CHAPTER- 1
INTRODUCTION

Globally, cancer results in 9.6 million deaths and is the principal factor of mortality because of its unregulated proliferation of cells[1]. Genetic and epigenetic alterations primarily drive the cell transformation, and the unchecked and abnormal cellular growth as it spreads, it affects other parts of the body from the primary site, referred to as metastasis. Although cancer therapies have made significant advancements, many challenges remain, including disease heterogeneity and resistance to anti-cancer drugs[2]. Breast cancer usually begins with cell proliferation in epithelium without control that occurs in the ducts or lobules of mammary glands, resulting in erroneous signalling between the cells[3]. Malignancies related to breast cancer are most commonly diagnosed in women in 154 countries, and the disease results in 630,00 deaths per year[4]. There are more deaths from breast cancer among women than any other predominant reason of mortality due to cancer in Americans, comprising 29% of all reported cancers[5,6]. According to statistics, the likelihood of breast cancer among Indian females has gradually risen over the years. According to estimates cancer developing risks for females is 25.8 out of every 100,000, and 12.7 female deaths are reported out of every 100,000 females[7].

In comparison, the mortality rates of breast cancer against every 100,000 females have been recorded to be 16.2 in Western Europe, 14.5 in Australia and New Zealand, 6.1 in Eastern Asia, and 14.8 in North America[8]. Breast cancer risk factors which extend beyond age comprise genetic mutations (BRCA1/BRCA2 mutations), environmental factors, and women's reproductive factors (onset of menarche or delayed menopause). In addition, the incidence of the disease is also influenced by

modifiable risk contributors, notably alcohol and fat-laden diet consumption. The most emerging risk factor for breast cancer rates for women in advanced and developing nations is lower vitamin D levels[9,10].

Most commonly the cases, breast cancerous rates increase afterwards the age of 50 years, and early-onset cancer of the breast are strongly affiliated with predisposing genetic mutations. Nearly 90% of hereditary breast cancers are predominantly caused by genetic changes in BRCA1 and BRCA2[11]. Breast cancers are classified into various molecular subtypes- normal breast-like, luminal A, having a basal-like appearance or being triple-negative, HER2 overexpressing, and luminal B. This classification is informed by the detection of hormonal molecules namely estrogen [ER], and progesterone [PR]) along with human epidermal growth factor receptors2 (HER2 or ERBB2) within mammary tissues[12,13]. The incidence, severity, treatment strategies, and prognosis differences determine the various subtypes, thereby attributing to the disease heterogeneity. Understanding the crosstalk between various steroid hormone receptor signalling pathways that contribute to breast cancer pathogenesis is highly significant due to the pivotal role of the hormonal milieu in this disease pathology. It has become evident over the past few decades that vitamin D is inversely correlated with breast cancer[14]. Epidemiological studies report lower occurrence and mortality levels from Breast cancer near geographic locations with a higher biologically effective solar ultraviolet B (280–315 nm) exposure[15]. UV light is essential for converting 7-dehydrocholesterol into pre-vitamin D3 in the cutaneous, and its isomers convert to vitamin D3.

Biologically active form, the hormone steroid 1,25-dihydroxyvitamin D (calcitriol) is essential to regulate a wide range of biological pathways that include

inhibiting cellular proliferation, angiogenesis, metastasis, invasion, and promotion of cellular differentiation and death[16]. Cancer of the breast has decreased significant risk that can be accomplished by increasing plasma levels of active vitamin D components, particularly during a post-menopause phase, indicating benefits of vitamin D in opposition to breast cancer[9,17,18]. Many factors play a role in its synthesis, including skin pigmentation, season, latitude, altitude, and sunscreen use (Sun Protection Factor- >15), and in the case of women, menopause, estrogen deficiency, Diabetes type 2, and aging trigger vitamin D deficiency[19,20]. Thus, the predominant target population for menopausal women is more likely to develop cancer of the breast and tend to be at greater vulnerability for lower vitamin D levels when compared to younger women[21]. Several case-control studies indicate that females having lower vitamin D status were at a higher likelihood of being positive for breast cancer, including mostly aggressive forms[22–24].

A receptor for Vitamin D (VDR), belonging being a family of nuclear receptors for steroid hormones, contributes a key role for the biological function of calcitriol. Apart in distinction to regulating the homeostasis of calcium and phosphate by a varied section of genes, their activities involve promoting the differentiation of cells and inhibiting the wide spread of certain cells that possess tumor-preventative properties[25]. VDR is located in cells of major organs of the body like the liver, mammary glands, kidneys, parathyroid glands, heart, bone, intestine, pancreas, prostate, colon, and vascular cells[26]. Due to the fact that the epithelial cells found in mammary glands have similar enzyme systems, consequently, those of the kidneys, vitamin D3 and its biological effects on cells of breast cancer are biologically reasonable. vitamin D is a vital component for optimal mammary glands

development. Calcitriol has a significant impact in opposing estrogen-driven proliferation and stimulating the differentiation regarding mammary epithelial cells through VDR gene expression regulation. Several evidence-based analyses have revealed that calcitriol represents a critical function in inhibiting estrogen-driven cell multiplication and angiogenesis in normal and malignant breast cells[27].

VDR gene located on chromosomal locus 12q13-14 has several common allelic variants[28]. The gene polymorphism in VDR possibly gives rise to substantial consequences of steroid receptor disruption, affecting the formation and function of calcitriol[29]. As part of routine breast cancer care, steroid receptor (estrogen and progesterone) status was evaluated[30]. Several studies have reported that approximately 50-70 percent of breast cancer were estrogen receptors positive (ER+). Additionally, ER+ tumors account for 50% of the cases that have progesterone-positive receptor (PR+) status as ER is involved in regulating gene transcription encoding the PR in the mammary gland. Since this finding, there is no doubt that mammary epithelial cells and breast cancer are dependent upon estrogen and/or progesterone to assist in proliferation and that this effect is mediated through ER with or without PR. Undoubtedly, ER and PR are overexpressed in malignant breast tissue[31]. Therefore, PR expression results in indirect ER activation[32].

Immunohistochemical (IHC) analysis is more accurate than biochemical assays in assessing ER status and predicting mortality risk among breast cancer patients. Following IHC assessment, hormonal therapy is initiated to control estrogen production and suppress its action. Most of the hormonal therapy drugs used for breast cancer either lower estrogen levels or block ER in malignant breast tissues[33]. Along with ER and PR, accruing evidence suggests a role for Androgen Receptor

(AR), in mammary epithelial cells in breast cancer. However, studies report conflicting results at high levels of circulating androgens[34]. Due to the tumor-suppressive action of AR in hormone-driven breast cancer, therapeutic drugs based on AR agonists, antagonists, and androgen synthesis inhibitors are under trials to treat breast cancer in clinical settings[35].

Hence, to understand the epidemiology, factors influencing and amounts of vitamin D corresponding to breast carcinoma, and the relationship between genetic polymorphisms in vitamin D receptors and receptors of ER, PR, and Her2 status among those affected with breast cancer, a study was conducted.

Hypothesis

Breast cancer is a multifactorial disease, wherein deficiency of vitamin D, its hormone receptors, and its polymorphisms are associated with the risk of breast cancer.

Considering, p-value <0.05, then the hypothesis can be considered statistically significant, and if p-value >0.05, then the hypothesis is said to be statistically insignificant.

1.1 Aim, Objectives, and Research Questions

1.1.1 Aim:

The aim of the study is to assess VDR gene polymorphisms and steroid receptor status among Breast Cancer patients.

1.1.2 Objectives:

1.1.3 Objective 1: To identify the different VDR gene polymorphisms among patients presenting Breast Cancer.

1.1.4 Objective 2: To identify the allele and genotype of individual VDR gene polymorphisms involved in the occurrence and prognosis of breast cancer.

1.1.5 Objective 3: To represent the odds ratio (OR) of estrogen receptor, progesterone, and Her2/neu receptor status in breast cancer.

1.1.6 Research Questions

1.1.7 RQ1: What serum levels of Vitamin D are associated with increased Breast Cancer risk?

1.1.8 RQ2: What are the distribution of VDR gene polymorphisms among patients having Breast Cancer?

1.1.9 RQ3: Which allele and genotype of VDR gene polymorphism are involved in increasing the risk of breast cancer?

1.1.10 RQ4: What percentage of positive and negative estrogen receptors, progesterone receptors, and Her2/new status are found in breast cancer cases?