CHAPTER -5

DISCUSSION

In India as well as around the world, breast cancer is mainly prevailing cancer in women. Among females in India, cancer of the breast is the utmost prevalent form of cancer, with an age-adjusted prevalence of up to 25.8 cases per 100,000 women and a fatality rate of 12.7 per 100,000 women[282]. The occurrence rate of breast cancers is still highest in developed regions, although emerging countries have shown a greater rate of death caused by breast cancer[283]. Cancer of the breast has a multifaceted origin in which vulnerability is affected by both environmental and genetic variables[284].

Globally, cancer of breasts is the main frequent variety of cancer, and deaths from cancer are due to this disease. In the back ten years, there have been significant advancements in the variety of therapeutic measures offered for breast cancer. Chemotherapy has demonstrated significant long-term advantages in primary breast cancer by preventing recurrence and extending patient survival. Various benefits have been documented for diverse hormonal interventions targeting steroid hormone receptors. Nevertheless, surgery has consistently been the favored option for treating breast cancer. Hence, timely detection of breast cancer is imperative for improved treatment. While mammograms and tissue biopsy have traditionally been the preferred methods of diagnosing cancer of the breasts, a molecular considering study has the potential to offer more accurate and detailed information regarding the stages of cancer in the breasts.

The incidence of breast cancer exhibits a notable age-related escalation, with advanced age constituting a recognized risk factor. Within the United States, an estimated five to seven percent of breast carcinomas manifest in women below the age of 40[285]. In contrast, our study reports a considerably higher prevalence, specifically 28.2%. This substantial variance may be attributed to the demographic composition of a predominantly youthful population in India, or alternatively, genetic and environmental factors may contribute to this observed distinction. Therapeutically, the association between the size and number of lymph nodes of the tumor engagement is widely acknowledged[286]. Significantly, this relationship serves as a pivotal prognostic determiner in cancer of the breast, with dimensions of the tumor and affected lymph nodes collectively representing the most influential indicators of an adverse prognosis[287,288]. Pathologic data entailing patients with breast cancer report that, at diagnosis, the detection of carcinoma-infiltrated lymph nodes is fairly widespread, with estimates ranging from thirty to fifty percent of cases, determined by tumor size[289].

In this study, the age group of women with cancer of the breast varied from 40 to 49 years old, with 37 (33.6%) of them being diagnosed, while the control group had a similar frequency of 40 (36.4%). Significantly, those aged 50–59 were prominently represented in both the cases (28.5%) and the controls (27.5%). The data additionally indicated that most individuals lived in metropolitan regions, with 77 (70% of cases) and 95 (86.4%) of controls coming from these locations. Conversely, rural and semi-urban areas exhibited lower frequencies. With respect to menopausal state, 64 (58.2%) of the cases and 68 (61.8%) of the controls were classified as pre-menopausal, demonstrating an equal distribution between the two groups.

The BMI categories analysis, as a result, most of the cases were 64(58.2%) in the normal BMI range, whereas controls had a greater percentage 94(85.5%). The frequency of overweight was markedly higher in patients, with 37 individuals (33.6%), compared to controls, with only 7 individuals (6.4%). Surprisingly, 14 cases (12.7%) exhibited a positive first-degree family history, while the control group did not show any such history.

Tumors in younger women had very distinct characteristics from tumors in older women, as well as exhibiting unfavorable biological characteristics. Based on our findings, the younger and older women have significantly different outcomes within stages. Progesterone receptors and higher stages of tumors were associated with most tumors occurring in young females. It has been observed that whole of these attributes are related to rapidly progressing tumors and poorer prognoses. The results of another study indicated that tumors females under the age of 40 show lower estrogen receptor positivity, higher HER-2/epidermal growth factor receptor expression, and a tendency toward inferior disease-free survival. Studies like these support the conclusion that tumors that develop in younger women have biological differences from those that develop in older women, and they also have stronger less favorable biological markers. There are a number of European and American studies that have shown that young age at diagnosis is an independent predictor of poor survival[290–293]. There is a higher risk factor profile for young women than for older women. The size of the tumors, number of positive lymph nodes, number of hormone receptors, and grade of tumors are all higher in young women than in older women[294–298]. There is controversy surrounding this issue, as in a retrospective study of Singaporean breast cancerous patients, Chia et al suggest that young women have a better chance of surviving than older women[299].

This is in contrast to our study findings that tumors with progesterone receptors were more common in young women. In addition, our results showed that negative progesterone receptor tumors often had overexpressed HER-2.

The previous study found that cancer of the breast is a prevalent disease that affects women, characterized by significant variation in its clinical presentation. Currently, the prediction and treatment approach for cancer of the breast are chosen on the basis of the indications of the disease's stage. This stage is assessed by considering pathological factors such as the tumor's dimensions, associated lymph nodes, and tumor grades, utilizing the TNM system. These three parameters are crucial indicators on the clinical front as they can offer significant prognostics and statistical data. Furthermore, a comprehensive analysis of clinical and pathological factors, including invasion of the vascular system, tumor cellular proliferation, Necrosis of tumors, degree of in-situ ductal carcinomas, and age can aid in prognostication and determining the necessity of adjuvant treatment[300]. Nevertheless, the likelihood of surviving cancer of the breast is linked to the timely identification of the disease, prompt medical intervention, and hereditary susceptibility. Hormone receptors such as estrogen receptors, progesterone receptors, and oncoprotein HER2/Neu are commonly analyzed immunologic biomarkers to guide plans for therapeutic interventions. However, the latest data highlight the prognosticative and predictive significance of markers contributing to regulating apoptosis and the proliferation of proteins p53, bcl-2, and ki-67 [301,302]. Hence, the current research looks for to determine the association between specific immunologic markers (PR, ER, and HER2/Neu) and conventional characteristics such as tumors size, lymph node invasions, grades, clinical stages, and menopause status. The size of a tumor has been widely acknowledged as a separate prognostic factor that

is directly associated with clinical outcomes. Tumors of a larger size are linked to a more unfavorable prognosis and a higher likelihood of nodal metastasis[303]. Additionally, the survival rate decreases as the tumor size increases. The findings of this study indicate that 43.6% of the tumors have a size ranging from 2 to 5 cm, while 30.9% of the tumors have a size beyond 5 cm.

Rivadeneira *et al.* found a strong correlation between the dimensions of the tumors and the presence of metastases in nodes of lymph in the axilla[304]. Furthermore, research has demonstrated that patients who possess lymph nodes in the axilla experience a much inferior prognosis compared to those who do not have nodal metastases[305]. Lymph nodes that are involved or not are a crucial prognostic factor in cancer of the breast. The current investigation found that the majority of the chosen tumors (87.3%) were positive for lymph node involvement. The findings align as reported in the past[306,307]. A clear correlation exists between axillary lymph nodes and the possibility of distant recurrence of breast cancer[308]. In addition, larger tumors have a higher probability of showing lymphatic invasion as well as lymph node positivity [309].

A diagnostic marker for pathological prognosis, such as size of tumor, histological grades, node status in lymph nodes, and stages of pathology and clinical presentation, have been found to be useful in assessing cancer. However, they have not been able to accurately predict the clinical outcomes of all patients due to the varying a cancer cell's inherent potential for metastatic spread[310]. Hence, the exploration of novel biomarkers has the potential to shed light on tumor metastasis by including supplementary predictive elements. The diagnostic protocol for breast carcinomas continues to be a crucial objective in clinical practice and treatment. In recent times, several possible tumor biomarkers have been identified in patient samples with

histological evidence of breast cancer. These biomarkers include progesterone and estrogen receptors, HER2/Neu (humans epidermal growth factor receptor HER1or HER2), tumors suppressor genes (p53, p63, and p73), and Bcl-2 (an anti-apoptotic oncogene). These markers are crucial for evaluating breast cancer and are detected through immune histochemical analysis[311].

According to many studies[312–314], the primary complaint of all female patients with cancer of the breast was a lump in the breast. None of the patients had a sole complaint of discomfort, nipple discharge, or nipple retraction. The higher prevalence of patients in stage II and stage III may be attributed to a lack of awareness concerning breast cancerous signs and symptoms, as determined during their interview. The issue of delayed presentation may primarily stem from factors such as rural upbringing, limited knowledge, economic hardship, social stigma surrounding breast cancer, and cultural norms that discourage women from seeking medical attention, particularly for treatments like mastectomy, due to the associated societal consequences. Therefore, by providing education to the general population regarding self-breast examination and screening tools, individuals can independently identify their own illness, thereby facilitating early warnings of the disease.

About cancer of breast, tumor grade is a helpful prognosis factor; tumors with greater grade expression typically have worse prognoses. The intricate histological nature of breast carcinoma makes tumor grading a reliable indicator for pathological cancer screening of breast cancer. The present study examined the histologic subtype, also known as tumor grade, of 110 patients. The analysis of these cases revealed the following distribution: Grade I was detected in two cases, accounting for 0.9% of the total. Grade II was the most prevalent, with 76 cases (34.5%), while Grade III was

recognized in 31 cases (14.1%). The results revealed a range of tumor grades among the individuals examined. Significantly, the majority of instances were classified as Grade II, indicating a moderate level of difference. Comparable findings were noted in additional investigations conducted in India[315], Pakistan[316], and Malaysia[317]. While pathological characteristics and conventional markers including size of the tumors, lymph nodal involvement, and tumor grade have prognostic value, the status of steroid receptors has consistently been shown to be one of the most significant prognostic markers. It has a direct impact on survival rates, mortality rates, and disease-free survival rates[318,319]. Research has extensively recorded that breast cancer patients who test positive for both estrogen receptors (ER) and progesterone receptors (PR) have demonstrated a favorable survival rate[320]. The expression of steroid hormone receptors plays a vital aspect in breast cancer, serving as the basis for the identification of various therapeutic approaches in adjuvant chemotherapy[321].

In this current investigation, the Fok1 FF polymorphism and ER had statistically signifiactory interactions with a significant p value of 0.024 with an odd ratio of 2.53 (95% CI: 1.163, 5.514). Also, ApaI AA polymorphism and ER show statistically significant interactions leading to a statistically significant p-value of 0.017 with an odd ratio of 1.26 (95% CI: 0.592, 2.70). In neither case did the Fok1 polymorphism or ApaI polymorphism have statistically significant interactions with PR or Her2. This is in contrast to Saudi women's breast cancer study where the f allele plays a crucial role in breast cancer risk[161]. The BsmI genotype, besides, did not show a remarkable correlation with ER, PR, or Her2 status. Based on these findings, it appears that estrogen poses an important part in binding to the ER at the plasma membrane. This binding then triggers the activation of various signaling pathways, including MAPK, protein kinases A and C, and calcium pathways.

This activation initiates a sequence of cell signaling events that ultimately result in cell proliferation. Tamoxifen, an anti-estrogen medication, competitively blocks the estrogen receptor (ER) to prevent the transcriptional activation of genes necessary for tumor growth[322]. This is one of several endocrine treatments that disrupt the effects of the estrogen hormone. Approximately 55–60% of breast cancer cases of tumors positive for ER have a good response to adjuvant therapy, but only about 8% of women with tumors that are ER-negative experience the same response. Hence, determining the steroid receptor status is advantageous for determining which patients have the highest chance of an advantage from endocrine therapy. Additionally, it offers valuable prognostic information on disease recurrence and patients' survival rates. Moreover, the expression of steroid receptors was found to be associated with the degree of tumor advancement and differentiation[323]. Thus, it indicates that the progesterone and estrogen receptors are likely to be significant prognostic and annotating biomarkers for the cures of breast cancerous patients.

In our current investigation, a noteworthy association was found between the Fok1 genotypes and the occurrence of cancer of the breast within our study population. Specifically, for the FF genotype, there were 60 cases and 64 controls, yielding an odds ratio (ORs) of 5.49 (95% confidence intervals: 1.72, 17.64) and a p-value of 0.004, indicating a substantial increase in breast cancerous risk associated with the FF genotype. Similarly, individuals with the Ff genotype showed a reduced occurrence in cases (32) compared to controls (42), with an odds ratios of 6.00 (95% confidence interval: 1.83, 19.67) and a p-value of 0.003, signifying a significant association. Conversely, the ff genotype exhibited a lower frequency within the control group (4) match up to the case group (18). Our study highlights an associations between the FokI genotypes FF and Ff and an increased risk of cancer of the breast. These findings

contributed to our understandings of the potential role of the FokI genotype in predisposing individuals to breast cancer within our studied population. It is worth noting that the allele probabilities and p values for Hardy-Weinberg equilibrium (HWE) were 0.15, suggesting equilibrium in both cases and controls.

In contrast, a previous study among Africans-Americans and Hispanics populations in a case-controls study (CCS) reported a heightened association for the Fok1 f allele with the risk of breast cancer[160]. However, no significant associations were observed for Bsm1, Taq1, and Apa1 polymorphisms in relation to the condition under study. Similarly, in a recent study within the Indian population, a notable association was found between the Fok1 FF and Ff genotypes and an elevated risk of breast cancer in a CCS design involving 130 cases and 130 controls[171].

A previous investigation conducted on the Caucasian population underwent examination in a Pilot-CCS with a focus on the Apa1 polymorphism. The study, which included 164 cases and 174 controls, revealed a notable elevation in the breast cancerous risk among individuals possessing the AA genotype[181]. Similarly, in the study among the African population, a CCS was conducted, examining BsmI, ApaI, and TaqI polymorphisms in a sample size of 100 cases and 50 controls. No significant association was found between these polymorphisms and the occurrence of the condition under investigation[176]. In another study which concentrated on the African population within a CCS exploring Fok1, Apa1, and Taq1 polymorphisms (392 cases and 193 controls), no significant association was accessed between the Apa1 and Taq1 polymorphisms and the condition under study. However, the study did identify a heightened risk of cancer of the breast connected to the Fok1 ff genotype[324]. In the ongoing study, the analysis of the ApaI genotype distribution unveils a lower frequency

of AA genotypes in cases (54) compared to controls (62), resulting in an odds ratios of 2.87 (95% CIs: 0.92, 8.97) and a p-value of 0.069. Similarly, the Aa genotype exhibits a slightly higher frequency in cases (45) than controls (43), with an odds ratio of 2.31 (95% CI: 0.72, 7.37) and a p-value of 0.157. ApaI genotypes show no significant linkage to the risk of breast cancer. The consistency with Hardy-Weinberg equilibrium (HWE) p-values of 0.15 in both cases and controls enhances the reliability of observed associations, and deviations from expected genotype frequencies may suggest underlying genetic or environmental influences.

In the current study, examining the BsmI genotype, the BB genotype was observed in 47 cases and 55 controls, resulting in an odds ratios of 1.76 (95% CIs: 0.36, 8.54) and a p-value of 0.482. The Bb genotype occurred in 59 cases and 52 controls, with an odds ratios of 1.30 (95% CIs: 0.27, 6.25) and a p-value of 0.743. Additionally, the bb genotype was identified in 4 cases and 3 controls, revealing an odds ratios of 0.056 and a p-value of 0.009 exhibiting minimal association. The Hardy-Weinberg equilibrium (HWE) p-value of 0.15 in both cases and controls suggest that the observed genotypic frequencies align with expectations. In previous research among Iranians using a CCS approach, Fok1 and Bsm1 polymorphisms were examined in 140 cases and 156 controls. The findings indicated an absence of significant interaction with cancer of the breast for the Fok1 polymorphism. However, the Bsm1 polymorphism showed a noteworthy connection, with the BsmI bb and Bb genotypes significantly involved in an increased risk of breast cancer[159]. Similarly, in the investigation within the African population using a CCS design, Fok I, Bsm I, Apa I, and Taq I polymorphisms were explored in 130 cases and 100 controls. The results revealed no significant association with cancer of the breast for the Fok1 and Taq1 polymorphisms. However, the BsmI bb

genotype and Apa1 aa genotype were found to be significantly associated with an hightened risk of breast cancer[169]. An other study conducted within the Iranian population using a CCS design, the polymorphisms Fok1 and Bsm1 were investigated in a sample comprising 203 cases and 214 controls. The findings indicated a lack of significant association with cancer of the breast for the Fok1 polymorphism. However, the BsmI bb and Bb genotypes were notably associated with an increased risk of breast cancer[178].

In the present study, mean vitamin D levels were examined concerning FokI, ApaI, and BsmI genotypes. However, the p-values for the FokI genotypes did not reach statistical significance, with respective values of 0.756 and 0.067. Concerning the ApaI genotype, significantly, the p-values for the ApaI genotypes were 0.316 and 0.001, indicating notable disparities in vitamin D levels between cases and controls for the Aa and aa genotypes. Moving to the BsmI genotype, the p-values for the BsmI genotypes were 0.006 and 0.001, respectively, indicating a significant statistical significance. Hence, ApaI genotypes (Aa and aa) and BsmI (BB and bb) revealed significant differences in vitamin D levels amongst cases and controls, proposing a potential relationship between these genotypes and vitamin D status within the context of breast cancer. This aligns with insights from the prior case-controls studies, which reported no modification of the effect of vitamin D by genetic polymorphisms of vitamin D-related genes[325–327]. Alternatively, a study reported there is an increased breast cancerous risk amongst Caucasian women living in the UK with lower levels of circulating 25-OHvitD (<50 nanomol/l) in combination with the VDR for the Bsm1 bb genotype[328]. Moreover, diseases and dosages of vitamin D3 differed in these studies, making them not directly in agreement with the current study's findings. In the study involving elderly subjects,

circulating 25-OHvitD, parathyroid hormone (PTH), ultra-sensitive C-reactive protein (us-CRP), and alpha acid glycoprotein (AGP-A) levels of those with the VDR ApaI AA/aa genotype were better responding to vitamin D3 megadose compared to the Aa genotype. However, this study lacked sufficient power to clarify the genotype-environment (GXE) interactions owing to its small sample size[329]. Additionally, variations in the frequency of VDR SNPs are observed between different populations, complicating the comparison of findings across studies.

Hence, there are both strengths and limitations to the present study as with any research study. For the logistic regression analysis, only a few factors were taken into account, and quite a few factors were unmatched. All breast cancer samples in this study could not be obtained for tissue blocks, particularly for androgen receptors. It is also likely that the sample size was small. However, despite these limitations, this study made a substantial contribution to advancing our understanding of the polymorphisms of vitamin D receptor gene(s) and the risk of developing cancer of the breast under this study.

The potential role of the most prevalent genetic polymorphisms within the vitamin D receptor (VDR) in modulating the risk of breast cancer has been explored. In the past decades, extensive research has indicated a relatedness between low sunlight exposure, vitamin D deficiency, and elevated risks of extra-skeletal diseases, including cancer[330,331]. VDR has been posited for the purpose of regulating expression of numerous genes linked to cellular proliferation and differentiation, suggesting a potentially key role in cancer prevention[332]. To date, merely a finite number of studies have unequivocally demonstrated the significance of gene variations as prognostic

markers. However, the exploration of single nucleotide polymorphisms (SNPs) remains active, as only a small proportion of identified genes have been thoroughly studied.