoietic stem cell proliferation. None of the protein expressions were strong independent predictors of overall survival in the study population. In the present study, IGFBP-2 protein was higher in old age patients, in all menopausal groups as compared to controls in node negative patients. Moreover, it was increased with the tumor size, linearly correlated with histologic grade but not associated with relapse or death.

A collaborative study by Sakauchi et al\(^{424}\) from Japan (2009) studied serum levels of IGF-1, -2 and IGFBP-3 in a nested case control study. IGF-2 concentration was lower in cases than in controls with marginal significance. In the present study, IGF-1 concentration was not associated with the breast cancer risk. This study supported that circulating IGFBP-3 concentration was inversely associated with the risk among pre-menopausal women. In pre-menopausal Japanese women, IGF-2 showed a marginal negative dose-dependent association with breast cancer risk, although significance disappeared by calculating with IGFBP-3, which was likely to be inversely associated with the risk. In post-menopausal women, IGFBP-3 showed a marginal dose-dependent association with risk. Dissimilar results were seen regarding IGF-2. The current study is also a case control study and it was observed that IGF-2 was significantly higher in controls as compared to patients. Moreover, it was higher in well differentiated tumors, not associated with aggressive characteristics as well as not associated with relapse or death.

A study by Gunter et al\(^{476}\) estimated insulin, glucose, total IGF-1, free IGF-1, IGFBP-3, and estradiol in non diabetic post-menopausal women. They found strong association between breast cancer risk and fasting insulin levels in post-menopausal women who were neither diabetic nor using hormonal therapy. Moreover, insulin and estradiol levels appeared to largely explain an association between obesity and post-menopausal breast cancer. A modest association was found between FIGF-1 level and breast cancer risk among non users of hormonal treatment. Free IGF-1 is supposed to be major bioactive component of circulating
IGF-1. Two previous studies\textsuperscript{428,477} showed that the level of free IGF-1 was more strongly associated with risk of post-menopausal breast cancer than total IGF-1 level.

Katherine Delellis et al\textsuperscript{478} (2004) studied dietary and lifestyle factors and their association in a multi-ethnic cohort. Plasma IGF-1 and IGFBP-3 levels were significantly associated with race, sex, age and body size. A direct association was seen between IGFBP-3 and alcohol consumption in women. Ecological evidence suggest association of IGF-1 and colorectal- but not breast- or prostate- cancer. Circulating IGF-1 in post-menopausal women significantly decreased in Latino women compared with African, American, Native, Hawaiian and Japanese women. We did not study dietary or lifestyle factors but it cannot be denied that they affect levels of IGFs. The only similarity with this study was association of IGF-1 and IGFBP-3 with age.

Keiko et al\textsuperscript{479} studied the expression of IGFBP-4 and IGFBP-5 by quantitative real-time reverse transcriptase-PCR. Expression of estrogen-regulated genes has been considered to provide predictive markers for endocrine therapy since their expression may indicate the presence of a functional estrogen signaling pathway. Amongst >100 estrogen regulated genes, IGFBP-4 is an early-responsive estrogen induced gene, and IGFBP-5, is an estrogen repressed gene. IGFBP-4 appears to be a potent inhibitor of IGF function in several human cell lines. IGFBP-4 mRNA expression was significantly correlated with histological grade. Positive associations were observed between IGFBP-4 mRNA expression and the expressions of ER and PR. An inverse correlation was found between IGFBP-4 mRNA expression and HER2 over expression. No association between IGFBP-4 and -5 mRNA expression and age, menopausal status, tumor size, or lymph node status was seen. High levels of IGFBP-4 mRNA were significantly associated with a reduced risk of recurrence than low levels of IGFBP-4 mRNA. It was demonstrated that IGFBP-4 mRNA expression is an independent prognostic factor of disease-free survival in ER-positive breast cancer. A higher IGFBP-4 mRNA suggests better overall survival than low levels. A recent study showed that IGFBP-4 is one of the key genes to correlate with tamoxifen resistance by gene expression array and IHC tissue micro arrays\textsuperscript{480}. In the present study, the circulatory IGFBP-4 was estimated. It was higher in patients than controls. It was also higher in patients of all age groups and all menopausal status. It was lower in stage IV tumors as compared to stage II and III, was higher in poorly differentiated tumors and in not relapsed patients. Thus, the factors which are not associated with IGFBP-4 as per the study of Keiko et al, almost with all of them were found to be associated in the current study. The higher levels in patients and in poorly differentiated tumors are suggestive of a poor prognostic role of IGFBP-4. On the other
Despite being the ‘gold-standard’ for antihormonal treatment of ERα-positive breast cancer, tamoxifen is still not effective in up to 30% ERα-positive breast tumors as shown by IHC. Extensive research revealed that the tamoxifen resistant breast cancer phenotype may also be due to altered cellular downstream pathways such as those involving IGFR1, ERα, EGFR and HER2. Some studies suggest that IGFR1 expression and activity of IGF axis molecules are important in maintaining a tamoxifen resistant phenotype. Several studies suggested that over expression of EGFR and HER2 in ERα-positive breast cancer cells can lead to resistance to anti-estrogens. Chong et al studied the expression of IGF-1, ERα, EGFR in two different groups; tamoxifen resistant and sensitive ERα-positive tumors by real time PCR. Further, they divided the resistant group in two more groups and compared them with each other as well as the two major groups. They did not find any difference in IGF-1, IGFR1, ERα, EGFR and HER2 mRNA levels between the two tamoxifen resistant groups and significantly lower expression of IGF-1 and ERα mRNA levels in both groups compared to tamoxifen sensitive group. In one of the resistant groups, IGF-1 and ERα mRNA levels were lower as compared to tamoxifen sensitive group. EGFR mRNA levels were higher in the same group compared to tamoxifen sensitive group. IGF-1 strongly correlated with ERα in tamoxifen sensitive group. A strong inverse correlation was seen between IGF-1 and ERα in tamoxifen resistant tumors. Inverse correlation between ERα and EGFR mRNA levels in tamoxifen resistant group was also seen. HER2 did not correlate with any other gene expression in either group. This suggests that IGFR1 expression and activity are maintained in tamoxifen resistant phenotype.

We also divided the patients in tamoxifen responders and non responder groups. Majority of our patients were responders. Amongst these patients, ER positivity and negativity was equally distributed. The effectiveness of ER positivity therefore was hard to assess in the current study cohort. Whereas amongst non responders, ER positivity was higher even though these patients did relapse; thus the ER positivity would increase the effectiveness of tamoxifen is not true for these patients. Moreover, HER2 positivity was also higher in both the groups. Looking at the expression of IGFs, IGF-1 negativity and IGFR1 positivity was
higher in both the groups. Therefore, the differences in ERα and IGFs expression were not found in the current study similar to these authors. Simultaneously, we could not define a reason for the non-responsiveness of tamoxifen. Possibly, the presence of HER2 may affect tamoxifen response in these patients as HER2 positivity was higher in non responders as compared to HER2 negativity which was higher than responder group. Moreover, we did not compare the expression of molecules in terms of copy numbers and have preferred to compare as percent positivity/negativity based on the ROC cutoffs.

This finding was consistent with Pariosot et al. who found that IGFR1 expression in tamoxifen resistant cell lines was up-regulated in response to estrogen. In addition to IGFR1, local tissue IGF-1 expression should also theoretically lead to stimulation of breast cancer cells via IGFR1 activation. ERα expression has long been considered an important factor in determining the response of ERα-positive breast carcinomas to endocrine/anti-estrogen therapy. Down-regulation of ERα expression can contribute to 'de novo' antiestrogen resistance. Several studies have established that over-expression of EGFR and HER2 in ERα-positive breast cancer cells can confer resistance to anti-estrogens. Dowsett et al. found that ERα-positive HER2 positive breast cancers have lower expression of ERα compared to ERα-positive HER2-negative breast carcinomas. In fact, HER2 has been found to be inversely related to ERα in other studies involving breast tumor tissue specimens. In ERα-positive breast cancer, IGF-1 is known to be a mediator of estrogen stimulation and ERα tend to correlate with IGFR1 and IGF-1 expression. ERα expression was a possible determinant of effectiveness to anti-estrogen treatment.

Voskuli et al. measured mRNA of IGF-1, IGF-2, IGFR1 and IGFR2 in breast tumors, adjacent normal tissues as well as from normal breast tissues obtained at the breast reduction surgery (mammoplasty). They compared sporadic and familial breast cancers. The tumors of familial breast cancer showed a higher receptor expression than sporadic breast tumors. A higher relative IGF-2 and IGFR2 expression in normal tissue of familial breast cancer as compared to the normal breast tissues obtained from sporadic breast cancers. These authors suggested that the differences in the expression may attributable to cell type present in the tissue samples. The expression also differed according to proportion of cells present in the tumors or normal epithelial cells; therefore, they compared the tumors and the adjacent normal tissues from same individuals. It was demonstrated a lower IGF-1 and IGF-2 expression where as a higher IGFR1 expression in tumors as compared to adjacent normal tissues. Similar results were obtained in the current study. However, the number of patients in their study was much lower than the current study. We have also assessed IGFs expression from breast tissue obtained at reduction mammoplasty. Expression of IGF-1, IGF-2 and
IGFBP-3 were higher in RMT as compared to tumors and ANT. However, we have not assessed familial breast cancer patients. Our results were still contradictory to this study. Several studies suggest that majority of normal breast specimen as well as tumors express IGF-1. IGF-2 mRNA was found to be expressed in both tumors and normal tissues, although inconsistency remains with respect to level of expression in both these tissue types. Earlier studies also showed lower IGFR1 levels in normal breast tissue as compared to breast tumors. IGFR2, thought to be a tumor suppressor was expressed in majority of breast tumors. Similar results were seen by Voskuli et al and the current study. Higher IGF-1 and IGF-2 levels in normal tissues create an environment which is conducive of stimulating cell proliferation and inhibit apoptosis. These authors have seen lower IGFR2 expression in tumors which contradicted our study. A higher expression in tumors indicates loss of tumor suppressor function of this receptor or the possibility of the mutation in gene which may up regulate expression. Moreover, all IGF molecules estimated in the current study were linearly associated with each other; suggestive of a co-regulation which was similar to the study of Voskuli et al. Additionally, higher protein levels of IGF-1 and IGFR1 in tumor as compared to normal breast tissue were found in most but not all studies using immunohistochemistry, radio immunoassays or binding assays.

The highest expression of both the growth factors (IGF-1 and IGF-2) in the reduction mammoplasty specimens reflect highest production of these factors at the transcript level in benign breast proliferative disorders which was substantiated by significantly reduced levels in ANT followed by the tumor tissue. Such direct observation in the current study is unique and being reported for the first time to the best of our knowledge. On the other hand, IGFBP-3 expression was highest in benign proliferative disease (RMT) followed by tumors and ANT; slightly different than IGFs. It can therefore be hypothesized that IGFs and IGFBP-3 are likely to act differently in breast tumorigenesis at least in the current cohort. For the IGFRs, a direct comparison between the three tissue types yielded similar results as of IGFs with the highest expression of IGFR1 in tumors followed by RMT and ANT strongly suggests a major role of IGFR1 in the breast tumorigenesis in this cohort.

Benjamin et al evaluated the role of free IGF-1 to examine its association with breast cancer. Free IGF-1 levels were compared between 40 newly diagnosed breast cancer patients and 40 age- and race- matched healthy controls. Plasma Free IGF-1, total IGF-1 and IGF-2, as well as total, intact and fragmented IGFBP-3 were measured using commercial immunoassay kits. Free IGF-1 was correlated with total IGF-1 and IGFBP-3 but not with IGF-2. A high ratio of IGF-1:IGFBP-3 was associated with breast cancer. Free IGF-1 levels in plasma were correlated with total IGF-1 levels both, for cases and controls. Free IGF-1
was also correlated with total IGFBP-3 as well as with intact and fragmented IGFBP-3. IGF-2 levels were correlated with total IGF-1 and all three IGFBP-3 variables, but not with free IGF-1. In the present study, free IGF-1 was higher in patients and linearly associated with IGF-1 and IGFBP-3.

Rinaldi et al.\textsuperscript{22} published a large case-control study nested within European Prospective Investigation into Cancer and Nutrition (EPIC). This study included 1081 incident breast cancers and 2098 matched control subjects. Both the molecules were measured by ELISA. Total IGF-1 was inversely correlated with age both in post- and pre-menopausal women. Total IGF-1 concentrations were positively correlated with IGFBP-3 concentrations. Moreover, it was suggested that overall breast cancer risk was increased with increase in IGF-1 and IGFBP-3. In women who had a diagnosis of breast cancer after 50 years of age, there was a direct relationship of breast cancer risk with increasing total IGF-1 and IGFBP-3 concentrations. Similar results were obtained for women who were post-menopausal at blood donation or who were aged 55 or more at blood donation. In this age group, cases had significantly higher mean levels of IGF-1 and IGFBP-3 compared with controls. Thus, among women who provided a blood sample at least two years before cancer diagnosis, increasing levels of IGF-1 were associated with increasing breast cancer risk before the age of 50 as well as after the age of 50. The similar association was found for IGFBP-3. Similarly, in our study, an inverse correlation of IGF-1 with age and linear correlation between IGF-1 and IGFBP-3 was evident. Moreover, a higher IGF-1 was evident in breast cancer patients than controls in the current study; this was similar to Rinaldi et al.\textsuperscript{22}. These authors observed augmentation in breast cancer risk with high IGFBP-3 in women >50 years while in the current study we observed higher IGFBP-3 in controls than breast cancer patients suggesting its protective role.

YUN ning\textsuperscript{49} (2010) studied IGFBP-4 and found that it inhibits IGF action 'in vitro' and 'in vivo'; it lacks the ability to associate with cell surface and it is generally co-expressed with IGF-2 during development. They showed for the first time that IGFBP-4 is required for prenatal growth and that its loss cannot be compensated by other IGFBPs expressed in the embryo. Moreover, it is also required for IGF-2 mediated prenatal and postnatal growth. Severe growth retardation accompanies deletion of PAPP-A (IGFBP-4 protease). Thus, it was concluded that the major role of PAPP-A is to adjust bioavailability of IGF-2 by proteolysis of IGFBP-4, which in itself is needed for normal growth. IGFBP-4 acts to localize and stabilize IGF-2 after secretion from fetal cells and its absence lead to increased IGF-2 degradation or diffusion directing to a growth deficit. IGFBP-4 serves as both positive and negative regulator of IGF activity and its function is critically regulated by PAPP-A 'in vivo'.

\textsuperscript{104}
In the present study, IGFBP-4 was higher in the patients; hence a similar role as of IGFBP-2 may be hypothesized demanding a conformation. Moreover, it was also higher in patients of all age and menopausal groups indicating a higher impact of the clinical factors than the pathological factors. However, in the current study no correlation was observed between IGFBP-4 and IGF-2.

Chong et al\textsuperscript{49} (2011) measured IGF-1 and IGFR1 expression in breast tumors and in adjacent normal tissues by real time PCR. They correlated the levels with clinico-pathology and disease free survival of patients. They found that patients who developed local recurrence or metastasis during the follow-up period had lower levels of IGF-1 mRNA in tumors and ANT compared to those who remained disease-free. However, there was no difference in IGFR1 mRNA levels between those who developed metastasis/recurrence compared to those who remained disease-free. Patients with lower IGF-1 mRNA levels had a shorter DFS compared to those with higher IGF-1 mRNA levels (Comparing tumor tissue and ANT expression). Same results were obtained in ER positive subgroup when patients were stratified according to ER positivity/negativity. Tumor IGFR1 mRNA levels did not predict DFS even when ER\textalpha-positive and ER\textalpha-negative subgroups were analyzed separately. It was demonstrated that histological grade and tumor IGF-1 mRNA levels were statistically independent predictors of DFS.

In the present study, IGF-1 was higher in ANT as compared to tumors. Moreover, there was a trend of IGF lowering as stage increases. IGF-1 was non-significantly higher in not relapsed patients and in alive patients as well as in the well differentiated tumors but showed a significantly inverse correlation with histologic grade. Thus, it was associated with the favorable prognostic features. IGFR1 was significantly higher in tumors. It was non-significantly higher in node positive tumors, showed significant linear association with disease extension and non significantly higher levels in relapsed and expired patients indicating an association of IGFR1 with unfavorable outcome. This study and our results were similar for IGF-1 but not for IGFR1. It was suggested that IGF-1 expression in tumor tissue and ANT should only be used as an intermediate prognostic marker rather than a potential therapeutic target. Two other studies explored similar prognostic potential by measuring IGF-1 mRNA levels only in tumor tissue but not in ANT\textsuperscript{496,497}. IGF-1 and IGFR1 expression showed no association with any of the clinico-pathological features like histological grading, lymph node group, LVI status and NPI. Law et al\textsuperscript{498} measured phosphorylated IGFR1/IR (P-IGFR1/ IR) and its downstream signaling partner phospho-S6 (P-S6) in 438 cases of invasive breast cancer using immunohistochemistry in tumor tissue microarrays. They found that levels of phospho-IGFR1/IR and total IR were associated with
poor survival while total IGFR1 was not. This finding was evident in ERα-positive tumors only and not in ERα-negative ones. This finding reinforces findings in other studies which emphasize the importance of estrogen (via ERα stimulation) which is thought to act as a cofactor to IGF-1 stimulation. Cancer cell line studies showed that IGF-1 is connected with more aggressive, fast growing, metastasizing tumors. Some other studies also suggested that IGF-1 can also increase cell differentiation in certain types of tumor cell lines which are usually associated with less aggressive tumors and hence, improved prognosis. The results of our study and those by Haffner et al496 and Shin et al497 suggest that unlike serum IGF-1 (which can be used as a biomarker of increased risk of secondary breast cancer), breast tumor tissue IGF-1 expression may be used as a marker of reduced secondary breast cancer risk.

Fu et al499 (2011) studied IGFR1 at gene copy number levels, and protein expression. IGFR1 was correlated with clinicopathological characteristics and prognosis was evaluated. These authors suggested that IGFR1 mRNA expression is a good prognostic feature and correlated with protein level. IGFR1 mRNA expression was higher in patients with negative lymph node status, low nuclear grade, hormone receptor positivity, negative HER2 status, ki67 and luminal tumor subtype. Moreover, there was no association between IGFR1, menopausal status and tumor size. This study showed slightly opposite results than the current study. The association of IGFR1 with the disease extension to axillary LNs, higher expression in relapsed and expired patients suggests that it was not a good prognosticator in our study cohort. We did not varify this result at the translational level and therefore, we cannot state it as a poor prognosticator. Several studies have tried to assess the prognostic and predictive value of IGFR1 in breast cancer patients370,372 but the correlation of IGFR1 over-expression with prognosis and clinico-pathologic factors was inconclusive375,500. IGFBP-3 has been reported to have no association with survival and was reported to be independent of other prognostic factors287,288.

Jun Ho Kim et al501 (2009) studied IGFR1, IGFBP-3 and their relation to overall- and relapse free- survival in 460 breast cancer patients using immunohistochemistry. The authors staged the patients with AJCC Staging Manual, 6th edition. IGFR1 expression exhibited a significant correlation with ER, PR and a significant inverse correlation with histological grade, N stage and HER2 over-expression which was similar to our study. Kaplan-Meier survival analyses showed a significantly better overall survival and RFS in IGFR1-positive group compared to IGFR1-negative group. In contrast, IGFBP-3-positive group showed poor overall survival and no difference in RFS compared to IGFBP-3-negative group and it was significantly correlated with HER2 over-expression. This study indicates that IGFR1 was a favorable prognostic factor in breast cancer. In addition, it was associated with tamoxifen-resistance...
and IGFBP-3 expression was associated with poor outcome of breast cancer patients. In the present study, IGFR1 was linearly correlated with disease extension and inversely associated with the histologic grade but higher levels were seen in grade II. We did not find any significant association of IGFR1 with RFS or OS. IGFBP-3 expression had no significant effect on OS or RFS.

Nielsen et al\textsuperscript{502} noted IGFR1 immunostaining in 87\% in breast cancer. They reported that expression of IGFR1 and urokinase plasminogen activator was associated with poor survival. Increased IGFR1 expression in breast cancer specimens was found to inhibit apoptosis and was associated with an increased risk of relapse after radiation therapy. The studies showed by immunoassay that IGFR1 expression was associated with a better overall survival and RFS\textsuperscript{370,373}. During tumorigenesis, over-expression of IGFR1 was presumed to increase cellular responsiveness to IGFs in terms of proliferation and inhibition of apoptosis\textsuperscript{472}. In breast cancer cells, estrogens enhance the mitogenic effect of IGF-1, induce expression of IGF-1 and stimulate production of IGFR1. The interaction between estrogens and IGF is reciprocal. IGF-1 enhances expression of ER in breast cancer cells, and ER levels in breast tissue are correlated with the levels of some IGFBPs\textsuperscript{219}.

Sarakbi W al et al\textsuperscript{503} (2006) selected 31 patients to study relationship between mRNA expression levels of IGF-1 and various clinico-pathological parameters (age, tumor size, grade, vascular invasion, estrogen receptor (ER) status, lymph node status and associated DCIS). They carried out the relative quantitation of IGF-1 and IGFR1. Majority of these patients had poorly differentiated tumors. The relative expression of IGF-1 mRNA was higher in tumors as compared to adjacent non cancerous breast tissue (ANCT); the difference was statistically not significant. A significant association was found between IGF-1 mRNA expression and lymph node status and it was not associated with any other factors. IGFR1 and type of surgery were significantly correlated but none of other factors showed significant association with IGFR1 similar to this study. In our study also, tumors had lower IGF-1 expression than ANT and was associated with the nodal status (N1, N2, N3-disease extension). Contradictory results were seen in case of IGFR1; we found higher expression in the tumors as compared to ANT.

Earlier studies looked into the association between IGF-1/IGFR1 expression and clinico-pathological features of breast cancer with conflicting results. Papa et al\textsuperscript{370} found no correlation between IGFR1 content and a variety of tumor parameters (tumor size, lymph node involvement, grade) and host characteristics (age, body mass index, menopausal status). Other studies, although looked at IGF-1 mRNA expression, failed to examine such relation to other clinical parameters\textsuperscript{490,491,503}. 

316
Chong et al (2006) also measured IGF-1 and IGFR1 expression in the tumors and adjacent non neoplastic tissue by IHC and RT-PCR. Higher IGF-1 mRNA levels in adjacent normal tissue were observed as compared to the tumors similar to our study; suggestive of a paracrine relationship within the local environment of cancerous breast tissue. This study did not find higher tumoral IGFR1 than their adjacent normal tissue while we observed the same in the present study. No positive correlation was observed between IGFR1 IHC (protein) and RT PCR (mRNA).

Gebauer et al used RT-PCR and deduced that IGF-1 was produced predominantly in the stromal component of tumor tissues. Further they found IGF-1 mRNA in tumor tissues but not in tumor cell cultures. Similar results were reported by Yee et al using ‘in situ hybridization’. Some studies have used ‘in situ hybridization’ to localize mRNA expression and found it to be limited to stromal cells. Other investigators demonstrated that it is the stromal-epithelial interaction that plays a central role in malignant progression and metastasis in breast cancer. Studies showed generally higher IGFR1 expression in breast tumor tissues. It is more activated with regard to normal or benign breast tissue. Happerfield et al further mentioned that IGFR1 molecule may be modulated between the cytosol and the cell membrane; cytoplasmic IGFR1 represents bound, internalized receptors. It is possible that, depending on the grade and pathological features of the invasive tumor, there may be a failure to transport IGFR1 to the surface and, hence, it cannot be concluded that the overall production of IGFR1 within the cell always correlates with the IGFR1 mRNA. Many other intracellular signals may modify the final protein product.

Shin et al (2007) quantified IGF-1 and IGFR1 mRNA in both benign and malignant breast tumor tissues and their adjacent normal tissues as well as their association with survival. They compared breast cancer and benign breast diseases (BBD). IGF-1 and IGFR1 expression was reduced in tumor tissues compared to adjacent normal tissues. The expression of these genes was further reduced in tumor tissues from more advanced cancer patients as compared to those obtained from early stage patients. The expression levels of IGF-1 gene were highest in tissues of benign proliferative tumors and lowest in malignant tissues. Although BBD tissue also had a higher expression of IGFR1 gene than cancer tissues, the expression of this gene was similar between proliferative and non-proliferative BBD. Moreover, the patients with a high expression of IGF-1 gene in cancer tissues have a more favorable overall- and disease free- survival. These findings suggest that expression of IGF-1 and IGFR1 genes may undergo substantial change over the course of breast tumorigenesis and the pattern of changes may be associated with breast cancer prognosis. In the present study, breast tumors and paired adjacent normal tissues were compared and found that IGF-1
expression was lower and IGFR1 was higher in malignant tissues. None of the two molecules were associated with DFS or OS. Mizukami et al. reported a higher percentage of IGF-1 stained cells in breast cancer than in benign tumors in a small study using immunochemical assays. Thus, a reduced IGFR1 expression in malignant tissues is supported by some, but not all earlier studies.\textsuperscript{492,500,504}

Peiro et al.\textsuperscript{506} determined the prevalence of IGFR1 in breast carcinoma subtypes and its impact on outcome. They studied 197 consecutive early breast carcinoma patients (stage I–II) treated conservatively. Phenotypic assessment was performed on the basis of expression of ER/PR, HER2, Ki67, p53, Bcl2, CK5/6 and EGFR. Luminal A was the most frequent subtype associated with good prognostic factors and patients' outcome. IGFR1 expression was detected in a higher proportion of luminal A tumors, followed by luminal B and HER2-positive tumors, with the lowest rate in basal/triple-negative tumors. This was in accordance with previous studies who reported co-expression of IGFR1 and ER signaling systems.\textsuperscript{507} The involvement of IGFR1 in luminal A and B tumors, as well as in a subgroup of HER2/HR-positive tumors might be related on one hand to a positive ER/PR cross-talk. On the other hand, its association with the lack of responsiveness to tamoxifen is supported by a poor patient outcome. In contrast, patients with basal/triple-negative tumors developed distant metastasis (and therefore, poor prognosis) more frequently as compared to previous reports. IGFR1 overexpression is not an obligatory need for cellular transformation, but its presence even at low levels is essential for the activation of the main substrate IRS1, which in turn activates the PI3K and Shc/Ras/ERKs pathways. In this study, it was observed that the patients with longer survival had low grade tumors, absence of vascular invasion and necrosis. Nevertheless, IGFR1 may have some relevance in a subgroup of aggressive basal/triple-negative tumors. IGFR1 mutations, only present at Tyr1131 (A3532G), were not associated with tumors that recurred or immunophenotypes; suggesting an unrelated gene event with a specific phenotype.

In the present study, there was less number of triple negative patients (on the basis of ROC cutoffs). We did observe the presence of IGFR1 in such tumors but as the expression was the lowest; these tumors may be good candidates for anti-IGFR1 therapy. Majority of the tumors in the current study cohort were triple positive; this may be the reason why majority of our patients responded to tamoxifen. In this tumor subtype, IGFR1 expression was the highest. Moreover, in the current cohort, histologic grade was inversely correlated with IGFR1 and was higher in moderately differentiated tumors. It was also higher, albeit non significantly, in relapsed and expired patients suggesting a link between higher expression to poor outcome. We did not correlate TNBC patients with survival due to less number.
Fiore et al. (2010) studied IGF-2 mRNA expression in breast cancer. These authors evaluated the impact of IGF-2 mRNA expression on 5-year survival in a group of 68 breast cancer patients and the relevance of other predictive parameters such as tumor size, axillary node status, proliferation index, expression of p53 oncoprotein, ER and PgR. IGF-2 expression had no significant impact on 5-year survival. Majority of the patients had tumors smaller than 2 cm and the survival was not significantly affected by tumor size. They bifurcated the patients in death group (DG) and survivor group (SG). At diagnosis, axillary node metastases were significantly more frequent in DG than SG patients. The 5-year survival rate was significantly higher in patients with no node metastases than in patients with metastatic axillary involvement. ER positivity was not significantly different in SG and DG. The 5-year survival was not significantly affected by ER and PgR expression. In IGF-2-positive group, Ki-67-positive patients had significantly poorer prognosis than Ki-67-negative patients. The 5-year survival rate was significantly higher in p53-negative than in p53-positive patients. While in IGF-2-positive group, p53-positive patients had a significantly poorer prognosis than p53-negative patients. The presence of node metastasis was the only independent factor associated with a significantly increased probability of death in breast cancer patients. IGF-2 is stromal in origin and in situ hybridization studies demonstrated mRNA signals in the stroma of higher number of breast tumors. Previous studies showed that IGF-2 mRNA in breast cancer is positively correlated with PgR and that IGF-2 protein is significantly related to both PgR and p21 oncogene expression. These data suggest that IGF-2 may be involved in the differentiation and proliferation of epithelial cancer cells. Presence of PgR in breast cancer is generally regarded as reflection of an intact estrogenic regulatory pathway and therefore, a valid and reliable marker of tumor sensitivity to hormone therapy. It is known that axilla is the major site of lymph node metastasis from breast cancer and histological analysis of axillary nodes provides a useful guide for prognosis. This study suggests that IGF-2 mRNA may represent an indication of poor prognosis when associated with the factors such as expression of p53 and Ki-67. In the present study, relatively higher expression of IGF-2 mRNA was seen in small sized, node negative tumors and in the patients who relapsed and alive. Moreover, it was lowest in IDC and in tumors with poor differentiation. In addition, a trend of its decrease was seen with an increase in disease stage and increase in stromal intensity. A higher expression was noted in ER PR positive tumors and was linearly associated with PR and with the combination of ER PR. No significant association was observed between the survival and IGF-2 expression.

Kalla et al. examined IGF-2 expression and regulation of anti-apoptotic proteins Bcl-2, Bcl-XL and survival in breast cancer cells from African-American (AA) and Caucasian (CA) women. They compared expression of these proteins in paired breast tissue samples in AA
and CA women by qRT PCR and Western blotting. They studied the impact of pro- and mature IGF-2 forms and IGF-2 siRNA on Bcl-2, Bcl-XL and survivin in women of different ethnicity. This study showed that the pro IGF-2 had a more potent effect than mIGF-2 in up-regulating Bcl-2, Bcl-XL and survivin regardless of estrogen receptor (ER) status. Survivin and Bcl-XL protein levels (like IGF-2), were significantly increased in AA tumors as compared to CA tumors. While comparing IGF-2 expression between AA and CA breast cancer cell lines, the expression was higher in cells originating from AA women with breast carcinoma. Similarly, IGF-2 protein expression was found to be higher in AA tumors as compared to Caucasian (CA) tumors. An increased IGF-2 expression was seen in AA tissue samples correlated with increased BclXL and survivin expression, while CA tissue samples expressed lower levels of IGF-2 and subsequently decreased levels of antiapoptotic proteins were observed in all age groups; pointing towards an important IGF-2 regulatory effect on antiapoptotic proteins 'in vivo'. Overall, IGF-2 expression was significantly higher in AA cell lines and tissue samples when compared to Caucasians. IGF-2 siRNA treatment decreased antiapoptotic protein levels in all cell lines (regardless of ER status). These effects were blocked by the addition of recombinant IGF-2. Of significance, IGF-2 expression and regulation of Bcl-XL and survivin in cell lines correlated with their expression in paired breast tissues. We have also found lower levels of IGF-2 mRNA in Indian patients similar to African-American women.

The same group\textsuperscript{510} carried out experiments for IGFR1 and IGFR2. They measured IGFR1, IGFR2 mRNA, protein expression and IGFR1 phosphorylation in paired breast tissue samples from AA and CA women by Real Time-PCR, western blot analysis, immunohistochemistry and ELISA. IGFR1 was higher in normal tissues of AA as compared to CA women whereas it was similar in tumors and normal tissues of AA women. IGFR1, IRS-1 and Shc phosphorylation was significantly higher in AA tumor samples. Tumors of CA women showed higher levels of IGFR2 as compared to AA tumors. The differential expression of both the receptors may cause higher risk of malignant transformation in young AA women and more aggressive breast cancer phenotype. This finding points towards their possible use as therapeutic target along with IGF-2. Thus, race plays an important role in determining the expression levels of IGF axis. The current study is the first report in Indian women from Western part of the country.

Tamini et al\textsuperscript{511} (2011) performed IGFR1 immunostaining on sections with tissue microarray from 312 women. IGFR1 staining was evaluable in normal TDLUs (terminal duct lobular units); in normal breast tissue from benign breast biopsies and subsequent risk of breast cancer was examined in women enrolled in the Nurses' Health Study. IGFR1 staining
positively correlated to PR status and with subsequent risk of breast cancer. It was found that women whose TDLUs were cytoplasm IGFR1 positive/membrane negative IGFR1 were 15 times more likely to develop subsequent breast cancer than women whose TDLUs were negative both for membranous and cytoplasmic IGFR1 expression. In this study, a higher risk was also shown in BBD women whose normal TDLU epithelium showed a cytoplasmic staining as compared to BBD women whose normal TDLU had a little or no membranous staining. These results raise a possibility that at least in a subset of patients with BBD, normal TDLU staining pattern may provide new opportunities for breast cancer chemoprevention. Moreover, location of IGFR1 staining reflects the activation status of the IGFR1. It was hypothesized that local production of IGFR1 plays an important role in malignant transformation.

Ren et al\textsuperscript{462} (2007) evaluated IGFBP3 mRNA expression in benign and malignant breast tumors and their adjacent normal tissues using real-time quantitative PCR. Tumor tissues had significantly lower IGFBP3 expression than benign tissues. IGFBP3 expression both in tumor and adjacent tissues were higher in patients who had proliferative benign tumors than in those who had non-proliferative benign tumors. Amongst patients with benign breast disease, IGFBP3 expression in the tumor was significantly higher than in adjacent normal tissue. There were no apparent associations of IGFBP3 expression in tumor tissues with either overall- or disease- free survival in a cohort of 521 patients with breast cancer. Tennant and colleagues\textsuperscript{33} compared expression of IGFBP-3 in prostate tissues containing benign epithelium, high-grade prostate intraepithelial neoplasia (PIN), and adenocarcinoma. They reported a significantly increased IGFBP-3 immunoreactivity in PIN regions compared with normal epithelium and a significant decrease in malignant cells. However, IGFBP3 mRNA levels remained virtually unchanged in benign epithelium, PIN, and adenocarcinoma cells, indicating a pre-translational and/or post-translational modification of IGFBP-3. IGFBP3 mRNA level was found to be higher in ER-negative than in ER-positive tumors. In the present study, the tumoral expression was non significantly higher as compared to ANT. It was also significantly higher in ER positive tumors as compared to ER negative tumors. We hypothesize that there may be a role of IGFBP-3 as a promoter at the transcript level; secondly, IGFBP-3 may act as a favorable indicator and the transcripts may have a protecting role.

Sayer et al\textsuperscript{512} (2005) studied IGF-2 gene expression in 109 advanced stage epithelial ovarian cancer. IGF-2 gene expression was 300 fold higher in ovarian tumors as compared to normal ovarian surface epithelial samples. High IGF-2 expression was associated with advanced stage disease at diagnosis, high-grade tumors and sub-optimal surgical cytoreduction.
Further, high IGF-2 gene expression emerged as an independent predictor of poor survival in patients with epithelial ovarian cancer. These observations suggest that IGF-2 is a molecular marker and potential therapeutic target for most aggressive epithelial ovarian cancers. Additionally, IGF-2 protein has been reported to be differentially expressed in gastric cancer cell lines compared to normal gastric cell cultures. Preliminary studies in breast, prostate, hepatocellular, pancreatic, and ovarian cancer cell lines suggest that agents that block the action of IGF-2 may have potential therapeutic utility. In the current study, IGF-2 expression in breast tumors and in adjacent normal tissues was almost similar; further, it was non significantly higher in small sized, node positive, early stage and moderately differentiated tumors. On the other hand, it was higher in relapsed and alive patients and decreased with increase in necrotic cells. Moreover, it was increased in the tumors and also in ANT with increased stromal reactivity. This indicates that the local production of IGF-2 increases with increased stromal cells (since they are produced from the stromal cells) and may have a role in the recurrence. IGF-2 expression is otherwise associated with favorable prognostic features.

Higher expression of IGF-2 in all ER, PR, HER-2 negative tumors and the highest expression in triple negative tumors suggest that in at least this subgroup of patients, IGF-2 is likely to take charge of IGF signaling or IGF-2 might change the biological behavior in absence of these receptors, ultimately leading to an adverse outcome. The cross talk between the hormone receptors, HER2 and IGFR1 is very well documented in literature but the complex interaction amongst all of them was less clear.

Bhargava et al (2011) studied IGFR1 expression in normal breast tissue, benign proliferative breast lesions and malignant breast lesions by IHC and its interaction with ER, PR and HER2. It was suggested that IGFR1 was universally expressed in normal breast tissue with slight to moderate increase in expression in benign and proliferative breast lesions. The hormonally driven lesions show higher expression of IGFR1 as compared to other benign lesions. Similarly, IGFR1 expression or over-expression was predominantly seen in ER positive tumors and lower expression was seen in ER negative tumors. After ligand binding, activated ER complexes bind to estrogen responsive elements in the promoter regions of the target genes that include IGFR1. In presence of unaltered ER pathway, ER ligands can promote cell growth through IGFR1. Further, two downstream pathways phosphorylate the serine residue in AF1 domain of ER. This synergy and crosstalk between ER and IGF system is seen in both normal and malignant breast tissues. Thus, inhibition of two pathways is likely to offer maximum therapeutic benefit.
The 'in vivo' data showed that ER+/HER2+ tumors are relatively resistant to tamoxifen therapy. This study also showed that a significantly larger number of ER+/HER2+ tumors show IGFR1 normal/over-expression as compared to ER+/HER2+ tumors which very rarely show IGFR1 normal/over-expression. In triple-negative tumors, IGFR1 normal/over-expression was seen in approximately one-third to one-half of tumors and may be used as a target for treatment of these tumors that lack any specific target. A consistent presence of IGFR1 in ER+ tumors may explain lower response rate to neoadjuvant chemotherapy in luminal tumors. These results suggest that IGFR1, when normally or over-expressed, provides a more conducive growth environment for the tumor and may be responsible for imparting resistance to chemotherapeutic regimens.

In the current study, the IGFR1 was significantly higher in ER positive group as compared to ER negative group but a lower expression was seen in HER2 positive subgroup than HER2 negative group where as in ER+HER2+ subgroup also, IGFR1 expression was non significantly higher than ER+HER2+. Our results were thus slightly different than these authors. We hypothesized that presence of ER and/or HER2 may enhance IGFR1 expression as it was also higher in ER+HER2+ tumors than ER+HER2+ tumors; while in triple negative tumors, IGFR1 was significantly lower as compared to triple positive tumors. This is again suggestive of enhancement of IGFR1 expression in presence of hormone receptors and growth factor receptor. However, it was not lowest amongst all the groups of combination of three molecules; this suggests that even with lower expression, anti IGFR1 therapy may be the choice of treatment for these aggressive tumors.

Berth et al514 (2003) studied IGFR2 in 42 benign breast diseases (BBD), 61 ‘in situ’ carcinomas (CIS) and 133 invasive carcinomas by immunohistochemistry and computerized image analyzer using specific polyclonal IGY antibodies. M6P/IGFR2 level varied markedly according to different patient samples, but median values and distributions were similar in lesions and normal adjacent glands. IGFR2 level was significantly increased in high grade ductal carcinomas ‘in situ’ (DCIS) and decreased in invasive carcinomas relative to adjacent normal tissue. M6P/IGFR2 protein concentration in invasive breast carcinomas was mostly independent of prognostic parameters: tumor size, histological grade, lymph node (N) invasiveness and estrogen receptor a (ERa) status. A positive association was observed with cathepsin D, progesterone receptor (PgR) and patients aged >60 years. These results do not support the hypothesis of a frequent and early inactivation of M6P/IGFR2 gene in breast cancer. Clinical follow-up of patients might reveal a prognostic value for one of the Cathepsin receptors. In the current study, IGFR2 expression was higher in tumors as compared to ANT. It was linearly correlated with age, lower in relapsed patients and was
significantly higher in patients who were relapse free and alive. Moreover, the lowest levels were seen in IDC and equivocal expression was seen in all grades. It was linearly correlated with ER and PR and with all other IGF axis molecules. Our results suggest that IGFR2 expression was not associated with any of the poor prognosticators and hence probably not a risk factor for breast cancer patients.

Hartog et al515 (2010) studied the effect of IGFR1 expression on prognosis in invasive ductal breast carcinoma (IDC) by IHC in a cohort of 429 breast cancer patients. Associations between IGFR1 expression with clinicopathological parameters, disease free survival (DFS) and breast cancer specific survival (BCSS) were evaluated by multivariate analyses focusing on ER-positive and triple negative IDC (TN-IDC). IHC showed a purely membranous IGFR1 expression that was present in 8%, pure cytoplasmic in 21% and both membranous and cytoplasmic staining in 51% whereas it was present in all 805 total breast cancer cases. Amongst all the clinicopathologic prognosticators, a strong association of ER with (i) cytoplasmic IGFR1 staining, (ii) overall IGF1 staining was evident. Only the cytoplasmic staining was correlated with prolonged DFS and increased BCCS. Further, cytoplasmic IGFR1 staining was associated with favorable prognosis in ER positive tumors while membranous staining in ER negative tumors was related to worse prognosis. IGFR1 was present in 45% triple negative cases and the cytoplasmic staining in these patients was related to unfavorable DFS. Different associations of IGFR1 expression and prognosis in ER-positive versus ER-negative carcinoma were observed in ‘in vitro’ studies also. IGFR1 and ER synergistically stimulate proliferation in breast cancer cell lines, but in absence of ER, IGFR1 activation fails to induce mitogenesis, while the migratory actions were retained. It was assumed that well-differentiated, ER-positive breast cancers retain physiological growth controlled by ER and IGFR1 signaling while in ER-negative carcinomas, IGFR1 expression confers metastatic capacities primarily. Hormone receptor positive invasive ductal breast cancers are treated with adjuvant tamoxifen. As IGFR1 and hormone receptors closely interact, a combination of hormonal therapy with IGFR1 targeted drugs may help block all mitogenic hormonal responses. The modulation/s of IGFR1 system has been concerned with the resistance to endocrine therapies.

Higher IGFR1 expression in ER positive tumors as compared to ER negative tumors demonstrate a synergy between these molecules/pathways in our study whereas a significant lower IGFR1 expression in TNBC was noted as compared to triple positive patients. As mentioned in this study, increased IGFR1 expression may suggest enhancement of metastatic capacities and retaining migratory effect in the absence of ER.
Davison et al\textsuperscript{516} (2011) investigated the importance of IGFs in the proliferation and survival of triple negative breast cancer cells. Estrogen and progesterone receptors, HER2, IGFR1, and insulin receptors were measured by Western blotting. Effects of IGF-1 on proliferation were assessed by DNA quantitation and on cell survival by poly (ADP-ribose) polymerase cleavage. It was demonstrated that there was a considerable variation in expression of IGF receptors but overall expression was similar in triple negative and estrogen responsive cells. Relatively high expression of IGF receptors in triple negative breast cancer cells suggests that IGF signal transduction pathway may be important in controlling cell proliferation and cell survival in this subtype of breast cancer. Moreover, it was clearly shown that IGF-1 increased cell growth of triple negative breast cancer cells and IGF-1 has a significant and, in some cases, a dramatic protective effect against staurosporine induced programmed cell death in TNBC cells. It was observed that IGF-1 increased cell division rather than decrease in cell death. Higher concentrations of downstream effector proteins may compensate low receptor concentrations from TNBC cells to respond to IGF-1. Similarly, growth and survival of triple negative breast cancer cells with very different concentrations of IGFR1 is increased by low IGF-1 concentrations. Sensitivity to IGF-1 stimulated autophosphorylation of IGFR1 is same for cells that express high and low IGFR1 concentrations; but cells that express higher levels of IGFR1 are more sensitive to the activation of downstream signaling molecules and towards the induction of biologic responses.

In the present study, significantly higher IGF-1 expression was evident in triple positive cases and lower in TNBC whereas, IGF-2 was the highest in TNBC suggesting a mitogenic stimulation from IGF2 rather than IGF-1. IGFR1 expression was lowest amongst all the subgroups, possibly connected to absence of ER; yet it remains the only available receptor for therapeutic targeting. Highest IGFR1 expression was seen in triple positive tumors. The same results were seen with IGFR2.

There is considerable overlap between triple-negative tumors, basal-like tumors, and tumors which express cytokeratins 5 and 6. Triple-negative tumors are more prevalent in younger and in black women. They account for 27% of breast cancer cases diagnosed in pre-menopausal African Americans, 25% in younger black British women, and 27% in all indigenous Africans\textsuperscript{516}.

Meit Bjo\textsuperscript{17} mdahl et al\textsuperscript{517} (2005) studied the role of IGF-1 and IGF-2 in lymphangiogenesis. They used IHC, RT-PCR, and Affymetrix Gene Chip microarray analysis. They showed that IGFRs are present in lymphatic endothelium. IGF-1 and IGF-2 act as lymphangiogenic factors and this effect is mediated directly by IGFRs present on lymphatic endothelium. It was also demonstrated that isolated primary lymphatic endothelial cells (LECs) express...
IGFR1 or IGFR2 mRNA and proteins. IGF-1 and IGF-2 were shown to stimulate proliferation and migration of primary human and mouse LECs. Both the growth factors were shown to induce phosphorylation of the protein kinases Akt, Src, and ERK; which are known transducers of cell growth signals in IGF responsive cells. Cell motility of LECs stimulated by IGF-1 or IGF-2 as well as IGF-1 induced lymphangiogenesis 'in vivo' was not affected by soluble VEGFR-3. Taken together, these results indicate that IGF-1 and IGF-2 might directly act on LECs to induce lymphangiogenesis. It was suggested that these growth factors may have indirect effects in the induction of lymphangiogenesis via other growth factor/receptor systems.

In the present study, expression of IGFR1 and IGFR2 was higher in node positive tumors as compared to node negative tumors. Lymphatic permeation was inversely correlated to IGFR1 transcript.

Chakraborty et al\textsuperscript{518a} (2010) studied two different ER+ cell lines [BT474 (HER2 over-expressing, low IGFR1)] and MCF7 (HER2 non over-expressing, high IGFR1) to examine the effect of combinations of IGFR1 antagonists (α-IR3, AG1024) and anti-estrogens (4-hydroxy tamoxifen, fulvestrant). Higher IGFR1 levels were seen in MCF7 cells and lower levels in BT474 cells. In BT474 cells, little or no growth inhibition was observed from IGFR1 antagonists α-IR3 or AG1024, while growth was inhibited in a dose-dependent fashion by ER inhibitors (4HT or Faslodex). In MCF7 cells, growth was inhibited by IGFR1 antagonists AG1024 and α-IR3 as well as ER antagonists 4HT and Faslodex. While combining both inhibitors, enhanced growth inhibition was observed. Studying the effect on cell cycle, anti-estrogens in BT474 cells increase percentage of cells in G1 phase and decrease percentage cells in S phase. IGFR1 antagonists exert similar effects, however the effect was not enhanced when anti-estrogens were added. In MCF7 cells, IGFR1 antagonists individually caused minimal increase in percentage cells in G1 and decrease in S phase, while anti-estrogens had slightly greater effect. Their combinations again caused a minimal enhancement of these effects. In both the cell lines, neither IGFR1 inhibitors nor anti-estrogens as single agents could induce apoptosis whereas the combination induced significant apoptosis. While studying ER transcriptional activity through ERE reporter assay, IGFR1 antagonists enhanced the ability of anti-estrogens to inhibit ER transcriptional activity in BT474 cells but not in MCF7 cells. In BT474 cell line, only the combination (anti-estrogens or IGFR1 inhibitors) showed inhibitory effect on ERK1/2 or AKT activity whereas in MCF7 cells, only IGFR1 antagonists, as single agents, showed partial inhibitory effect; but combination with ER inhibitor (4HT or Faslodex) profoundly inhibited ERK1/2 and AKT activity.
The same authors studied the signaling interactions of IGFR1 and HER2 in breast cancer as well as the effect of combinations of antagonists using same cell lines [BT474 (high HER2, low IGFR1) and MCF7 (low HER2, high IGFR1)]. Little or no growth inhibition from IGFR1 antagonists (α-IR3 or AG1024) in BT474 cell line was seen whereas a dose dependent inhibition was seen with HER2 antagonist (Herceptin or AG825); addition of IGFR1 antagonists significantly enhanced the growth inhibition with Herceptin or AG825 alone. Growth of MCF-7 cells was inhibited by IGFR1 antagonists (AG1024 and α-IR3) but not with HER2 antagonists (Herceptin or AG825) as expected. On addition of IGFR1 antagonists to HER2 antagonists, enhanced growth inhibition was elicited. In addition, synergistic inhibition of soft agar growth was also observed.

In BT474 cells, Herceptin increased percentage of cells in G1 phase and decreased cells in S phase. IGFR1 antagonists exert similar effects and slightly increase the effect of HER2 antagonists. In MCF7 cells, HER2 and IGFR1 antagonists individually caused minimal increase in percentage cells in G1 and decrease in cells in S phase; their combinations caused a small increment. While studying the effect on apoptosis, HER2 antagonists did not induce apoptosis in BT474 cells. They dramatically enhanced the otherwise minimal level of apoptosis induced by IGFR1 antagonists. Similarly, in MCF7 cells, minimal apoptosis is induced by any single drug. However, massive induction of apoptosis was observed with the combination of IGFR1 plus HER2 inhibitors (especially with Herceptin). Co-immunoprecipitation experiments showed that in both the cell lines, inhibition of HER2 phosphorylation with HER2 antagonists was significantly augmented by IGFR1 antagonists; this suggests that IGFR1 contributes to HER2 signaling, and HER2 signaling can be more completely inhibited by combining Herceptin with IGFR1 inhibitor. Similarly, the inhibition of IGFR1 phosphorylation was noted in MCF7 cells when Herceptin was added to IGFR1 inhibitors; this suggests that there exists a cross talk between HER2 and IGFR1 in both these cell lines. Moreover, targeting both receptors gave the maximal inhibition of their downstream extracellular signal-regulated kinase 1/2 and AKT signaling pathways. Such drug combinations should be used to treat the patients in which single agents were likely to be inactive/less active.

Ohtani et al (2009) used MCF-7 breast cancer cells to investigate the effects of anti-IGFR1 mAbs with various epitope specificities on IGFR1 down-regulation and signaling pathways. Despite their differential effect on IGFR1 signaling, all the five mAbs demonstrated down-regulation of IGFR1. Inhibitor experiments indicated that anti-IGFR1 mAbs induced internalization of IGFR1 from clathrin coated-pits. Pretreatment of MCF-7 cells with methylamine substantially reduced the antibody mediated IGFR1 down-regulation, while
MG115 did not. Ubiquitination of IGFR1 did not occur in MCF-7 cells after mAb treatment. These results suggest that anti-IGFR1 antibodies with different epitope-specificities can cause internalization of IGFR1 from clathrin-coated pits and down-regulation via a lysosome-dependent pathway in IGFR1 activation-independent manner. They assumed that these antibodies act through different epitopes and cause internalization and down-regulation via a lysosome-dependent pathway, this was the only reason of differences in the results antibodies and associated with treatment failure.

Yerushalmi et al (2010) studied a large cohort to assess the prognostic impact of IGFR1 expression in patients with early breast cancer and also amongst the breast cancer subtypes. A total 2,871 patients with early breast cancer along with the complete scoring data for IGFR1 and an intrinsic subtype assignment based on immunohistochemistry were studied. These patients were divided in 5 subtypes: (i) Luminal A (ER and/or PR, and HER2- and Ki-67 status; <14%), (ii) Luminal B (ER and/or PR and Her2+ and Ki-67 ≥14%) (iii) Luminal/HER2 (ER+ and/or PR+ and HER2+, regardless of Ki67 status), (iv) HER2 enriched (ER' and PR and HER2+), and (v) Basal-like (ER' and PR and HER2+) and (EGFR+ and/or CK 5/6+). The uni- and multivariate-analysis was performed to define its prognostic impact. While comparing the benign and malignant cases, IGFR1 was highly expressed in the cohort and was associated with improved outcomes. While correlating with other prognostic variables, IGFR1 positivity was associated with ER positivity, HER2 negative status, high Bcl2 score and was weakly correlated with age ≥50 years, lower histology grade and high p27 score. In breast cancer subtype, high IGFR1 was seen in luminal A, B and Luminal/HER2+ patients and it was negative in HER2 enriched and basal like. IGFR1 positivity was associated with superior relapse-free survival, breast cancer specific survival (BCSS) and overall survival (OS). Among Luminal B patients, IGFR1+ conferred an improved BCSS. A trend for superior outcomes was also observed among Luminal/HER2 tumors. An opposite effect was observed in patients with HER2-enriched subtype where IGFR1 positivity conferred a trend of inferior outcomes. These results were also confirmed in the multivariate analysis. IGFR1 was a good prognostic marker for Lumina B subtype and a bad prognostic marker for HER2-enriched patients. In our study we have observed a higher expression of IGFR1 and not IGFR2 in HER-2 positive tumors. This subgroup therefore is most likely to benefit by the addition of IGFR1 targeted therapies.

In the present study, a trend of lower IGFR1 with advancing age was observed. IGFR1 was linearly correlated with nodal status and inversely associated with histologic grade. Higher IGFR1 expression was connected to higher relapse and death. Moreover, IGFR1 had a linear correlation with ER and not with HER2; Higher IGFR1 copy numbers were seen in ER
positive and HRE2 negative groups as compared to ER negative and HER2 positive patients. It may therefore be hypothesized that the patients with such gene expression patterns are more likely to benefit with antiestrogen + IGFR1 antagonist rather than HER2 antagonist + IGFR1 antagonist. Further studies are indicated to confirm the hypothesis.

Cui et al\textsuperscript{21} (2003) studied the interactions of the growth factor signaling and estrogen and mechanisms for growth factor regulation of PR using IGF-1 and MCF-7 cells in breast cancer. IGF-1 lowers PR levels in breast cancer cells. IGF-1 repression of PR transcriptional activity occurs through down-regulation of PR in MCF-7 cells. HRG and EGF at similar concentrations as of IGF-1 also markedly down-regulated PR. Insulin required 100-fold higher concentrations than IGF-1 to achieve the same PR degree of reduction. This suggests that the reduction of PR expression by IGF-1 is a common effect shared by other growth factors in MCF-7 cells. IGF-1 does not lower PR protein stability consistent with the results from proteasome inhibitor assay. IGF-1 down-regulates PR mRNA transcription and is dependent upon the original PR promoter context. The data demonstrate that PI3K/Akt/mTOR pathway uniquely mediates alteration of PR expression elicited by IGF-1 in breast cancer cells. These data also indicate that IGF-1 regulates ER activity and negates ER dependent PR levels; IGF-1 down-regulation of PR is independent of ER levels. Collectively, low PR levels may serve as an indicator of activated growth factor signaling in breast tumor cells and therefore point towards an aggressive tumor phenotype and resistance to hormonal therapy.

Kibbey et al\textsuperscript{52} (2006) reported that recombinant human IGFBP-2 significantly attenuated IGF-1 stimulated MCF-7 cell proliferation with addition of 20 or 100nM IGFBP-2. They provided initial evidence that the region downstream of the CWCV motif of IGFBP-2 may contribute to high-affinity IGF-1 binding activity. The binding analysis with IGFBP-2\textsubscript{1-248} exhibited and ~20-fold reduction in binding activity compared to intact IGFBP-2 (EC\textsubscript{50} = 0.35 nM). IGFBP-2\textsubscript{1-190} has the same property as IGFBP-2\textsubscript{1-248}. The finding that IGFBP-2\textsubscript{1-248} and IGFBP-2\textsubscript{1-190} reduced abilities to inhibit IGF-1 binding to IGFR1 on cells compared to full-length IGFBP-2. Kinetic studies indicated that the region downstream of CWCV motif (249–289 a.a.) provides stability to IGFBP-2/IGF-1 complex, accounting for the slow dissociation rates. It was also suggested that the region upstream of the CWCV motif is important for maintaining IGF-1 binding and for contributing to binding efficacy. IGFBP-2\textsubscript{1-190} combined with the cell binding data suggest that the region upstream of the CWCV motif (residues 191-248) contribute to blocking IGF-1 from binding to IGFR1. Deletion of the entire C-terminal domain significantly reduces the ability to block IGF-1 action. It was inferred from these results that binding of different IGFBPs at different motifs by IGF-1 and IGF-2 may have different signal transduction potential.
Wang et al. studied IGFBP-2 and IGFBP-5 through IHC. More T1N1 carcinomas had HER-2/neu protein over-expression than T1N0 carcinomas whereas very little or no expression of IGFBP-2 and IGFBP-5 were found in normal or benign breast tissue containing fibrocystic change. IGFBP-2 expression was significantly higher in T1 breast carcinoma than in benign breast epithelium. Moderate to strong cytoplasmic staining for IGFBP2 was detected in overall T1 carcinoma and T1N1 ductal carcinoma groups. IGFBP-2 expression also correlated positively with modified Black’s nuclear grade in overall T1 carcinoma group and T1N0 carcinoma group. Similar observation holds true for ductal carcinoma. IGFBP-2 expression was positively correlated with PR expression in overall T1 carcinoma group and T1N0 group, but not in T1N1 group. IGFBP-2 expression was also positively associated with HER2/neu expression status. Moderate to strong staining for IGFBP-2 was more common in T1 carcinomas with HER2/neu protein over-expression than in those with negative or borderline HER2/neu. IGFBP-2 was differentially expressed in T1 carcinoma of different histologic type. IGFBP-2 expression was significantly higher in lobular carcinoma than ductal carcinoma. It was demonstrated that IGFBP-2 and IGFBP-5 were implicated in development of metastasis of T1 carcinoma and they may predict lymph node metastasis.

In the present study, higher IGFBP-2 was seen in breast cancer patients as compared to normal individuals; showed linear association with tumor size and was higher in node negative patients as compared to node positive patients. IGFBP-2 levels increases as stage increases and showed a linear association with histologic grade.

So et al. (2008) studied IGFBP-2 and its behavior in breast cancer and the effect of OGX-225 (an antisense oligonucleotide drug candidate) on IGFBP-2 level through 'in vitro' and 'in vivo' experiments. They included 4,181 primary invasive breast tumors and 120 benign breast tissues samples were identified for tumor tissue microarray construction and immunostained with IGFBP-2 antibody. A functional role for IGFBP-2 expression was attempted in promoting neoplastic growth and the effect of OGX-225 on the growth of IGFBP-2 expressing human breast cancer cells was examined. On comparison of IGFBP-2 with clinico-pathologic parameters and outcome of the patients, only positive association between IGFBP-2 and the hormone receptors was found. Patients who were negative for the hormone receptors had worst disease specific survival. Further, MDA-MB-231 cells constitutively over-expressing IGFBP-2 (MDA-MB-231BP-2) were created to assess the effect of IGFBP-2 gain-of-function. Naturally IGFBP-2 expressing MDA-MB-468 cells were used to determine the effect of IGFBP-2 loss-of-function using OGX-225. IGFBP-2 over-expression was noted in the cell line with stable expression of IGFBP-2 cDNA (MDA-MB-231BP-2) as compared to empty vector controls (MDA-MB-231mock). MDA-MB-231BP-2 cells grew more rapidly and were more resistant to paclitaxel both ‘in vitro’ and ‘in vivo’ compared to parental cells.
It was observed OGX-225 decreased IGFBP-2 expression and attenuated associated aggressive phenotype of MDA-MB-231BP-2 cells both 'in vitro' and 'in vivo'. Further, OGX-225 inhibited the growth of MDA-MB-468 cells 'in vitro' and 'in vivo.'