CHAPTER -4

RESULTS

4.1 Demographic Characteristics.

In **Table 13**, the distribution of demographic characteristics and risk factors for breast cancer patients was meticulously examined in both cases that were matched with controls within the study population. In terms of age, the majority of cases fell within the 40–49 age range, comprising 37 (33.6%), while controls showed a comparable frequency of 40 (36.4%). Notably, the age group of 50–59 also exhibited substantial representation in both cases (28.5%) and controls (27.5%). The data further revealed that the majority resided in urban areas, with 77 (70% of cases) and 95 (86.4%) of controls originating from these settings.

Table 13: Distribution of demographic determinants and breast cancerous risk factors among cases and matched controls within the study population

Demographic factors		**Cases (%)**	**Controls (%)**
Age	30-39	31(28.2%)	34(30.9%)
	40-49	37(33.6%)	40(36.4%)
	50-59	28(25.5%)	27(24.5%)
	60-69	13(11.8%)	9(8.2%)
	≥70	1(0.9%)	0(0%)
Areas	Rural	22(20%)	6(5.5%)
	Urban	77(70%)	95(86.4%)
	Semi-Urban	11(10%)	9(8.2%)
Menopausal	Pre-menopausal	64(58.2%)	68(61.8%)
	Post-menopausal	46(41.8%)	42(38.2%)
BMI Category	Normal	64(58.2%)	94(85.5%)
	Underweight	5(4.5%)	9(8.2%)
	Overweight	37(33.6%)	7(6.4%)
	Obese class I	4(3.6%)	0(0%)
First Degree Family history	Yes	14(12.7%)	0
Total		110(100%)	110(100%)

In contrast, rural and semi-urban areas displayed lower frequencies. Regarding menopausal status, 64 (58.2%) of cases and 68 (61.8%) of controls were pre-menopausal, indicating a balanced distribution between the two groups.

Analysis of BMI categories underscored the prevalence of individuals within the normal BMI range, constituting 64 (58.2%) of cases and a higher percentage of 94 (85.5%) in controls. The prevalence of the overweight category shown significantly greater in patients 37 (33.6%) compared to controls 7 (6.4%). Remarkably, 14 (12.7%) of cases had a positive first-degree family history, whereas no such history was identified in the control group.

The study included 110 cases and 110 controls, which represented the entire population. The absence of familial background for the control group and its existence in the case group suggest a possible hereditary susceptibility to breast cancer. In general, the data offers a thorough analysis of the demographic traits and risk variables in the group being studied. It suggests possible connections between age, geographic location, menopausal state, BMI, and family history with the occurrence of breast cancer.

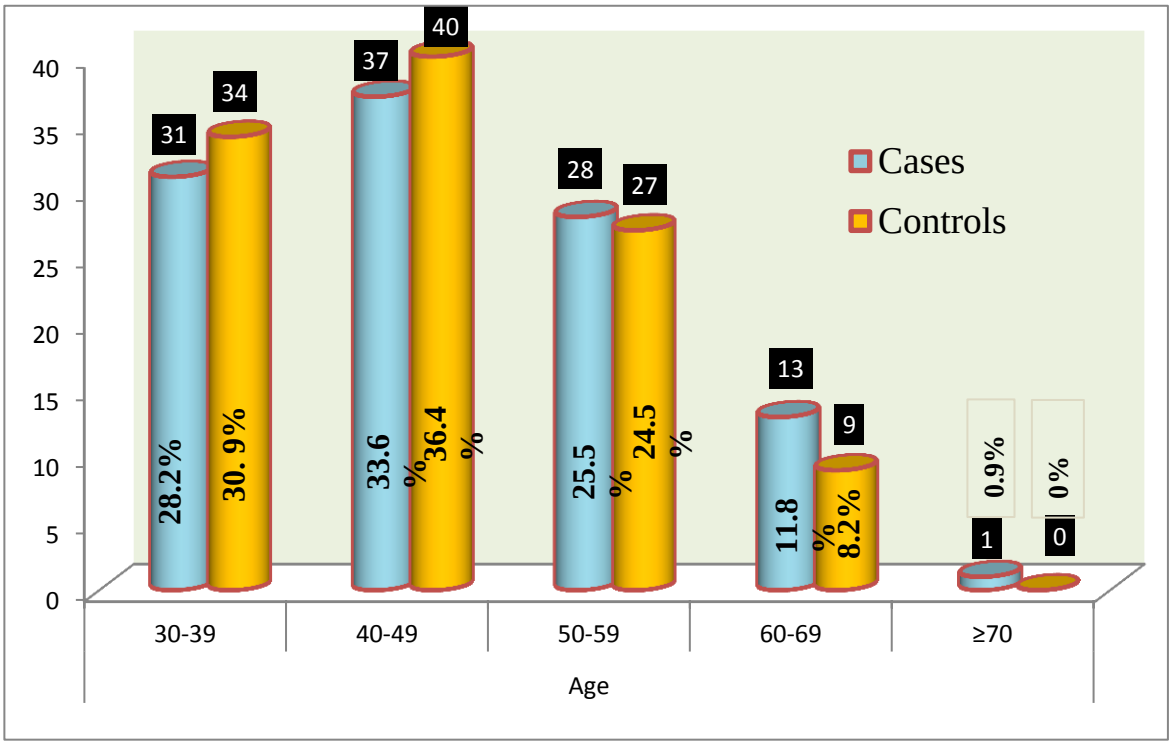


Figure 9 : Distribution of the study population according to their Age Group

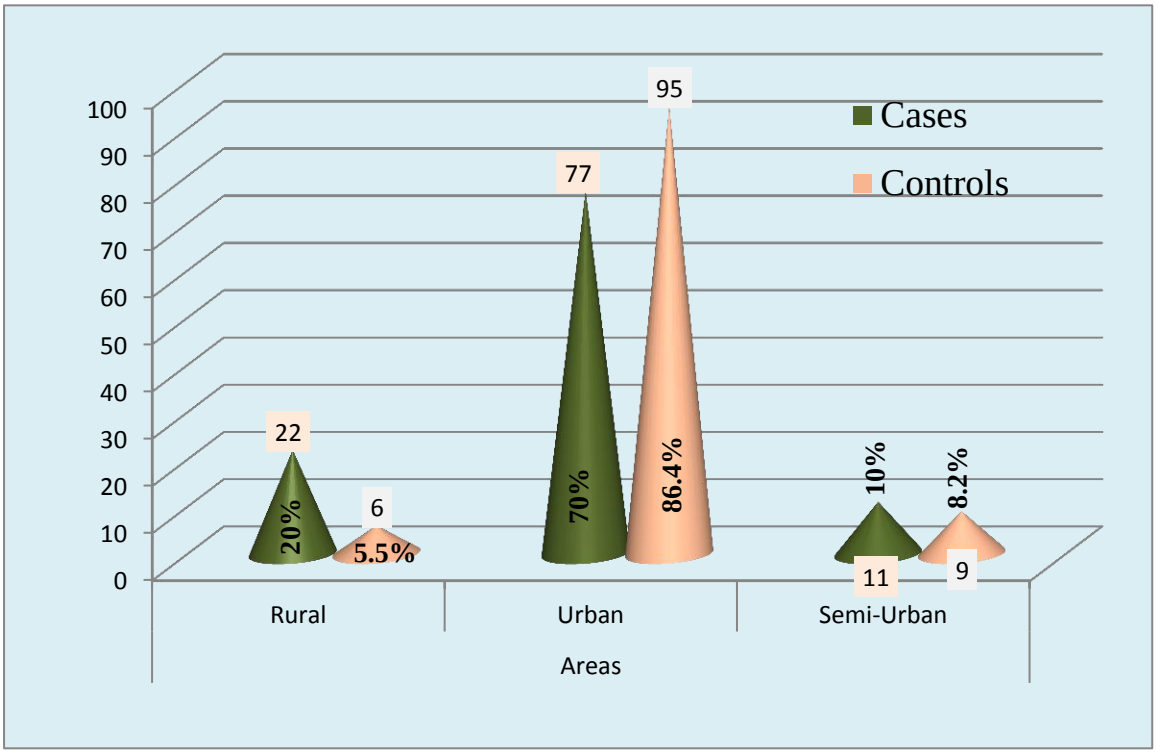


Figure 10: Distribution of study population according to their Areas

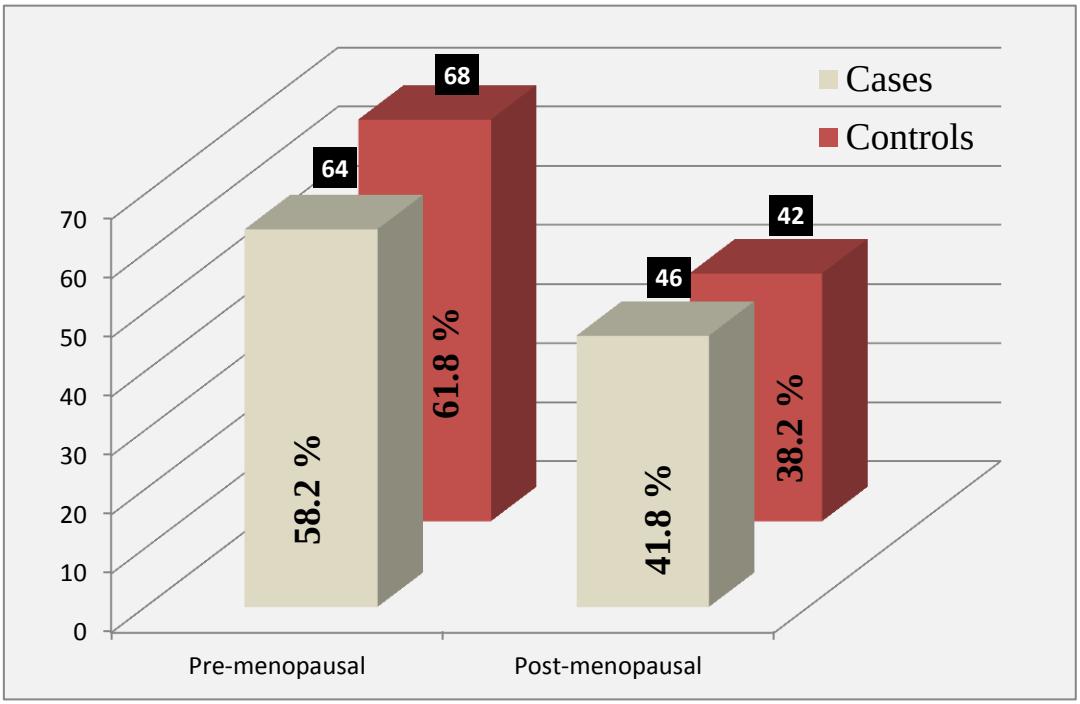


Figure 11: Distribution of the study population according to their Menopausal stage

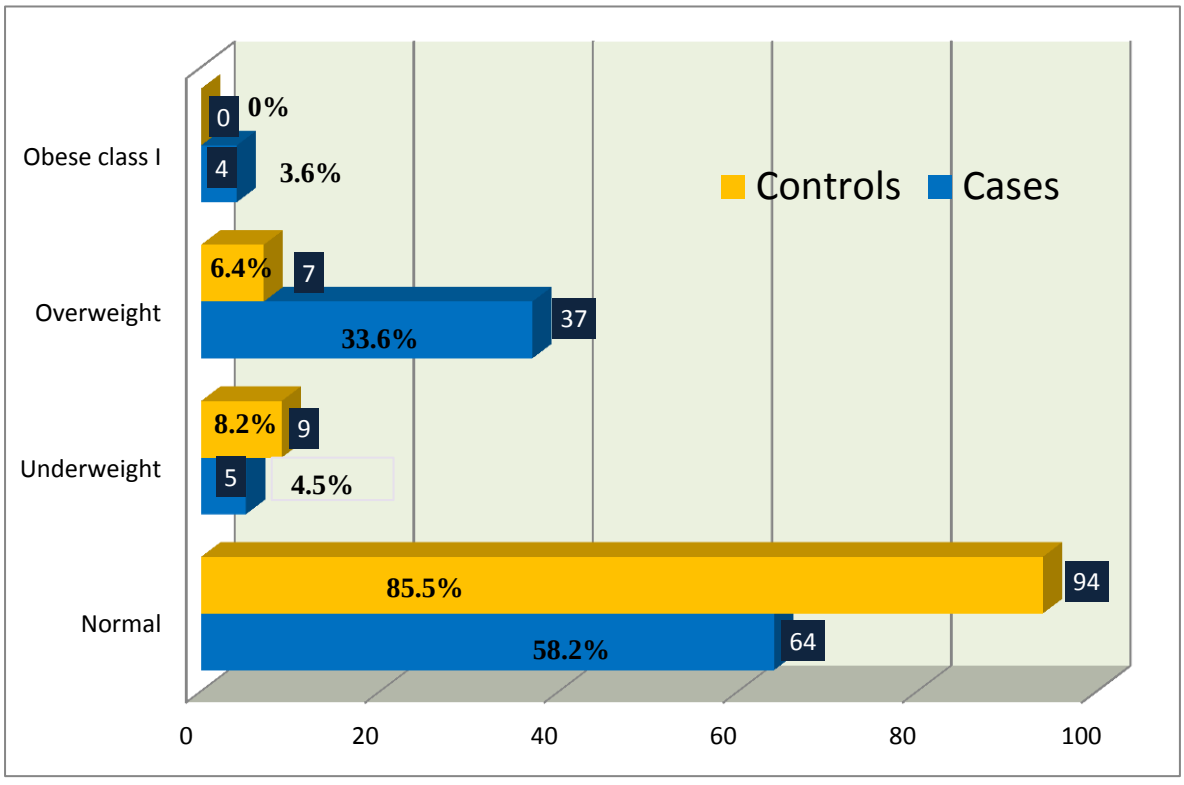


Figure 12: Distribution of study population according to their BMI Category

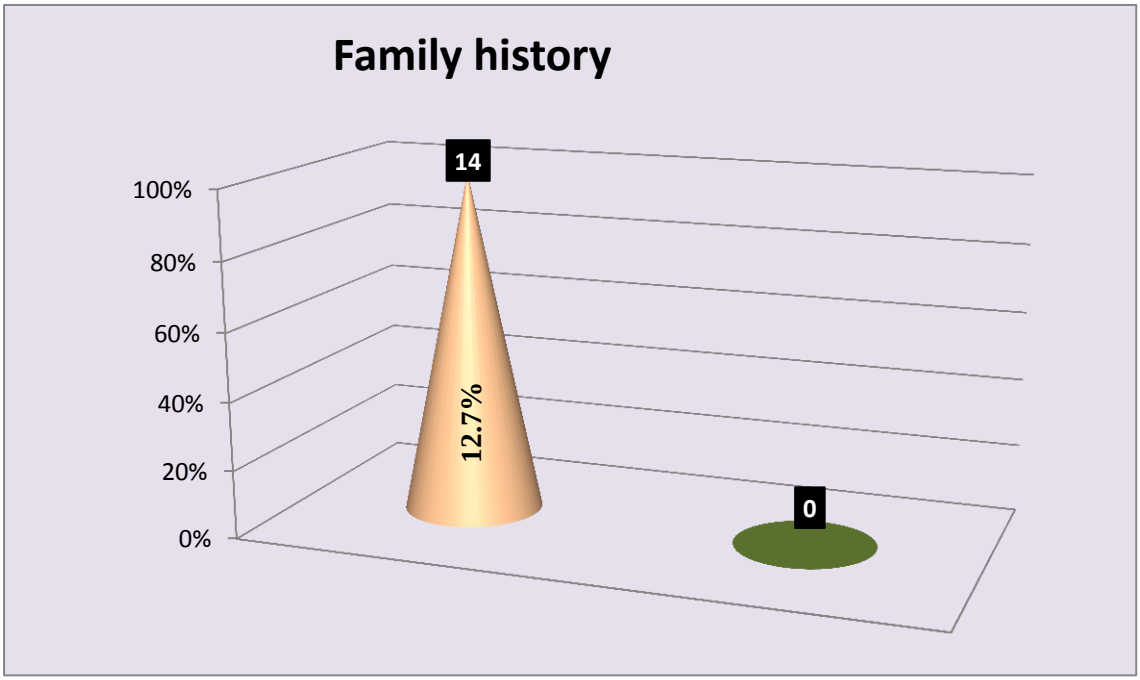


Figure 13: Distribution of the study population according to their family history

4.2 Clinicopathological features.

Table 14, depicts that, in the examination of clinicopathological features among breast cancer patients, the age at diagnosis plays a pivotal role. The data illustrates that the majority of cases fall within the age range of 40–49, comprising 37 (33.6%), closely followed by the 31–40 age group at 31 (28.2%). The distribution indicates a diverse age profile among patients, with 28 (25.5%) diagnosed between 50 and 59, and 14 (12.7%) aged more than 60.

Table 14: The Clinicopathological features of breast cancer patients

Clinicopathological		**Cases (%)**
Age at Diagnosis	30-39	31(28.2 %)
	40-49	37(33.6 %)
	50-59	28(25.5 %)
	≥ 60	14(12.7 %)
Co-Morbid Conditions	Type-2 Diabetes	8(7.3%)
	Hypertension	14(12.7%)
Breast Cancer laterality	Left	51(46.4%)
	Right	59(53.6%)
Tumor size	Upto 2cm, >2	18(16.4%)
	Upto 5cm	70(63.6%)
	>5cm	22(20%)
Tumor Grade	Well-differentiated	4(3.6%)
	Moderately differentiated	76(69.1%)
	Poorly differentiated	30(27.3%)
Hormone receptor Positive/Present Status	Lymph node status	64(58.2%)
	Angiolymphatic Invasion	98(89.1%)
	Nipple/Skin Involvement	12(10.9%)
	ER Status	64(58.2%)
	PR Status	70(63.6%)
	Her-2 Status	31(28.2%)
Total		110(100%)

Co-morbid conditions add another layer to the clinical profile, with 8 (7.3%) of cancerous breast patients presenting along type-2 diabetes and 14 (12.7%) having hypertension. The presence of these co-morbidities highlights the complexity of managing breast cancer cases, as these conditions may influence treatment decisions and the overall prognosis. Monitoring and addressing these co-morbidities are crucial aspects of providing comprehensive healthcare for breast cancer patients.

Examining breast cancer laterality, the data reveals a relatively balanced distribution, with 51 (46.4%) of cases affecting the left breast and 59 (53.6%) affecting the right breast. While laterality might not always have direct clinical implications, understanding the patterns of breast cancer occurrence in each breast can contribute to a more nuanced approach in diagnostics and treatment planning.

The data reveals diverse patterns in tumor size, grade, hormone receptor status, and other critical factors. Tumor size, a fundamental parameter in cancer staging, displays a range of distribution among the studied cases. Notably, 70 (63.6%) of cases present with tumors up to 5 cm, while 22 (20%) exhibit larger tumors >5 cm. An additional 18 (16.4%) cases have tumors categorized as up to 2 cm or >2 cm. This distribution emphasizes the variability in tumor sizes, which is crucial information for determining the extent of disease progression and guiding therapeutic strategies.

Tumor grade, a key determinant of cancer aggressiveness, demonstrates variability as well. The majority of cases, at 76 (69.1%), fall within the category of moderately differentiated tumors, indicating an intermediate level of aggressiveness. Meanwhile, 30 (27.3%) are classified as poorly differentiated, and a smaller proportion 4(3.6%) is classified as well differentiated. These findings underscore the

heterogeneity in tumor grades among breast cancer patients, influencing prognostic considerations and treatment planning.

Examining hormone receptor status, pivotal in understanding the molecular characteristics of breast cancer, reveals varying frequencies. Lymph node involvement, a crucial indicator of cancer spread, is present in 64 (58.2%) of cases. Angiolymphatic invasion, a potential marker of tumor aggressiveness, is highly prevalent at 98 (89.1%). Nipple or skin involvement, indicative of advanced disease, is noted in 12 (10.9%) of cases. Hormone receptor stature, amongst ER (estrogen receptors) and PR (progesterone receptors), is positive in 64 (58.2%) and 70 (63.6%) of cases, respectively. In contrast, the Her-2 status is positive in 31 (28.2%) cases.

The clinicopathological features of breast cancer patients, including age at diagnosis, co-morbid conditions, laterality, etc., offer valuable insights into the diverse characteristics of this complex disease. These findings emphasize the vitality of personalized and multidisciplinary approaches in managing breast cancer cases, taking into account individual patient profiles and associated medical conditions.

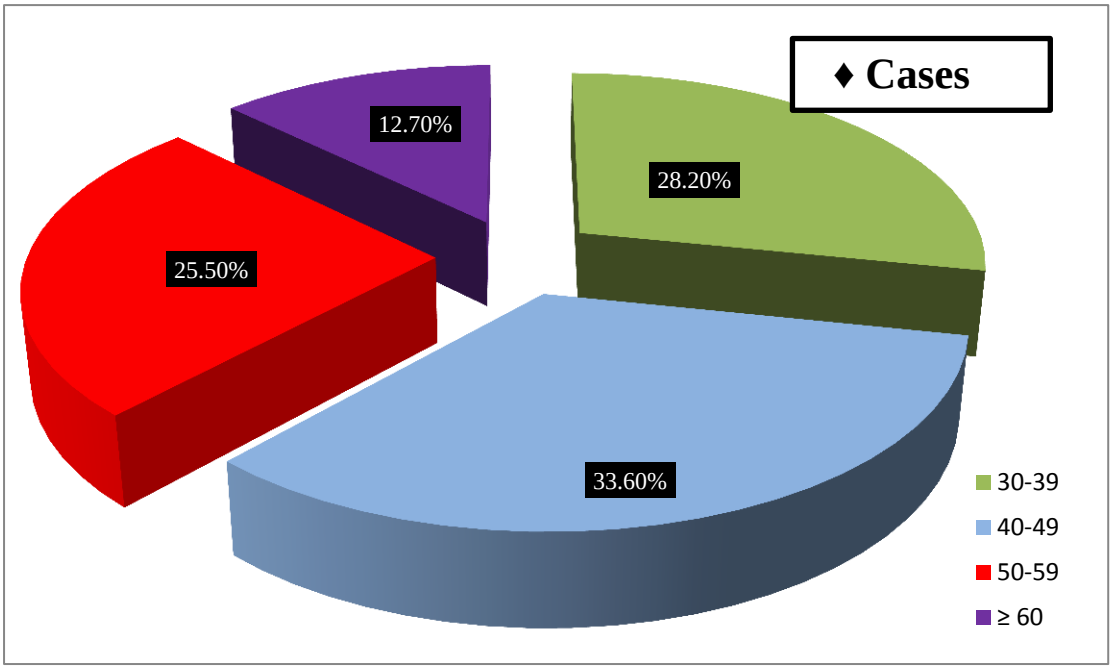


Figure 14: Distribution of the study population according to their Age at Diagnosis

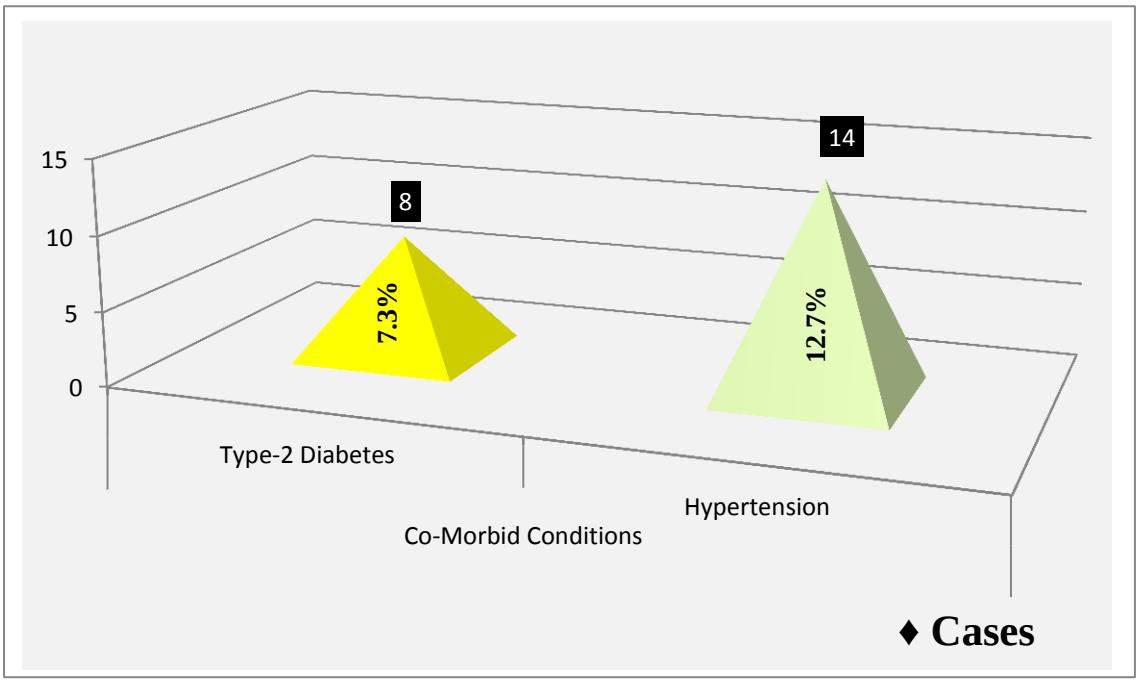


Figure 15: Distribution of the study population according to their Co-morbid conditions

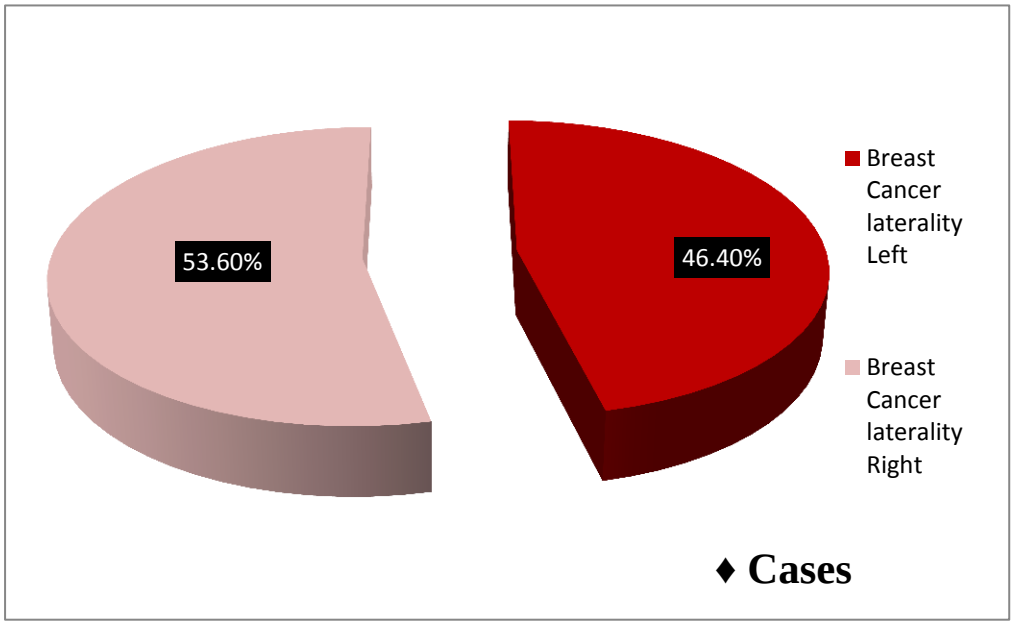


Figure 16: Distribution of the study population according to their Breast Cancer laterality

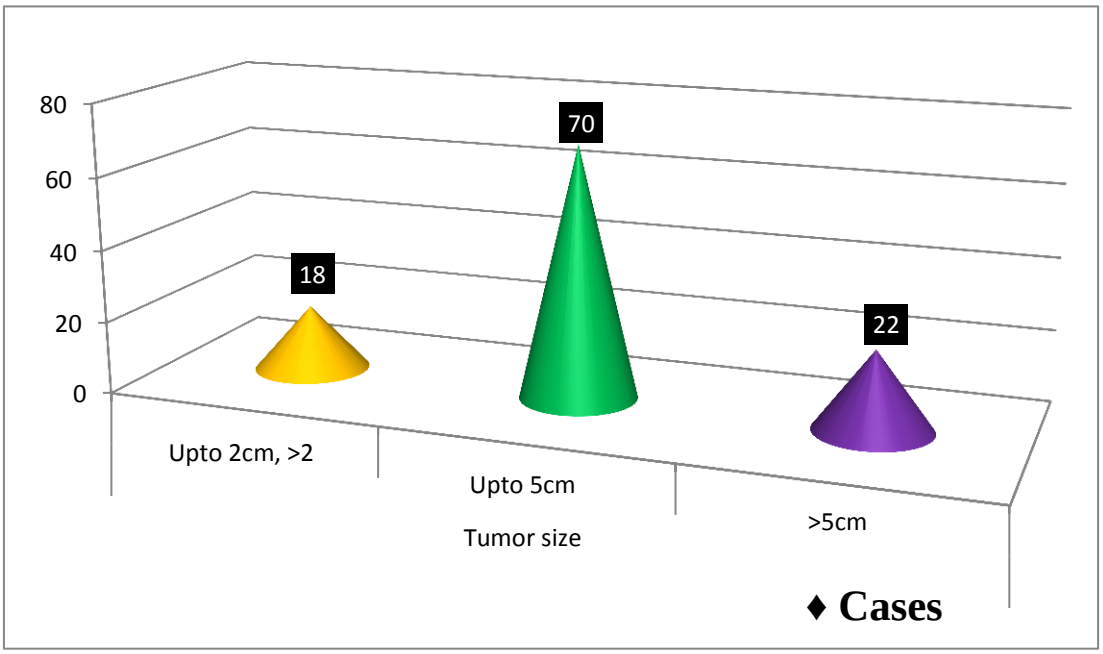


Figure 17: Distribution of the study population according to their Tumor size

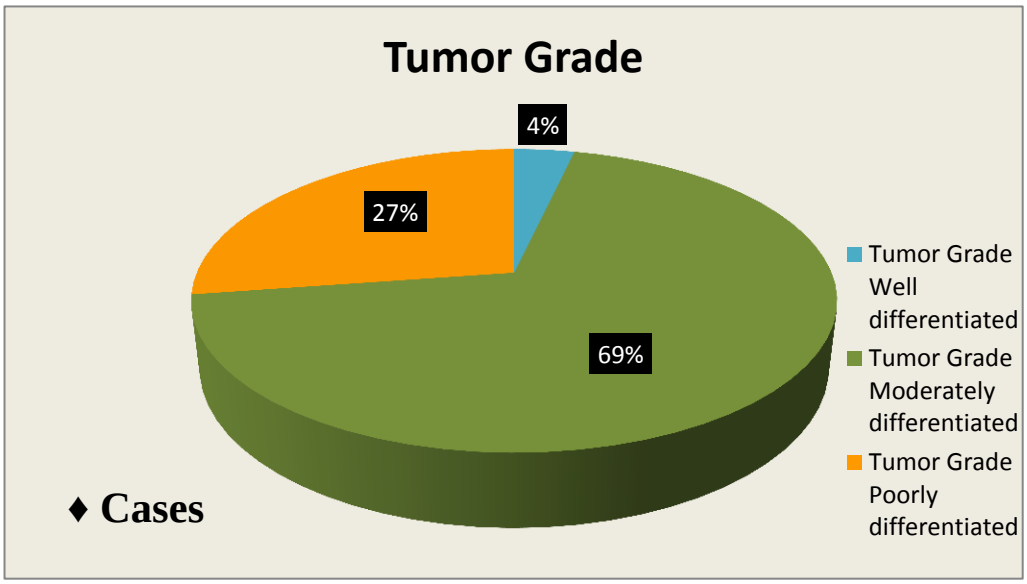


Figure 18: Distribution of the study population according to their Tumor grade

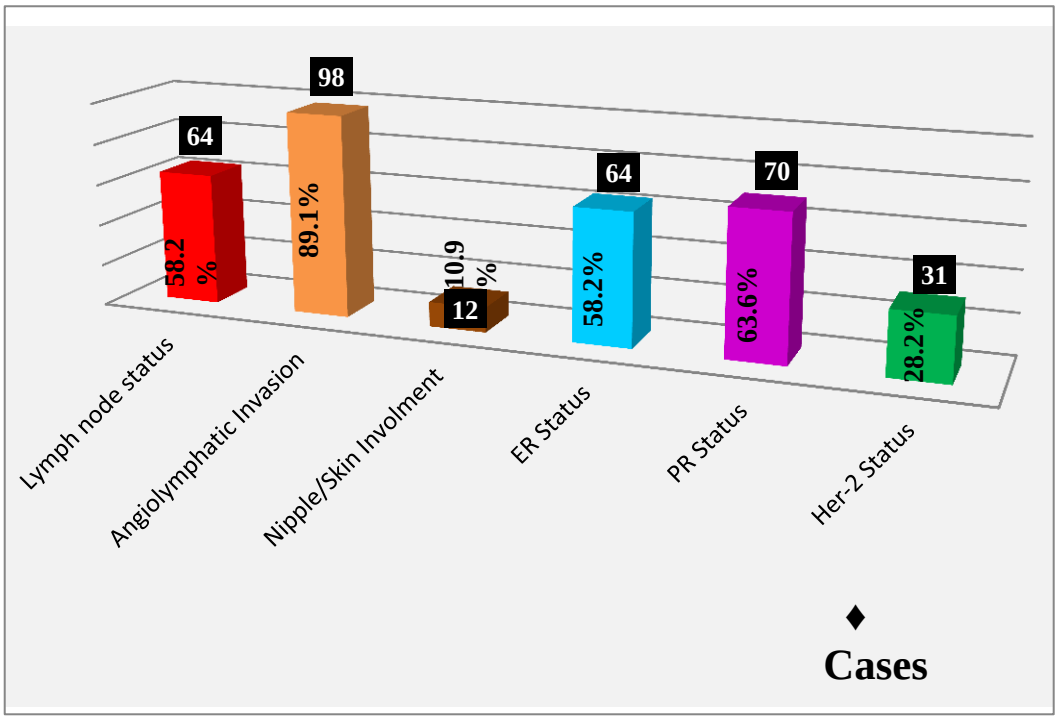


Figure 19: Distribution of the study population according to their Hormone receptor Positive/Present Status

4.3 Anthropometric Characteristics, Vitamin D Levels, and DNA Ratios Assessment.

In **Table 15**, a comparative assessment of anthropometric characteristics, vitamin D levels, and DNA ratios amongst breast cancer patients (cases) and theirs matched controls is presented.

Anthropometric characteristics, including weight, height, and BMI, are crucial factors in understanding the overall health profile of individuals. The mean weight of breast cancerous patients is 58.95 kg with a standard deviation of 7.660, while in the control group, it is slightly lower at 54.36 kg with a standard deviation of 5.07. Similarly, the mean height of breast cancerous patients is 158.06 cm with a standard deviation of 5.761, whereas controls exhibit a slightly taller mean height of 160.85 cm with a standard deviation of 3.70. The BMI, estimated by dividing weight in kilograms by height in meters squared, is higher in breast cancer patients 23.60 kg/m^2 compared to controls 21.02 kg/m^2. These anthropometric differences suggest potential associations between body composition and breast cancer, indicating the relevance of considering such characteristics in cancer risk assessments.

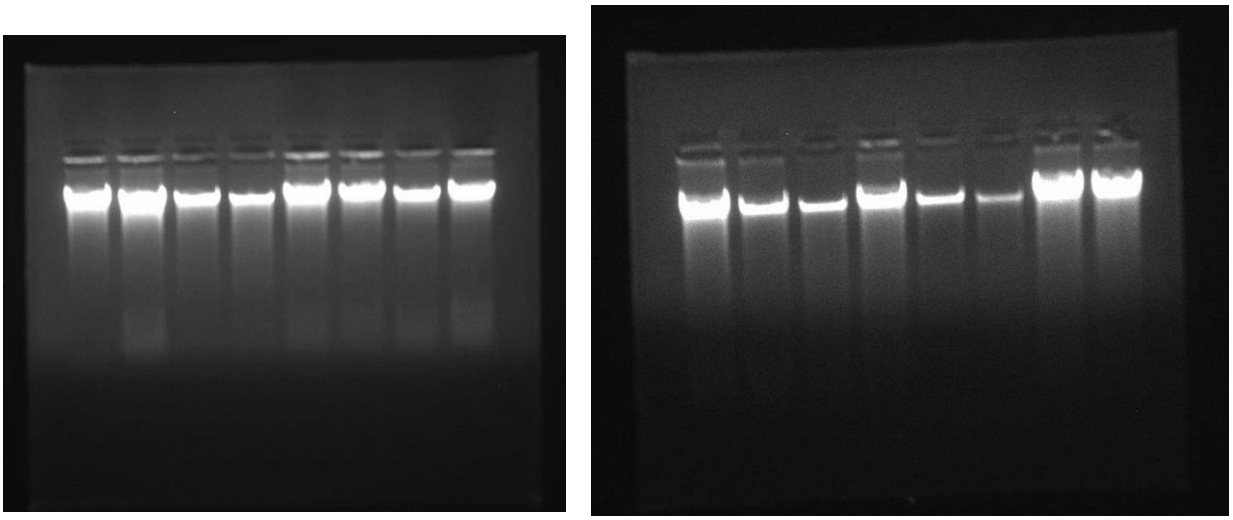
Table 15: Comparative assessment of Anthropometric Characteristics, Vitamin D Levels, and DNA Ratios along Breast Cancer Patients (Cases) and their Matched Controls

Anthropometric Characteristics	Cases (%)	Control (%)
	Mean ± S.D.	
Weight(kg)	58.95 ± 7.660	54.36 ± 5.07
Height(cm)	158.06 ± 5.761	160.85 ± 3.70
BMI(kg/m²)	23.60 ± 3.014	21.02 ± 1.99
Vitamin D level(nmol/L)	43.54 ± 19.525	89.89 ± 26.13
DNA A260	0.130 ± 0.028	0.14 ± 0.026
DNA A280	0.073 ± 0.015	0.08 ± 0.015
Ratio A260/A280	1.78 ± 0.034	1.76 ± 0.039

The vitamin D levels, a critical factor in various physiological processes, exhibit notable differences between breast cancerous patients and controls. Breast cancerous patients show a mean vitamin D level of 43.54 nanomol/l with a standard deviation of 19.525, whereas controls have a substantially higher mean level of 89.89 nmol/L with a standard deviation of 26.13. This disparity suggests a potential correlation amongst vitamin D deficit and breast cancer incidence, emphasizing the need for further exploration into the influence of vitamin D in the progress of cancer of the breast.

The DNA ratios, specifically A260, A280, and the A260/A280 ratio, provide insights into nucleic acid purity and quality. The A260 value represents nucleic acid

absorbance at 260 nm, and A280 at 280 nm, and the A260/A280 ratio indicates the purity of the DNA sample. Breast cancer patients exhibit a mean A260 value of 0.130 with a standard deviation of 0.028, while controls have a slightly higher mean value of 0.14 with a standard deviation of 0.026. The A280 values are 0.073 ± 0.015 in breast cancer patients and 0.08 ± 0.015 in controls. The A260/A280 ratio, reflecting DNA purity, is 1.78 ± 0.034 in breast cancer patients and 1.76 ± 0.039 in controls. These DNA ratio differences may be indicative of variations in DNA quality between breast cancer patients (A) and controls (B), suggesting potential implications for genetic analyses. The comparative assessment of anthropometric characteristics, vitamin D levels, and DNA ratios reveals intriguing differences between breast cancerous patients and controls. These findings taking part in the understanding of potential risk factors and molecular markers for breast cancer-related.



A B

4.4 Analysis of FokI (rs 2228570) polymorphisms of the VDR and breast cancer.

In the case of the Fok1 polymorphism, the amplification product had a size of 272bp (Figure 20). Fok1 restriction site (F) was absent from an entire amplification product, whereas two or three fragments marked the presence of Fok1 (f) restriction site.

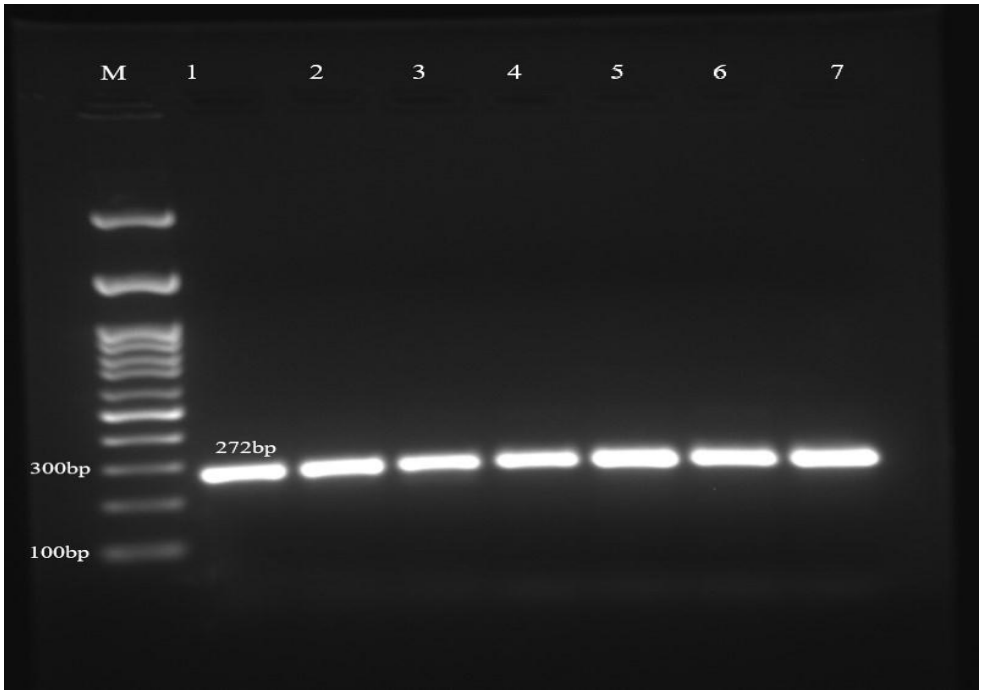


Figure 20: The FokI amplified PCR products of VDR gene stained with ethidium bromide on a 2% agarose gel. A 272bp amplification product was obtained for the Fok1 polymorphism. Lane M indicates 100bp Ladder. On lanes, 1-7, single band related to 272bp FokI amplified PCR products are shown.

An agarose gel analysis of the non-digested, single 272bp bands genotype FF as homozygous for the common allele. Those carrying the homozygote of the uncommon allele (ff) produced 198 bp and 72 bp fragments, whereas, heterozygotes (Ff) presented three fragments as 272 bp, 198 bp, and 72 bp. The distribution of polymorphism in VDR FokI in cases and controls were shown in Figure 21.

There were 110 breast cancer cases and 110 controls covered in the analysis. Table 13, illustrates the genotypes and alleles frequencies among breast cancerous patients and controls. There were 29.1% constituted the Heterozygous Ff, 54.5% found to be Homozygous FF, and 16.4% presented as homozygous ff cases related to the Fok1 polymorphism. The corresponding Control group genotype frequencies were 58.2%, 38.2%, and 3.6%, respectively.

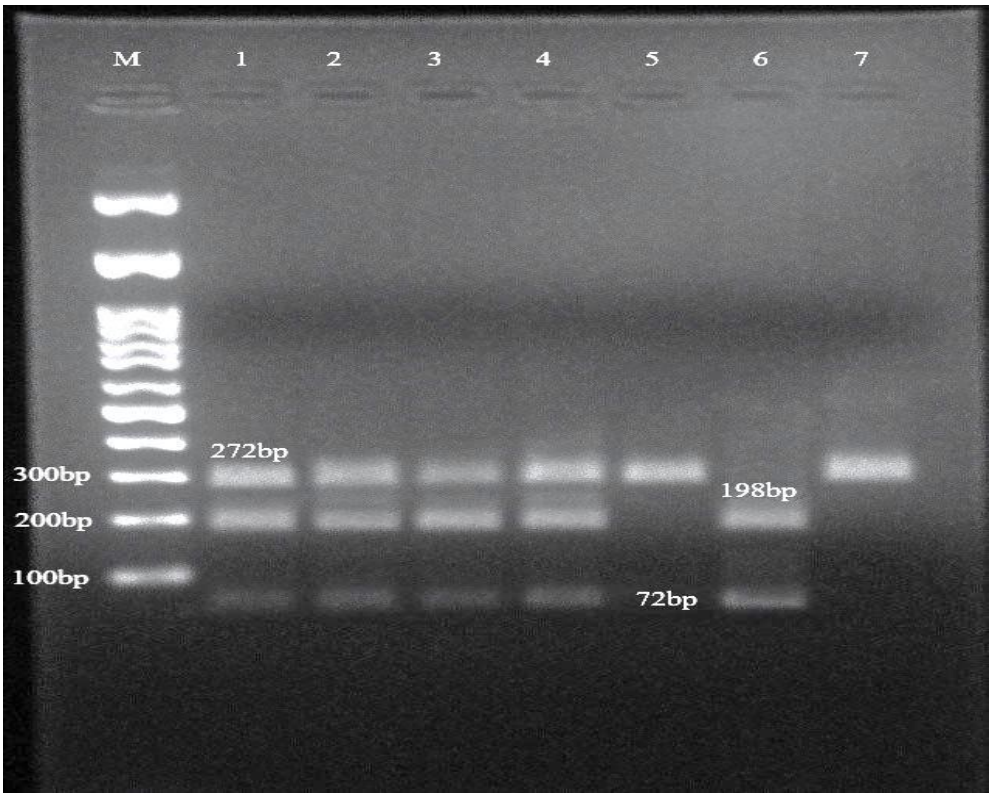


Figure 21: A picture of two percent agarose gel stained with ethidium bromide illustrating amplification products digested by Fok1. The upper band corresponds to F (T allele), while the lower band corresponds to f (C allele). Lane M indicates 100bp Ladder. Lanes, 1-3, represent Ff heterozygotes, Lanes 4-6, represents FF homozygotes and Lane 7 represent ff homozygotes.

4.5 Analysis of ApaI (rs 7975232) VDR polymorphisms with Breast Cancer.

According to the Apa1 polymorphism, the amplification product had a size of 352bp (Figure 22). The Apa1 restriction site (A) was absent from an entire amplification product, whereas two or three fragments correspond to the existence of the Apa1 restriction site (a).

The undigested 352bp bands on agarose gels were genotyped as homozygous alleles for AA. Those carrying the homozygote of the infrequent allele (aa) produced 216bp and 135bp fragments, whereas, heterozygotes (Aa) displayed three fragments as 352bp, 216bp, and 135bp. A comparison of polymorphism distributions in VDR Apa1 between cases and controls were shown in Figure 23.

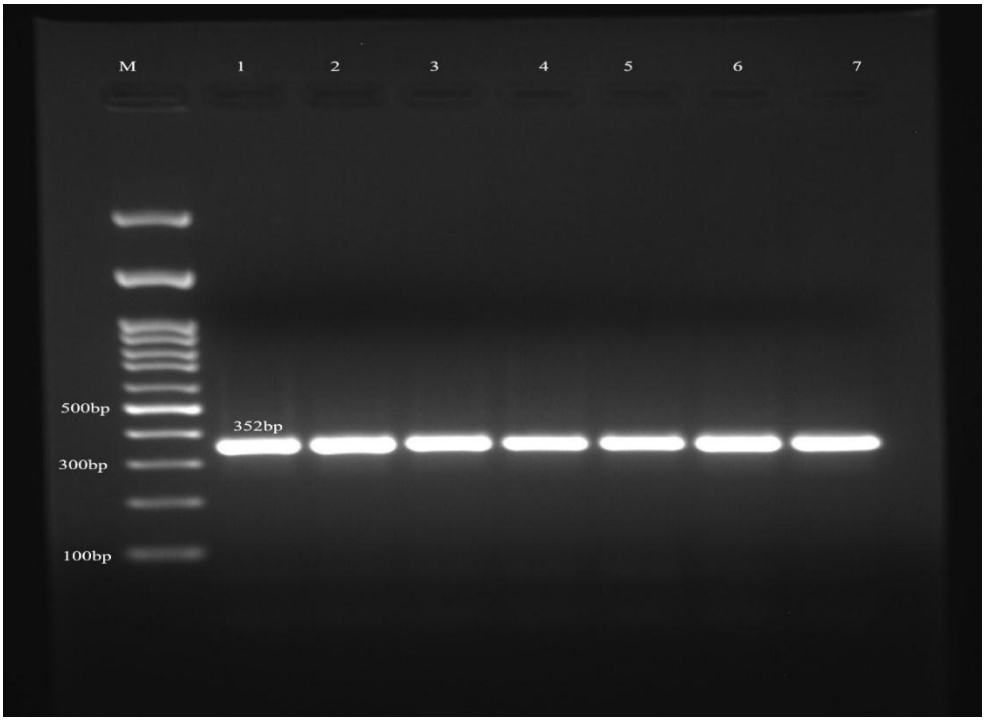


Figure 22: The Apa1 amplified PCR products of VDR gene stained with ethidium bromide on a 2% agarose gel. A 352bp amplification product was obtained for the Apa1 polymorphism. Lane M indicates 100bp Ladder. On lanes, 1-7, single bands related to 352bp Apa1 PCR products are shown.

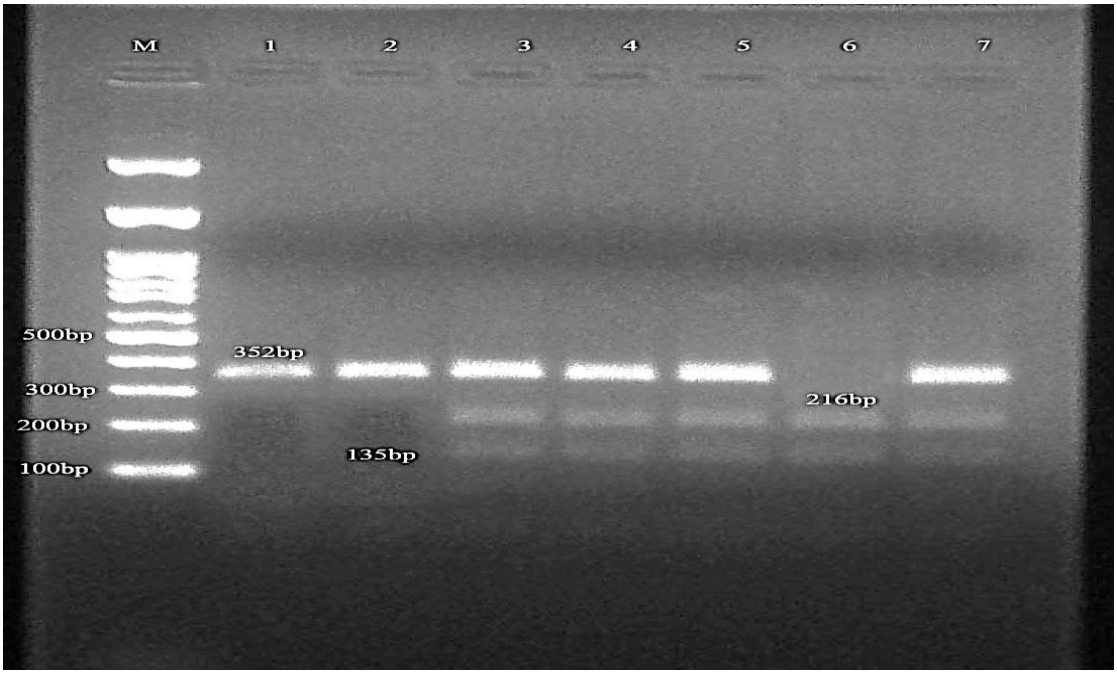


Figure 23: This image shows Apa1 digested amplification products of two percent agarose gel stained with ethidium bromide. The upper band corresponds to A (C allele), while the lower band corresponds to a (A allele). Lane M indicates 100bp Ladder. Lanes 1,2 represent AA homozygotes, Lanes 3,4,5,7, represents Aa heterozygotes and Lane 6 represent aa homozygotes.

4.6 Analysis of BsmI (rs1544410) VDR polymorphisms with Breast Cancer.

An amplification product of approximately 825bp was obtained for the Bsm1 polymorphism (Figure 24). The product of intact amplification exhibited the lack of the Bsm1 restriction site (B), while two or three fragments revealed the presence of the Bsm1 restriction site (b).

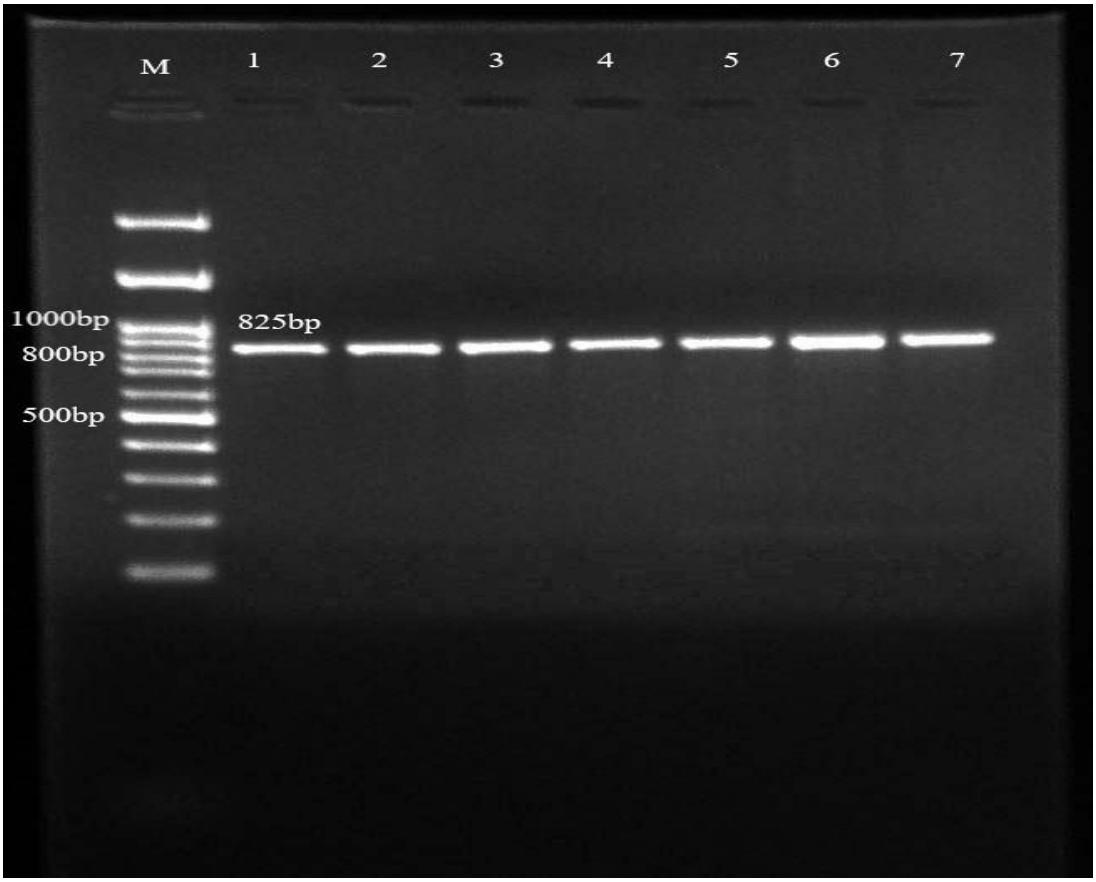


Figure 24: The PCR amplification of the Bsm1 gene is shown in this picture with the ethidium bromide stain on a 2% agarose gel. As a result of amplification of the Bsm1 polymorphism, 825bp of DNA was obtained. The 100bp ladder M shown in Lane 1. A single-band PCR product of 825bp were visible in lanes 1-7.

VDR Bsm1 polymorphism distributions in cases and controls are shown in Figure 25. Agarose gel genotyping of the single 825 bp undigested bands suggested the BB genotype as homozygous for the common allele. The homozygote of the infrequent allele depicting the bb genotype produced two fragments (650bp and 175bp) and heterozygotes displaying the Bb genotype produced three fragments (825bp, 650bp, and 175bp).

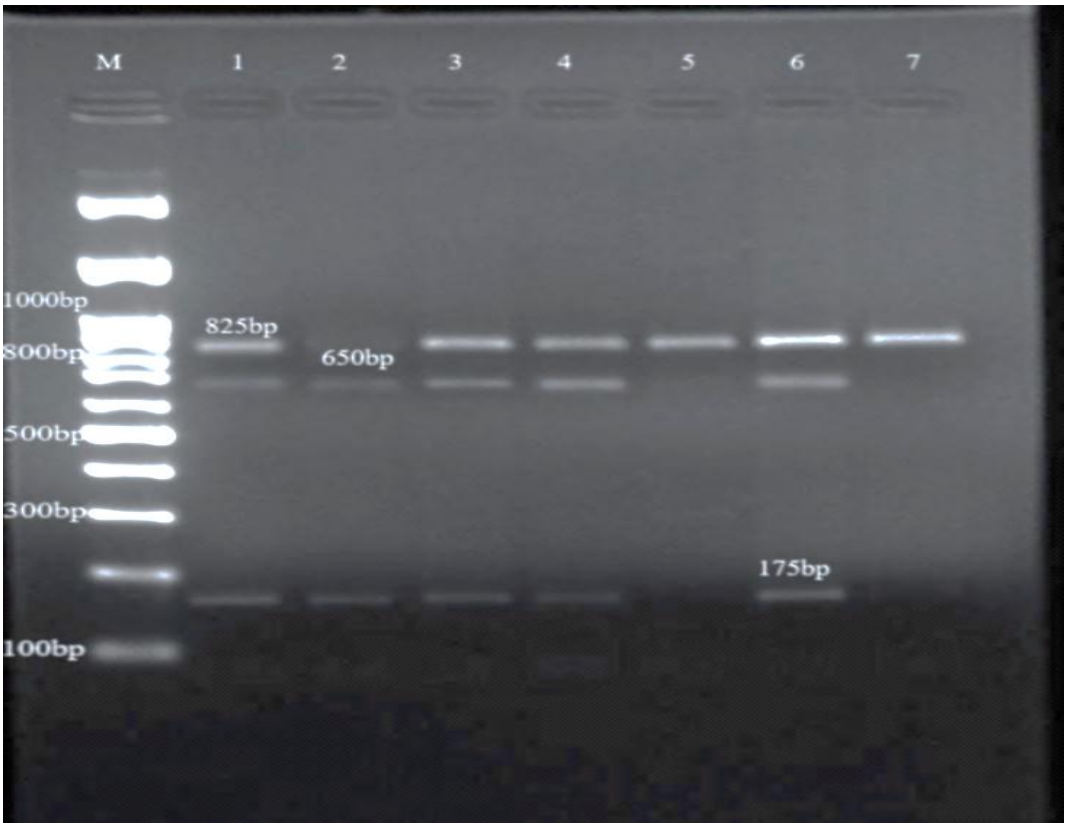


Figure 25: Picture of two percent agarose gel visualized with ethidium bromide showing PCR products of VDR gene digested by Bsm1. In this illustration, the upper bands represent the B allele (A) and the lower bands represent the b allele (G). Lane M displays a 100bp ladder M. Lanes 1,3,4 and 6 represent Bb heterozygotes, Lanes 5,7 represent BB homozygotes and Lane 2 represents bb homozygotes.

A total of 110 cases of breast cancer and 110 controls were analyzed. Table 13, provides information about the genotypes and allele frequencies among patients of cancer of the breast and controls. There were 53.6% constituted the Heterozygous Bb, 42.7% found to be Homozygous BB, and 3.6% presented homozygous aa as cases referring to the Bsm1 polymorphism. Likewise, the Control group genotype frequencies were 47.3%, 50%, and 2.7%, respectively.

4.7 Vitamin D levels and genotypes distribution.

As shown in **Table 16,** The Vitamin D levels and genotypic distributions alongside breast cancerous patients and matched controls yield insightful information regarding potential associations between genetic factors and Vitamin D status in the context of breast cancer. 73(66.4%) of breast cancerous cases exhibit Vitamin D levels below 50, a stark contrast to the mere 7(6.4%) observed in controls. This significant difference suggests a potential link connecting vitamin D deficit and an increased risk of cancer of the breast. On the contrary, a noteworthy 86(78.2%) of controls showcase Vitamin D levels within the 75-250 range, indicating a potential protective role of adequate Vitamin D levels against breast cancer development.

Table 16: Distribution of Vitamin D Levels & Genotypes (FokI, BsmI, and ApaI) Linking Patients of Cancer of the Breast and Matched Controls in the Study Population

		Cases (%)	**Control (%)**
Vitamin D level	<50	73(66.4%)	7(6.4%)
	50 to <75	30(27.3%)	17(15.5%)
	75-250	7(6.4%)	86(78.2%)
Genotypes (FokI)	FF	60(54.5%)	64(58.2%)
	Ff	32(29.1%)	42(38.2%)
	ff	18(16.4%)	4(3.6%)
Genotypes (ApaI)	AA	54(49.1%)	62(56.4%)
	Aa	45(40.9%)	43(39.1%)
	aa	11(10%)	5(4.5%)
Genotypes (BsmI)	BB	47(42.7%)	55(50%)
	Bb	59(53.6%)	52(47.3%)
	bb	4(3.6%)	3(2.7%)
Total		110(100%)	110(100%)

Examining genotypic variations, The FF genotype in the FokI gene is slightly more prevalent in controls 64(58.2%) than in cases 60(54.5%), while the ff genotype demonstrates a higher frequency in cases 18(16.4%) compared to controls 4(3.6%). This discrepancy implies a potential connection between specific FokI genotypes and breast cancerous risk, shedding light on the genetic factors that might contribute to the disease.

Similarly, in the ApaI gene, the AA genotype is more often in controls 62(56.4%) than in cases 54(49.1%), suggesting a potential protective effect associated with this genotype. Regarding the BsmI genotype, the BB genotype is more prevalent in cases at 59(53.6%), whereas it constituted 52(47.3%) in controls. The bb genotype, representing the homozygous recessive form, was present in 4(3.6%) of cases and 3(2.7%) of controls.

The data underscores the intricate relationship between Vitamin D levels, specific genotypes (FokI, ApaI, BsmI), and breast cancer risk. The observed patterns prompt further exploration into the underlying mechanisms and potential interactions between genetic factors and Vitamin D in the context of cancer of the breast.

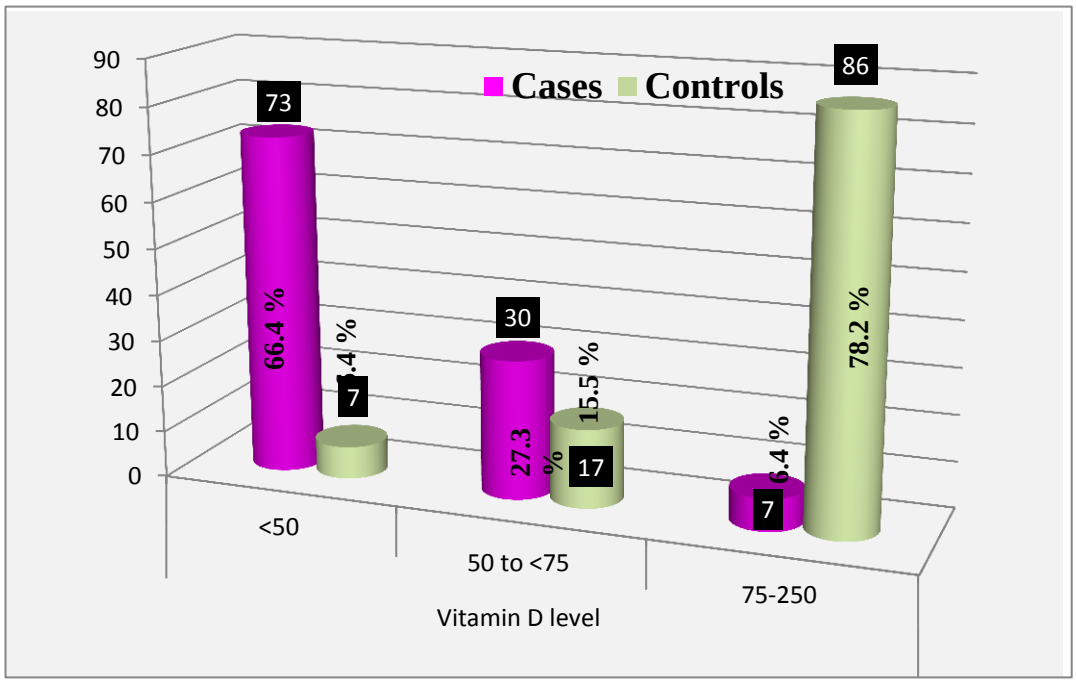


Figure 26: Distribution of the study population as per their Vitamin D levels

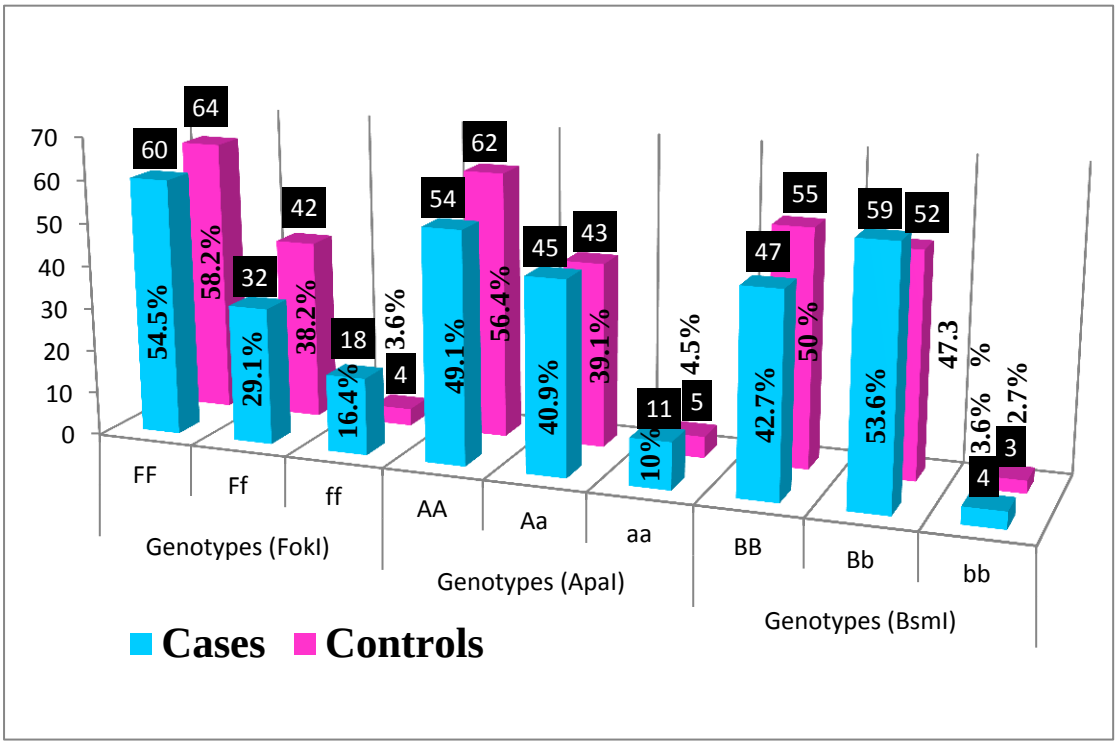


Figure 27: Distribution of the study population according to their Genotypes

4.8 Association of VDR genotypes.

In **Table 17**, it presents the association between genotypes among patients of breast cancer and controls in the study population, providing valuable insights into the potential genetic factors contributing to breast cancer susceptibility.

The table includes information on three specific genetic markers: FokI, ApaI, and BsmI, along with their respective genotypic distributions in cases and controls, odds ratios (OR) with 95% confidence intervals, p-values, and $\chi 2$ values.

For the FokI genotype, For the FF genotype, there were 60 cases and 64 controls. The odds ratios (ORs) with a 95% confidence intervals (CIs) was 5.49 (1.72, 17.64), and the p-value was 0.004. This suggests an association of significance between the FF genotype and cancer of breast in the study population (p=0.004). Similarly, individuals with the Ff genotype showed a smaller occurrence in cases (32) compared to controls (42), based on the odds ratio of 6.00 (95% confidence interval: 1.83, 19.67) and a p-value of 0.003.

Table 17: Association between Genotypes along with breast cancerous patients and controls in the study population

Genotypes		Cases	Allele probabilities (P-value HWE)	Control	Allele probabilities (p-value HWE)	ORs (95% CI)	*p-value**	*χ2 value*
Genotypes (FokI)	FF	60	F= 0.69, f = 0.30 (0.15)	64	F= 0.77, f = 0.22 (0.15)	5.49 (1.72,17.64)	0.004	0.006
	Ff	32		42		6.00 (1.83,19.67)	0.003	
	ff	18		4		-	0.181	
Genotypes (ApaI)	AA	54	A= 0.69, a = 0.30 (0.15)	62	A= 0.75, a = 0.24 (0.15)	2.87 (0.92,8.97)	0.069	0.241
	Aa	45		43		2.31 (0.72,7.37)	0.157	
	aa	11		5		-	0.518	
Genotypes (BsmI)	BB	47	B= 0.69, b = 0.30 (0.15)	55	B= 0.73, b = 0.26 (0.15)	1.76 (0.36,8.54)	0.482	0.546
	Bb	59		52		1.30 (0.27,6.25)	0.743	
	bb	4		3		0.056	0.009	
Total		110		110				

***Hardy Weinberg equilibrium , *p-value <0.05, Significant**

Nevertheless, the ff genotype is fewer in the control group (4) contrary to the case group (18), and this difference in frequency fails to achieve statistical significance. The allele probabilities and p-values for Hardy Weinberg equilibrium (HWE) 0.15 indicate equilibrium in both cases and controls. This adherence to HWE supports the reliability of the genotypic data, suggesting that factors such as selection or genetic drift do not significantly impacting the distribution.

In the ApaI genotype, the distribution of genotypes reveals a lower frequency of AA genotypes in cases (54) compared to controls (62), with an odds ratios of 2.87 (95% CI: 0.92, 8.97) and a p-value of 0.069. The Aa genotype also shows a slightly higher frequency in cases (45) than in controls (43), with an odds ratios of 2.31 (95% CI: 0.72, 7.37) and a p-value of 0.157. Again, The equilibrium is strengthened by the Hardy-Weinberg equilibrium, p-values of 0.15 in both cases and controls. This consistency with HWE enhances the reliability of the observed associations, and deviations from expected genotype frequencies may be indicated by underlying genetic or environmental influences.

Considering the BsmI genotype, the BB genotype was there 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. The bb genotype was identified in 4 cases and 3 controls, in an odds ratios of 0.056 and a p value of 0.009. The Hardy Weinberg equilibrium, p-values of 0.15 in both cases and controls suggest that the observed genotypic frequencies align with expectations

The analysis of genotypes reveals significant associations between the FokI genotypes FF and Ff and the risk of breast cancer. While the ApaI and BsmI

genotypes show trends toward association, these findings emphasize the potential part of genetic factors in predisposing individuals to cancer of the breast within the studied population. The odds ratios and associated statistical values provide a quantitative measure of these associations. The Hardy-Weinberg equilibrium indicated potential links between specific genotypes and the probability of progressing breast cancer in the population under study. The adherence to Hardy-Weinberg Equilibrium (HWE) has enhanced the dependability of the results, establishing a strong basis for additional research into the impact of genetic and environmental factors on the vulnerability to breast cancer. Researchers frequently employed statistical tests, such as the chi-square test, to evaluate if observed genotype frequencies deviated considerably from the frequencies expected under Hardy-Weinberg equilibrium. This illustrates the significance of HWE in genetic association studies.

4.9 Menopausal Status and Genotypes Association.

Table 18, depicts the association between menopausal status and genotypes among breast cancer patients, exploring the connection between FokI, ApaI, and BsmI genotypes and menopausal status. The frequencies and percentages of genotypes are reported for including pre- and postmenopausal breast cancer patients, with associated p-values indicating statistical significance.

Table 18: Association between Menopausal Status and Genotypes among breast cancer patients

Genotypes		Menopausal status		*p-value**
		Pre-menopausal	Post-menopausal	
FokI	FF	40	20	0.053
	Ff	13	19	
	ff	11	7	
ApaI	AA	30	24	0.840
	Aa	27	18	
	aa	7	4	
BsmI	BB	24	23	0.363
	Bb	38	21	
	bb	2	2	

p <0.05, Significant

Examining the FokI genotype distribution, the FF genotype was found in 40 pre-menopausal cases, and in 20 post-menopausal cases. The p-value for the association between the FokI FF genotype and menopausal status was 0.053, suggesting a marginally significant difference. The Ff genotype was observed in 13

pre-menopause cases and 19 post-menopause cases, while the ff genotype occurred in 11 pre-menopause cases and 7 post-menopause cases. The association between the FokI genotypes Ff and ff and menopausal status is not significant in terms of statistical significance (p-value > 0.05).

For the ApaI genotypes distribution, the AA genotype was identified in 30 pre-menopause cases and 24 post-menopause cases. The Aa genotype was observed in 27 pre-menopause cases and 18 post-menopause cases, while the aa genotype occurred in 7 pre-menopausal cases and 4 post-menopausal cases. The p-value for the association between ApaI genotypes and menopausal stature was 0.840, indicating no statistically significant difference in distribution.

In the case of the BsmI genotype, the BB genotype was found in 24 pre-menopause cases and 23 post-menopause cases. The Bb genotype occurred in 38 pre-menopause cases and 21 post-menopause cases, while the bb genotype was identified in 2 pre-menopause cases and 2 post-menopause cases. The p-value for the association between BsmI genotypes and menopausal stature was 0.363, indicating no significant difference in distribution.

It suggests a potential association between the FokI FF genotype and pre-menopausal status, although the p-value falls slightly above the conventional significance threshold. The ApaI and BsmI genotypes do not show a significant association with menopausal status. These findings provide insight into the connection between genetic factors and menopausal status in breast cancer patients within the studied population.

4.10 Tumor Grade and Genotypes Association.

Table 19, elucidates the association between tumor grade and genotypes among breast cancer patients, examining the distribution of FokI, ApaI, and BsmI genotypes across well-differentiated, moderately differentiate, and poorly differentiate tumor grades. The frequencies and percentages of genotypes are reported for each tumor grade, accompanied by p-values indicating statistical significance.

In the FokI genotype, the FF genotype shows no occurrences in well-differentiated tumor cases, while it is observed in 42 cases and 18 cases for moderately and poorly differentiated tumors, respectively. The p-value for the association between the FokI FF genotype and tumor grade was 0.160, suggesting no statistically significant difference in distribution. The Ff genotype was found in 3 cases with well-differentiated tumors, 20 cases with moderately differentiated tumors, and 9 cases with poorly differentiated tumors. The ff genotype occurred in 1 case with well-differentiated tumors, 14 cases with moderately differentiated tumors, and 3 cases with poorly differentiated tumors. The association between FokI genotypes and tumor grade achieved no significance (p-value > 0.05).

For the ApaI genotype, the AA genotype was observed in 2 cases with well-differentiated tumors, 42 cases with moderately differentiated tumors, and 10 cases with poorly differentiated tumors. The Aa genotype was found in 2 cases (1.8%) with well-differentiated tumors, 34 cases with moderately differentiated tumors, and 9 cases (with poorly differentiated tumors. Interestingly, the aa genotype was not present in well-differentiated or moderately differentiated tumors but occurred in 11 cases with poorly differentiated tumors. The p-value for the association between ApaI

genotypes and tumor grade was 0.000, indicating a significant difference in distribution.

Table 19: Association between Tumor Grade and Genotypes among breast cancer patients

Genotypes		Histologic Subtype (Tumor Grade)			*p-value**
		Well-differentiated (Grade I)	Moderately differentiated (Grade II)	Poorly differentiated (Grade III)	
FokI	FF	0	42	18	0.160
	Ff	3	20	9	
	ff	1	14	3	
ApaI	AA	2	42	10	0.000
	Aa	2	34	9	
	aa	0	0	11	
BsmI	BB	2	35	10	0.779
	Bb	2	38	19	
	bb	0	3	1	

p <0.05, Significant

Turning to the BsmI genotype, the BB genotype occurred in 2 cases with well-differentiated tumors, 35 cases with moderately differentiated tumors, and 10 cases with poorly differentiated tumors. The Bb genotype was observed in 2 cases with well-differentiated tumors, 38 cases with moderately differentiated tumors, and 19 cases with poorly differentiated tumors. The bb genotype was not present in well-differentiated tumors, occurred in 3 cases with moderately differentiated tumors, and

1 case with poorly differentiated tumors. The p-value for the association between BsmI genotypes and tumor grade was 0.779, indicating no statistically significant difference in distribution.

A significant correlation between the ApaI genotype and tumor grade, specifically, the AA genotype was significantly associated with Grade II tumors, while the Aa genotype showed a significant association with Grade III tumors. The findings suggest that, within the examined cohort, the ApaI genotype may exhibit a correlation with specific tumor grades in breast cancerous patients. These findings contribute valuable insights into the prospective significance of genetic factors in the differentiation of breast cancerous tumors within the studied population.

4.11 Lymph Node Status and Genotypes Association.

In **Table 20**, an investigating the relationship between lymph node status and genotypes among breast cancerous patients, the analysis encompassed the examination of three distinct genotypes: FokI, ApaI, and BsmI. The distribution of these genotypes was assessed concerning an absence or presence of lymph nodes involvement among the patients.

Table 20: Association between Lymph node status and Genotypes among breast cancer patients

Genotypes		**Lymph Node**		***p-value****
		Present	Absent	
FokI	FF	36	24	0.423
	Ff	20	12	
	ff	8	10	
ApaI	AA	25	29	0.030
	Aa	30	15	
	aa	9	2	
BsmI	BB	29	18	0.790
	Bb	33	26	
	bb	2	2	

p-value <0.05, Significant

In the case of the FokI genotype, individuals with the genotype of FF exhibited a distribution of 36 cases with present lymph nodes and 24 cases with absent lymph nodes. The statistical analysis yielded a non-significant p-value of

0.423. Similarly, for the Ff genotype, there were 20 cases with present lymph nodes and 12 cases with absent lymph nodes, with a non-significant p-value of the same magnitude. The ff genotype was found not to have shown a significant association, with 8 cases being presented with lymph nodes, and 10 cases were found not to have lymph nodes.

Moving to the ApaI genotype, individuals with the AA genotype exhibited a distribution of 25 cases with present lymph nodes and 29 cases with absent lymph nodes, resulting in a p-value of 0.030. For the Aa genotype, 30 cases had present lymph nodes, while 15 cases had absent lymph nodes, with a significant p-value of less than 0.05. The aa genotype also displayed a significant association, with 9 cases presenting with lymph nodes and 2 cases without, yielding a p-value below the significance threshold at 0.030.

Finally, for the BsmI genotype, individuals with the BB genotype had a distribution of 29 cases with present lymph nodes and 18 cases with absent lymph nodes, resulting in a non-significant p-value of 0.790. The Bb genotype exhibited 33 cases with present lymph nodes and 26 cases with absent lymph nodes, again yielding a non-significant p-value. The bb genotype showed 2 cases with present lymph nodes and 2 cases with absent lymph nodes, resulting in a non-significant p-value of 0.790.

The associations between lymph node status and the ApaI genotype, specifically with the AA and Aa genotypes, while no significant associations were observed for the FokI and BsmI genotypes among breast cancerous patients. The findings suggest a potential role of the ApaI genotype in influencing lymph node involvement in the examined cohort.

4.12 Stage Groupings and Vitamin D Levels Association.

As shown in **Table 21**, the association between stage groupings and levels of vitamin D in patients with cancer of the breast was investigated in the study. The results, based on different vitamin D level categories (<50, 50 to <75, and 75-250 nmol/l) for each stage grouping (I, IIA, IIB, IIIA, IIIC, IV, NA), are summarized.

For stage I, there were 3 cases with vitamin D levels <50 nmol/l, 3 cases with levels between 50 to <75 nanomol/l, and 1 case with levels amongst 75-250 nmol/L. The p-value was 0.388, indicating no statistically significant correlation exists between stage I and vitamin D levels (p=0.388). Similarly, For stage IIA, there were 22 cases with vitamin D levels less than 50 nanomol/l, 8 cases with levels between 50 to <75 nmol/l, and 1 case with levels between 75-250 nmol/L. The analysis did not reveal a statistically significant interrelation, with a p-value above 0.050.

For stage IIB, there were 27 cases with concentrations of vitamin D less than 50 nanomol/l, 6 cases with levels between 50 to <75 nanomol/l, and 2 cases with levels between 75-250 nmol/l. The association was not significative, with a p-value exceeding 0.05. For stage IIIA, there were 15 cases with amounts of vitamin D less than 50 nanomol/l, 11 cases with levels between 50 to <75 nanomol/l, and 1 case with levels between 75-250 nmol/l. The analysis did not yield a statistically significant correlation, with a p-value above 0.05.

Table 21: Association between Stage Groupings and Vitamin D levels (nmol/l) among Breast cancer patients

Stage Groupings	Vitamin D levels (nmol/L)			*p-value**
	< 50	**50 to < 75**	**75 - 250**	
I	3	3	1	0.388
IIA	22	8	1	
IIB	27	6	2	
IIIA	15	11	1	
IIIC	4	2	2	
IV	1	0	0	
NA	1	0	0	
N=110	73	30	7	

p <0.05, Significant, NA-not applicable.

For stage IIIC, there were 4 cases with levels of vitamin D less than 50 nanomol/l, 2 cases with levels between 50 to <seventy-five nmol/l, and 2 cases with levels between 75-250 nmol/l. The p-value was not statistically significant, exceeding 0.05. For stage IV, there was 1 case of vitamin D levels <50 nanomol/l, and no cases were reported for the other two vitamin D level categories.

The analysis did not reveal any significant associations between stage groupings and vitamin D levels among patients of breast cancer. The findings suggest that, within the examined cohort, vitamin D levels may not exhibit a strong correlation with the various stage groupings of breast cancer.

4.13 Tumor Grade and Vitamin D Levels Association.

Table 22, depicts the examination of the association between tumor grade and levels of vitamin D (nmol/l) in patients with cancer of the breast involved in a review of patients distributed across different vitamin D level categories (<50, 50 to <75, and 75-250) within each histologic subtype (Grade I, Grade II, Grade III, and NA). An inclusive of 110 patients with breast cancer were incorporated in the analysis.

For Grade I tumors, no cases were observed for vitamin D levels less than of 50 nanomol/l, 2 cases had levels between 50 to <75 nanomol/l, and no cases had levels between 75-250 nmol/l. The p-value was 0.433, indicating that an insignificant statistical association was found between Grade I tumors and levels of vitamin D (p=0.433).

Table 22: Association between the Tumor Grade and Vitamin D Levels (nmol/l) among Breast cancer patients

Histologic Subtype (Tumor Grade)	**Vitamin D levels (nmol/L)**			***p-value****
	< 50	**50 to < 75**	**75 - 250**	
Grade I	0	2	0	0.433
Grade II	51	20	5	
Grade III	21	8	2	
NA	1	0	0	
N = 110	73	30	7	

p <0.05, Significant, NA- not applicable.

For Grade II tumors, there were 51 cases for vitamin D levels less than 50 nanomol/l, 20 cases with levels between 50 to <75 nanomol/l, and 5 cases with levels between

75-250 nmol/l. The analysis did not reveal a statistically significant correlation, with a p-value above 0.05. For Grade III tumors, there were 21 cases with vitamin D levels less than fifty nanomol/l, 8 cases with levels between 50 to <75 nanomol/l, and 2 cases with levels between 75-250 nmol/l. The association was not statistically significant, with a p-value exceeding 0.05.

The study did not reveal any statistically significant associations between tumor grades (grades I, II, and III) and vitamin D levels among breast cancer patients. These findings advance our comprehension of the potential association between tumor grade and vitamin D status in this patient population.

4.14 Hormone Receptor Status and Genotypes Association.

In **Table 23,** it shows that in the investigation of the connection between hormone receptor status and genotypes among breast cancer patients, an analysis was conducted on the distribution of genotypes (FF, Ff, ff for Fok1; AA, Aa, aa for ApaI; BB, Bb, bb for BsmI) for estrogen receptor (ER) status. The study included a total of cases with positive ER status and cases with negative ER status.

Table 23: Association between Hormone receptor status and Genotypes among Breast cancer patients

Genotypes		ER Status			
		Cases		Odds Ratio (95% of C.I.)	*p-value**
		Positive	Negative		
FokI	FF	41(68.3%)	19(31.7%)	2.53 (1.163, 5.514)	0.024
	Ff	17(53.1%)	15(46.9%)	0.74 (0.326, 1.713)	
	ff	6(33.3%)	12(66.7%)	0.29 (0.100, 0.852)	
ApaI	AA	33(61.1%)	21(38.9%)	1.26 (0.592, 2.70)	0.017
	Aa	29(64.4%)	16(35.6%)	1.55 (0.711, 3.393)	
	aa	2(18.2%)	9(81.8%)	0.13 (0.027, 0.647)	
BsmI	BB	26(55.3%)	21(44.7%)	0.81 (0.379, 1.750)	0.793
	Bb	36(61%)	23(39%)	1.28 (0.601, 2.749)	
	bb	2(50%)	2(50%)	0.70 (0.096, 5.231)	
Genotypes		**PR Status**			
		Cases			*p-value**
		Positive	Negative		
FokI	FF	43(71.7%)	17(28.3%)	2.15 (0.977, 4.749)	0.091
	Ff	19(59.4%)	13(40.6%)	0.77 (0.332, 1.802)	
	ff	8(44.4%)	10(55.6%)	0.75 (0.261, 2.153)	
ApaI	AA	35(64.8%)	19(35.2%)	1.10 (0.507, 2.405)	0.128

	Aa	31(68.9%)	14(31.1%)	1.47 (0.661, 3.29)	
	aa	4(36.4%)	7(63.6%)	0.28 (0.078, 1.045)	
BsmI	BB	28(59.6%)	19(40.4%)	0.73 (0.336, 1.613)	0.578
	Bb	40(67.8%)	19(32.2%)	1.47 (0.675, 3.216)	
	bb	2(50%)	2(50%)	0.55 (0.075, 4.128)	
Genotypes		**Her-2 Status**			
		Cases			***p-value****
		Positive	Negative		
FokI	FF	17(28.3%)	43(71.7%)	1.01 (0.441, 2.342)	0.438
	Ff	7(21.9%)	25(78.1%)	0.63 (0.239, 1.655)	
	ff	7(38.9%)	11(61.1%)	1.80 (0.627, 5.182)	
ApaI	AA	15(27.8%)	39(72.2%)	0.96 (0.418, 2.207)	0.691
	Aa	14(31.1%)	31(68.9%)	1.27 (0.550, 2.951)	
	aa	2((18.2%)	9(81.8%)	0.53 (0.109, 2.636)	
BsmI	BB	14(29.8%)	33(70.2%)	1.14 (0.497, 2.650)	0.945
	Bb	16(27.1%)	43(72.9%)	0.89 (0.388, 2.052)	
	bb	1(25%)	3(75%)	0.51 (0.050, 5.146)	

p <0.05, Significant

In **ER Status,**

For the FokI genotype, the FF genotype exhibited a distribution of 41(68.3%) cases with positive ER status and 19(31.7%) cases with negative ER status, resulting in a significant p-value of 0.024. Similarly, for the Ff genotype, there were 17(53.1%) cases with positive ER status and 15 (46.9%) cases with negative ER status. The ff genotype showed 6(33.3%) cases with positive ER status and 12(66.7%) cases with negative ER status. The associated p-value is 0.024, indicating a statistically significant association between the FF genotype and ER positivity. Concerning the ApaI genotype, individuals with the AA genotype had a distribution of 33(61.1%) cases with positive ER status and 21(38.9%) cases with negative ER status, resulting

in a significant p-value of 0.017. For the Aa genotype, 29(64.4%) cases had positive ER status, while 16(35.6%) cases had negative ER status. The aa genotype exhibited 2(18.2%) cases with positive ER status and 9(81.8%) cases with negative ER status. The associated p-value is 0.017, indicating a statistically significant association between the AA genotype and ER positivity.

The BsmI genotype did not reveal a significant association with ER status. Individuals with the BB genotype had a distribution of 26(55.3%) cases with positive ER status and 21(44.7%) cases with negative ER status, resulting in a non-significant p-value of 0.793. The Bb genotype exhibited 36(61%) cases with positive ER status and 23(39%) cases with negative ER status, while the bb genotype showed 2(50%) cases with positive ER status and 2(50%) cases with negative ER status.

The association between hormone receptor status and genotypes among breast cancerous patients was investigated in the study, focusing on estrogen receptor (ER) stature, progesterone receptor (PR) stature, and human epidermal growth factor receptor 2 (Her-2) stature. The results are presented for each genotype at the FokI, ApaI, and BsmI loci, and the p-values are provided for ER, PR, and Her-2 statuses.

In **PR Status,**

For the FokI genotype, the FF genotype showed 43(71.7%) cases with PR positive status and 17 (28.3%) cases with PR negative status. The p-value was 0.091, indicating a trend but not reaching statistical significance (p=0.091). For the Ff genotype, there were 19(59.4%) cases with PR-positive status and 13(40.6%) cases with PR negative status. For PR status, the p-value was 0.128, indicating no significant association. For the ff genotype, there were 8(44.4%) cases with PR-

positive status and 10(55.6%) cases with PR negative status. The analysis yielded a p-value of 0.091 for PR-status, suggesting no statistically significant association (p=0.091).

Moving on to the ApaI genotype, the AA genotype showed 35(64.8%) cases with PR-positive status and 19(59.4%) cases with PR-negative status. The p-value was 0.128, indicating no statistically significant association (p=0.128). For the Aa genotype, there were 31(68.9%) cases with PR-positive status and 14(31.1%) cases with PR-negative status. The analysis yielded a p-value of 0.128 for PR status, indicating no statistically significant association (p=0.128). For the aa genotype, there were 4(36.4%) cases with PR-positive status and 7(63.6%) cases with PR-negative status. The p-value was 0.128 for PR status, indicating no statistically significant association.

Moving to the BsmI genotype, the BB genotype showed 28(59.6%) cases with PR-positive status and 19(40.4%) cases with PR-negative status. The p-value was 0.578, indicating no statistically significant association (p=0.578). For PR status, the BB genotype exhibited a p-value of 0.578, suggesting no significant association (p=0.578). For the Bb genotype, there were 40(67.8%) cases with PR-positive status and 19(40.4%) cases with PR-negative status. The analysis yielded a p-value of 0.578 for PR status, indicating no statistically significant association (p=0.578). For the bb genotype, there were 2(50%) cases with PR-positive status and 2(50%) cases with PR-negative status. The p-value was 0.578 for PR status, indicating no statistically significant association (p=0.578).

In **Her-2 Status,**

For Her-2 status, the analysis revealed that the FF genotype exhibited a distribution of 17(28.3%) cases with positive Her-2 status and 43(71.7%) cases with negative Her-2 status, resulting in a non-significant p-value of 0.438. Similarly, for the Ff genotype, there were 7(21.9%) cases with positive Her-2 status and 25(78.1%) cases with negative Her-2 status, while the ff genotype showed 7(21.9%) cases with positive Her-2 status and 11(61.1%) cases with negative Her-2 status. The statistical analysis for Ff and ff genotypes did not yield significant p-values.

Concerning ApaI genotypes, individuals with the AA genotype had a distribution of 15(27.8%) cases with positive Her-2 status and 39(72.2%) cases with negative Her-2 status, resulting in a non-significant p-value of 0.691. For the Aa genotype, 14(31.1%) cases had positive Her-2 status, while 31(68.9%) cases had negative Her-2 status. The aa genotype exhibited 2(18.2%) cases with positive Her-2 status and 9(81.8%) cases with negative Her-2 status.

The BsmI genotype did not reveal a significant association with Her-2 status. Individuals with the BB genotype had a distribution of 14(29.8%) cases with positive Her-2 status and 33(70.2%) cases with negative Her-2 status, resulting in a non-significant p-value of 0.945.

Overall, the investigation did not unveil significant associations between hormonal receptor stature and the examined genotypes among breast cancerous patients. The findings suggest that, within the examined cohort, these specific genotypes may not strongly correlate with the estrogen, progesterone, or human epidermal growth factor receptor 2 stature.

4.15 Stage Groupings and Genotypes Association.

Table 24, depicts the association between stage groupings and genotypes among breast cancer patients, the analysis involved examining the distribution of genotypes (FF, Ff, ff for Fok1; AA, Aa, aa for Apa1; BB, Bb, bb for BsmI) across different stages (I, IIA, IIB, IIIA, IIIC, IV, and NA). The study covered a total of one hundred ten breast cancerous patients, and a statistical analysis was designed to examine the significance of associations.

For the FokI genotype, the FF genotype had 2 cases in stage I, 17 cases in stage IIA, 21 cases in stage IIB, 15 cases in stage IIIA, 4 cases in stage IIIC, 1 case in stage IV, and there were no cases in NA category. The p-value was 0.464, indicating no statistically significant association between the FokI genotype and stage groupings (p-value 0.464). For the Ff genotype at the FokI locus, there were 2 cases in stage I, 9 cases in stage IIA, 7 cases in stage IIB, 9 cases in stage IIIA, 4 cases in stage IIIC, 0 cases in stage IV, and 1 case in the NA category. The p-value was not statistically significant (p-value>0.05). For the ff genotype at the FokI locus, there were 3 cases in stage I, 5 cases in stage IIA, 7 cases in stage IIB, 3 cases in stage IIIA, 0 cases in stage IIIC, 0 cases in stage IV, and 0 cases in the NA category. The p-value indicates no statistical significance (p-value>0.05).

Moving on to the ApaI locus, the AA genotype had 4 cases in stage I, 16 cases in stage IIA, 21 cases in stage IIB, 10 cases in stage IIIA, 2 cases in stage IIIC, 0 cases in stage IV, and 1 case in the NA category. The p-value was 0.602, indicating no statistically significant association between the ApaI genotype and stage groupings (p=0.602). For the Aa genotype at the ApaI locus, there were 3 cases in stage I, 13 cases in stage IIA, 11 cases in stage IIB, 12 cases in stage IIIA, 5 cases in stage IIIC,

1 case in stage IV, and 0 cases in the NA category. The p-value did not reach statistical significance (p-value>0.05). For the aa genotype at the ApaI locus, there were 0 cases in stage I, 2 cases in stage IIA, 3 cases in stage IIB, 5 cases in stage IIIA, 1 case in stage IIIC, 0 cases in stage IV, and 0 cases in the NA category. The p-value was not statistically significant (p-value>0.05).

Table 24: Association between the Stage Groupings and Genotypes among Breast cancer patients

Genotypes		**Stage Groupings**							***p-value****
		I	**IIA**	**IIB**	**IIIA**	**IIIC**	**IV**	**NA**	
FokI	FF	2	17	21	15	4	1	0	0.464
	Ff	2	9	7	9	4	0	1	
	ff	3	5	7	3	0	0	0	
ApaI	AA	4	16	21	10	2	0	1	0.602
	Aa	3	13	11	12	5	1	0	
	aa	0	2	3	5	1	0	0	
BsmI	BB	3	13	15	11	3	1	1	0.878
	Bb	4	16	19	16	4	0	0	
	bb	0	2	1	0	1	0	0	

p <0.05, Significant, NA- not applicable.

Finally, for the BsmI locus, the BB genotype had 3 cases in stage I, 13 cases in stage IIA, 15 cases in stage IIB, 11 cases in stage IIIA, 3 cases in stage IIIC, 1 case in stage IV, and 1 case in the NA category. The p-value was 0.878, indicating no statistically significant association between the BsmI genotype and stage groupings (p-value 0.878). For the Bb genotype at the BsmI locus, there were 4 cases in stage I,

16 cases in stage IIA, 19 cases in stage IIB, 16 cases in stage IIIA, 4 cases in stage IIIC, 0 cases in stage IV, and 0 cases in the NA category. The p-value was not statistically significant ($p>0.05$). For the bb genotype at the BsmI locus, there were 0 cases in stage I, 2 cases in stage IIA, 1 case in stage IIB, 0 cases in stage IIIA, 1 case in stage IIIC, 0 cases in stage IV, and 0 cases in the NA category. The p-value was not statistically significant ($p>0.05$).

The analysis did not identify significant associations between genotypes and stage groupings among patients of breast cancer. These findings suggest that, within the examined cohort, the specific genotypes investigated may not exhibit a strong correlation with the different stages of breast cancer.

4.16 Comparative analysis of vitamin D levels

Table 25, showed that in the comparative analysis of vitamin D levels and the A260/A280 ratio between breast cancerous patients and the control group, the mean values and standard deviations for both parameters were assessed. The mean level of vitamin D for breast cancerous patients was 43.54 ± 19.58 nmol/L, whereas the control group displayed a higher mean level of 89.89 ± 26.13 nmol/L. The independent t-test conducted for vitamin D levels revealed a highly significant p-value of 0.000, indicating a statistically significant difference in both groups.

Table 25: Comparison of Vitamin D levels (nmol/l) and Ratio among Breast cancer patients and control groups

	Cases	**Control**	***p-value****
Vitamin D level	**Mean ± S.D.**		
	43.54 ± 19.58	89.89 ± 26.13	0.000
Ratio A260/A280	1.78 ± 0.034	1.766 ± 0.039	0.000

Independent t-test, *p <0.05, Significant

Similarly, the mean A260/A280 ratio for breast cancer patients was 1.78 ± 0.034, while the control group had a slightly lower mean ratio of 1.766 ± 0.039. The independent t-test for the A260/A280 ratio also resulted in a highly significant p-value of 0.000, signifying statistically significant differences between the patients with breast cancer and the control group in terms of this ratio.

The study reported highly significant variations in both vitamin D concentrations and the A260/A280 ratio between breast cancer patients and the control group. These findings contribute to the understanding of potential biomarkers associated with breast cancer and might have indications for diagnostic and prognostic considerations in this patient population.

4.17 Comparative analysis of genotypes and vitamin D levels amongst breast cancer patients and the control group.

In **Table 26,** the comparative analysis of genotypes and vitamin D concentrations between breast cancerous patients and the control group, the investigation involved three specific genotypes (FF, Ff, ff for Fok1; AA, Aa, aa for ApaI; BB, Bb, bb for BsmI) and their corresponding vitamin D levels categorized as <50, 50 to <75, and 75 - 250 nmol/L. The mean vitamin D levels with standard deviations were assessed for both cases and controls and these mean values provide insights into the central tendency of vitamin D levels for each specific genotype within each group.

Table 26: Comparison of Genotypes & Vitamin D levels (nmol/L) among Breast cancer patients and Control groups

Genotypes		**Cases**	**Control**
		Mean ± S.D.	
FokI	FF	42.98 ± 21.32	46.33 ± 26.08
	Ff	55.58 ± 19.04	72.96 ± 25.59
	ff	81.77 ± 16.40	114.71 ± 18.66
	p-value*	**0.756**	**0.067**
ApaI	AA	43.66 ± 17.37	48.90 ± 19.14
	Aa	65.39 ± 21.56	67.90 ± 27.14
	aa	89.54 ± 19.43	102.67 ± 21.42
	p-value*	**0.316**	0.001
BsmI	BB	44.76 ± 22.41	87.70 ± 24.24
	Bb	56.71 ± 15.52	88.02 ± 22.23
	bb	72.23 ± 16.79	162.63 ± 6.05
	p-value*	0.006	0.001

One-way *ANOVA test*, *p <0.05, *significant*

In breast cancer patients (cases), individuals possessing the FF genotype exhibit an average Vitamin D level of 42.98 nmol/L ± 21.32, those with the Ff genotype have a mean level of 55.58 nmol/L ± 19.04, and those with the ff genotype show a mean level of 81.77 nmol/L ± 16.40. However, these distinctions do not achieve statistical significance (p-value = 0.756). In the control group, individuals with the FF genotype display an average Vitamin D level of 46.33 nmol/L ± 26.08, those with the Ff genotype have a mean level of 72.96 nmol/L ± 25.59, and those with the ff genotype have a mean level of 114.71 nmol/L ± 18.66, and once again, statistical significance is not attained (p-value = 0.067).

Similarly, those with the AA genotype among breast cancer patients demonstrate an average Vitamin D level of 43.66 nmol/L ± 17.37, individuals with the Aa genotype show a mean level of 65.39 nmol/L ± 21.56, and those with the aa genotype have a mean level of 89.54 nmol/L ± 19.43, with no substantial difference noted (p-value = 0.316). In the control group, individuals with the AA genotype present an average Vitamin D level of 48.90 nmol/L ± 19.14, those with the Aa genotype have a mean level of 67.90 nmol/L ± 27.14, and those with the aa genotype display a mean level of 102.67 nmol/L ± 21.42. A significant variation in mean Vitamin D levels is evident across different genotypes (p-value = 0.001).

Among breast cancer patients, those with the BB genotype exhibit an average Vitamin D level of 44.76 nmol/L ± 22.41, individuals with the Bb genotype show a mean level of 56.71 nmol/L ± 15.52, and those with the bb genotype have a mean level of 72.23 nmol/L ± 16.79. In the control group, individuals with the BB genotype display an average Vitamin D level of 87.70 nmol/L ± 24.24, those with the Bb genotype have a mean level of 88.02 nmol/L ± 22.23, and those with the bb genotype

show a mean level of 162.63 nmol/L ± 6.05. Significant differences in mean Vitamin D levels are observed among both cases (p-value = 0.006) and controls (p-value = 0.001). The data suggested a varying degrees of association between genotypes and vitamin D levels.