REFERENCES

1. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019 Apr 15;144(8):1941–53.

2. Liu Z, Delavan B, Roberts R, Tong W. Lessons Learned from Two Decades of Anticancer Drugs. Trends Pharmacol Sci. 2017 Oct;38(10):852–72.

3. Brunetti A, Manfioletti G. Editorial: Hormone Receptors and Breast Cancer. Front Endocrinol (Lausanne). 2019 Apr 2;10.

4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018 Nov;68(6):394–424.

5. DeSantis C, Ma J, Bryan L, Jemal A. Breast cancer statistics, 2013. CA Cancer J Clin. 2014 Jan;64(1):52–62.

6. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019 Jan 8;69(1):7–34.

7. Malvia S, Bagadi SA, Dubey US, Saxena S. Epidemiology of breast cancer in Indian women. Asia Pac J Clin Oncol. 2017 Aug;13(4):289–95.

8. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012 Jan;62(1):10–29.

9. Bauer SR, Hankinson SE, Bertone-Johnson ER, Ding EL. Plasma Vitamin D Levels, Menopause, and Risk of Breast Cancer. Medicine. 2013 May;92(3):123–31.

10. Kelsey JL, Gammon MD, John EM. Reproductive Factors and Breast Cancer. Epidemiol Rev. 1993;15(1):36–47.

11. Mehrgou A, Akouchekian M. The importance of BRCA1 and BRCA2 genes mutations in breast cancer development. Med J Islam Repub Iran. 2016;30:369.

12. Tavera-Mendoza LE, Westerling T, Libby E, Marusyk A, Cato L, Cassani R, et al. Vitamin D receptor regulates autophagy in the normal mammary gland and in luminal breast cancer cells. Proceedings of the National Academy of Sciences. 2017 Mar 14;114(11).

13. Harbeck N, Penault-Llorca F, Cortes J, Gnant M, Houssami N, Poortmans P, et al. Breast cancer. Nat Rev Dis Primers. 2019 Sep 23;5(1):66.

14. Atoum M, Alzoughool F. Vitamin D and Breast Cancer: Latest Evidence and Future Steps. Breast Cancer (Auckl). 2017 Jan 1;11:117822341774981.

15. Grigalavicius M, Moan J, Dahlback A, Juzeniene A. Daily, seasonal, and latitudinal variations in solar ultraviolet A and B radiation in relation to vitamin D production and risk for skin cancer. Int J Dermatol. 2016 Jan;55(1):e23–8.

16. de La Puente-Yagüe M, Cuadrado-Cenzual MA, Ciudad-Cabañas MJ, Hernández-Cabria M, Collado-Yurrita L. Vitamin D: And its role in breast cancer. Kaohsiung J Med Sci. 2018 Aug;34(8):423–7.

17. Chen P, Hu P, Xie D, Qin Y, Wang F, Wang H. Meta-analysis of vitamin D, calcium and the prevention of breast cancer. Breast Cancer Res Treat. 2010 Jun 23;121(2):469–77.

18. Mohr SB, Gorham ED, Alcaraz JE, Kane CJ, Macera CA, Parsons JK, et al. Serum 25-hydroxyvitamin D and prevention of breast cancer: pooled analysis. Anticancer Res. 2011 Sep;31(9):2939–48.

19. Holick MF. Photobiology of Vitamin D. [Internet]. In: Vitamin D . 2018 [cited 2023 Jun 8]. p. 45–55. Available from: Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780128099650000045.

20. Fondjo LA, Sakyi SA, Owiredu WKBA, Laing EF, Owiredu EW, Awusi EK, et al. Evaluating Vitamin D Status in Pre- and Postmenopausal Type 2 Diabetics and Its Association with Glucose Homeostasis. Biomed Res Int. 2018;2018:1–12.

21. WELSH J. Vitamin D and prevention of breast cancer. Acta Pharmacol Sin. 2007 Sep;28(9):1373–82.

22. Yao S, Ambrosone CB. Associations between vitamin D deficiency and risk of aggressive breast cancer in African-American women. J Steroid Biochem Mol Biol. 2013 Jul;136:337–41.

23. Shekarriz-Foumani R, Khodaie F. The Correlation of Plasma 25-Hydroxyvitamin D Deficiency With Risk of Breast Neoplasms: A Systematic Review. Iran J Cancer Prev. 2016 Apr 24;In Press(In Press).

24. Park S, Lee DH, Jeon JY, Ryu J, Kim S, Kim JY, et al. Serum 25-hydroxyvitamin D deficiency and increased risk of breast cancer among Korean women: a case–control study. Breast Cancer Res Treat. 2015 Jul 3;152(1):147–54.

25. Wang Y, Zhu J, DeLuca HF. Where is the vitamin D receptor? Arch Biochem Biophys. 2012 Jul;523(1):123–33.

26. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects. Physiol Rev. 2016 Jan;96(1):365–408.

27. Welsh J, Wietzke JA, Zinser GM, Byrne B, Smith K, Narvaez CJ. Vitamin D-3 Receptor as a Target for Breast Cancer Prevention. J Nutr. 2003 Jul;133(7):2425S-2433S.

28. Ruan L, Zhu J guo, Pan C, Hua X, Yuan D bo, Li Z ming, et al. Association between Single Nucleotide Polymorphism of Vitamin D Receptor Gene FokI Polymorphism and Clinical Progress of Benign Prostatic Hyperplasia. The Scientific World Journal. 2015;2015:1–5.

29. Li K, Shi Q, Yang L, Li X, Liu L, Wang L, et al. The association of vitamin D receptor gene polymorphisms and serum 25-hydroxyvitamin D levels with generalized vitiligo. British Journal of Dermatology. 2012 Oct;167(4):815–21.

30. Ramteke P, Seenu V, Prashad R, Gupta S, Iyer V, Deo S, et al. Alteration in steroid hormone and Her-2/neu receptor status following neoadjuvant chemotherapy in locally advanced breast cancer: Experience at a tertiary care centre in India. Indian J Cancer. 2016;53(3):366.

31. Hammond MEH, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer. Journal of Clinical Oncology. 2010 Jun 1;28(16):2784–95.

32. Huszno J, Badora A, Nowara E. The influence of steroid receptor status on the cardiotoxicity risk in HER2-positive breast cancer patients receiving trastuzumab. Archives of Medical Science. 2015;2:371–7.

33. Society AC. Hormone Therapy for Breast Cancer [Internet]. American Cancer Society. 2019 [cited 2023 Jun 8]. Available from: https://www.cancer.org/cancer/breast-cancer/treatment/hormone-therapy-for-breast-cancer.html

34. Giovannelli P, Di Donato M, Galasso G, Di Zazzo E, Bilancio A, Migliaccio A. The Androgen Receptor in Breast Cancer. Front Endocrinol (Lausanne). 2018 Aug 28;9.

35. Bleach R, McIlroy M. The Divergent Function of Androgen Receptor in Breast Cancer; Analysis of Steroid Mediators and Tumor Intracrinology. Front Endocrinol (Lausanne). 2018 Oct 26;9.

36. Malvia S, Bagadi SA, Dubey US, Saxena S. Epidemiology of breast cancer in Indian women. Asia Pac J Clin Oncol. 2017 Aug;13(4):289–95.

37. Dikshit R, Gupta PC, Ramasundarahettige C, Gajalakshmi V, Aleksandrowicz L, Badwe R, et al. Cancer mortality in India: a nationally representative survey. The Lancet. 2012 May;379(9828):1807–16.

38. Arnold M, Morgan E, Rumgay H, Mafra A, Singh D, Laversanne M. Current and future burden of breast cancer: Global statistics for 2020 and 2040 [Internet]. Breast. 2022 [cited 2023 Jun 12]. p. 15–23. Available from: http://dx.doi.org/10.1016/j.breast.2022.08.010

39. National Cancer registry Programme: Consolidated report of the population based cancer registries 1990–1996. New Delhi; 2001.

40. Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB. Cancer Incidence in Five Continents [Internet]. Vol. VIII. IARC Scientific Publication No. 155; 2002 [cited 2023 Aug 7]. 1–781 p. Available from: <http://www.iarc.fr/IARCPress/pdfs/ci5v8/index.php>.

41. Yersal O. Biological subtypes of breast cancer: Prognostic and therapeutic implications. World J Clin Oncol. 2014;5(3):412.

42. Arpino G, Generali D, Sapino A, Del Matro L, Frassoldati A, de Laurentis M, et al. Gene expression profiling in breast cancer: A clinical perspective. The Breast. 2013 Apr;22(2):109–20.

43. Eliyatkin N, Yalcin E, Zengel B, Aktaş S, Vardar E. Molecular Classification of Breast Carcinoma: From Traditional, Old-Fashioned Way to A New Age, and A New Way. Journal of Breast Health. 2015 Apr 7;11(2):59–66.

44. West AKV, Wullkopf L, Christensen A, Leijnse N, Tarp JM, Mathiesen J, et al. Division Induced Dynamics in Non-Invasive and Invasive Breast Cancer. Biophys J. 2017;112.

45. Clauser P, Marino MA, Baltzer PAT, Bazzocchi M, Zuiani C. Management of atypical lobular hyperplasia, atypical ductal hyperplasia, and lobular carcinoma in situ. Expert Rev Anticancer Ther. 2016 Mar 3;16(3):335–46.

46. Ziperstein MJ, Guzman A, Kaufman LJ. Evaluating Breast Cancer Cell Morphology as a Predictor of Invasive Capacity. Biophys J. 2016;110.

47. Stevanovic A, Lee P, Wilcken N. Metastatic breast cancer. Aust Fam Physician. 2006 May;35(5):309–12.

48. Arpino G, Bardou VJ, Clark GM, Elledge RM. Infiltrating lobular carcinoma of the breast: tumor characteristics and clinical outcome. Breast Cancer Research. 2004 Jun 17;6(3):R149.

49. Somiari RI, Sullivan A, Russell S, Somiari S, Hu H, Jordan R, et al. High-throughput proteomic analysis of human infiltrating ductal carcinoma of the breast. Proteomics. 2003 Oct;3(10):1863–73.

50. Mateo AM, Pezzi TA, Sundermeyer M, Kelley CA, Klimberg VS, Pezzi CM. Chemotherapy Significantly Improves Survival for Patients with T1c-T2N0M0 Medullary Breast Cancer: 3739 Cases From the National Cancer Data Base. Ann Surg Oncol. 2017 Apr 2;24(4):1050–6.

51. Anuradha D, Lakshmi AB. Mucinous carcinoma of breast with neuroendocrine differentiation: a rare case report with review of literature [Internet]. Vol. 2 No. 4. 2017 [cited 2023 Jun 13]. p. 1751–4. Available from: https://www.msjonline.org/index.php/ijrms/article/view/2505

52. Priya VSL, Prasaad PR. Tubulo- lobular carcinoma: a rare mixed invasive carcinoma of breast. Int J Res Med Sci. 2017;5:2818–20.

53. Hubalek M, Czech T, Müller H. Biological Subtypes of Triple-Negative Breast Cancer. Breast Care. 2017;12(1):8–14.

54. Godoy-Ortiz A, Sanchez-Muñoz A, Chica Parrado MR, Álvarez M, Ribelles N, Rueda Dominguez A, et al. Deciphering HER2 Breast Cancer Disease: Biological and Clinical Implications. Front Oncol. 2019 Oct 29;9.

55. Goldberg M, D'Aloisio AA, O'Brien KM, Zhao S, Sandler DP. Pubertal timing and breast cancer risk in the Sister Study cohort. Breast Cancer Research. 2020 Dec 27;22(1):112.

56. Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. Lancet Oncol. 2012 Nov;13(11):1141–51.

57. Kinlen LJ. Breast cancer and ages at first marriage and first birth. European Journal of Cancer Prevention. 2014 Jan;23(1):53–7.

58. Lipworth L. History of Breast-Feeding in Relation to Breast Cancer Risk: a Review of the Epidemiologic Literature. J Natl Cancer Inst. 2000 Feb 16;92(4):302–12.

59. Lord SJ, Bernstein L, Johnson KA, Malone KE, McDonald JA, Marchbanks PA, et al. Breast Cancer Risk and Hormone Receptor Status in Older Women by Parity, Age of First Birth, and Breastfeeding: A Case-Control Study. Cancer Epidemiology, Biomarkers & Prevention. 2008 Jul 1;17(7):1723–30.

60. Milanese TR, Hartmann LC, Sellers TA, Frost MH, Vierkant RA, Maloney SD, et al. Age-Related Lobular Involution and Risk of Breast Cancer. JNCI: Journal of the National Cancer Institute. 2006 Nov 15;98(22):1600–7.

61. Bernstein L. Exercise and breast cancer prevention. Curr Oncol Rep. 2009 Nov 14;11(6):490–6.

62. Shridhar K, Singh G, Dey S, Singh Dhatt S, Paul Singh Gill J, Goodman M, et al. Dietary Patterns and Breast Cancer Risk: A Multi-Centre Case Control Study among North Indian Women. Int J Environ Res Public Health. 2018 Sep 6;15(9):1946.

63. Picon-Ruiz M, Morata-Tarifa C, Valle-Goffin JJ, Friedman ER, Slingerland JM. Obesity and adverse breast cancer risk and outcome: Mechanistic insights and strategies for intervention. CA Cancer J Clin. 2017 Sep;67(5):378–97.

64. Xu M, Ren Z, Wang X, Comer A, Frank JA, Ke Z ji, et al. ErbB2 and p38γ MAPK mediate alcohol-induced increase in breast cancer stem cells and metastasis. Mol Cancer. 2016 Dec 14;15(1):52.

65. Goldvaser H, Gal O, Rizel S, Hendler D, Neiman V, Shochat T, et al. The association between smoking and breast cancer characteristics and outcome. BMC Cancer. 2017 Dec 6;17(1):624.

66. Using HRT (Hormone Replacement Therapy) [Internet]. [cited 2023 Jun 14]. Available from: https:// www.breastcancer.org/risk/factors/hrt

67. Oral Contraceptives and Breast Cancer. Washington, D.C.: National Academies Press; 1991.

68. "BRCA gene mutations: cancer risk and genetic testing fact sheet [Internet]. [cited 2023 Jun 14]. Available from: https://www.cancer.gov/ about-cancer/causes-prevention/genetics/brca-fact-sheet.

69. Gray JM, Rasanayagam S, Engel C, Rizzo J. State of the evidence 2017: an update on the connection between breast cancer and the environment. Environmental Health. 2017 Dec 2;16(1):94.

70. Huss L, Butt ST, Borgquist S, Elebro K, Sandsveden M, Rosendahl A, et al. Vitamin D receptor expression in invasive breast tumors and breast cancer survival. Breast Cancer Research. 2019 Dec 29;21(1):84.

71. Voutsadakis IA. Vitamin D receptor (VDR) and metabolizing enzymes CYP27B1 and CYP24A1 in breast cancer. Mol Biol Rep. 2020 Dec 1;47(12):9821–30.

72. Wacker M, Holick MF. Sunlight and Vitamin D. Dermatoendocrinol. 2013 Jan 27;5(1):51–108.

73. Henry HL. Vitamin D hydroxylases. J Cell Biochem. 1992 May;49(1):4–9.

74. Nebert DW, Gonzalez FJ. P450 GENES: STRUCTURE, EVOLUTION, AND REGULATION. Annu Rev Biochem. 1987 Jun;56(1):945–93.

75. Zhu J, DeLuca HF. Vitamin D 25-hydroxylase – Four decades of searching, are we there yet? Arch Biochem Biophys. 2012 Jul;523(1):30–6.

76. Jones G, Prosser DE, Kaufmann M. Cytochrome P450-mediated metabolism of vitamin D. J Lipid Res. 2014 Jan;55(1):13–31.

77. Köstner K, Denzer N, Müller CSL, Klein R, Tilgen W, Reichrath J. The relevance of vitamin D receptor (VDR) gene polymorphisms for cancer: a review of the literature. Anticancer Res. 2009 Sep;29(9):3511–36.

78. Iqbal M un N, Khan TA. Vitamin D, Its Receptor Gene Polymorphism and Breast Cancer. In: A Critical Evaluation of Vitamin D - Clinical Overview. InTech; 2017.

79. KHAN MI, BIELECKA ZF, NAJM MZ, BARTNIK E, CZARNECKI JS, CZARNECKA AM, et al. Vitamin D receptor gene polymorphisms in breast and renal cancer: Current state and future approaches. Int J Oncol. 2014 Feb;44(2):349–63.

80. Orlov I, Rochel N, Moras D, Klaholz BP. Structure of the full human RXR/VDR nuclear receptor heterodimer complex with its DR3 target DNA. EMBO J. 2012 Jan 18;31(2):291–300.

81. Dogra A, Prakash A, Gupta S, Gupta M, Bhat Dr showkat. Genetic variations of vitamin D receptor gene and steroid receptors status in breast cancer risk: An updated review. Adv Biomark Sci Technol. 2022 Jun;4.

82. Feldman D, Krishnan A V., Swami S, Giovannucci E, Feldman BJ. The role of vitamin D in reducing cancer risk and progression. Nat Rev Cancer. 2014 May 4;14(5):342–57.

83. Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. Nat Rev Cancer. 2007 Sep;7(9):684–700.

84. Iqbal MUN, Khan T. Vitamin D, Its Receptor Gene Polymorphism and Breast Cancer. In 2017.

85. Jeon SM, Shin EA. Exploring vitamin D metabolism and function in cancer. Exp Mol Med. 2018 Apr 16;50(4):1–14.

86. Jensen SS, Madsen MW, Lukas J, Binderup L, Bartek J. Inhibitory Effects of 1α,25-Dihydroxyvitamin D3 on the G1–S Phase-Controlling Machinery. Molecular Endocrinology. 2001 Aug 1;15(8):1370–80.

87. Saunders DE, Christensen C, Wappler NL, Schultz JF, Lawrence WD, Malviya VK, et al. Inhibition of c-myc in breast and ovarian carcinoma cells by 1,25-dihydroxyvitamin D3, retinoic acid and dexamethasone. Anticancer Drugs. 1993 Apr;4(2):201–8.

88. Campbell MJ, Gombart AF, Kwok SH, Park S, Koeffler HP. The anti-proliferative effects of 1α,25(OH)2D3 on breast and prostate cancer cells are associated with induction of BRCA1 gene expression. Oncogene. 2000 Oct 19;19(44):5091–7.

89. Pervin S, Hewison M, Braga M, Tran L, Chun R, Karam A, et al. Down-Regulation of Vitamin D Receptor in Mammospheres: Implications for Vitamin D Resistance in Breast Cancer and Potential for Combination Therapy. PLoS One. 2013 Jan 14;8(1):e53287.

90. Ben-Shoshan M, Amir S, Dang DT, Dang LH, Weisman Y, Mabjeesh NJ. 1α,25-dihydroxyvitamin D3 (Calcitriol) inhibits hypoxia-inducible factor-1/vascular endothelial growth factor pathway in human cancer cells. Mol Cancer Ther. 2007 Apr 1;6(4):1433–9.

91. Koli K, Keski-Oja J. 1alpha,25-dihydroxyvitamin D3 and its analogues down-regulate cell invasion-associated proteases in cultured malignant cells. Cell Growth Differ. 2000 Apr;11(4):221–9.

92. Krishnan A V., Swami S, Peng L, Wang J, Moreno J, Feldman D. Tissue-Selective Regulation of Aromatase Expression by Calcitriol: Implications for Breast Cancer Therapy. Endocrinology. 2010 Jan 1;151(1):32–42.

93. Wang D, DuBois RN. Cyclooxygenase-2: a potential target in breast cancer. Semin Oncol. 2004 Feb;31:64–73.

94. Welsh D, Ramadani V. Internationalization Strategies of Family Firms: Challenges and Perspectives (Editorial Notes). Journal of Small Business Strategy. 2018 Jun;28.

95. Nemere I, Garbi N, Hammerling G, Hintze KJ. Role of the 1,25D3-MARRS receptor in the 1,25(OH)2D3-stimulated uptake of calcium and phosphate in intestinal cells. Steroids. 2012 Aug;77(10):897–902.

96. Sequeira VB, Rybchyn MS, Tongkao-on W, Gordon-Thomson C, Malloy PJ, Nemere I, et al. The Role of the Vitamin D Receptor and ERp57 in Photoprotection by 1α,25-Dihydroxyvitamin D3. Molecular Endocrinology. 2012 Apr 1;26(4):574–82.

97. Heaney RP. Functional indices of vitamin D status and ramifications of vitamin D deficiency. Am J Clin Nutr. 2004 Dec;80(6):1706S-1709S.

98. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D from the Institute of Medicine: What Clinicians Need to Know. J Clin Endocrinol Metab. 2011 Jan;96(1):53–8.

99. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2011 Jul;96(7):1911–30.

100. Garland CF, Garland FC, Gorham ED, Lipkin M, Newmark H, Mohr SB, et al. The Role of Vitamin D in Cancer Prevention. Am J Public Health. 2006 Feb;96(2):252–61.

101. Bilinski K, Boyages J. Association between 25-hydroxyvitamin D concentration and breast cancer risk in an Australian population: an observational case–control study. Breast Cancer Res Treat. 2013 Jan 14;137(2):599–607.

102. Peppone LJ, Rickles AS, Janelsins MC, Insalaco MR, Skinner KA. The Association Between Breast Cancer Prognostic Indicators and Serum 25-OH Vitamin D Levels. Ann Surg Oncol. 2012 Aug 24;19(8):2590–9.

103. Karthikayan A, Sureshkumar S, Kadambari D, Vijayakumar C. Low serum 25-hydroxy vitamin D levels are associated with aggressive breast cancer variants and poor prognostic factors in patients with breast carcinoma. Arch Endocrinol Metab. 2018 Aug;62(4):452–9.

104. Machado MRM, de Sousa Almeida-Filho B, De Luca Vespoli H, Schmitt EB, Nahas-Neto J, Nahas EAP. Low pretreatment serum concentration of vitamin D at breast cancer diagnosis in postmenopausal women. Menopause. 2019 Mar;26(3):293–9.

105. Crew KD. Vitamin D: Are We Ready to Supplement for Breast Cancer Prevention and Treatment? ISRN Oncol. 2013 Feb 26;2013:1–22.

106. Forrest KYZ, Stuhldreher WL. Prevalence and correlates of vitamin D deficiency in US adults. Nutrition Research. 2011 Jan;31(1):48–54.

107. Manousaki D, Richards JB. Low vitamin D levels as a risk factor for cancer. BMJ. 2017 Oct 31;j4952.

108. O'Brien KM, Sandler DP, Taylor JA, Weinberg CR. Serum Vitamin D and Risk of Breast Cancer within Five Years. Environ Health Perspect. 2017 Jul 24;125(7).

109. Skaaby T, Husemoen LLN, Thuesen BH, Pisinger C, Jørgensen T, Roswall N, et al. Prospective Population-Based Study of the Association between Serum 25-Hydroxyvitamin-D Levels and the Incidence of Specific Types of Cancer. Cancer Epidemiology, Biomarkers & Prevention. 2014 Jul 1;23(7):1220–9.

110. Yao S, Kwan ML, Ergas IJ, Roh JM, Cheng TYD, Hong CC, et al. Association of Serum Level of Vitamin D at Diagnosis With Breast Cancer Survival. JAMA Oncol. 2017 Mar 1;3(3):351.

111. Hossain S, Beydoun MA, Beydoun HA, Chen X, Zonderman AB, Wood RJ. Vitamin D and breast cancer: A systematic review and meta-analysis of observational studies. Clin Nutr ESPEN. 2019 Apr;30:170–84.

112. Tommie JL, Pinney SM, Nommsen-Rivers LA. Serum Vitamin D Status and Breast Cancer Risk by Receptor Status: A Systematic Review. Nutr Cancer. 2018 Jul 4;70(5):804–20.

113. Lope V, Castelló A, Mena-Bravo A, Amiano P, Aragonés N, Fernández-Villa T, et al. Serum 25-hydroxyvitamin D and breast cancer risk by pathological subtype (MCC-Spain). J Steroid Biochem Mol Biol. 2018 Sep;182:4–13.

114. Villaseñor A, Ballard-Barbash R, Ambs A, Bernstein L, Baumgartner K, Baumgartner R, et al. Associations of serum 25-hydroxyvitamin D with overall and breast cancer–specific mortality in a multiethnic cohort of breast cancer survivors. Cancer Causes & Control. 2013 Apr 30;24(4):759–67.

115. Vanhevel J, Verlinden L, Doms S, Wildiers H, Verstuyf A. The role of vitamin D in breast cancer risk and progression. Endocr Relat Cancer. 2022 Feb 1;29(2):R33–55.

116. Jiang X, O'Reilly PF, Aschard H, Hsu YH, Richards JB, Dupuis J, et al. Genome-wide association study in 79,366 European-ancestry individuals informs the genetic architecture of 25-hydroxyvitamin D levels. Nat Commun. 2018 Jan 17;9(1):260.

117. Jiang X, Dimou NL, Al-Dabhani K, Lewis SJ, Martin RM, Haycock PC, et al. Circulating vitamin D concentrations and risk of breast and prostate cancer: a Mendelian randomization study. Int J Epidemiol. 2019 Oct 1;48(5):1416–24.

118. Jiang X, Ge T, Chen CY. The causal role of circulating vitamin D concentrations in human complex traits and diseases: a large-scale Mendelian randomization study. Sci Rep. 2021 Jan 8;11(1):184.

119. Manson JE, Cook NR, Lee IM, Christen W, Bassuk SS, Mora S, et al. Vitamin D Supplements and Prevention of Cancer and Cardiovascular Disease. New England Journal of Medicine. 2019 Jan 3;380(1):33–44.

120. Lappe J, Watson P, Travers-Gustafson D, Recker R, Garland C, Gorham E, et al. Effect of Vitamin D and Calcium Supplementation on Cancer Incidence in Older Women. JAMA. 2017 Mar 28;317(12):1234.

121. Arnaout A, Robertson S, Pond GR, Vieth R, Jeong A, Hilton J, et al. Randomized window of opportunity trial evaluating high-dose vitamin D in breast cancer patients. Breast Cancer Res Treat. 2019 Nov 9;178(2):347–56.

122. Colston KW, Chander SK, MaCkay AG, Coombes RC. Effects of synthetic vitamin d analogues on breast cancer cell proliferation in vivo and in vitro. Biochem Pharmacol. 1992 Aug;44(4):693–702.

123. Binderup L. Comparison of Calcipotriol with Selected Metabolites and Analogues of Vitamin D_3: Effects on Cell Growth Regulation in Vitro and Calcium Metabolism in Vivo. Pharmacol Toxicol. 1993 Apr 5;72(4–5):240–4.

124. Matthews D, LaPorta E, Zinser GM, Narvaez CJ, Welsh J. Genomic vitamin D signaling in breast cancer: Insights from animal models and human cells. J Steroid Biochem Mol Biol. 2010 Jul;121(1–2):362–7.

125. Boucher BJ. Why do so many trials of vitamin D supplementation fail? Endocr Connect. 2020 Sep;9(9):R195–206.

126. Beaudin SG, Robilotto S, Welsh J. Comparative regulation of gene expression by 1,25-dihydroxyvitamin D3 in cells derived from normal mammary tissue and breast cancer. J Steroid Biochem Mol Biol. 2015 Apr;148:96–102.

127. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. American Journal of Physiology-Renal Physiology. 2005 Jul;289(1):F8–28.

128. Hughes MR, Malloy PJ, Kieback DG, Kesterson RA, Pike JW, Feldman D, et al. Point mutations in the Human Vitamin D Receptor Gene Associated with Hypocalcemic Rickets. Science (1979). 1988 Dec 23;242(4886):1702–5.

129. Miyamoto K ichi, Kesterson RA, Yamamoto H, Taketani Y, Nishiwaki E, Tatsumi S, et al. Structural Organization of the Human Vitamin D Receptor Chromosomal Gene and Its Promoter. Molecular Endocrinology. 1997 Jul;11(8):1165–79.

130. Norman AW. Vitamin D Receptor: New Assignments for an Already Busy Receptor. Endocrinology. 2006 Dec;147(12):5542–8.

131. Haussler MR, Whitfield GK, Haussler CA, Hsieh JC, Thompson PD, Selznick SH, et al. The Nuclear Vitamin D Receptor: Biological and Molecular Regulatory Properties Revealed. Journal of Bone and Mineral Research. 1998 Mar 1;13(3):325–49.

132. Li WH, Gu Z, Wang H, Nekrutenko A. Evolutionary analyses of the human genome. Nature. 2001 Feb 15;409(6822):847–9.

133. Shastry BS. SNP alleles in human disease and evolution. J Hum Genet. 2002 Nov;47(11):0561–6.

134. Wall JD, Pritchard JK. Haplotype blocks and linkage disequilibrium in the human genome. Nat Rev Genet. 2003 Aug 1;4(8):587–97.

135. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, et al. The Structure of Haplotype Blocks in the Human Genome. Science (1979). 2002 Jun 21;296(5576):2225–9.

136. Fuhrman BJ, Freedman DM, Bhatti P, Doody MM, Fu YP, Chang SC, et al. Sunlight, polymorphisms of vitamin D-related genes and risk of breast cancer. Anticancer Res. 2013 Feb;33(2):543–51.

137. Tizaoui K, Hamzaoui K. Association between VDR polymorphisms and rheumatoid arthritis disease: Systematic review and updated meta-analysis of case–control studies. Immunobiology. 2015 Jun;220(6):807–16.

138. Tizaoui K, Kaabachi W, Hamzaoui A, Hamzaoui K. Contribution of VDR polymorphisms to type 1 diabetes susceptibility: Systematic review of case–control studies and meta-analysis. J Steroid Biochem Mol Biol. 2014 Sep;143:240–9.

139. Valdivielso JM, Fernandez E. Vitamin D receptor polymorphisms and diseases. Clinica Chimica Acta. 2006 Sep;371(1–2):1–12.

140. Mishra DK, Wu Y, Sarkissyan M, Sarkissyan S, Chen Z, Shang X, et al. Vitamin D Receptor Gene Polymorphisms and Prognosis of Breast Cancer among African-American and Hispanic Women. PLoS One. 2013 Mar 12;8(3):e57967.

141. Halsall JA, Osborne JE, Potter L, Pringle JH, Hutchinson PE. A novel polymorphism in the 1A promoter region of the vitamin D receptor is associated with altered susceptibilty and prognosis in malignant melanoma. Br J Cancer. 2004 Aug 6;91(4):765–70.

142. Arai H, Miyamoto KI, Yoshida M, Yamamoto H, Taketani Y, Morita K, et al. The Polymorphism in the Caudal-Related Homeodomain Protein Cdx-2 Binding Element in the Human Vitamin D Receptor Gene. Journal of Bone and Mineral Research. 2001 Jul 1;16(7):1256–64.

143. Gong YL. Vitamin D receptor gene *Tru* 9I polymorphism and risk for incidental sporadic colorectal adenomas. World J Gastroenterol. 2005;11(31):4794.

144. Arai H, Miyamoto KI, Taketani Y, Yamamoto H, Iemori Y, Morita K, et al. A Vitamin D Receptor Gene Polymorphism in the Translation Initiation Codon: Effect on Protein Activity and Relation to Bone Mineral Density in Japanese Women. Journal of Bone and Mineral Research. 1997 Jun 1;12(6):915–21.

145. Ferrari S, Rizzoli R, Manen D, Slosman D, Bonjour JP. Vitamin D Receptor Gene Start Codon Polymorphisms (FokI) and Bone Mineral Density: Interaction with Age, Dietary Calcium, and 3′-End Region Polymorphisms. Journal of Bone and Mineral Research. 1998 Jun 1;13(6):925–30.

146. Eccleshall TR, Garnero P, Gross C, Delmas PD, Feldman D. Lack of Correlation Between Start Codon Polymorphism of the Vitamin D Receptor Gene and Bone

Mineral Density in Premenopausal French Women: The OFELY Study. Journal of Bone and Mineral Research. 1998 Jan 1;13(1):31–5.

147. Ergul E, Simsek T, Uren N, Korak T, Simsek H, Cine N, et al. Haplotype Analysis of Vitamin D Receptor (VDR) Gene in Breast Cancer Patients. Clin Lab. 2021;67(06/2021).

148. Jurutka PW, Whitfield GK, Hsieh JC, Thompson PD, Haussler CA, Haussler MR. Molecular nature of the vitamin D receptor and its role in regulation of gene expression. Rev Endocr Metab Disord. 2001;2(2):203–16.

149. Bretherton-Watt D, Given-Wilson R, Mansi JL, Thomas V, Carter N, Colston KW. Vitamin D receptor gene polymorphisms are associated with breast cancer risk in a UK Caucasian population. Br J Cancer. 2001 Jul 17;85(2):171–5.

150. Ingles SA, Haile RW, Henderson BE, Kolonel LN, Nakaichi G, Shi CY, et al. Strength of linkage disequilibrium between two vitamin D receptor markers in five ethnic groups: implications for association studies. Cancer Epidemiol Biomarkers Prev. 1997 Feb;6(2):93–8.

151. Morita A, Iki M, Dohi Y, Ikeda Y, Kagamimori S, Kagawa Y, et al. Effects of the Cdx-2 Polymorphism of the Vitamin D Receptor Gene and Lifestyle Factors on Bone Mineral Density in a Representative Sample of Japanese Women: The Japanese Population-based Osteoporosis (JPOS) Study. Calcif Tissue Int. 2005 Dec 5;77(6):339–47.

152. Fang Y, Van Meurs JB, Bergink AP, Hofman A, Van Duijn CM, Van Leeuwen JP, et al. Cdx-2 Polymorphism in the Promoter Region of the Human Vitamin D Receptor

Gene Determines Susceptibility to Fracture in the Elderly. Journal of Bone and Mineral Research. 2003 Sep 1;18(9):1632–41.

153. Arai H, Miyamoto KI, Yoshida M, Yamamoto H, Taketani Y, Morita K, et al. The Polymorphism in the Caudal-Related Homeodomain Protein Cdx-2 Binding Element in the Human Vitamin D Receptor Gene. Journal of Bone and Mineral Research. 2001 Jul 1;16(7):1256–64.

154. Halsall JA, Osborne JE, Potter L, Pringle JH, Hutchinson PE. A novel polymorphism in the 1A promoter region of the vitamin D receptor is associated with altered susceptibilty and prognosis in malignant melanoma. Br J Cancer. 2004 Aug 6;91(4):765–70.

155. Gong YL. Vitamin D receptor gene *Tru* 9I polymorphism and risk for incidental sporadic colorectal adenomas. World J Gastroenterol. 2005;11(31):4794.

156. Guy M, Lowe LC, Bretherton-Watt D, Mansi JL, Peckitt C, Bliss J, et al. Vitamin D Receptor Gene Polymorphisms and Breast Cancer Risk. Clinical Cancer Research. 2004 Aug 15;10(16):5472–81.

157. Chen WY, Bertone-Johnson ER, Hunter DJ, Willett WC, Hankinson SE. Associations Between Polymorphisms in the Vitamin D Receptor and Breast Cancer Risk. Cancer Epidemiology, Biomarkers & Prevention. 2005 Oct 1;14(10):2335–9.

158. Tang C, Chen N, Wu M, Yuan H, Du Y. Fok1 polymorphism of vitamin D receptor gene contributes to breast cancer susceptibility: a meta-analysis. Breast Cancer Res Treat. 2009 Sep 6;117(2):391–9.

159. Shahbazi S, Alavi S, Majidzadeh-A K, GhaffarPour M, Soleimani A, Mahdian R. BsmI but not FokI polymorphism of VDR gene is contributed in breast cancer. Medical Oncology. 2013 Mar 1;30(1):393.

160. Mishra DK, Wu Y, Sarkissyan M, Sarkissyan S, Chen Z, Shang X, et al. Vitamin D Receptor Gene Polymorphisms and Prognosis of Breast Cancer among African-American and Hispanic Women. PLoS One. 2013 Mar 12;8(3):e57967.

161. Nemenqani DM, Karam RA, Amer MG, Abd El Rahman TM. Vitamin D receptor gene polymorphisms and steroid receptor status among Saudi women with breast cancer. Gene. 2015 Mar;558(2):215–9.

162. Amadori D, Serra P, Masalu N, Pangan A, Scarpi E, Maria Bugingo A, et al. Vitamin D receptor polymorphisms or serum levels as key drivers of breast cancer development? The question of the vitamin D pathway. Oncotarget. 2017 Feb 21;8(8):13142–56.

163. Anderson LN, Cotterchio M, Cole DEC, Knight JA. Vitamin D-Related Genetic Variants, Interactions with Vitamin D Exposure, and Breast Cancer Risk among Caucasian Women in Ontario. Cancer Epidemiology, Biomarkers & Prevention. 2011 Aug 1;20(8):1708–17.

164. Shaker OG, Senousy MA. Association of SNP-SNP Interactions Between RANKL, OPG, CHI3L1, and VDR Genes With Breast Cancer Risk in Egyptian Women. Clin Breast Cancer. 2019 Feb;19(1):e220–38.

165. Fuhrman BJ, Freedman DM, Bhatti P, Doody MM, Fu YP, Chang SC, et al. Sunlight, polymorphisms of vitamin D-related genes and risk of breast cancer. Anticancer Res. 2013 Feb;33(2):543–51.

166. Deschasaux M, Souberbielle JC, Latino-Martel P, Sutton A, Charnaux N, Druesne-Pecollo N, et al. Weight Status and Alcohol Intake Modify the Association between Vitamin D and Breast Cancer Risk. J Nutr. 2016 Mar;146(3):576–85.

167. Engel LS, Orlow I, Sima CS, Satagopan J, Mujumdar U, Roy P, et al. Vitamin D Receptor Gene Haplotypes and Polymorphisms and Risk of Breast Cancer: A Nested Case–Control Study. Cancer Epidemiology, Biomarkers & Prevention. 2012 Oct 1;21(10):1856–67.

168. Ahmed J, Makonnen E, Fotoohi A, Yimer G, Seifu D, Assefa M, et al. Vitamin D Status and Association of VDR Genetic Polymorphism to Risk of Breast Cancer in Ethiopia. Nutrients. 2019 Jan 29;11(2):289.

169. Abd-Elsalam EAE, Ismaeil NA, Abd-Alsalam HS. Vitamin D receptor gene polymorphisms and breast cancer risk among postmenopausal Egyptian women. Tumor Biology. 2015 Aug 25;36(8):6425–31.

170. Raza S, Dhasmana A, Bhatt ML, Lohani M, Arif JM. Molecular Mechanism of Cancer Susceptibility Associated with Fok1 Single Nucleotide Polymorphism of VDR in Relation to Breast Cancer. Asian Pacific Journal of Cancer Prevention. 2019 Jan 1;20(1):199–206.

171. Chakraborty M, Arora M, Ramteke A, Yadav V, Naaz H, Muntakhab M, et al. FokI polymorphism of Vitamin D receptor gene and deficiency of serum Vitamin D increases the risk of breast cancer in North Indian women. Endocrine. 2023 Mar 1;81(1):168–74.

172. Iqbal M un N, Khan TA. Association between Vitamin D receptor (Cdx2, Fok1, Bsm1, Apa1, Bgl1, Taq1, and Poly (A)) gene polymorphism and breast cancer: A

systematic review and meta-analysis. Tumor Biology. 2017 Oct 26;39(10):101042831773128.

173. Rollison DE, Cole AL, Tung KH, Slattery ML, Baumgartner KB, Byers T, et al. Vitamin D intake, vitamin D receptor polymorphisms, and breast cancer risk among women living in the southwestern U.S. Breast Cancer Res Treat. 2012 Apr 1;132(2):683–91.

174. Akilzhanova A, Abilova Z, Sikhayeva N, Shtefanov I, Makishev A, Adylkhanov T, et al. Vitamin D Receptor Gene Polymorphisms and Breast Cancer Risk in Kazakhstan. Cent Asian J Glob Health. 2014 Jan 24;2.

175. Guo B, Jiang X, Hu X, Li F, Chen X. Association between vitamin D receptor gene polymorphisms and breast cancer in a Chinese population. Int J Clin Exp Med. 2015;8(5):8020–4.

176. El-Shorbagy HM, Mahmoud NH, Sabet S. Association of vitamin D receptor gene polymorphisms with breast cancer risk in an Egyptian population. Tumor Biology. 2017 Oct 12;39(10):101042831772773.

177. Rashid MU, Muzaffar M, Khan FA, Kabisch M, Muhammad N, Faiz S, et al. Association between the BsmI Polymorphism in the Vitamin D Receptor Gene and Breast Cancer Risk: Results from a Pakistani Case-Control Study. PLoS One. 2015 Oct 30;10(10):e0141562.

178. Shahabi A, Alipour M, Safiri H, Tavakol P, Alizadeh M, Milad Hashemi S, et al. Vitamin D Receptor Gene Polymorphism: Association with Susceptibility to Early-Onset Breast Cancer in Iranian, BRCA1/2-Mutation Carrier and non-carrier Patients. Pathology & Oncology Research. 2018 Jul 6;24(3):601–7.

179. Matini AH, Jafarian-Dehkordi N, Bahmani B, Sharifi M, Jahantigh D, Mazoochi T. Association of ApaI and TaqI polymorphisms in VDR Gene with Breast Cancer. Asian Pacific Journal of Cancer Prevention. 2020 Sep 1;21(9):2667–72.

180. Atoum MF, Al-Khatib YM. Association between Serum 25-hydroxy Vitamin D Concentration and TaqI Vitamin D Receptor Gene Polymorphism among Jordanian Females with Breast Cancer. Chin Med J (Engl). 2017 May 5;130(9):1074–8.

181. Dalessandri KM, Miike R, Wiencke JK, Farren G, Pugh TW, Manjeshwar S, et al. Vitamin D Receptor Polymorphisms and Breast Cancer Risk in a High-Incidence Population: A Pilot Study. J Am Coll Surg. 2012 Nov;215(5):652–7.

182. Ingles SA, Garcia DG, Wang W, Nieters A, Henderson BE, Kolonel LN, et al. Vitamin D receptor genotype and breast cancer in Latinas (United States). Cancer Causes and Control. 2000;11(1):25–30.

183. Colagar AH, Firouzjah HM, Halalkhor S. Vitamin D Receptor Poly(A) Microsatellite Polymorphism and 25-Hydroxyvitamin D Serum Levels: Association with Susceptibility to Breast Cancer. J Breast Cancer. 2015;18(2):119.

184. Xu J, Li H, Gu L, Zhou X. Association between vitamin D receptor poly(A) polymorphism and breast cancer risk: a meta-analysis. Tumor Biology. 2014 Jan 15;35(1):589–93.

185. Yao S, Zirpoli G, Bovbjerg DH, Jandorf L, Hong CC, Zhao H, et al. Variants in the vitamin D pathway, serum levels of vitamin D, and estrogen receptor negative breast cancer among African-American women: a case-control study. Breast Cancer Research. 2012 Apr 4;14(2):R58.

186. Iqbal M un N, Khan TA, Maqbool SA. Vitamin D Receptor Cdx-2 Polymorphism and Premenopausal Breast Cancer Risk in Southern Pakistani Patients. PLoS One. 2015 Mar 23;10(3):e0122657.

187. Iqbal M un N, Maqbool SA, Khan TA. Association of low penetrance vitamin D receptor *Tru* 9I (rs757343) gene polymorphism with risk of premenopausal breast cancer. Journal of International Medical Research. 2018 May 12;46(5):1801–14.

188. Dogra AK. An Updated Insights into Vitamin D Receptor Gene Variation and Steroid Receptor Status in Breast Cancer Risk. In: New Advances in Medicine and Medical Science Vol 2. B P International (a part of SCIENCEDOMAIN International); 2023. p. 64–89.

189. Whirledge S, Cidlowski JA. Steroid Hormone Action. In: Yen and Jaffe's Reproductive Endocrinology. Elsevier; 2019. p. 115-131.e4.

190. Papatheodorou I, Moreno P, Manning J, Fuentes AMP, George N, Fexova S, et al. Expression Atlas update: from tissues to single cells. Nucleic Acids Res. 2019 Oct 30;

191. Brunetti A, Manfioletti G. Editorial: Hormone Receptors and Breast Cancer. Front Endocrinol (Lausanne). 2019 Apr 2;10.

192. Paik S, Tang G, Shak S, Kim C, Baker J, Kim W, et al. Gene Expression and Benefit of Chemotherapy in Women With Node-Negative, Estrogen Receptor–Positive Breast Cancer. Journal of Clinical Oncology. 2006 Aug 10;24(23):3726–34.

193. Althuis MD, Fergenbaum JH, Garcia-Closas M, Brinton LA, Madigan MP, Sherman ME. Etiology of hormone receptor-defined breast cancer: a systematic review of the literature. Cancer Epidemiol Biomarkers Prev. 2004 Oct;13(10):1558–68.

194. Miller K. Estrogen and DNA Damage: The Silent Source of Breast Cancer? JNCI Journal of the National Cancer Institute. 2003 Jan 15;95(2):100–2.

195. Dunnwald LK, Rossing MA, Li CI. Hormone receptor status, tumor characteristics, and prognosis: a prospective cohort of breast cancer patients. Breast Cancer Research. 2007 Feb 19;9(1):R6.

196. Swain SM, Wilson JW, Mamounas EP, Bryant J, Wickerham DL, Fisher B, et al. Estrogen Receptor Status of Primary Breast Cancer Is Predictive of Estrogen Receptor Status of Contralateral Breast Cancer. JNCI Journal of the National Cancer Institute. 2004 Apr 7;96(7):516–23.

197. Duffy MJ. Estrogen Receptors: Role in Breast Cancer. Crit Rev Clin Lab Sci. 2006 Jan 10;43(4):325–47.

198. Hayashi SI, Eguchi H, Tanimoto K, Yoshida T, Omoto Y, Inoue A, et al. The expression and function of estrogen receptor alpha and beta in human breast cancer and its clinical application. Endocr Relat Cancer. 2003 Jun;193–202.

199. Zhou Y, Liu X. The role of estrogen receptor beta in breast cancer. Biomark Res. 2020 Dec 7;8(1):39.

200. van Kruchten M, Glaudemans AWJM, de Vries EFJ, Beets-Tan RGH, Schröder CP, Dierckx RA, et al. PET Imaging of Estrogen Receptors as a Diagnostic Tool for Breast Cancer Patients Presenting with a Clinical Dilemma. Journal of Nuclear Medicine. 2012 Feb;53(2):182–90.

201. Kasielska-Trojan A, Danilewicz M, Strużyna J, Bugaj M, Antoszewski B. The role of oestrogen and progesterone receptors in gigantomastia. Archives of Medical Science. 2019 Sep 26;

202. Haque MdM, Desai K V. Pathways to Endocrine Therapy Resistance in Breast Cancer. Front Endocrinol (Lausanne). 2019 Aug 21;10.

203. Bhattacharjee A, Hossain MU, Chowdhury ZM, Rahman SMA, Bhuyan ZA, Salimullah Md, et al. Insight of druggable cannabinoids against estrogen receptor β in breast cancer. J Biomol Struct Dyn. 2021 Mar 24;39(5):1688–97.

204. Crandall DL, Busler DE, Novak TJ, Weber R V., Kral JG. Identification of Estrogen Receptor β RNA in Human Breast and Abdominal Subcutaneous Adipose Tissue. Biochem Biophys Res Commun. 1998 Jul;248(3):523–6.

205. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase–AKT pathway in human cancer. Nat Rev Cancer. 2002 Jul 1;2(7):489–501.

206. Fuqua SAW, Schiff R, Parra I, Moore JT, Mohsin SK, Osborne CK, et al. Estrogen receptor beta protein in human breast cancer: correlation with clinical tumor parameters. Cancer Res. 2003 May 15;63(10):2434–9.

207. Singhal H, Greene ME, Zarnke AL, Laine M, Al Abosy R, Chang YF, et al. Progesterone receptor isoforms, agonists and antagonists differentially reprogram estrogen signaling. Oncotarget. 2018 Jan 12;9(4):4282–300.

208. Mote PA, Bartow S, Tran N, Clarke CL. Loss of Co-ordinate Expression of Progesterone Receptors A and B is an Early Event in Breast Carcinogenesis. Breast Cancer Res Treat. 2002 Mar;72(2):163–72.

209. Grimm SL, Hartig SM, Edwards DP. Progesterone Receptor Signaling Mechanisms. J Mol Biol. 2016 Sep;428(19):3831–49.

210. Cenciarini ME, Proietti CJ. Molecular mechanisms underlying progesterone receptor action in breast cancer: Insights into cell proliferation and stem cell regulation. Steroids. 2019 Dec;152:108503.

211. Li CI, Beaber EF, Chen Tang MT, Porter PL, Daling JR, Malone KE. Effect of Depo-Medroxyprogesterone Acetate on Breast Cancer Risk among Women 20 to 44 Years of Age. Cancer Res. 2012 Apr 15;72(8):2028–35.

212. Lyytinen H, Dyba T, Ylikorkala O, Pukkala E. A CASE-CONTROL STUDY ON HORMONE THERAPY AS A RISK FACTOR FOR BREAST CANCER IN FINLAND. Maturitas. 2009 May;63:S50.

213. Banks E, Beral V, Bull D, Reeves G, Austoker J, English R, et al. Breast cancer and hormone-replacement therapy in the Million Women Study. The Lancet. 2003 Aug;362(9382):419–27.

214. Piasecka D, Składanowski AC, Kordek R, Romańska HM, Sądej R. [Aspects of progesterone receptor (PR) activity regulation - impact on breast cancer progression]. Postepy Biochem. 2015;61(2):198–206.

215. Olivotto IA, Truong PT, Speers CH, Bernstein V, Allan SJ, Kelly SJ, et al. Time to Stop Progesterone Receptor Testing in Breast Cancer Management. Journal of Clinical Oncology. 2004 May 1;22(9):1769–70.

216. Fuqua SAW, Cui Y, Lee A V., Osborne CK, Horwitz KB. Insights Into the Role of Progesterone Receptors in Breast Cancer. Journal of Clinical Oncology. 2005 Feb 1;23(4):931–2.

217. Colozza M, Larsimont D, Piccart MJ. Progesterone Receptor Testing: Not the Right Time to Be Buried. Journal of Clinical Oncology. 2005 Jun 1;23(16):3867–8.

218. Osborne CK, Yochmowitz MG, Knight WA, McGuire WL. The value of estrogen and progesterone receptors in the treatment of breast cancer. Cancer. 1980 Dec 15;46(12 Suppl):2884–8.

219. Hanley KZ, Birdsong GG, Cohen C, Siddiqui MT. Immunohistochemical detection of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 expression in breast carcinomas. Cancer Cytopathol. 2009 Aug 25;117(4):279–88.

220. Hammond MEH, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer. Journal of Clinical Oncology. 2010 Jun 1;28(16):2784–95.

221. Houghton J. Radiotherapy and tamoxifen in women with completely excised ductal carcinoma in situ of the breast in the UK, Australia, and New Zealand: randomized controlled trial. The Lancet. 2003 Jul;362(9378):95–102.

222. Cummings SR, Eckert S, Krueger KA, Grady D, Powles TJ, Cauley JA, et al. The Effect of Raloxifene on Risk of Breast Cancer in Postmenopausal Women. JAMA. 1999 Jun 16;281(23):2189.

223. Vogel VG, Costantino JP, Wickerham DL, Cronin WM. National surgical adjuvant breast and bowel project update: prevention trials and endocrine therapy of ductal carcinoma in situ. Clin Cancer Res. 2003 Jan;9(1 Pt 2):495S-501S.

224. Dowsett M, Cuzick J, Ingle J, Coates A, Forbes J, Bliss J, et al. Meta-Analysis of Breast Cancer Outcomes in Adjuvant Trials of Aromatase Inhibitors Versus Tamoxifen. Journal of Clinical Oncology. 2010 Jan 20;28(3):509–18.

225. van de Velde CJ, Rea D, Seynaeve C, Putter H, Hasenburg A, Vannetzel JM, et al. Adjuvant tamoxifen and exemestane in early breast cancer (TEAM): a randomised phase 3 trial. The Lancet. 2011 Jan;377(9762):321–31.

226. Mamounas EP, Tang G, Fisher B, Paik S, Shak S, Costantino JP, et al. Association Between the 21-Gene Recurrence Score Assay and Risk of Locoregional Recurrence in Node-Negative, Estrogen Receptor–Positive Breast Cancer: Results From NSABP B-14 and NSABP B-20. Journal of Clinical Oncology. 2010 Apr 1;28(10):1677–83.

227. Dowsett M, Sestak I, Lopez-Knowles E, Sidhu K, Dunbier AK, Cowens JW, et al. Comparison of PAM50 Risk of Recurrence Score With Onco *type* DX and IHC4 for Predicting Risk of Distant Recurrence After Endocrine Therapy. Journal of Clinical Oncology. 2013 Aug 1;31(22):2783–90.

228. Mauri D, Pavlidis N, Ioannidis JPA. Neoadjuvant Versus Adjuvant Systemic Treatment in Breast Cancer: A Meta-Analysis. JNCI Journal of the National Cancer Institute. 2005 Feb 2;97(3):188–94.

229. Boughey JC, Wagner J, Garrett BJ, Harker L, Middleton LP, Babiera G V., et al. Neoadjuvant Chemotherapy in Invasive Lobular Carcinoma May Not Improve Rates of Breast Conservation. Ann Surg Oncol. 2009 Jun 12;16(6):1606–11.

230. Kurebayashi J, Sonoo H, Inaji H, Nishimura R, Iino Y, Toi M, et al. Endocrine Therapies for Patients with Recurrent Breast Cancer: Predictive Factors for Responses to First- and Second-Line Endocrine Therapies. Oncology. 2000;59(Suppl. 1):31–7.

231. Fossati R, Confalonieri C, Torri V, Ghislandi E, Penna A, Pistotti V, et al. Cytotoxic and hormonal treatment for metastatic breast cancer: a systematic review of published

randomized trials involving 31,510 women. Journal of Clinical Oncology. 1998 Oct;16(10):3439–60.

232. Tan ME, Li J, Xu HE, Melcher K, Yong E leong. Androgen receptor: structure, role in prostate cancer and drug discovery. Acta Pharmacol Sin. 2015 Jan 9;36(1):3–23.

233. Kensler KH, Beca F, Baker GM, Heng YJ, Beck AH, Schnitt SJ, et al. Androgen receptor expression in normal breast tissue and subsequent breast cancer risk. NPJ Breast Cancer. 2018 Sep 21;4(1):33.

234. Niemeier LA, Dabbs DJ, Beriwal S, Striebel JM, Bhargava R. Androgen receptor in breast cancer: expression in estrogen receptor-positive tumors and in estrogen receptor-negative tumors with apocrine differentiation. Modern Pathology. 2010 Feb;23(2):205–12.

235. Collins LC, Cole KS, Marotti JD, Hu R, Schnitt SJ, Tamimi RM. Androgen receptor expression in breast cancer in relation to molecular phenotype: results from the Nurses' Health Study. Modern Pathology. 2011 Jul;24(7):924–31.

236. Doane AS, Danso M, Lal P, Donaton M, Zhang L, Hudis C, et al. An estrogen receptor-negative breast cancer subset characterized by a hormonally regulated transcriptional program and response to androgen. Oncogene. 2006 Jun 29;25(28):3994–4008.

237. Hickey TE, Robinson JLL, Carroll JS, Tilley WD. Minireview: The Androgen Receptor in Breast Tissues: Growth Inhibitor, Tumor Suppressor, Oncogene? Molecular Endocrinology. 2012 Aug 1;26(8):1252–67.

238. Bozovic-Spasojevic I, Zardavas D, Brohée S, Ameye L, Fumagalli D, Ades F, et al. The Prognostic Role of Androgen Receptor in Patients with Early-Stage Breast

Cancer: A Meta-analysis of Clinical and Gene Expression Data. Clinical Cancer Research. 2017 Jun 1;23(11):2702–12.

239. Kensler KH, Poole EM, Heng YJ, Collins LC, Glass B, Beck AH, et al. Androgen Receptor Expression and Breast Cancer Survival: Results From the Nurses' Health Studies. JNCI: Journal of the National Cancer Institute. 2019 Jul 1;111(7):700–8.

240. Park S, Koo JS, Kim MS, Park HS, Lee JS, Lee JS, et al. Androgen receptor expression is significantly associated with better outcomes in estrogen receptor-positive breast cancers. Annals of Oncology. 2011 Aug;22(8):1755–62.

241. Venema CM, Bense RD, Steenbruggen TG, Nienhuis HH, Qiu SQ, van Kruchten M, et al. Consideration of breast cancer subtype in targeting the androgen receptor. Pharmacol Ther. 2019 Aug;200:135–47.

242. Gerratana L, Basile D, Buono G, De Placido S, Giuliano M, Minichillo S, et al. Androgen receptor in triple negative breast cancer: A potential target for the targetless subtype. Cancer Treat Rev. 2018 Jul;68:102–10.

243. Hu R, Dawood S, Holmes MD, Collins LC, Schnitt SJ, Cole K, et al. Androgen Receptor Expression and Breast Cancer Survival in Postmenopausal Women. Clinical Cancer Research. 2011 Apr 1;17(7):1867–74.

244. Choi JE, Kang SH, Lee SJ, Bae YK. Androgen Receptor Expression Predicts Decreased Survival in Early Stage Triple-Negative Breast Cancer. Ann Surg Oncol. 2015 Jan 22;22(1):82–9.

245. Loibl S, Müller BM, von Minckwitz G, Schwabe M, Roller M, Darb-Esfahani S, et al. Androgen receptor expression in primary breast cancer and its predictive and

prognostic value in patients treated with neoadjuvant chemotherapy. Breast Cancer Res Treat. 2011 Nov 12;130(2):477–87.

246. Wang C, Pan B, Zhu H, Zhou Y, Mao F, Lin Y, et al. Prognostic value of androgen receptor in triple negative breast cancer: A meta-analysis. Oncotarget. 2016 Jul 19;7(29):46482–91.

247. Xu M, Yuan Y, Yan P, Jiang J, Ma P, Niu X, et al. Prognostic Significance of Androgen Receptor Expression in Triple Negative Breast Cancer: A Systematic Review and Meta-Analysis. Clin Breast Cancer. 2020 Aug;20(4):e385–96.

248. Wada T, Qian X, Greene MI. Intermolecular association of the p185 protein and EGF receptor modulates EGF receptor function. Cell. 1990 Jun;61(7):1339–47.

249. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol. 2001 Feb;2(2):127–37.

250. van de Vijver MJ, Peterse JL, Mooi WJ, Wisman P, Lomans J, Dalesio O, et al. Neu-Protein Overexpression in Breast Cancer. New England Journal of Medicine. 1988 Nov 10;319(19):1239–45.

251. Lovekin C, Ellis I, Locker A, Robertson J, Bell J, Nicholson R, et al. c-erbB-2 oncoprotein expression in primary and advanced breast cancer. Br J Cancer. 1991 Mar 1;63(3):439–43.

252. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, et al. Studies of the HER-2/ *neu* Proto-Oncogene in Human Breast and Ovarian Cancer. Science (1979). 1989 May 12;244(4905):707–12.

253. Miles DW, Harris WH, Gillett CE, Smith P, Barnes DM. Effect of c-erbB2 and estrogen receptor status on survival of women with primary breast cancer treated with adjuvant cyclophosphamide/methotrexate/fluorouracil. Int J Cancer. 1999 Aug 20;84(4):354–9.

254. Stål O, Sullivan S, Wingren S, Skoog L, Rutqvist LE, Carstensen JM, et al. c-erbB-2 expression and benefit from adjuvant chemotherapy and radiotherapy of breast cancer. Eur J Cancer. 1995 Dec;31(13–14):2185–90.

255. Perez EA, Suman VJ, Davidson NE, Martino S, Kaufman PA, Lingle WL, et al. *HER2* Testing by Local, Central, and Reference Laboratories in Specimens From the North Central Cancer Treatment Group N9831 Intergroup Adjuvant Trial. Journal of Clinical Oncology. 2006 Jul 1;24(19):3032–8.

256. Paik S, Bryant J, Tan-Chiu E, Romond E, Hiller W, Park K, et al. Real-World Performance of HER2 Testing--National Surgical Adjuvant Breast and Bowel Project Experience. JNCI Journal of the National Cancer Institute. 2002 Jun 5;94(11):852–4.

257. Fendly BM, Winget M, Hudziak RM, Lipari MT, Napier MA, Ullrich A. Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product. Cancer Res. 1990 Mar 1;50(5):1550–8.

258. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of Chemotherapy plus a Monoclonal Antibody against HER2 for Metastatic Breast Cancer That Overexpresses HER2. New England Journal of Medicine. 2001 Mar 15;344(11):783–92.

259. Vogel C, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, et al. First-line, single-agent Herceptin(R) (trastuzumab) in metastatic breast cancer. a preliminary report. Eur J Cancer. 2001 Jan;37 Suppl 1:25–9.

260. LAN KH, LU CH, YU DH. Mechanisms of Trastuzumab Resistance and Their Clinical Implications. Ann N Y Acad Sci. 2005 Nov;1059(1):70–5.

261. Konecny G, Pauletti G, Pegram M, Untch M, Dandekar S, Aguilar Z, et al. Quantitative Association Between HER-2/neu and Steroid Hormone Receptors in Hormone Receptor-Positive Primary Breast Cancer. JNCI Journal of the National Cancer Institute. 2003 Jan 15;95(2):142–53.

262. Barone I, Brusco L, Fuqua SAW. Estrogen Receptor Mutations and Changes in Downstream Gene Expression and Signaling. Clinical Cancer Research. 2010 May 15;16(10):2702–8.

263. Campbell EJ, Tesson M, Doogan F, Mohammed ZM, Mallon E, Edwards J. The combined endocrine receptor in breast cancer, a novel approach to traditional hormone receptor interpretation and a better discriminator of outcome than ER and PR alone. Br J Cancer. 2016 Oct 22;115(8):967–73.

264. Montaser R, Coley H. Crosstalk between ERα and Receptor Tyrosine Kinase Signalling and Implications for the Development of Anti-Endocrine Resistance. Cancers (Basel). 2018 Jun 20;10(6):209.

265. Tolaney SM, Wardley AM, Zambelli S, Hilton JF, Troso-Sandoval TA, Ricci F, et al. Abemaciclib plus trastuzumab with or without fulvestrant versus trastuzumab plus standard-of-care chemotherapy in women with hormone receptor-positive, HER2-

positive advanced breast cancer (monarcHER): a randomised, open-label, phase 2 trial. Lancet Oncol. 2020 Jun;21(6):763–75.

266. Ditsch N, Toth B, Mayr D, Lenhard M, Gallwas J, Weissenbacher T, et al. The Association between Vitamin D Receptor Expression and Prolonged Overall Survival in Breast Cancer. Journal of Histochemistry & Cytochemistry. 2012 Feb 21;60(2):121–9.

267. Siregar KB. Vitamin D Receptor Gene TAQ 1 (rs 731236) PolymorphismIn Breast Cancer Patients ATH Adam Malik Hospital Medan and its Relationship with Histopathologygrading. Biomed J Sci Tech Res. 2018 Jun 26;6(1).

268. Bretherton-Watt D, Given-Wilson R, Mansi JL, Thomas V, Carter N, Colston KW. Vitamin D receptor gene polymorphisms are associated with breast cancer risk in a UK Caucasian population. Br J Cancer. 2001 Jul 17;85(2):171–5.

269. Guy M, Lowe LC, Bretherton-Watt D, Mansi JL, Peckitt C, Bliss J, et al. Vitamin D Receptor Gene Polymorphisms and Breast Cancer Risk. Clinical Cancer Research. 2004 Aug 15;10(16):5472–81.

270. Abbas S, Nieters A, Linseisen J, Slanger T, Kropp S, Mutschelknauss EJ, et al. Vitamin D receptor gene polymorphisms and haplotypes and postmenopausal breast cancer risk. Breast Cancer Research. 2008 Apr 17;10(2):R31.

271. Younus A, Faiz M, Yasmeen A. Breast Cancer Prognostic Indicators and Vitamin D Receptor Gene Polymorphism (FokI and TaqI) in Pakistani Women. Pak J Zool. 2019 Apr;51(3).

272. Shaikh F, Alamgir M, Ahmed S. Hormonal and genetic risk factors for breast cancer in a subset of the Karachi population. J Taibah Univ Med Sci. 2022 Aug;17(4):694–700.

273. Weir CB, Jan A. BMI Classification Percentile And Cut Off Points [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [cited 2023 Aug 7]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK541070/

274. Elston EW, Ellis IO. Method for grading breast cancer. J Clin Pathol. 1993 Feb 1;46(2):189–90.

275. Yap KKL, Efiom-Ekaha DN. Is modified Bloom-Richardson grade a reliable predictor of breast cancer recurrence? Journal of Clinical Oncology [Internet]. 2011 Sep 20;29(27_suppl):52. Available from: https://doi.org/10.1200/jco.2011.29.27_suppl.52

276. Ahn HJ, Jung SJ, Kim TH, Oh MK, Yoon HK. Differences in Clinical Outcomes between Luminal A and B Type Breast Cancers according to the St. Gallen Consensus 2013. J Breast Cancer. 2015;18(2):149.

277. Dogra AK, Prakash A, Gupta S, Gupta M. An Effective and Rapid Method of DNA Extraction Protocol from Samples of Human Blood. Asian J Biol Life Sci. 2023 Jun 6;12(1):187–91.

278. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr. 2008 Apr;87(4):1080S-1086S.

279. Lorenz TC. Polymerase Chain Reaction: Basic Protocol Plus Troubleshooting and Optimization Strategies. Journal of Visualized Experiments. 2012 May 22;(63).

280. Raza S, Jahan R, Dhasmana A, Brahma Bhatt M. L, Lohani M, Arif J. M. Vitamin D receptor gene Bsm1 polymorphism and risk of breast cancer. Biochem Cell Arch [Internet]. 2017 [cited 2023 Aug 10];17(2):657–63. Available from: https://scholarworks.utrgv.edu/som_pub

281. Sambrook J, Fritsch E.F, Maniatis T. Gel Electrophoresis of DNA. In Molecular Cloning: A Laboratory Manual, Chapter 6. Cold Spring Harbor Laboratory Press, New York; 1989.

282. Malvia S, Bagadi SA, Dubey US, Saxena S. Epidemiology of breast cancer in Indian women. Asia Pac J Clin Oncol. 2017 Aug;13(4):289–95.

283. Ghoncheh M, Pournamdar Z, Salehiniya H. Incidence and Mortality and Epidemiology of Breast Cancer in the World. Asian Pacific Journal of Cancer Prevention. 2016 Jun 1;17(sup3):43–6.

284. E. Ellsworth R, J. Decewicz D, D. Shriver C, L. Ellsworth D. Breast Cancer in the Personal Genomics Era. Curr Genomics. 2010 May 1;11(3):146–61.

285. Carter CL, Allen C, Henson DE. Relation of tumor size, lymph node status, and survival in 24,740 breast cancer cases. Cancer. 1989 Jan 1;63(1):181–7.

286. McGuire WL. Prognostic factors for recurrence and survival in human breast cancer. Breast Cancer Res Treat. 1987 Oct;10(1):5–9.

287. Foster RS. The biologic and clinical significance of lymphatic metastases in breast cancer. Surg Oncol Clin N Am. 1996 Jan;5(1):79–104.

288. Vinh-Hung V, Verschraegen C, Promish DI, Cserni G, Van de Steene J, Tai P, et al. Ratios of involved nodes in early breast cancer. Breast Cancer Research. 2004 Dec 6;6(6):R680.

289. Jatoi I, Hilsenbeck SG, Clark GM, Osborne CK. Significance of Axillary Lymph Node Metastasis in Primary Breast Cancer. Journal of Clinical Oncology. 1999 Aug;17(8):2334–2334.

290. Rodríguez-Cuevas S, Macías CG, Franceschi D, Labastida S. Breast carcinoma presents a decade earlier in Mexican women than in women in the United States or European countries. Cancer. 2001 Feb 15;91(4):863–8.

291. Nissan A, Spira RM, Hamburger T, Badrriyah M, Prus D, Cohen T, et al. Clinical profile of breast cancer in Arab and Jewish women in the Jerusalem area. The American Journal of Surgery. 2004 Jul;188(1):62–7.

292. El Saghir NS, Shamseddine AI, Geara F, Bikhazi K, Rahal B, Salem ZMK, et al. Age distribution of breast cancer in Lebanon: increased percentages and age adjusted incidence rates of younger-aged groups at presentation. J Med Liban. 2002;50(1–2):3–9.

293. Swanson GM, Lin CS. Survival patterns among younger women with breast cancer: the effects of age, race, stage, and treatment. J Natl Cancer Inst Monogr. 1994;(16):69–77.

294. Rapiti E, Fioretta G, Verkooijen HM, Vlastos G, Schäfer P, Sappino AP, et al. Survival of young and older breast cancer patients in Geneva from 1990 to 2001. Eur J Cancer. 2005 Jul;41(10):1446–52.

295. Albain KS, Allred DC, Clark GM. Breast cancer outcome and predictors of outcome: are there age differentials? J Natl Cancer Inst Monogr. 1994;(16):35–42.

296. Shannon C, Smith IE. Breast cancer in adolescents and young women. Eur J Cancer. 2003 Dec;39(18):2632–42.

297. de la Rochefordière A, Campana F, Fenton J, Vilcoq JR, Fourquet A, Asselain B, et al. Age as prognostic factor in premenopausal breast carcinoma. The Lancet. 1993 Apr;341(8852):1039–43.

298. Kroman N. Factors influencing the effect of age on prognosis in breast cancer: population based study Commentary: much still to learn about relations between tumour biology, prognosis, and treatment outcome in early breast cancer. BMJ. 2000 Feb 19;320(7233):474–9.

299. Chia KS, Du WB, Sankaranarayanan R, Sankila R, Wang H, Lee J, et al. Do younger female breast cancer patients have a poorer prognosis? Results from a population-based survival analysis. Int J Cancer. 2004 Feb 20;108(5):761–5.

300. Hanby AM, walker C. Tavassoli FA, Devilee P: Pathology and Genetics: Tumours of the Breast and Female Genital Organs. WHO Classification of Tumours series - volume IV. Lyon, France: IARC Press. Breast Cancer Research. 2004 Jun 31;6(3):133.

301. Bast RC, Ravdin P, Hayes DF, Bates S, Fritsche H, Jessup JM, et al. 2000 Update of Recommendations for the Use of Tumor Markers in Breast and Colorectal Cancer: Clinical Practice Guidelines of the American Society of Clinical Oncology*. Journal of Clinical Oncology. 2001 Mar 15;19(6):1865–78.

302. Kröger N, Milde-Langosch K, Riethdorf S, Schmoor C, Schumacher M, Zander AR, et al. Prognostic and Predictive Effects of Immunohistochemical Factors in High-Risk Primary Breast Cancer Patients. Clinical Cancer Research. 2006 Jan 1;12(1):159–68.

303. Carter CL, Allen C, Henson DE. Relation of tumor size, lymph node status, and survival in 24,740 breast cancer cases. Cancer. 1989 Jan 1;63(1):181–7.

304. Rivadeneira DE, Simmons RM, Christos PJ, Hanna K, Daly JM, Osborne MP. Predictive factors associated with axillary lymph node metastases in T1a and T1b breast carcinomas: analysis in more than 900 patients. J Am Coll Surg. 2000 Jul;191(1):1–6; discussion 6-8.

305. Swenson KK, Nissen MJ, Ceronsky C, Swenson L, Lee MW, Tuttle TM. Comparison of side effects between sentinel lymph node and axillary lymph node dissection for breast cancer. Ann Surg Oncol. 2002 Oct;9(8):745–53.

306. Ambroise M, Ghosh M, Mallikarjuna VS, Kurian A. Immunohistochemical profile of breast cancer patients at a tertiary care hospital in South India. Asian Pac J Cancer Prev. 2011;12(3):625–9.

307. Azizun-Nisa, Bhurgri Y, Raza F, Kayani N. Comparison of ER, PR and HER-2/neu (C-erb B 2) reactivity pattern with histologic grade, tumor size and lymph node status in breast cancer. Asian Pac J Cancer Prev. 2008;9(4):553–6.

308. Nemoto T, Natarajan N, Bedwani R, Vana J, Murphy GP. Breast cancer in the medial half. Results of 1978 National Survey of the American College of Surgeons. Cancer. 1983 Apr 15;51(8):1333–8.

309. Port ER, Tan LK, Borgen PI, Van Zee KJ. Incidence of axillary lymph node metastases in T1a and T1b breast carcinoma. Ann Surg Oncol. 1998 Jan;5(1):23–7.

310. Michaelson JS, Cheongsiatmoy JA, Dewey F, Silverstein MJ, Sgroi D, Smith B, et al. Spread of human cancer cells occurs with probabilities indicative of a nongenetic mechanism. Br J Cancer. 2005 Nov 8;93(11):1244–9.

311. Nielsen TO, Hsu FD, Jensen K, Cheang M, Karaca G, Hu Z, et al. Immunohistochemical and Clinical Characterization of the Basal-Like Subtype of Invasive Breast Carcinoma. Clinical Cancer Research. 2004 Aug 15;10(16):5367–74.

312. Butt Z, Haider SF, Arif S, Khan MR, Ashfaq U, Shahbaz U, et al. Breast cancer risk factors: a comparison between pre-menopausal and post-menopausal women. J Pak Med Assoc. 2012 Feb;62(2):120–4.

313. Lopez AM. Telemedicine, Telehealth, and e-Health Technologies in Cancer Prevention. In: Fundamentals of Cancer Prevention. Cham: Springer International Publishing; 2019. p. 333–52.

314. Bhoo Pathy N, Yip CH, Taib NA, Hartman M, Saxena N, Iau P, et al. Breast cancer in a multi-ethnic Asian setting: Results from the Singapore–Malaysia hospital-based breast cancer registry. The Breast. 2011 Apr;20:S75–80.

315. Desai SB, Moonim MT, Gill AK, Punia RS, Naresh KN, Chinoy RF. Hormone receptor status of breast cancer in India: a study of 798 tumours. The Breast. 2000 Oct;9(5):267–70.

316. Ahmad Z, Khurshid A, Qureshi A, Idress R, Asghar N, Kayani N. Breast carcinoma grading, estimation of tumor size, axillary lymph node status, staging, and nottingham prognostic index scoring on mastectomy specimens. Indian J Pathol Microbiol. 2009;52(4):477.

317. Choridah L, Icanervilia AV, Rengganis AA, At Thobari J, Postma MJ, D.I. van Asselt A. Comparing the performance of three modalities of breast cancer screening within a combined programme targeting at-risk women in Indonesia: An implementation study. Glob Public Health. 2023 Jan 2;18(1).

318. Brinton LA, Sherman ME, Carreon JD, Anderson WF. Recent Trends in Breast Cancer Among Younger Women in the United States. JNCI: Journal of the National Cancer Institute. 2008 Nov 19;100(22):1643–8.

319. Mamoon N, Hassan U, Mushtaq S. Breast carcinoma in young women aged 30 or less in Northern Pakistan - the Armed Forces Institute of Pathology experience. Asian Pac J Cancer Prev. 2009;10(6):1079–82.

320. Parise CA, Bauer KR, Brown MM, Caggiano V. Breast Cancer Subtypes as Defined by the Estrogen Receptor (ER), Progesterone Receptor (PR), and the Human Epidermal Growth Factor Receptor 2 (HER2) among Women with Invasive Breast Cancer in California, 1999-2004. Breast J. 2009 Nov;15(6):593–602.

321. Goldhirsch A, Glick JH, Gelber RD, Coates AS, Thürlimann B, Senn HJ. Meeting Highlights: International Expert Consensus on the Primary Therapy of Early Breast Cancer 2005. Annals of Oncology. 2005 Oct;16(10):1569–83.

322. Campbell RA, Bhat-Nakshatri P, Patel NM, Constantinidou D, Ali S, Nakshatri H. Phosphatidylinositol 3-Kinase/AKT-mediated Activation of Estrogen Receptor α. Journal of Biological Chemistry. 2001 Mar;276(13):9817–24.

323. Fan Y, Zhong X, Wang Y, Wang Z, Luo T, Wang Y, et al. A prospective cohort study of clinical characteristics and outcomes in Chinese patients with estrogen receptor-

negative/progesterone receptor-positive early breast cancer. Breast Cancer Res Treat. 2023 Jul;200(2):171–82.

324. Ahmed J, Makonnen E, Fotoohi A, Yimer G, Seifu D, Assefa M, et al. Vitamin D Status and Association of VDR Genetic Polymorphism to Risk of Breast Cancer in Ethiopia. Nutrients. 2019 Jan 29;11(2):289.

325. Ogunkolade BW, Boucher BJ, Prahl JM, Bustin SA, Burrin JM, Noonan K, et al. Vitamin D Receptor (VDR) mRNA and VDR Protein Levels in Relation to Vitamin D Status, Insulin Secretory Capacity, and VDR Genotype in Bangladeshi Asians. Diabetes. 2002 Jul 1;51(7):2294–300.

326. Rao Vupputuri M, Goswami R, Gupta N, Ray D, Tandon N, Kumar N. Prevalence and functional significance of 25-hydroxyvitamin D deficiency and vitamin D receptor gene polymorphisms in Asian Indians. Am J Clin Nutr. 2006 Jun;83(6):1411–9.

327. Kazemian E, Akbari ME, Moradi N, Gharibzadeh S, Mondul AM, Jamshidi-Naeini Y, et al. Vitamin D Receptor Genetic Variation and Cancer Biomarkers among Breast Cancer Patients Supplemented with Vitamin D3: A Single-Arm Non-Randomized Before and After Trial. Nutrients. 2019 Jun 4;11(6).

328. Lowe LC, Guy M, Mansi JL, Peckitt C, Bliss J, Wilson RG, et al. Plasma 25-hydroxy vitamin D concentrations, vitamin D receptor genotype and breast cancer risk in a UK Caucasian population. Eur J Cancer. 2005 May;41(8):1164–9.

329. Cavalcante IG de M, Silva AS, Costa MJC, Persuhn DC, Issa CI, Freire TL de L, et al. Effect of vitamin D3 supplementation and influence of BsmI polymorphism of the

VDR gene of the inflammatory profile and oxidative stress in elderly women with vitamin D insufficiency. Exp Gerontol. 2015 Jun;66:10–6.

330. Bikle DD. Extraskeletal actions of vitamin D. Ann N Y Acad Sci. 2016 Jul 20;1376(1):29–52.

331. Wang H, Chen W, Li D, Yin X, Zhang X, Olsen N, et al. Vitamin D and Chronic Diseases. Aging Dis. 2017;8(3):346.

332. Gil Á, Plaza-Diaz J, Mesa MD. Vitamin D: Classic and Novel Actions. Ann Nutr Metab. 2018;72(2):87–95.

APPENDIX I

Swami Rama Himalayan University
Swami Ram Nagar,
P.O. Jolly Grant, Dehra dun 248016 (INDIA)
Phone: 91-135-2471111, Extn. 328, Fax 910135-24711122

"Ethics Committee"
(Reg. No. ECR/483/Inst/UK/2013/RR-16)

SRHU/HIMS/ETHICS/2020//93 Dated: 28.02.2020

"Ethics Committee"
(Registration No. : ECR/483/Inst/UK/ 2013/RR-16, Dt. 23.8.2017)

The Ethics committee in its meeting held on 21.12.2019 approved the Research Project entitled:

Vitamin D Receptor (VDR) gene polymorphisms and steroid receptor status in breast cancer patients.

Submitted by Principal investigator, Ashok Kumar Dogra, Ph.D scholar, Under the guidance of Dr. Archana Prakash, Professor, Deptt. of Biochemistry, HIMS, Swami Rama Himalayan University.

Dt: 28.02.2020

Dr. D.C. Dhasmana
Member Secretary,
Ethics Committee

APPENDIX II

Thesis Protocol, Dated:

INFORMED CONSENT DOCUMENT

(To be administered before recruiting/ screening of the study subject/patient. A signed copy of it must be given back to the subject/patient.)

Part I:Subject/Patient Information document

(*To be written in non technical language understandable to a layman;Strike off which ever point is not applicable*)

Title of the study/protocol: Vitamin D Receptor (VDR) gene polymorphisms and steroid receptor status in Breast Cancer patients

Ethics committee approval letter no dated

CTRI/UHID Registration no, (if applicable)…….

Principal Investigator with qualifications: Dr. Archana Prakash, Professor, Dept. of Biochemistry.

Name & address of institution: SRHU, Jolly Grant, Dehradun

Contact No:

Subject's /Patient's Name…….

Introduction: matched case-control study.

Purpose: The study is to assess VDR gene polymorphisms and steroid receptor status among Breast Cancer patients

Methods: Blood Sampling (~5 ml) for DNA extraction and vit D level. PCR-RFLP Methodology for identify VDR gene polymorphisms and assess the Estrogen, progesterone and Her2 receptors from medical records
Risk involved: *None.*

Potential benefits: The information collated from this research work can contribute to improve health and healthcare of patients and population affected with breast cancer or having high prognosis for breast cancer occurrence.

Reasonable alternatives/ possible variant treatment available: None.

Subject's responsibility: To provide the relevant information being asked

Compensation: No

Confidentiality: Yes

Voluntary participation: Yes

Financial cost of participation involved: *None*

Contact person: Dr. Archana Prakash, Professor, Dept. of Biochemistry, Dr. Sanjay Gupta, Prof. and Head, Dept. of Biosciences. Dr. Meenu Gupta, Professor, Dept. of Radiotherapy & Ethics committee (Member secretary) for further information on any query at any time in an event of a problem.

Patient /legal representative initials

……Date……..

(Thank you for taking time to read this document .If you decide to take part in this study, you will be given a copy of this information document and signed consent form to keep with you)

Principal Investigator' Name: Dr. Archana Prakash, Professor, Dept. of Biochemistry.

Name of the Institute: SRHU, Jolly Grant, Dehradun.

Part II: Informed consent form

Name of the study/trial: The study is to assess VDR gene polymorphisms and steroid receptor status among Breast Cancer patients.

(1) Name of the Investigator: Ashok Kumar Dogra under the guidance of Dr. Archana Prakash, Professor, Dept. of Biochemistry and the Co-Supervisor-Dr. Sanjay Gupta, Prof. and Head, Dept. of Biosciences. Dr. Meenu Gupta, Professor, Dept. of Radiotherapy.

Study code: …….
Patient Name ……..
Date of birth…… age…….

1. I confirm that I have read and understood the patient information sheet dated…….. for the above study on ……(drugs /procedures etc)…. and had the opportunity to ask questions which were answered to my satisfaction
2. I have been well informed about the potential anticipated risks, discomfort and side effects associated with ….(the trial drugs/procedures etc)…. and what I will be expected to do?
3. I understand that my participation is voluntary and I am free to withdraw from the study at any time without giving any reason, without affecting my future medical care or legal rights. I shall inform the principal investigator in this regard for any precaution/ medical care required to follow.
4. I understand that the principal investigator, others workers on the principal investigator's behalf and the ethics committee HIHT University will not need my permission to look at my health record both in the respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the study/ trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published.
5. I agree not restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose.

6. I am aware that investigator will inform, whenever the situation arises, about any new finding that develop anywhere in the world, related to my treatment which may affect any decision to continue participation in the study.
7. I had have time to make my decision whether or not to take part in this study/trial. I agree to take part in the above study; I have received a signed and dated copy of this consent form for my records.

Patient/ Legally Acceptable Representative(LAR) Name	Patient's LAR's Signature	Date and Time
Investigator/ Designee Name	Investigator/ Designee's Name	Date and Time
Witness Name	Witness's Signature	Date and Time

APPENDIX III

Patient Information Form - Breast Cancer

1. Name of the Participating Centre_____

2. Registration
   ID/UHID_____
   2.1. Name of source of Registration_____
   2.2. Name of the Department_____
   2.3. Name of the Physician_____
   2.4. Case Registered as Out Patient (OP) ☐ In Patient (IP) ☐
   Others
   (specify)_____
3. Date of First
   Diagnosis_____
   (Date of first attendance to any hospital for this diseases)
4. Patient's
   Name_____
5. Name of the Spouse/Father/Mother/Caretaker.

   _____ _____ _____ _____

   Name Mobile No. Name Mobile No.

6. Age_____ 7.
   Sex_____

8. Menopausal
Status_____

9. Address_____

10. Details of Socioeconomic status, Family income, Occupation, etc.

---

11. Co-Morbid Conditions

- Tuberculosis/Diabetes/Bronchial Asthma/Hepatitis/HBsAg +ve/AIDS/HIV+ve /Hypertension/Ischaemic Heart Disease/Allergic Conditions
  (Specify)_____
  Others(Specify)_____

12. Method of Diagnosis

i. Clinical only ii. Microscopic iii. X-Ray/Imaging Techniques iv. Others

Microscopic (If ii above) X-Ray/Imaging Techniques(If iii above) Others(If iv. above)

a) Histology of Primary	a) X-Ray	a) Surgery or Autopsy
b) Histology of Metastasis	b) Isotopes	Without Histology
c) Cytology of Primary	c) Angiography	b) Biochemical/Immuno-
d) Cytology of Metastasis	d) Ultrasonogram	logical tests.
		Specify test(s)_____

_____

13. Pathological Diagnosis (With complete description of Primary site of tumour and morphological Diagnosis)

13.1 Primary/Secondary site of Tumour -
Topology_____

_____

13.2 Morphology_____

14. Details of Stage

14.1 Staging System Followed:

a) TNM staging b) Others
(Specify)_____

14.2 Staging Done at

a) Reporting Institution b) Previous Institution c) Others (specify)_____

15. Clinical Stage – UICC

15.1 TNM with Description

I. Tumour size (in cms) _____×_____

II. Axillary Lymph Node(s): 1) Not Present 2) Present
If Present, Number_____ Size (in cms) of largest node
_____×_____
Whether Matted a) No b) Yes
Whether Fixed a) No b) Yes

III. Supra-Clavicular Node(s): 1) Not Present 2) Present
If Present, Number_____ Size (in cms) of largest node
_____×_____

Whether Matted a) No b) Yes
Whether Fixed a) No b) Yes

IV. Skin Involvement a) No b) Yes
If Yes, Not Present Present
Ulcer
Infiltration
Satellite nodule
Others
(specify)_____

V. TNM Stage (Tick (√) as appropriate)

T TX T0 Tis Tis(DCIS) Tis(LCIS) Tis(Paget) T1 T1a T1b T1c T2 T3

T4 T4a T4b T4c T4d Unknown

N NX N0 N1 N2 N2a N2b N3 N3a N3b N3c Unknown

M MX M0 M1 (e.g. PUL) Unknown

VI. Stage Grouping (Tick (√) as appropriate)

I IA IB IIA IIB IIIA IIIB IIIC IV Unknown

16. Investigations for Staging

1. Mammography* 2. Chest X-ray film 3. Ultasound-Abdomen & Pelvis

4. Bone Scan 5. Others
(specify)_____

*If Mammography is done 'Normal' 'Abnormal' 'Suspicious/Inconclusive'

Specify any relevant abnormal
findings_____

_____

17. Details of Cancer directed treatment (CDT) (Tick (√) as appropriate)

17.1 Treatment given prior to registration at reporting institution (RI)

a. No b. Yes c. Unknown

If Yes,

17.2 Type of Prior Treatment given

1.Surgery Yes No Unknown If Yes, Date of completion of treatment_____

2.Radiotherapy Yes No Unknown If Yes, Date of completion of treatment_____

3.Chemotherapy Yes No Unknown If Yes, Date of completion of treatment_____

4.Others(Specify) Yes No Unknown If Yes, Date of completion of treatment_____

(Including Harmonal therapy)_____

18. Treatment at reporting institution (RI)

18.1 Intention to treat

1) Curative/Radical 2) Palliative 3) No Treatment 4) Unknown

If Palliative Yes,

1) Palliative RT only 2) Palliative RT+CT 3) Palliative CT only 4) Palliative Surgery

5) Pain & Symptoms Relief Drugs (specify)_____

6) Others (specify)_____

18.2 Type of Cancer Directed Treatment Planned at Reporting Institution:

1) Surgery Yes No Unknown

2) Chemotherapy Yes No Unknown

3) Radiotherapy Yes No Unknown

4) Others (specify)_____

19. Performance Status (WHO) Before Treatment

1) Able to carry out all normal activity without restriction.

2) Restricted in physically strenuous activity but ambulatory and able to carry out light work.

3) Ambulatory and capable of all self-care but unable to carry out any work; up and about more than 50% of waking hours.

4) Capable of only limited self-care; confined to bed or chair more than 50% of waking hours.

5) Completely disabled; cannot carry on any self-care; totally confined to bed or chair.

6) Unknown

20. Surgery

1) Surgery not planned 2) Yes, done as planned 3) Surgery planned but not taken

4) Others
(specify)_____

20.1 If Surgery done, Type of Surgical Procedure (specify)

_____

_____

_____

21. Surgical Histopathology Findings

21.1pT size (cms)_____×_____

21.2 Tumour origin: Single Multicentric

21.3 Modified Richardson Bloom
Score_____

21.4 Extensive Intraductal Component(EIC)	Not Applicable	Positive	Negative	Unknown
21.5 Lymphatic/Vasculuar Invasion	Not Applicable	Positive	Negative	Unknown
21.6 Nipple/Skin Involvement	Not Applicable	Positive	Negative	Unknown
21.7 Oestrogen Receptor Status	Not Applicable	Positive	Negative	Unknown
21.8 Progesterone Receptor Status	Not Applicable	Positive	Negative	Unknown
21.9 Androgen Receptor Status	Not Applicable	Positive	Negative	Unknown
21.10 C-erb – B2/HER – 2	Not Applicable	Positive	Negative	Unknown

21.11 Number of Axillary nodes removed [ ] Number showing Tumour

21.12 Pathological Stage

pT pTX pT0 pTis pTis(DCIS) pTis(LCIS) pTis(Paget) pT1 pT1a pT1b pT1c

pT2 pT3 pT4 pT4a pT4b pT4c pT4d Unknown

pN pNX pN0 pN1 pN1mi pN1a pN1b pN1c

pN2 pN2a pN2b pN3 pN3a pN3b pN3c Unknown

pM pMX pM0 pM1 (specify)_____ Unknown

21.13 R Classification RX R0 R1 R2 Unknown

APPENDIX IV

Patient Information Form - Controls

7. Name of the Participating Centre_____

8. Registration ID/UHID_____
   8.1. Name of the Department_____
   8.2. Name of the Physician_____
   8.3. Case Registered as Out Patient (OP) ☐ In-Patient (IP) ☐
   Others
   (specify)_____

9. _____
   (Date of first attendance to hospital for Routine Checkup/ Sample collection)

10. Patient's Name_____

11. Name of the Spouse/Father/Mother/Caretaker.

Name	Mobile No.	Name	Mobile No.

12. Age_____ 7.
    Sex_____

8. Menopausal Status_____

9. Address_____

10. Details of Socioeconomic status, Family income, Occupation, etc.

_____
_____
_____
_____

11. Co-Morbid Conditions

- Tuberculosis/Diabetes/Bronchial Asthma/Hepatitis/HBsAg +ve/AIDS/HIV+ve /Hypertension/Ischemic Heart Disease/Allergic Conditions
  (Specify)_____
  Others (Specify)_____

12. Biochemical/Immuno-logical tests.

Specify test(s)_____

APPENDIX V

LIST OF PUBLICATIONS

1. Dogra AK, Prakash A, Gupta S, Gupta M. An effective and rapid method of DNA extraction protocol from samples of human blood. Asian J Biol Life Sci [Internet]. 2023;12(1):187–91. Available from: http://dx.doi.org/10.5530/ ajbls.2023.12.25 **Impact factor:1.36**
2. Dogra AK, Prakash A, Gupta S, Gupta M, Bhat SA. Genetic variations of vitamin D receptor gene and steroid receptors status in breast cancer risk: An updated review. Adv Biomark Sci Technol [Internet]. 2022;4:1–11. Available from: http://dx.doi.org/10.1016/j.abst.2022.01.001 **Impact factor:5.1**
3. Ashok Kumar Dogra, Pranav Prakash, Sanjay Gupta, Meenu Gupta, Archana Prakash, Haamid Bashir, Role of Vitamin D Receptor Gene Polymorphism with Steroid Receptors in Breast Cancer: an Update. (2022).Int. J. Life Sci. Pharma Res.12(3), 1-6 http://dx.doi.org/10.22376/ijpbs/lpr.2022.12.2.L1-6 **Impact factor:7.1**
4. Dogra AK. Prakash A, Gupta S, Gupta M, Vitamin D and Vitamin D Receptor FokI, ApaI and BsmI gene polymorphisms and their relation with the risk of breast carcinoma: A case-control Study. J Clin Diagn Res. 2024; Available from:http;//dx.doi.org/10.7860/jcdr/2024/69296.19241 **Impact factor:1.14**

APPENDIX VI

LIST OF PAPER/POSTER PRESENTATION

1. Paper presentation at International Conference on Health and Medicine (ICHM- 21) held in Chandigarh, India on 12th December 2021, entitled "Polymorphisms of the Vitamin D Receptor Gene and Their Relationship with Steroid Receptor Status An Update"
2. Paper presentation at International Conference on Biology, Applied Science & Medicine 28-Jan-2022, entitled "The Association of Vitamin D Receptor Gene Polymorphisms in Women With Breast Cancer"
3. Presented a poster in the *CME & Workshop on "Recent Advances in Breast Cancer Management" at Cancer Research Institute, HIMS, Dehradun.*

APPENDIX VII

Awards/Certificates

SAIRAP

CERTIFICATE

OF PARTICIPATION

(ICHM-21)

International Conference on Health and Medicine

12th December 2021,Chandigarh,India

This is to certify that Ashok Kumar Dogra

of Swami Rama Himalayan University, Dehradun, Uttarakhand.

has done his/her excellence in presenting the research paper titled "Polymorphisms of the vitamin D receptor gene and their relationship with steroid receptor status. An Update."

on 12th December 2021 at Chandigarh,India.

Dr. Joe Franklin
President

SAIRAP

Sanjay D' Souza
Program Co-ordinator


Brainy Meet
International Conference on
Biology, Applied Science & Medicine
28-Jan-2022 | Webinar
CERTIFICATE OF PARTICIPATION
This is to certify that
Mr. ASHOK KUMAR DOGRA
PHD SCHOLAR,
SWAMI RAMA HIMALAYAN UNIVERSITY,
DEHRADUN, INDIA.
Has Presented a Paper titled on
"THE ASSOCIATION OF VITAMIN D RECEPTOR GENE POLYMORPHISMS IN WOMEN WITH BREAST CANCER"
In the International Conference on Biology, Applied Science & Medicine held on 28-Jan-2022
Organized by VDGOOD Professional Association.
Mr.M.Dinesh
Association Director
VDGOOD ASSOCIATION PROFESSIONAL


Asoma
Asian Society of Mastology
SRHU
SWAMI RAMA HIMALAYAN UNIVERSITY
LEARN. APPLY. CREATE.
ACS Chapter | India
CME and Workshop
Recent Advances in Breast Cancer Management
Organized by
Department of Surgical Oncology, Cancer Research Institute, Himalayan Institute of Medical Sciences, SRHU
Asian Society Of Mastology (ASOMA)
American Society of Surgeons – India Chapter
Certificate
This is to certify that
Ashok Kumar Dogra
has participated as Delegate and Poster Presentation in this
CME & Workshop on
"Recent Advances in Breast Cancer Management"
at Cancer Research
Institute, HIMS, Dehradun.
Prof. Sunil Saini
Organizing Chairman
Prof. Chintamani
President-ASOMA
Dr. Anshika Arora
Organizing Secretary
Conference accredited from Uttarakhand Medical Council for 3 Hours (U.K.M.C./2022-23/1479)

NATIONAL TESTING AGENCY

Excellence in Assessment

E-certificate No.: JUN22C05942

University Grants Commission

ज्ञान-विज्ञान विमुक्तये

JOINT CSIR-UGC TEST

NATIONAL ELIGIBILITY TEST FOR ASSISTANT PROFESSOR

NTA Ref. No: 221610228410 Roll No.: UK0102430100

Certified that ASHOK KUMAR DOGRA

Son/Daughter of GEETA DEVI

and LAKSHMAN DASS has qualified

in June 2022 Joint CSIR-UGC Test for eligibility for Assistant Professor in the subject

Life Sciences

As per information provided by the candidate, he/she had completed/appeared or was pursuing his/her Master's degree or equivalent examination in the concerned/related subject at the time of applying for Joint CSIR-UGC Test.

The date of eligibility for Assistant Professor is the date of declaration of Joint CSIR-UGC Test result, i.e., 28-10-2022, or the date of completion of Master's degree or equivalent examination with required percentage of marks within two years from the date of declaration of Joint CSIR-UGC Test result, i.e. by 27-10-2024, whichever is later.

This is an electronic certificate only, its authenticity and category in which the candidate had appeared should be verified from National Testing Agency (NTA) by the institution/appointing authority. This electronic certificate can also be verified by scanning the QR Code.

The validity of this electronic certificate is forever.

Date of issue: 29.11.2022

Senior Director, NTA

Note: NTA has issued the electronic certificate on the basis of information provided by the candidate in his/her online Application Form. The appointing authority should verify the original records/certificates of the candidate while considering him/her for appointment, as the NTA will not be liable for any false information provided by the candidate. The NTA is only responsible for the result which can be verified from the repository available in the website of NTA (csirnet.nta.nic.in). The candidate must fulfil the minimum eligibility conditions as laid down in the notification for Joint CSIR-UGC Test.

Research Article

An Effective and Rapid Method of DNA Extraction Protocol from Samples of Human Blood

Ashok Kumar Dogra[1,*], Archana Prakash[1], Sanjay Gupta[2], Meenu Gupta[3]

[1]Department of Biochemistry, Swami Rama Himalayan University, Dehradun, Uttarakhand, INDIA.
[2]Department of Biosciences, Swami Rama Himalayan University, Dehradun, Uttarakhand, INDIA.
[3]Department of Radiation Oncology, Swami Rama Himalayan University, Dehradun, Uttarakhand, INDIA.

Submission Date: 19-02-2023; Revision Date: 13-03-2023; Accepted Date: 12-04-2023.

ABSTRACT

Introduction: Several different protocols ranging from a variety of manual and automated DNA extraction protocols, are available to extract nucleic acids from whole blood samples, one of the primary sources of DNA. These methods have one or more limitations in terms of low yield, Quality issues, cost, and time efficacy, utilization of toxic organic solvents, and others as well. This study aims to develop an effective protocol for extracting DNA from 500 µl of human blood. **Materials and Methods:** The extraction procedure was standardized using 500 microliters of fresh human blood samples. The disruption and cell lysis done by Lysis Buffers R (RBC) and N (Nucleic acid) contain detergents and salts, followed by the removal of proteins and other contaminants and recovery of DNA. The DNA samples were investigated for quality and quantity by measuring their absorbance at 260 and 280 nm, respectively (A260/A280). **Results:** DNA was checked by Gel docking on 0.8% Agarose gels. According to our protocol, we yielded 19 to 25 µg DNA, respectively, from 500 µL of fresh blood. **Conclusion:** Furthermore, our protocol yields bulk amounts of DNA while avoiding toxic organic solvents like Phenol. Consequently, downstream applications can be performed with the DNA because its quality has not been affected.

Keywords: Buffers, DNA extraction, Human blood.

Correspondence:
Dr. Ashok Kumar Dogra,
Department of Biochemistry, Swami Rama Himalayan University, Dehradun, Uttarakhand, INDIA.

Email: akbhagat.pu@gmail.com

INTRODUCTION

In molecular biology, DNA, RNA, and proteins are needed for human health studies. DNA of high quantity and quality is crucial for successful downstream applications.[1-3] Furthermore, the rapid and cost-effective DNA extraction method would make it much more research-friendly.[4]

Blood DNA isolation protocols have been published in several publications. Albarino *et al.*[5] Parzer *et al.*[6] Robbins *et al.*[7] Rudbeck *et al.*[8] Sambrook *et al.*[9] Wang *et al.*[10] and Lahiri *et al.*[11] However, some of these methods require significant amounts of blood samples, making them unsuitable for low volume DNA extraction procedures. Lahiri and Nurnberger *et al.*[12] and Miller *et al.*[13] There were some protocols that used enzymes and organic solvents to get high-quality, PCR-inhibitor-free DNA, while others incorporated salting-out procedures to increase the yield of DNA. Castella *et al.*[14] and Cattaneo *et al.*[15] Therefore, the costs and time involved in some protocols are high by Nasiri *et al.*[16] Besides, in some cases, the quality of the DNA has been compromised. El Bali *et al.*[17] Chacon-Cortes *et al.*[18] and Santos *et al.*[19]

As a result, to meet the requirement for rapid, low volume and cost-effective genomic DNA extraction, our objective was to developed a protocol that would reflect the wide range of scientific interest pertaining to this field for extracting pure DNA from fresh human blood without costly enzymes and toxic organic solvents.

SCAN QR CODE TO VIEW ONLINE

www.ajbls.com

DOI: 10.5530/ajbls.2023.12.25

MATERIALS AND METHODS

Blood Collection

An EDTA-containing vacutainer tube containing blood samples was collected from 30 healthy individuals randomly selected from areas nearby the Swami Rama Himalayan University's campus situated at Jolly Grant, Dehradun, Uttarakhand, India. In order to participate in this study, the volunteers provided consent prior to participation, and no prevalent diseases were present in the volunteers. As a part of the research work, ethics committee approval was obtained prior to the study. The DNA extraction process was performed on fresh blood after 1 hr after collection. While handling the blood samples, appropriate precautions was taken to prevent biohazards. Troutman *et al*.[20]

Chemicals and Reagents

The chemicals used in this method are standard chemicals found at major suppliers, Himedia Chemicals, for this study.

Reagents Preparation

The reagents were prepared using different concentrations as RBC lysis buffer denoted as Lysis buffer R (10X) containing NH_4Cl (1.54 M), $NaHCO_3$ (0.14 M) and 0.5 M EDTA (pH~8) dissolved in 100 mL distilled water and pH adjusted to 8. The solution was converted to the 1X working solution. Another lysis buffer, nucleic acid lysis buffer denoted as Lysis buffer N containing Tris-Cl (50 mM), $MgCl_2$ (50 mM), EDTA (2 mM), NaCl (0.5M), 1% Triton X-100 and 2% of 2- mercaptoethanol dissolved in 25 mL of autoclaved distilled water and pH was adjusted to 8. This protocol also involves 10% SDS followed by high salt concentration preparation of 6M NaCl. Further, reagents preparation involves chemicals like TAE buffer, Chloroform: Isoamyl alcohol (24:1), Isopropanol and 70% Ethanol.

Methodology for DNA extraction

The standardization of the DNA extraction method was done for the fresh blood samples.

Step 1. A whole Blood sample of only 500 µL from the vacutainer was centrifuged at 2500 rpm for three minutes at 4°C and the serum was carefully aspirated.

Step 2. Approximately 1mL of Lysis buffer R (1X) was added to the pellet, mixed gently with periodic inversions, and was kept for 5 min at room temperature.

Step 3. Centrifugation of the cell's mixture was done for 5 min at 5000 rpm and carefully supernatant was discarded. Repeated the steps 2 and 3 for the pellet becomes white.

Step 4. Approximately, 500µL of Lysis Buffer N was added to the white pellet obtained from the previous steps, was mixed gently with a wide bore pipette, followed by adding 50µL of 10% SDS. The mixture was then incubated at 55-60°C for 30 min.

Step 5. At the end of incubation, 200µL of NaCl was added, vortexed vigorously, and centrifugation was done at 8000 rpm for 5 min.

Step 6. The Supernatant was then taken in a clean Eppendorf tube and added an equal volume of Chloroform: Isoamyl alcohol (24:1). The mixture was mixed well by gentle inversions and centrifuged at 12,000 rpm for 1 min.

Step 7. The Aqueous phase (DNA present) was removed carefully without disturbing the base layer and transferred into a clean Eppendorf tube containing an equal volume of chilled Isopropanol. Then the tube was vigorously shaken for few seconds resulted in fine white threads that appeared in the solution.

Step 8. The floating precipitate was transferred into a clean Eppendorf tube, washed with chilled 70% ethanol to remove any salts trapped with DNA, and was centrifuged at 13,000 rpm for 3 min.

Step 9. The tube was drained and evaporated to remove the ethanol completely. The pellet was then allowed to dried at 37°C.

Step 10. Finally, added sufficient (1X) TAE solution and dissolved the precipitate by light fingertip vibration. A solution of DNA was then stored at -20°C for further use.

RESULTS

A total of thirty human whole blood samples were used for DNA extraction and samples prepared manually showed no failures. Our optimized method was accelerated and economized by using red cell and nucleic acid lysis instead of separation of buffy coats and replacement of proteinase K. Our method observed human whole blood samples with A260/A280 absorbance ratios, a consistent range of 1.8. This effectively indicates that the samples were pure and successfully deproteinized. (Figure 1) It was also assumed that RNA was not found in the extracted DNA

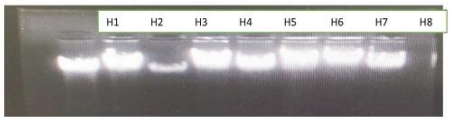


Figure 1: DNA extraction was demonstrated by electrophoresis on agarose gel containing 0.8 percent agarose.

Table 1: Ratios of Optical Density (OD) and the amount of DNA that can be extracted from each 500 µL Human blood DNA.

Coding sample	DNA quantity in ug/500ul blood	OD260/OD280 ratio
H1	23.00	1.78-0.02
H2	24.12	1.82-0.04
H3	19.11	1.72-0.05
H4	25.19	1.81-0.02
H5	22.12	1.85-0.03
H6	25.16	1.89-0.04
H7	22.14	1.87-0.07
H8	23.18	1.86-0.02

samples. In accordance with our protocol, we yielded an average of 25 µg of genomic DNA per 500 µL of fresh blood. The mean concentrations were calculated and tabulated using the experiment results (Table 1).

There were varying intensities of bands in DNA samples run on 0.8% agarose gels. Despite this, all DNA bands were prominent and homogeneous, with minimal lane smearing. It appears that none of the DNA has degraded, despite being exposed to several chemical washes.

At this point, DNA can be quantified by spectrophotometer and diluted to a working concentration, or you can simply use 1-2 µL per for the PCR reaction by Huberman *et al.*[21]

DISCUSSION

Genomic DNA extracted from whole blood samples is being used for diagnostic and genotype studies, thereby facilitating personalized medicine. DNA extraction protocols are designed according to the chemical activity of the reagents on various organelles of cells by Push *et al.*[22] and Tan *et al.*[23] The RBC Lysis Buffer contains NH_4Cl, $NaHCO_3$, and EDTA. NH_4Cl produces an increase in osmotic pressure within RBCs until they burst due to water influx. Despite this, it has a negligible effect on other cellular contents of the blood, especially on leucocytes. EDTA and $NaHCO_3$ act as buffer components, and $NaHCO_3$ increases the RBCs' swelling rate by Thoms *et al.*[24] A higher concentration of Tris buffer was used for the DNA extraction buffer, which maintains the buffer's pH at a steady level. Following this, EDTA was added to the extraction buffer to bind calcium and divalent magnesium cations that maintain the membrane integrity. Triton X-100 is a non-ionic surfactant to lyse cells and maintains DNA integrity, but higher concentrations lead to cell death by Yee *et al.*[25] NaCl forms ionic bonds with the phosphates in DNA that neutralize the negative charges, otherwise causing DNA molecules to repel one another and keep the DNA in solution. Anionic detergents like SDS can solubilize lipids and proteins by assisting in breaking down the membranes and nuclear envelopes, exposing the DNA-containing chromosomes. 2-mercaptoethanol is a powerful reducing agent used in DNA extraction buffer through disulfide bond breaking. It linearizes proteins, causing the molecules' denaturation and removal during the centrifugation by Koley *et al.*[26] The next step involves the addition of chloroform and isoamyl alcohol, which help to bind and precipitate proteins and lipids of cell membranes. DNA was generated in an aqueous phase, and lipids and proteins were formed in a non-aqueous phase. The hydration shell is formed by water molecules surrounding DNA at this stage. The DNA can therefore be separated from the remaining soluble components by centrifugation by adding Isopropanol, which acts as a dehydrating agent and disrupts the hydration shell.

Thomas *et al.*[27] published an earlier DNA extraction protocol using CTAB, but the buffer compositions and the sequences of steps differed considerably from our experiment. However, our present method is unique because it is simpler, faster, and more robust than many other methods for separating DNA from human blood samples. Additionally, there are no toxic reagents used in this protocol., so extractions are safe. Ness *et al.*[28] The average DNA yield was within the normal range using our protocol, and it takes roughly 2-3 hr for the protocol to be successfully completed.

CONCLUSION

This protocol could prove to be efficient in obtaining considerable quantities of genomic DNA from fresh human blood samples. The versatility of this universal method can be extended from fresh to frozen samples. Additionally, eliminating time-consuming steps like enzymatic incubation of Proteinase K and RNAase treatment and the absence of toxic organic solvents such as Phenol permitted an efficient and time-saving protocol in a way that could be used for advanced molecular biological techniques. Further, laboratories with limited funds may benefit from it by pursuing basic molecular biological research.

ACKNOWLEDGEMENT

The Authors like to thank all the volunteers who provided their consent to participate in our study and

the medical staff of Swami Rama Himalayan University, Dehradun, who assisted us in collecting samples.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

µl: Microlitre; **nm:** Nanogram; **µg:** Microgram; **A260/A280:** Absorbance; **OD:** Optical density; **EDTA:** Ethylenediamine tetraacetic acid; **NaCl:** Sodium Chloride; **$NaHCO_3$:** Sodium bicarbonate; **NH_4Cl:** Ammonium chloride; **SDS:** Sodium Dodecyl Sulfate; **TAE:** Tris-acetate-EDTA.

SUMMARY

A successful application of this method has been made in freshly isolated human whole blood samples. Genomic DNA isolated by this method has an average quantity of 25 µg, and according to the measurements, this DNA had a quality of 1.7 to 1.8. Further agarose gel electrophoresis was performed to ensure that the DNA obtained was of high quality, without RNA and protein contamination (Figure 1). In this method, however, only a few chemicals were used in addition to ethanol (tris-HCl, EDTA, NaCl, and SDS), and they are readily available in every routine laboratory worldwide.

REFERENCES

1. Carpi FM, Di Pietro F, Vincenzetti S, Mignini F, Napolioni V. Human DNA extraction methods: patents and applications. Recent Pat DNA Gene Seq. 2011;5(1):1-7. doi: 10.2174/187221511794839264, PMID 21303346.
2. Phillips HA, Howard GC, Miller WR. p53 mutations as a marker of malignancy in bladder washing samples from patients with bladder cancer. Br J Cancer. 2000;82(1):136-41. doi: 10.1054/bjoc.1999.0890, PMID 10638980.
3. Angelini A, Di Febbo C, Rullo A, Di Ilio C, Cuccurullo F, Porreca E. New method for the extraction of DNA from white blood cells for the detection of common genetic variants associated with thrombophilia. Pathophysiol Haemost Thromb. 2002;32(4):180-3. doi: 10.1159/000070424, PMID 12759519.
4. Guha P, Das A, Dutta S, Chaudhuri TK. A rapid and efficient DNA extraction protocol from fresh and frozen human blood samples. J Clin Lab Anal. 2018;32(1):e22181. doi: 10.1002/jcla.22181, PMID 28233351.
5. Albariño CG, Romanowski V. Phenol extraction revisited: a rapid method for the isolation and preservation of human genomic DNA from whole blood. Mol Cell Probes. 1994;8(5):423-7. doi: 10.1006/mcpr.1994.1060, PMID 7877639.
6. Parzer S, Mannhalter C. A rapid method for the isolation of genomic DNA from citrated whole blood. Biochem J. 1991;273(1);1(1):229-31. doi: 10.1042/bj2730229, PMID 1671203.
7. Robbins V, Aguinaga MP, Valenzuela MS. Efficient isolation of whole genomic DNA from cell cultures and blood samples. BioTechniques. 1995;18(3):414-8. PMID 7779389.
8. Rudbeck L, Dissing J. Rapid, simple alkaline extraction of human genomic DNA from whole blood, buccal epithelial cells, semen and forensic stains for PCR. BioTechniques. 1998;25(4):588-92. doi: 10.2144/98254bm09, PMID 9793639.
9. Sambrook J, Russell DW. Molecular cloning: A laboratory manual. New York, NY. Cold Spring Harbor; 2001.
10. Wang L, Hirayasu K, Ishizawa M, Kobayashi Y. Purification of genomic DNA from human whole blood by isopropanol-fractionation with concentrated NaI and SDS. Nucleic Acids Res. 1994;22(9):1774-5. doi: 10.1093/nar/22.9.1774, PMID 8202389.
11. Lahiri DK, Nurnberger JI Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res. 1991;19(19):5444. doi: 10.1093/nar/19.19.5444, PMID 1681511.
12. Lahiri DK, Bye S, Nurnberger JI Jr, Hodes ME, Crisp M. A non-organic and non-enzymatic extraction method gives higher yields of genomic DNA from whole-blood samples than do nine other methods tested. J Biochem Biophys Methods. 1992;25(4):193-205. doi: 10.1016/0165-022x(92)90014-2, PMID 1494032.
13. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16(3):1215. doi: 10.1093/nar/16.3.1215, PMID 3344216.
14. Castella V, Dimo-Simonin N, Brandt-Casadevall C, Mangin P. Forensic evaluation of the QIAshredder/QIAamp DNA extraction procedure. Forensic Sci Int. 2006;156(1):70-3. doi: 10.1016/j.forsciint.2005.11.012, PMID 16326058.
15. Cattaneo C, Craig OE, James NT, Sokol RJ. Comparison of three DNA extraction methods on bone and blood stains up to 43 years old and amplification of three different gene sequences. J Forensic Sci. 1997;42(6):1126-35. doi: 10.1520/JFS14273J, PMID 9397557.
16. Nasiri H, Forouzandeh M, Rasaee MJ, Rahbarizadeh F. Modified salting-out method: high-yield, high-quality genomic DNA extraction from whole blood using laundry detergent. J Clin Lab Anal. 2005;19(6):229-32. doi: 10.1002/jcla.20083, PMID 16302208.
17. El Bali L, Diman A, Bernard A, Roosens NH, De Keersmaecker SC. Comparative study of seven commercial kits for human DNA extraction from urine samples suitable for DNA biomarker-based public health studies. J Biomol Tech. 2014;25(4):96-110. doi: 10.7171/jbt.14-2504-002, PMID 25365790.
18. Chacon-Cortes D, Haupt LM, Lea RA, Griffiths LR. Comparison of genomic DNA extraction techniques from whole blood samples: a time, cost and quality evaluation study. Mol Biol Rep. 2012;39(5):5961-6. doi: 10.1007/s11033-011-1408-8, PMID 22228086.
19. Santos EM, Paula JF, Motta PM, Heinemann MB, Leite RC, Haddad JP, *et al*. Comparison of three methods of DNA extraction from peripheral blood mononuclear cells and lung fragments of equines. Genet Mol Res. 2010;9(3):1591-8. doi: 10.4238/vol9-3gmr818, PMID 20730710.
20. Troutman T, Prasauckas KA, Kennedy MA, Stevens J, Davies MG, Dadd AT. How to properly use and maintain laboratory equipment. Molecular biology problem solver. Vol. 2. New York: John Wiley and Sons, Inc; 2002;49-111.
21. Huberman JA. Importance of measuring nucleic acid absorbance at 240 nm as well as at 260 and 280 nm. BioTechniques. 1995;18(4):636. PMID 7598897.

22. Pusch C. A simple and fast procedure for high quality DNA isolation from gels using laundry detergent and inverted columns. Electrophoresis. 1997;18(7):1103-4. doi: 10.1002/elps.1150180713, PMID 9237563.
23. Tan SC, Yiap BC. DNA, RNA, and protein extraction: the past and the present. J Biomed Biotechnol. 2009:574398. doi: 10.1155/2009/574398, PMID 20011662 [published correction appears in J Biomed Biotechnol. 2013;2013:628968].
24. Ciulla TA, Sklar RM, Hauser SL. A simple method for DNA purification from peripheral blood. Anal Biochem. 1988;174(2):485-8. doi: 10.1016/0003-2697(88)90047-4.
25. Yee W, Kumar JN, Muthusamy PD. Inclusion of 2-mercaptoethanol in lysis buffer could interfere with isolation of high molecular weight DNA from freshwater microalgae. Indian J Microbiol. 2018;58(1):109-13. doi: 10.1007/s12088-017-0698-5, PMID 29434405.
26. Koley D, Bard AJ. Triton X-100 concentration effects on membrane permeability of a single HeLa cell by Scanning Electrochemical Microscopy (SECM). Proc Natl Acad Sci U S A. 2010;107(39):16783-7. doi: 10.1073/pnas.1011614107, PMID 20837548.
27. Thomas JC, Khoury R, Neeley CK, Akroush AM, Davies EC. A fast CTAB method of human DNA isolation for polymerase chain reaction applications. Biochem Educ. 1997;25(4):233-5. doi: 10.1016/S0307-4412(97)00122-2.
28. Ness JV, Cimler BM, Rich B, Meyer J, Vermeulen NMJ, and. Inventors; 1995. Nontoxiccompositions and methods useful for the extraction of nucleic acids. US patent US. p. 5393672.

Cite this article: Dogra AK, Prakash A, Gupta S, Gupta M. An Effective and Rapid Method of DNA Extraction Protocol from Samples of Human Blood. Asian J Biol Life Sci. 2023;12(1):187-91.

Contents lists available at ScienceDirect

Advances in Biomarker Sciences and Technology

journal homepage: www.keaipublishing.com/ABST

Genetic variations of vitamin D receptor gene and steroid receptors status in breast cancer risk: An updated review

Ashok Kumar Dogra [a,*], Archana Prakash [a], Sanjay Gupta [b], Meenu Gupta [c], Showkat Ahmad Bhat [d]

[a] Department of Biochemistry, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Swami Ram Nagar, Jolly Grant, Dehradun, Uttarakhand, 248016, India
[b] Department of Biosciences, Swami Rama Himalayan University, Swami Ram Nagar, Jolly Grant, Dehradun, Uttarakhand, 248016, India
[c] Department of Radiotherapy, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Swami Ram Nagar, Jolly Grant, Dehradun, Uttarakhand, 248016, India
[d] Department of Biochemistry, Govt. Medical College, Doda, India

ARTICLE INFO

Keywords:
Breast cancer
Steroid receptors
Vitamin D
Vitamin D receptor (VDR) gene polymorphism

ABSTRACT

Breast cancer, the most predominant type of cancer reported in females, is a heterogeneous disease classified into various subcategories depending on the presence of hormone receptors. Epidemiological studies show a strong correlation between reduced 1,25 dihydroxy vitamin D3 (1,25(OH)2D3) levels, the active component of vitamin D, and increased breast cancer risk in diverse populations. In a ligand-dependent manner, vitamin D receptor (VDR) transcriptionally modulates its target genes belonging to cell proliferation, differentiation, and apoptosis pathways, thus imparting protective function against cancer growth and progression. The coding and regulatory regions of the VDR gene contain several polymorphisms (BsmI, Fok1, Taq1, Apa1, Cdx2, poly (A), etc.) that modulate its transcription, translation, and mRNA stability. Despite this, research in this area has not yet led to many conclusions. In this review, we analyzed in a systematic way that the association of VDR allelic variants with breast cancer risk among patients from various populations. This analysis has revealed that Fok1, Bsm1, Apa1were to some extent associated with breast cancer risk, Taq1 shows no association, and Cdx2, poly(A), Tru91 gene polymorphisms may be susceptible for breast cancer development. We have highlighted the new insights of the current understanding of molecular mechanisms of the VDR gene polymorphisms related to breast cancer risk and also examined the interaction between VDR polymorphisms and steroid hormone (estrogen, progesterone, and androgen) receptors and their modifying effects on breast cancer risk, cancer severity, progression rate, and disease outcome. Therefore, with a lack of studies and inconsistent results, we recommend that further studies focus on genetic variations of the VDR gene that should be integrated with the assessment of steroid hormone receptor status in breast cancer.

* Corresponding author.
E-mail addresses: akbhagat.pu@gmail.com (A.K. Dogra), prakashap@hotmail.com (A. Prakash), sanjaygupta@srhu.edu.in (S. Gupta), meenugupta@srhu.edu.in (M. Gupta), biochemistrygmcsrinagar@gmail.com (S.A. Bhat).

https://doi.org/10.1016/j.abst.2022.01.001
Received 12 August 2021; Received in revised form 21 November 2021; Accepted 31 January 2022

1. Introduction

Cancer is a multifaceted disease characterized by the unregulated proliferation of cells and is the primary cause of mortality globally, resulting in 9.6 million deaths.[1] Despite tremendous progress in cancer therapy, heterogeneity of the disease and development of resistance to anti-cancer drugs continue to pose challenges for effective treatment.[2] Breast cancer commonly arises from the uncontrolled proliferation of the epithelial cells present within the ducts or lobules of the mammary gland, leading to deregulated cellular signaling.[3] It is the most commonly diagnosed malignancy noted among women in nearly 154 countries and results in ~630, 000 deaths annually.[4] Breast cancer contributes to 29% of the reported cancers in the United States and is a significant cause of cancer-related mortality among women.[5,6] Over the recent years, statistical analyses have documented a steady rise in breast cancer diagnosis among the Indian female population, wherein 25.8 females against every 100,000 females are at risk of developing cancer, and 12.7 female deaths against every 100,000 females reported in India.[7] In addition to age, the well-known risk factors of breast cancer include genetic (BRCA1 and BRCA2 mutations), environmental, and reproductive factors (early menarche and late menopause). Also, modifiable risk factors such as alcohol consumption and a high-fat diet contribute to the disease incidence.[8] Some of the risk factors that account for the differences between patients are nulliparity or first pregnancy after 30, combined estrogen and progesterone use, breastfeeding, drinking alcohol, obesity, and insufficient exercise.[9]

Vitamin D and Vitamin D receptor (VDR) has gained increasing relevance during the past two decades and are well-known for their roles in calcium homeostasis and metabolism.[10] Apart from regulation in calcium homeostasis, they promote cell differentiation and inhibit the proliferation of specific cells exhibiting cancer prevention properties.[11] Growing evidence demonstrates the inverse correlation between vitamin D and the development of breast cancer.[12] Several evidence-based studies have shown that calcitriol, an active form of vitamin D, plays a crucial role in inhibiting cell proliferation and angiogenesis and inducing cell differentiation and cell death in breast cancer through the intervention of VDR.[13–15] We have illustrated new insights into the current understanding of molecular mechanisms concerning breast cancer risk.

The polymorphisms of the VDR gene highly contribute to the risk of breast cancer, as per several studies conducted by researchers to identify its association.[16–18] Studies concerning the association of VDR gene polymorphisms and steroid receptors and their modifying effects on breast cancer risk are limited.[19–21] The purpose of this study is to provide a comprehensive review of the genetic variations of the VDR gene that may elucidate the association of VDR polymorphism with steroid hormone receptor status in breast cancer.

2. Search strategy

We conducted a systematic analysis of published literature using the National Library of Medicine (NLM) of PubMed database

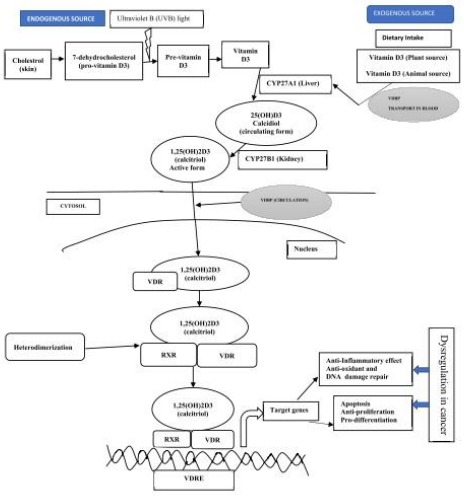


Fig. 1. Diagrammatic pathway representation of the endogenous vitamin D synthesis and downstream signaling. The active hormone 1,25(OH)2D3 is synthesized from precursor cholesterol mediated by ultraviolet-B (UVB) light. Vitamin D receptor (VDR) is activated by its ligand 1,25(OH)2D3 and it later forms a heterodimeric complex with retinoid X receptor (RXR). The 100-kDa sized complex identifies the vitamin D response element (VDRE) sequences in the 5′ end of its target genes in the nucleus. Vitamin D signaling transcriptionally modulates genes functioning in cellular processes such as cell proliferation, apoptosis, and angiogenesis etc., which are cellular pathways pivotal for cancer development. The key enzymes and intermediate metabolites involved in the pathway are indicated.

search from the year 2010–2020. In the search, keyword combinations were used as 'vitamin D' or 'vitamin D receptor gene polymorphisms' and 'Breast cancer' also, keywords: 'VDR gene polymorphisms' and 'Steroid receptors' concerning breast cancer. Our study included articles investigating VDR gene polymorphisms and steroid hormone receptor status related to breast cancer risk and incidence. The animal studies and studies focused on breast cancer in men or adolescents were excluded from this study.

3. Vitamin D and vitamin D receptor (VDR) concerning breast cancer

Vitamin D is a lipophilic secosteroid that is endogenously synthesized from cholesterol in a multi-step process under the skin or can be obtained through dietary sources (exogenously). Via photolytic reaction, ultraviolet B (UVB) light mediates the formation of

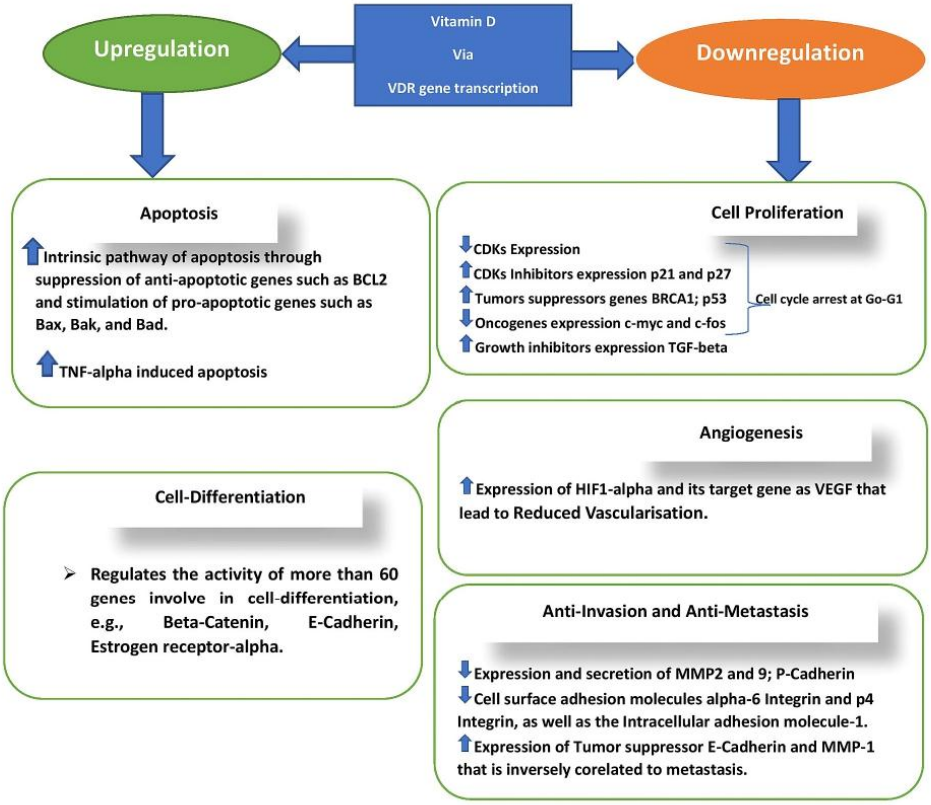


Fig. 2. Diagrammatic representation of Vitamin Ds effects on Breast cancer. 1,25 (OH)2D3–VDR complex activates the transcription of CDK inhibitors like p21 and p27 and represses cell cycle promoting factors including cyclin D1, cyclin A1, and cyclin E1. These transcriptional changes induce G1 cell cycle arrest, promote cell cycle exit, and limit the expansion of the cell cycle. Vitamin D signaling suppresses the expression of *c*-myc and *c*-fos oncogenes and upregulates tumor suppressor BRCA1, both of which regulate cell proliferation. Furthermore, 1,25(OH)2D3 hormone exerts its control on the apoptosis process by stimulating pro-apoptotic factors (BAX, BAK, BAD) and by downregulating anti-apoptotic factors (BCL-2, BCL-XL). In addition to controlling cellular proliferation, 1,25(OH)2D3 represses the hypoxia-inducible factor (HIF)-1 pathway and inhibits angiogenesis. Vitamin D signaling suppresses the HIF-1 and its target pro-survival genes such as Glut-1, ET-1, and vascular endothelial growth factor (VEGF). Vitamin D's anti-invasion or anti-metastasis properties are attained by inhibiting the expression of matrix metalloproteinase (MMP), urokinase-type plasminogen activator (uPA), and P-cadherin promotes metastasis, accompanied by stimulation of cell adhesion molecules such as E-cadherin and MMP inhibitor 1. Vitamin D, in addition to restricting cell proliferation, also promotes cellular differentiation that facilitates normal development. This is achieved through inhibition of epidermal growth factor receptor (EGFR) and insulin-like growth factor 1 (IG-1) that suppress downstream mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) 1 and 2 pathways essential for cell proliferation. These data imply that VDR is necessary and sufficient to carry out the anticarcinogenic functions mediated by vitamin D, specifically in breast malignancies, yet, the mechanism of such functions is still unknown. (Up arrows indicates stimulation and down arrows indicate repression in the boxes).

vitamin D3 (cholecalciferol) from 7-dehydrocholesterol via pre-vitamin D3 intermediate-generation step.[22,23] Further, 1, 25-dihydroxy vitamin D3 (1,25(OH)2D3; calcitriol), the biologically relevant active molecule, is derived by the action of two key CYP450-mediated hydroxylase enzymes - CYP27A1 and CYP27B1.[24] The intermediate metabolite 25-hydroxyvitamin D3 (25(OH)D3) is synthesized from vitamin D3 via the first hydroxylation step mediated by CYP27A1 in the liver.[25] The levels of the stable and most circulating 25(OH)D3 metabolite are commonly measured as an indicator of the vitamin D levels of an individual. CYP27B1 mediates the second hydroxylation reaction and catalyzes the conversion of circulating 25(OH)D3 to the potent steroid hormone 1,25(OH)2D3 in the kidney.[26] After entering the circulation, calcitriol binds to vitamin D binding protein (VDBP). It is administered to target tissues via interaction of its high-affinity receptor, vitamin D receptor, and it heterodimerizes with auxiliary proteins such as retinoid X receptors (RXR) to carry out diverse biologic functions of vitamin D[27] (Fig. 1).

The VDR protein belongs to the nuclear receptor superfamily, and its gene comprises at least 11 exons that stretch over 60 kb of DNA region on long arm of chromosome 12 (12q 12–14).[28] However, exon 1 constitutes a non-coding region, and exon 2–9 codes for the VDR protein.[29] Multiple studies have shown that vitamin D regulates vital cellular pathways that prevent cancer, such as apoptosis, differentiation, cell proliferation, and promote the inhibition of angiogenesis, metastasis, invasion, and inflammation.[30–32] Therefore, Vitamin Ds anti-cancer properties suggest that as an agent that could be used in therapeutics. Moreover, VDRs can be found in a large number of tumors, suggesting the receptor affects the aetiology of cancer. In a normal mammary gland, VDR expression is observed, and epithelial cells of mammary glands possess the same enzyme system as the kidneys; hence, vitamin D is essential for normal mammary gland development.[33] Therefore, the effects of vitamin D on breast cancer are biologically reasonable. We have illustrated updated information on molecular mechanisms of the Vitamin Ds effects of breast cancer (Fig. 2), and in relation to this figure, further studies pertain to a complete understanding of the mechanism of action concerning breast cancer in the vitamin D research is required.[34] The literature has provided much information concerning vitamin D preventive function in breast cancer.[35–37] The correlation between circulating 25(OH)D levels and breast cancer risk has been highlighted in case-control studies, showing that higher levels of 25(OH)D may reduce breast cancer risk.[38,39] In addition, a significant reduction in 25(OH) levels were observed in women diagnosed with highly aggressive triple-negative and basal-like breast cancer.[40] Accordingly, these studies suggest that there is an increased risk of breast cancer with the deficiency of vitamin D and is associated with more aggressive cancers. On the other hand, different studies showed no link between the plasma levels of 25(OH)D or supplementation with the risk of breast cancer.[41,42] According to these contradictory findings, vitamin D might only exert its effects on distinct breast cancer subtypes with different designs of tumors, which may reflect the disease's heterogeneity. As a result, further research is needed to fully understand this issue.

4. Findings in VDR gene polymorphisms and breast cancer

4.1. Polymorphic sites in the human VDR gene

Multiple research groups have identified more than 200 variations in the DNA sequences of the VDR gene, known as polymorphisms, including restriction fragment length polymorphisms (RFLPs) and a variable number of tandem repeats (VNTRs).[43] Numerous population-based studies demonstrate a link between breast cancer and VDR gene polymorphisms as major polymorphisms in the coding region are Fok1 and Taq1 in exons 2 and 9, respectively, whereas Cdx2 polymorphism in exon 1, Bsm1, Apa1 in intron 8, and poly (A) repeats in the 3′ UTR are found in the regulatory regions. Also, some of the rarer polymorphisms include Tru91 in intron 8 and A-1012G near the transcription start-site in exon 1.[44–47] The Fok1 and Cdx2 are near the 5′ UTR region, and the rest of all polymorphisms are near the 3′ UTR region. Allele frequencies differ widely over populations for polymorphisms in the 3′-UTR region, suggesting that these polymorphisms were linked to linkage disequilibrium (LD). (Fig. 3).

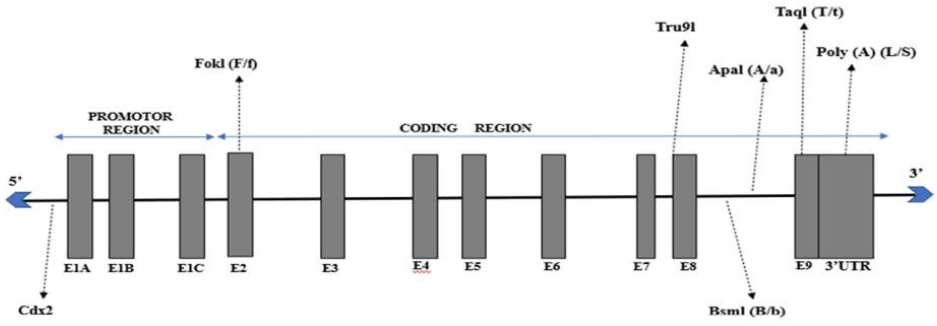


Fig. 3. Schematic of the human Vitamin D receptor (VDR) gene structure marked with the location and alleles of the different VDR gene polymorphisms. E1 to E9 indicate the exons of the VDR gene.

4.2. Fok1 polymorphism and breast cancer

In exon 2, Fok1 polymorphism consists of T to C mutation (T/C), leading to an alternate upstream start codon (ACG to ATG) that creates a differently sized VDR protein and has shown differences in the activity of the two isoforms of the transcription factor. The polymorphism results in F (absence of Fok1 restriction site) and f (presence of Fok1 restriction site) alleles resulted in three genotypes FF, Ff, and ff.[48] Numerous case-control studies on different ethnic populations have investigated the relationship between VDR gene polymorphism of Fok1 with breast cancer, summarized in Table 1. Only one study found a significant inverse relationship between Fok1ff genotype and breast cancer (OR = 0.71; 95%CI: 0.57–0.88).[49] This study examines a large sample size with estimates age-adjusted and shows a marked deviation from Hardy-Weinberg equilibrium in this polymorphism.

In contrast, six studies[21,50–54] significantly positive associations were reported between Fok1 ff genotype and risk for breast cancer. In the other nine studies,[43,46,55–61] no significant associations were found. One study (Amadori et al.) was conducted on a mixed population, i.e., African and Caucasian, but the effect was only seen in Africans, and a relatively limited sample size (53/50) may have influenced the results. Most of the studies have been done in African and Caucasian populations and have shown inconclusive results. As far the populations and ethnicities of the Indian subcontinent are pretty diverse, there is limited information available about the variations found in the Fok1 VDR gene. A recent study in India shows that the Fok1 ff allele shows a strong association with breast cancer.[53] A meta-analysis of 34 studies including 26,372 cases and 32,883 controls reaffirmed the risk posed by Fok1 in taking with other gene variants like BsmI, ApaI, and poly (A) VDR variants are susceptible to develop breast cancer.[62] There is still a need for further studies to be conducted in more ethnic groups in order to determine its functional polymorphism effects related to the risk of breast cancer.

4.3. Bsm1 polymorphism and breast cancer

The Bsm1 polymorphism (A/G) assigned the genotypes BB, Bb, bb considered silent polymorphism (SNPs) that do not alter the amino acid sequence as in Fok1, and it may affect gene expression via modulation of mRNA stability.[16] Several research studies have been conducted, but some conclusions vary by ethnic group and author, as shown in Table 2. Most of the studies have found no association between Bsm1 and breast cancer risk.[43,49–51,56,59,63,64] Few studies in this regard show Bsm1 bb and Bb genotype associations.[46,57,58,60,61] A study by shahbazi in Iranians shows significantly increased breast cancer risk between Bsm1 bb and BB genotypes (OR 1.74, CI 1.06–2.87 and OR 2.08, CI 1.31–3.29) in the BRCA1/2 mutation of the non-carriers subgroup. In an Egyptian

Table 1
Fok1 polymorphism and Breast cancer.

Author's Studies (Year)	Study Population	Type of study	Polymorphism studied	Number of Cases/ Controls	Study Findings
Anderson et al (2011)	Caucasian	PCCS	Fok1, Bsm1, Apa1, Taq1, Cdx2	1777/1839	The Fok1 ff genotype is inversely related to breast cancer risk.
Engel et al (2012)	Caucasian	NCCS	27 SNPs including Fok1	270/554	No association
Rollison et al (2012)	Hispanic and Non-Hispanic	PCCS	Fok1, Bsm1, Poly(A)	Hispanic-1527/791 Non-Hispanic-1599/922	No association
Fuhrman et al (2013)	Caucasian	PCCS	6 SNPs in CYP24A1 including Fok1, Bsm1	484/845	No association
Mishra et al (2013)	African-American and Hispanic	CCS	Fok1, Bsm1, Apa1, Taq1	232/349	The Fok1 f allele shows an increased association with breast cancer risk.
Shahbazi et al (2013)	Iranians	CCS	Fok1, Bsm1	140/156	No association
Akilzhanova et al (2014)	Other	CCS	Fok1, Bsm1	315/604	The Fok1 ff genotype is related to increase breast cancer risk.
Abd-Elsalam et al (2015)	African	CCS	Fok1, Bsm1, Apa1, Taq1	130/100	No association
Nemenqani et al (2015)	Asian	CCS	Fok1, Taq1	95/100	The Fok1 ff genotype is related to breast cancer risk.
Rashid et al (2015)	Other	CCS	Fok1, Bsm1	463/1012	No association
Deshasaux et al (2016)	Caucasian	NCCS	Fok1, Bsm1	233/466	No association
Amadori et al (2017)	African and Caucasian	PCCS	Fok1, A1012G, Cdx2	53/50	The Fok1 ff genotype is related to increase breast cancer risk. (African)
Shahabi et al (2018)	Other	CCS	Fok1, Bsm1	203/214	No association
Shaker et al (2019)	African	CCS	Fok1, Bsm1	115/120	No association
Raza et al (2019)	Indian	CCS	Fok1	125/125	The Fok1 ff genotype is related to increase breast cancer risk.
Ahmed et al (2019)	African	CCS	Fok1, Apa1, Taq1	392/193	The Fok1 ff genotype is related to breast cancer risk

PCCS: population-based case-control study, CCS: case-control study, NCCS: nested case-control study.

Table 2
Bsm1 polymorphism and Breast cancer.

Author's Studies (Year)	Study Population	Type of study	Polymorphism studied	Number of Cases/ Controls	Study Findings
Anderson et al (2011)	Caucasian	PCCS	Bsm1, Fok1, Apa1, Taq1, Cdx2	1777/1839	No association
Rollison et al (2012)	Hispanic and Non-Hispanic	PCCS	Bsm1, Fok1, Poly(A)	Hispanic-1527/791 Non-Hispanic-1599/ 922	No association
Fuhrman et al (2013)	Caucasian	PCCS	6 SNPs in CYP24A1 including Bsm1,Fok1	484/845	No association
Mishra et al (2013)	African-American and Hispanic	CCS	Bsm1, Fok1, Apa1, Taq1	232/349	No association
Shahbazi et al (2013)	Iranians	CCS	Bsm1, Fok1	140/156	BsmI bb and Bb genotype is significantly associated with an increased risk of breast cancer.
Akilzhanova et al (2014)	Other	CCS	Bsm1, Fok1	315/604	No association
Abd-Elsalam et al (2015)	African	CCS	Bsm1, Fok1, Apa1, Taq1	130/100	BsmI bb genotype is significantly associated with an increased risk of breast cancer.
Guo et al (2015)	Chinese	CCS	Bsm1, Apa1, Taq1	219/391	No association
Rashid et al (2015)	Other	CCS	Bsm1, Fok1	463/1012	BsmI bb genotype is associated with an increased risk of breast cancer.
Deshasaux et al (2016)	Caucasian	NCCS	Bsm1, Fok1	233/466	No association
El-Shorbagy et al (2017)	African	CCS	Bsm1, Apa1, Taq1	100/50	No association
Shahabi et al (2018)	Other	CCS	Bsm1, Fok1	203/214	BsmI bb and Bb genotype is significantly associated with an increased risk of breast cancer.
Shaker et al (2019)	African	CCS	Bsm1, Fok1	146/130	BsmI bb and Bb genotype is significantly associated with an increased risk of breast cancer.

PCCS: population-based case-control study, CCS: case-control study, NCCS: nested case-control study.

study of 130 cases and 100 controls, it was found that the B allele was shown to be protective for risk of breast cancer with significant risk reduction by 44 and 60% those with genotypes Bb and BB in comparison to bb genotype.[46] Studies conducted in Kazakhstan,[51] Pakistan,[58] and Iran[61] also found that the BB genotype significantly reduced breast cancer risk by 32, 33, and 47%, respectively. In short, on average, it appears there is no association between Bsm1 and breast cancer risk. Some recent studies have found significant differences among estimates even in groups of similar ethnicities, which should be investigated further and maybe through subgroup analysis in the future.

4.4. Taq1 polymorphism and breast cancer

The Taq1 is the ATT nucleotide substitution for ATC in exon nine, resulting in a synonym change in codon 252 (isoleucine) and its polymorphism assigned genotypes as TT, Tt, and tt.[16] An analysis of a case-control study (PCCS) has found a significant association

Table 3
Taq1 polymorphism and Breast cancer.

Author's Studies (Year)	Study Population	Type of study	Polymorphism studied	Number of Cases/ Controls	Study Findings
Anderson et al (2011)	Caucasian	PCCS	Taq1, Fok1, Bsm1, Apa1, Cdx2	1777/1839	No association
Engel et al (2012)	Caucasian	NCCS	27 SNPs including Taq1	270/554	No association
Mishra et al (2013)	African-American and Hispanic	CCS	Taq1, Apa1, Fok1, Bsm1	232/349	No association.
Abd-Elsalam et al (2015)	African	CCS	Taq1, Fok1, Bsm1, Apa1	130/100	No association.
Nemenqani et al (2015)	Asian	CCS	Taq1, Fok1	95/100	No association.
Guo et al (2015)	Chinese	CCS	Taq1, Bsm1, Apa1	219/391	No association.
Atoum et al (2017)	Other	CCS	Taq1	122/100	No association.
Ahmed et al (2019)	African	CCS	Taq1, Fok1, Apa1	392/193	No association
Matini et al (2020)	Other	CCS	Taq1, Apa1	150/150	The tt genotype is significantly associated with increased breast cancer risk.

PCCS: population-based case-control study, CCS: case-control study, NCCS: nested case-control study.

among premenopausal women for VDR Taq1 Tt (OR = 1.33; 95% CI: 1.01–1.74) and no association for vitamin D or calcium intake.[49] A nested case-control study (NCCS) review haplotypes analysis defines different haplotypes blocks according to their study population and showed that block B, which includes Taq1, was associated with a decreased risk of breast cancer (OR = 0.5).[55] Another study in African- Americans and Hispanics observed the Taq1 is in the strongest linkage disequilibrium with Apa1 in Hispanics than in African-Americans.[50] The Jordanian population found that Taq1 tt, TT, Tt genotypes and 25(OH)D levels are significantly associated.[65] Only one study found a significant association of Taq1 tt genotype with the breast cancer risk.[66] In conclusion, Taq1 VDR polymorphism has not been associated with breast cancer risk in many studies. Additional studies with a larger sample size in conjunction with different environmental and genetic factors are required to obtain more accurate findings. An analysis of the association of the Taq1 polymorphism with Breast cancer is shown in Table 3.

4.5. Apa1 polymorphism and breast cancer

The Apa1 vitamin D receptor gene assigned genotypes are AA, Aa, aa and contain a C to A base substitution in Intron 8 considered silent polymorphism (SNPs) that do not alter the amino acid sequence as in Fok1, to influence mRNA stability.[16] A large population-based case-control study shows higher risk of breast cancer is associated with the AA genotype.[49] The Apa1 is in linkage disequilibrium and is associated with 50% reduced breast cancer risk in a nested case-control study.[55] A pilot case-control study conducted on Caucasian women of Marin country revealed that the frequency of Apa1 AA genotype has increased by 1.9 times greater than that of the population as a whole, which is statistically significant in an increased breast cancer risk. Also present were Aa and aa genotypes, although at a lower frequency.[67] On the other hand, Mishra et al. show no association with Apa1 and breast cancer risk but explore a significant association between VDR Apa1 aa genotype and poorly differentiated tumors (p = 0.04). Also, the frequency of the aa allele is more common in Hispanics/Latinas in younger age groups than African-Americans indicates that VDR-Apa1 plays a role in advancing cancer in younger breast cancer patients.[50]

Similarly, Guo et al. have found no association in the Chinese population[63,] and Ahmed et al. have found no association in the African population.[54] Abd-Elsalam et al. observed a significantly increased risk for breast cancer in Apa1 aa genotype (OR = 2.2).[46] A recent study in Iran shows that the Apa1 aa allele appears to be associated with an increased risk of breast cancer, and Taq1 variations did not significantly correlate with tumor size or lymph node metastasis based on a stratified analysis.[66] Accordingly, although the Apa1 allele association has not been studied extensively across races, most studies have noted it is associated with breast cancer. An analysis of the association of the Apa1 polymorphism with Breast cancer is shown in Table 4.

4.6. Poly (A), Cdx2, Tru91 polymorphism, and breast cancer

The Poly (A) polymorphism yields two alleles – S (short) allele containing less than 18 A nucleotides and L (long) allele containing more than 168 A nucleotides. Differing number of adenine (A) repeats in the 3′ UTR, deviant from the ordinarily occurring poly (A) repeats in this loci, generated the poly (A) polymorphism that can potentially influence the post-transcriptional regulation of the VDR gene.[68] The Cdx2 polymorphism referred to G to A sequence variation in the VDR promoter and suggested modulation of promoter activity.[16] The Tru91 polymorphism in the VDR gene is a G (U allele) to A (u allele) is thought to modify gene expression via its effects on the affinity of enhancer regions.[69] A population-based case-control study found that reduction in risk of breast cancer was associated with the Cdx2 AG genotype (OR = 0.83), and a higher risk of cancer is associated with the Cdx2 AA genotype (OR = 1.36).[49] Another population-based case-control study for Poy(A) genotype is not associated with increased breast cancer risk but with dietary intake of vitamin D; women with LS or SS Poly (A) genotypes are at greater risk of breast cancer with higher vitamin D intake (OR = 1.41).[56] The study by Yao et al. revealed that the Cdx2 G allele is marginally associated with an increased risk of breast cancer.[70] A case-control study in southern Pakistani females for Cdx2 polymorphism observed that breast cancer risk was not significantly associated with VDR Cdx2 polymorphism, but the risk was associated with the GG genotype (OR = 1.8).[44] Another case-control study

Table 4
Apa1 polymorphism and Breast cancer.

Author's Studies (Year)	Study Population	Type of study	Polymorphism studied	Number of Cases/ Controls	Study Findings
Anderson et al (2011)	Caucasian	PCCS	Apa1, Fok1, Bsm1, Taq1, Cdx2	1777/1839	The AA genotype shows an increased risk for breast cancer.
Engel et al (2012)	Caucasian	NCCS	27 SNPs including Apa1	270/554	No association
Dalessandri et al (2012)	Caucasian	Pilot-CCS	Apa1	164/174	The AA genotype shows a significantly increased risk for breast cancer.
Mishra et al (2013)	African-American and Hispanic	CCS	Apa1, Taq1, Fok1, Bsm1	232/349	No association
Abd-Elsalam et al (2015)	African	CCS	Apa1, Taq1, Fok1, Bsm1	130/100	The aa genotype shows a significantly increased risk for breast cancer.
Guo et al (2015)	Chinese	CCS	Apa1, Taq1, Bsm1	219/391	No association
Ahmed et al (2019)	African	CCS	Apa1, Fok1, Taq1	392/193	No association
Matini et al (2020)	Other	CCS	Apa1, Taq1	150/150	The aa genotype is significantly associated with increased breast cancer risk.

PCCS: population-based case-control study, CCS: case-control study, NCCS: nested case-control study.

in Iranian females assessed an increased risk of breast cancer in women carrying the Poly(A) LS and LL alleles (OR = 1.8), and women with a low 25(OH)D level and the L carrier genotype are at greater risk.[60] Amadori et al. found that the Cdx2 AA genotype was linked to breast cancer susceptibility in Africans only.[52] In the VDR Tru9I polymorphism study, premenopausal breast cancer was not significantly associated.

In contrast, breast cancer risk was associated with the 'uu' genotype (OR = 1.1) in a case-control study.[47] In conclusion, poly(A) seems controversial, but the variant L allele apparently increases breast cancer risk, although further verification is undoubtedly warranted. Also, further studies are required for Cdx2, Tru91 VDR polymorphisms as the data are still limited. An analysis of the association of the Poly(A), Cdx2, Tru91 polymorphism with Breast cancer is shown in Table 5.

5. Steroid hormone receptor status and breast cancer

Like VDR, hormone receptors ER, PR, and AR (Estrogen, Progesterone, and Androgen Receptors) are part of the nuclear receptor superfamily. Binding to the respective ligands (estrogen, progesterone, and androgen) induces the dimerization of the receptors and translocation to the nucleus. The hormone-receptor complex transcriptionally modulates the expression of its downstream target genes by identifying the DNA sequences corresponding to hormone response elements (HREs) in the promoter region of these genes.[71]

Two forms of ER, encoded by different genes – Erα, ERβ, are of interest in the context of breast cancer. Depending on the presence of ERα, breast cancers categorized as ER-positive (ER+) or ER-negative (ER-) and ER-cancers can be positive for PR. Therefore, ER+ cancers positively respond to hormone therapy and are less aggressive with better survival outcome.[72] Notably, studies have demonstrated that ERα transcriptionally regulates the expression of PR in the mammary gland.[73,74] Accordingly, it becomes clear that mammary epithelial cells depend on estrogen and/or progesterone for proliferation, which significantly contributes to different subtypes of breast cancer. The Androgen receptor (AR) is strongly linked to prostate cancer pathology, but evaluation of AR expression in primary breast cancer samples showed reduced levels of AR in triple-negative cancers compared with the levels in HR+/HER2-or HER2+ cancers.[75] However, increased AR expression was noted in less aggressive forms (I/II) of tumors. Furthermore, AR expression was positively associated with the co-expression of several luminal genes, steroid hormone genes, and HER2 pathway members.[76] In triple-negative breast cancer, the presence of AR with the concurrent expression of luminal genes is also characterized as the luminal-androgen receptor (LAR) subtype.[77]

A study conducted in India recorded 49.2% of patients to be ER+, and 50.8% to be ER-including 24.8% of triple-negative tumor cases.[78] However, ER-breast cancer patients were double in proportion (50.8%) in comparison to ER+ patients (23.4%), as per another independent report. Further, 15% of patients were diagnosed with triple-negative tumors. The study also revealed that the ER negativity rates increased by 63.5% among young women (less than 50 years), and this trend diminished with the rise in age. However, in the United States, the majority of the cases were ER+, and the distribution of the patients was found to be 67.2% ER+ PR+, 19.0% ER+ PR-, 1.6% ER- PR+, and ER- PR-, 12.2%.[79] Further, individuals suffering from ER+ PR+ breast cancer had 30%–60% lower mortality risk from the disease. Individuals showing ER positivity within the range of 1%–9% are more likely to receive chemotherapy as they are considered to have lower ER expression than patients that show more than 10% ER positive rates and are treated with endocrine therapy.[80] Although ERα, PR, and HER2 are the commonly studied prognosis biomarkers, current efforts have been intensified to investigate the role of other less-studied receptors such as ERβ and AR.[81]

6. VDR polymorphisms and hormone receptor status in breast cancer

The steroid hormone receptor status is known to influence the relationship between vitamin D and breast cancer risk. Reduced levels of 25(OH)D show association with increased risk of advanced forms of breast cancers, and to a lesser extent for ER+ PR+ breast cancers with a higher prognosis.[82] Treatment with $1,25(OH)_2D_3$ halts explicitly the cell cycle progression in ER expressing breast cancer cells.[83] Multiple observational studies have highlighted the pronounced reduction of 25 (OH)D metabolite levels in triple-negative breast cancers, unlike other molecular subtypes, demonstrating that vitamin D is negatively correlated with this subtype.[84] Supporting this finding, increased circulating 25 (OH)D concentrations exerted maximum protective effect in triple-negative breast cancer patients.[85]

Epidemiological studies that evaluate the relationship between VDR polymorphism with different molecular subtypes of breast cancer will provide more detailed insights into the heterogeneity of this malignancy. VDR polymorphism alone was not earlier considered a breast cancer prognostic marker owing to lack of significant association of VDR with various factors or markers of breast cancer checked such as tumor type, lymph node status, hormone receptors, Ki-67 expression, p53 levels, etc.[86] However, another study reported a negative association between VDR expression and factors including hormone receptor status, Ki-67, triple-negative status, tumor size etc.[87] Due to the conflicting observations, further conclusive studies are warranted in this aspect. It is proposed that interactions of VDR with the steroid receptor status can possibly modify the breast cancer risk and further our understanding of the disease pathogenesis and the resistance to therapies noted in patients.[88]

Due to insufficient power and low sample size, minimal studies have considered the hormone receptor's status when assessing the link between VDR polymorphism and breast cancer. A study demonstrated a significant interaction of FokI polymorphisms (carrying at least one copy of 'f' allele) on ER+ cancer development in Saudi women patients.[21] A study assessing the effects of common VDR variations Taq1, Apa1, and Bsm1 on breast cancer susceptibility among Egyptian women showed a significant correlation for ApaI and TaqI variants and the absence of a link between the VDR genotypes and ER and PR expression levels in the tumor tissues.[64] In summary, these studies highlight the contribution of VDR variant alleles in response to treatments, specifically targeting hormone receptors such as estrogen modulators in breast cancer patients.

Table 5
Poly(A), Cdx2, Tru91 polymorphism, and Breast cancer.

Author's Studies (Year)	Study Population	Type of study	Polymorphism studied	Number of Cases/ Controls	Study Findings
Anderson et al (2011)	Caucasian	PCCS	Cdx2, Fok1, Bsm1, Apa1, Taq1	1777/1839	The Cdx2 AA genotype is related to increase breast cancer risk.
Rollison et al. (2012)	Hispanic and Non-Hispanic	PCCS	Poly(A), Fok1, Bsm1	Hispanic-1527/791 Non-Hispanic-1599/922	No association
Yao et al. (2012)	African- American, and Caucasian	PCCS	Cdx2	928/843	The Cdx2 G allele is related to a lower risk of breast cancer in African- Americans.
Iqbal et al (2015)	Other	CCS	Cdx2	103/161	The Cdx2 GG genotype is related to breast cancer susceptibility.
Colagar et al (2015)	Other	CCS	Poly(A)	134/127	The Long Poly (A) L allele is significantly associated with an increased risk for breast cancer.
Amadori et al. (2017)	African and Caucasian	PCCS	Cdx2, Fok1, A1012G	53/50	The Cdx2 AA genotype is related to breast cancer susceptibility.
Iqbal et al (2018)	Other	CCS	Tru91	228/503	The Tru91 uu genotype is related to increased breast cancer risk.

PCCS: population-based case-control study, CCS: case-control study, NCCS: nested case-control study.

7. Conclusions

Breast cancer is caused by the complex interplay of genetic, epigenetic, environmental, and reproductive factors and is the most predominant malignancy among women. In the past two decades, a number of large population-based studies have been conducted to determine whether variants in the VDR gene polymorphism are associated with breast cancer risk. However, studies have yielded conflicting results. On the basis of the data collected from different studies conducted on different ethnicities, we cannot make a definitive statement regarding the role of VDR polymorphisms in breast cancer and the interaction of steroid hormone receptor status. However, our illustrated overview of vitamin D-related effects on breast cancer that will provide new insights. Nevertheless, because vitamin D via VDR acts in a multitude of ways on cancer cell biology, including the cell cycle regulation, apoptosis, invasion, and metastasis, as well as angiogenesis, it has brought together a wide-ranging impact on tumor development under its domain. Well-powered epidemiological studies with a large sample size accompanied by molecular investigations are warranted in the future to understand the relationship between VDR variants, hormone receptor status, and 1,25(OH)2D3 concentrations, and the underlying molecular mechanisms that contribute to the pathophysiology of breast cancer and would enable the development of new prevention strategies.

Author's contribution

All the authors have contributed equally. All the authors read and approved the final manuscript.

Declaration of competing interest

Authors declare that they have no conflict of interests.

References

1 Ferlay J, Colombet M, Soerjomataram I, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer*. 2019; 144(8):1941–1953.
2 Liu Z, Delavan B, Roberts R, Tong W. Lessons learned from two decades of anti-cancer drugs. *Trends Pharmacol Sci*. 2017;38(10):852–872.
3 Brunetti A, Manfioletti G. Hormone receptors and breast cancer. *Front Endocrinol*. 2019;10:205.
4 Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimatesp of incidence and mortality worldwide for 36 cancers in 185 countries. *CA A Cancer J Clin*. 2018;68(6):394–424. https://doi.org/10.3322/caac.21492.
5 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA A Cancer J Clin*. 2019;69(1):7–34.
6 DeSantis C, Ma J, Bryan L, Jemal A. Breast cancer statistics, 2013. *CA A Cancer J Clin*. 2014;64(1):52–62.
7 Malvia S, Bagadi SA, Dubey US, Saxena S. Epidemiology of breast cancer in Indian women. *Asia Pac J Clin Oncol*. 2017;13(4):289–295. https://doi.org/10.1111/ajco.12661.
8 Bauer SR, Hankinson SE, Bertone-Johnson ER, Ding EL. Plasma vitamin D levels, menopause, and risk of breast cancer: dose-response meta-analysis of prospective studies. *Medicine*. 2013;92(3):123.
9 American Cancer Society. *Breast Cancer Facts and Figures 2009-2010*. Atlanta, GA: American Cancer Society; 2010.
10 Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr*. 2008;87:1080s–1086s.
11 Wang Y, Zhu J, DeLuca HF. Where is the vitamin D receptor? [Online] *Arch Biochem Biophys*. 2012;523(1):123–133. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0003986112001324.
12 Atoum M, Alzoughool F. Vitamin D and breast cancer: latest evidence and future steps. *Breast Cancer Basic Clin Res*. 2017;11, 1178223417749816.
13 de La Puente-Yagüe M, Cuadrado-Cenzual MA, Ciudad-Cabañas MJ, Hernández-Cabria M, Collado-Yurrita L. Vitamin D: and its role in breast cancer. *Kaohsiung J Med Sci*. 2018;34(8):423–427. https://doi.org/10.1016/j.kjms.2018.03.004.
14 Murray A, Madden SF, Synnott NC, et al. Vitamin D receptor as a target for breast cancer therapy. *Endocr Relat Cancer*. 2017;24(4):181–195. https://doi.org/10.1530/ERC-16-0463.

15 Zhalehjoo N, Shakiba Y, Panjehpour M. Gene expression profiles of CYP24A1 and CYP27B1 in malignant and normal breast tissues. *Mol Med Rep*. 2017;15(1):467–473. https://doi.org/10.3892/mmr.2016.5992.
16 Khan MI, Bielecka ZF, Najm MZ, et al. Vitamin D receptor gene polymorphisms in breast and renal cancer: current state and future approaches (review). *Int J Oncol*. 2014;44(2):349–363. https://doi.org/10.3892/ijo.2013.2204.
17 Xu Y, He B, Pan Y, et al. Systematic review and meta-analysis on vitamin D receptor polymorphisms and cancer risk. *Tumour Biol*. 2014;35(5):4153–4169. https://doi.org/10.1007/s13277-013-1544-y.
18 Li J, Li B, Jiang Q, et al. Do genetic polymorphisms of the vitamin D receptor contribute to breast/ovarian cancer? A systematic review and network meta-analysis. *Gene*. 2018;677:211–227. https://doi.org/10.1016/j.gene.2018.07.070.
19 McCullough ML, Bostick RM, Mayo TL. Vitamin D gene pathway polymorphisms and risk of colorectal, breast, and prostate cancer. *Annu Rev Nutr*. 2009;29:111–132. https://doi.org/10.1146/annurev-nutr-080508-141248.
20 Köstner K, Denzer N, Müller CS, Klein R, Tilgen W, Reichrath J. The relevance of vitamin D receptor (VDR) gene polymorphisms for cancer: a review of the literature. *Anticancer Res*. 2009;29(9):3511–3536.
21 Nemenqani Dalal M, Karam Rehab A, Amer Mona G, Tamer M, Abd El Rahman. Vitamin D receptor gene polymorphisms and steroid receptor status among Saudi women with breast cancer. *Gene*. 2015;558(2):215–219. https://doi.org/10.1016/j.gene.2014.12.065. ISSN 0378-1119.
22 Carlberg C. The physiology of vitamin D-far more than calcium and bone. *Front Physiol*. 2014;5:335. https://doi.org/10.3389/fphys.2014.00335. Published 2014 Sep. 2.
23 Ross AC, Manson JE, Abrams SA, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metabol*. 2011;96(1):53–58.
24 Jones G, Prosser DE, Kaufmann M. Cytochrome P450-mediated metabolism of vitamin D. *J Lipid Res*. 2014;55(1):13–31.
25 Zhu J, DeLuca HF. Vitamin D 25-hydroxylase Four decades of searching, are we there yet? *Arch Biochem Biophys*. 2012;523(1):30–36.
26 Schuster I. Cytochromes P450 are essential players in the vitamin D signaling system. *Biochim Biophys Acta*. 2011;1814(1):186–199. https://doi.org/10.1016/j.bbapap.2010.06.022.
27 Haussler MR, Whitfield GK, Kaneko I, et al. Molecular mechanisms of vitamin D action. *Calcif Tissue Int*. 2013;92(2):77–98. https://doi.org/10.1007/s00223-012-9619-0.
28 Miyamoto K, Kesterson RA, Yamamoto H, et al. Structural organization of the human vitamin D receptor chromosomal gene and its promoter. *Mol Endocrinol*. 1997;11(8):1165–1179.
29 Zmuda JM, Cauley JA, Ferrell RE. Molecular epidemiology of vitamin D receptor gene variants. *Epidemiol Rev*. 2000;22(2):203–217. https://doi.org/10.1093/oxfordjournals.epirev.a018033.
30 Feldman D, Krishnan AV, Swami S, Giovannucci E, Feldman BJ. The role of vitamin D in reducing cancer risk and progression. *Nat Rev Cancer*. 2014;14(5):342–357.
31 Haussler MR, Whitfield GK, Kaneko I, et al. Molecular mechanisms of vitamin D action. *Calcif Tissue Int*. 2013;92(2):77–98. https://doi.org/10.1007/s00223-012-9619-0.
32 Fleet JC, DeSmet M, Johnson R, Li Y. Vitamin D and cancer: a review of molecular mechanisms. *Biochem J*. 2012;441(1):61–76. https://doi.org/10.1042/BJ20110744.
33 Lopes N, Sousa B, Martins D, et al. Alterations in Vitamin D signalling and metabolic pathways in breast cancer progression: a study of VDR, CYP27B1 and CYP24A1 expression in benign and malignant breast lesions. *BMC Cancer*. 2010;10:483. https://doi.org/10.1186/1471-2407-10-483. Published 2010 Sep. 11.
34 Lopes N, Paredes J, Costa JL, Ylstra B, Schmitt F. Vitamin D and the mammary gland: a review on its role in normal development and breast cancer. *Breast Cancer Res*. 2012;14(3):211. https://doi.org/10.1186/bcr3178. Published 2012 May 31.
35 van der Rhee H, Coebergh JW, de Vries E. Is prevention of cancer by sun exposure more than just the effect of vitamin D? A systematic review of epidemiological studies. *Eur J Cancer*. 2013;49(6):1422–1436. https://doi.org/10.1016/j.ejca.2012.11.001.
36 Morrison MA, Silveira AC, Huynh N, et al. Systems biology-based analysis implicates a novel role for vitamin D metabolism in the pathogenesis of age-related macular degeneration. *Hum Genom*. 2011;5(6):538–568. https://doi.org/10.1186/1479-7364-5-6-538.
37 Romieu II , Amadou A, Chajes V. The role of diet, physical activity, body fatness, and breastfeeding in breast cancer in young women: epidemiological evidence. *Rev Invest Clin*. 2017;69(4):193–203. https://doi.org/10.24875/ric.17002263.
38 Kim Y, Franke AA, Shvetsov YB, et al. Plasma 25-hydroxyvitamin D3 is associated with decreased risk of postmenopausal breast cancer in whites: a nested case-control study in the multiethnic cohort study. *BMC Cancer*. 2014;14:29. https://doi.org/10.1186/1471-2407-14-29. Published 2014 Jan 17.
39 Fedirko V, Torres-Mejía G, Ortega-Olvera C, et al. Serum 25-hydroxyvitamin D and risk of breast cancer: results of a large population-based case-control study in Mexican women. *Cancer Causes Control*. 2012;23(7):1149–1162. https://doi.org/10.1007/s10552-012-9984-z.
40 Peppone LJ, Rickles AS, Janelsins MC, Insalaco MR, Skinner KA. The association between breast cancer prognostic indicators and serum 25-OH vitamin D levels. *Ann Surg Oncol*. 2012;19(8):2590–2599. https://doi.org/10.1245/s10434-012-2297-3.
41 Anderson LN, Cotterchio M, Vieth R, Knight JA. Vitamin D and calcium intakes and breast cancer risk in pre- and postmenopausal women. *Am J Clin Nutr*. 2010;91(6):1699–1707. https://doi.org/10.3945/ajcn.2009.28869.
42 Eliassen AH, Spiegelman D, Hollis BW, Horst RL, Willett WC, Hankinson SE. Plasma 25-hydroxyvitamin D and risk of breast cancer in the nurses' health study II. *Breast Cancer Res*. 2011;13(3):R50. https://doi.org/10.1186/bcr2880. Published 2011 May 11.
43 Fuhrman BJ, Freedman DM, Bhatti P, et al. Sunlight, polymorphisms of vitamin D-related genes and risk of breast cancer. *Anticancer Res*. 2013;33(2):543–552.
44 Mu Iqbal, Khan TA, Maqbool SA. Vitamin D receptor Cdx-2 polymorphism and premenopausal breast cancer risk in southern Pakistani patients. *PLoS One*. 2015;10(3), e0122657. https://doi.org/10.1371/journal.pone.0122657. Published 2015 Mar 23.
45 Reimers LL, Crew KD, Bradshaw PT, et al. Vitamin D-related gene polymorphisms, plasma 25-hydroxyvitamin D, and breast cancer risk. *Cancer Causes Control*. 2015;26:187–203. https://doi.org/10.1007/s10552-014-0497-9.
46 Abd-Elsalam EA, Ismaeil NA, Abd-Alsalam HS. Vitamin D receptor gene polymorphisms and breast cancer risk among postmenopausal Egyptian women. *Tumour Biol*. 2015;36(8):6425–6431. https://doi.org/10.1007/s13277-015-3332-3.
47 Iqbal MUN, Maqbool SA, Khan TA. Association of low penetrance vitamin D receptor Tru9I (rs757343) gene polymorphism with risk of premenopausal breast cancer. *J Int Med Res*. 2018;46(5):1801–1814. https://doi.org/10.1177/0300060518761304.
48 Shaikh F, Baig S, Jamal Q. Do VDR gene polymorphisms contribute to breast cancer? *Asian Pac J Cancer Prev APJCP*. 2016;17(2):479–483. https://doi.org/10.7314/apjcp.2016.17.2.479.
49 Anderson LN, Cotterchio M, Cole DE, Knight JA. Vitamin D-related genetic variants, interactions with vitamin D exposure, and breast cancer risk among Caucasian women in Ontario [published correction appears in Cancer Epidemiol Biomarkers Prev. 2014 Jul;23(7):1440-1442]. *Cancer Epidemiol Biomarkers Prev*. 2011;20(8):1442–1708. https://doi.org/10.1158/1055-9965.EPI-11-0300.
50 Mishra DK, Wu Y, Sarkissyan M, et al. PLoS ONE. In: Ling MT, ed. *Vitamin D Receptor Gene Polymorphisms and Prognosis of Breast Cancer Among African-American and Hispanic Women*. vol. 8. 2013. https://doi.org/10.1371/journal.pone.0057967, 3, e57967.
51 Akilzhanova A, Abilova Z, Sikhayeva N, et al. Vitamin D receptor gene polymorphisms and breast cancer risk in Kazakhstan. *Cent Asian J Global Health*. 2014;2(Suppl):95. https://doi.org/10.5195/cajgh.2013.95. Published 2014 Jan 24.
52 Amadori D, Serra P, Masalu N, et al. Vitamin D receptor polymorphisms or serum levels as key drivers of breast cancer development? The question of the vitamin D pathway. *Oncotarget*. 2017;8(8):13142–13156. https://doi.org/10.18632/oncotarget.14482.
53 Raza S, Dhasmana A, Bhatt MLB, Lohani M, Arif JM. Molecular mechanism of cancer susceptibility associated with Fok1 single nucleotide polymorphism of VDR in relation to breast cancer. *Asian Pac J Cancer Prev APJCP*. 2019;20(1):199–206. https://doi.org/10.31557/APJCP.2019.20.1.199. Published 2019 Jan 25.
54 Ahmed JH, Makonnen E, Fotoohi A, et al. Vitamin D status and association of VDR genetic polymorphism to risk of breast cancer in Ethiopia. *Nutrients*. 2019;11(2):289. https://doi.org/10.3390/nu11020289. Published 2019 Jan 29.

55 Engel LS, Orlow I, Sima CS, et al. Vitamin D receptor gene haplotypes and polymorphisms and risk of breast cancer: a nested case-control study. *Cancer Epidemiol Biomarkers Prev*. 2012;21(10):1856–1867. https://doi.org/10.1158/1055-9965.EPI-12-0551.
56 Rollison DE, Cole AL, Tung KH, et al. Vitamin D intake, vitamin D receptor polymorphisms, and breast cancer risk among women living in the southwestern U.S. *Breast Cancer Res Treat*. 2012;132(2):683–691. https://doi.org/10.1007/s10549-011-1885-4.
57 Shahbazi S, Alavi S, Majidzadeh -AK, Ghaffarpour M, Soleimani A, Mahdian R. BsmI but not FokI polymorphism of VDR gene is contributed in breast cancer. *Med Oncol*. 2013;30(1):393. https://doi.org/10.1007/s12032-012-0393-7.
58 Rashid MU, Muzaffar M, Khan FA, et al. Association between the BsmI polymorphism in the vitamin D receptor gene and breast cancer risk: results from a Pakistani case-control study. *PLoS One*. 2015;10(10), e0141562. https://doi.org/10.1371/journal.pone.0141562. Published 2015 Oct 30.
59 Deschasaux M, Souberbielle JC, Latino-Martel P, et al. Weight status and alcohol intake modify the association between vitamin D and breast cancer risk. *J Nutr*. 2016;146(3):576–585. https://doi.org/10.3945/jn.115.221481.
60 Shaker OG, Senousy MA. Association of SNP-SNP interactions between RANKL, OPG, CH13L1, and VDR genes with breast cancer risk in Egyptian women. *Clin Breast Cancer*. 2019;19(1):e220–e238. https://doi.org/10.1016/j.clbc.2018.09.004.
61 Shahabi A, Alipour M, Safiri H, et al. Vitamin D receptor gene polymorphism: association with susceptibility to early-onset breast cancer in Iranian, BRCA1/2-mutation carrier and non-carrier patients. *Pathol Oncol Res*. 2018;24(3):601–607. https://doi.org/10.1007/s12253-017-0281-8.
62 Iqbal MUN, Khan TA. Association between vitamin D receptor (Cdx2, Fok1, Bsm1, Apa1, Bgl1, Taq1, and poly (A)) gene polymorphism and breast cancer: a systematic review and meta-analysis. *Tumour Biol*. 2017;39(10). https://doi.org/10.1177/1010428317731280, 1010428317731280.
63 Guo B, Jiang X, Hu X, Li F, Chen X. Association between vitamin D receptor gene polymorphisms and breast cancer in a Chinese population. *Int J Clin Exp Med*. 2015;8(5):8020–8024.
64 El-Shorbagy HM, Mahmoud NH, Sabet S. Association of vitamin D receptor gene polymorphisms with breast cancer risk in an Egyptian population. *Tumour Biol*. 2017;39(10). https://doi.org/10.1177/1010428317727738, 1010428317727738.
65 Atoum MF, Al-Khatib YM. Association between serum 25-hydroxy vitamin D concentration and TaqI vitamin D receptor gene polymorphism among Jordanian females with breast cancer. *Chin Med J (Engl)*. 2017;130(9):1074–1078. https://doi.org/10.4103/0366-6999.204933.
66 Matini AH, Jafarian-Dehkordi N, Bahmani B, Sharifi M, Jahantigh D, Mazoochi T. Association of ApaI and TaqI polymorphisms in VDR gene with breast cancer. *Asian Pac J Cancer Prev APJCP*. 2020;21(9):2667–2672. https://doi.org/10.31557/APJCP.2020.21.9.2667. Published 2020 Sep. 1.
67 Dalessandri KM, Miike R, Wiencke JK, et al. Vitamin D receptor polymorphisms and breast cancer risk in a high-incidence population: a pilot study. *J Am Coll Surg*. 2012;215(5):652–657. https://doi.org/10.1016/j.jamcollsurg.2012.06.413.
68 Colagar AH, Firouzjah HM, Halalkhor S. Vitamin D receptor poly(A) microsatellite polymorphism and 25-hydroxyvitamin D serum levels: association with susceptibility to breast cancer. *J Breast Cancer*. 2015;18(2):119–125. https://doi.org/10.4048/jbc.2015.18.2.119.
69 He L, Wang M. Association of vitamin d receptor-a gene polymorphisms with coronary heart disease in Han Chinese. *Int J Clin Exp Med*. 2015;8(4):6224–6229. Published 2015 Apr 15.
70 Yao S, Zirpoli G, Bovbjerg DH, et al. Variants in the vitamin D pathway, serum levels of vitamin D, and estrogen receptor negative breast cancer among African-American women: a case-control study. *Breast Cancer Res*. 2012;14(2):R58. https://doi.org/10.1186/bcr3162. Published 2012 Apr 4.
71 Brunetti A, Manfioletti G. Hormone receptors and breast cancer. *Front Endocrinol*. 2019;10:205.
72 Piperigkou Z, Karamanos NK. Estrogen receptor-mediated targeting of the extracellular matrix network in cancer. *Semin Cancer Biol*. 2020;62:116–124. https://doi.org/10.1016/j.semcancer.2019.07.006.
73 Tanos T, Rojo L, Echeverria P, Brisken C. ER and PR signaling nodes during mammary gland development. *Breast Cancer Res*. 2012;14(4):210. https://doi.org/10.1186/bcr3166. Published 2012 Jul 19.
74 Carroll JS. Mechanisms of oestrogen receptor (ER) gene regulation in breast cancer. *Eur J Endocrinol*. 2016;175(1):R41–R49. https://doi.org/10.1530/EJE-16-0124.
75 Vidula N, Yau C, Wolf D, Rugo HS. Androgen receptor gene expression in primary breast cancer. *NPJ Breast Cancer*. 2019;5(1):1–7.
76 Giovannelli P, Di Donato M, Galasso G, Di Zazzo E, Bilancio A, Migliaccio A. The androgen receptor in breast cancer. *Front Endocrinol*. 2018;9:492. https://doi.org/10.3389/fendo.2018.00492. Published 2018 Aug 28.
77 Lehmann BD, Bauer JA, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest*. 2011;121(7):2750–2767.
78 Manjunath S, Prabhu JS, Kaluve R, Correa M, Sridhar TS. Estrogen receptor negative breast cancer in India: do we really have higher burden of this subtype? *Ind J Surg Oncol*. 2011;2(2):122–125.
79 Mlole AT. *Expression of Estrogen, Progesterone Receptors and Human Epidermal Growth Factor Receptor 2 in Women with Breast Cancer*. Published online. 2018.
80 Yi M, Huo L, Koenig KB, et al. Which threshold for ER positivity? A retrospective study based on 9639 patients. *Ann Oncol*. 2014;25(5):1004–1011.
81 Louie MC, Sevigny MB. Steroid hormone receptors as prognostic markers in breast cancer. *Am J Canc Res*. 2017;7(8):1617.
82 de Sousa Almeida-Filho B, Vespoli HDL, Pessoa EC, Machado M, Nahas-Neto J, Nahas EAP. Vitamin D deficiency is associated with poor breast cancer prognostic features in postmenopausal women. *J Steroid Biochem Mol Biol*. 2017;174:284–289.
83 Estebanez N, Gomez-Acebo I, Palazuelos C, Llorca J, Dierssen-Sotos T. Vitamin D exposure and risk of breast cancer: a meta-analysis. *Sci Rep*. 2018;8(1):1–13.
84 Blasiak J, Pawlowska E, Chojnacki J, Szczepanska J, Fila M, Chojnacki C. Vitamin D in triple-negative and BRCA1-deficient breast cancer-implications for pathogenesis and therapy. *Int J Mol Sci*. 2020;21(10):3670.
85 Lope V, Castello A, Mena-Bravo A, et al. Serum 25-hydroxyvitamin D and breast cancer risk by pathological subtype (MCC-Spain). *J Steroid Biochem Mol Biol*. 2018;182:4–13.
86 Xu H, Liu Z, Shi H, et al. Prognostic role of vitamin D receptor in breast cancer: a systematic review and meta-analysis. *BMC Cancer*. 2020;1051:20. https://doi.org/10.1186/s12885-020-07559-w.
87 Al-Azhri J, Zhang Y, Bshara W, et al. Tumor expression of vitamin D receptor and breast cancer histopathological characteristics and prognosis. *Clin Cancer Res*. 2017;23(1):97–103.
88 Ditsch N, Toth B, Mayr D, et al. The association between vitamin D receptor expression and prolonged overall survival in breast cancer. *J Histochem Cytochem*. 2012;60(2):121–129. https://doi.org/10.1369/0022155411429155.

ijlpr 2022; doi 10.22376/ijpbs/lpr.2022.12.2.L1-6

International Journal of Life science and Pharma Research

ISSN 2250-0480

Review Article

Biochemistry for better diagnosis and therapy

Role of Vitamin D Receptor Gene Polymorphism with Steroid Receptors in Breast Cancer: an Update

Ashok Kumar Dogra[1*], Pranav Prakash[2], Sanjay Gupta[3], Meenu Gupta[4], Archana Prakash[5] and Haamid Bashir[6]

[1]Department of Biochemistry, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun, Uttarakhand, India, [2]Department of Surgery, Swami Rama Himalayan University, Dehradun, Uttarakhand, India. [3]Department of Biosciences, Swami Rama Himalayan University, Dehradun, Uttarakhand, India. [4]Department of Radiotherapy, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun, Uttarakhand, India. [5]Department of Biochemistry, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun, Uttarakhand, India. [6]Department of Biochemistry, Govt Medical College, Srinagar, Kashmir, India.

Abstract: This review touches on new insights into the possible role of Vitamin D receptors genetic polymorphisms, and steroid receptors in the patients diagnosed with breast cancer. This is the most common cancer in women; further, it creates diverse illnesses among the diseased patients, and the prognosis is linked with the different subtypes present in the hormone receptors. Most of the review studies focus on the epidemiology of the disease. However, fewer studies are done on the genes polymorphism prognosis, which plays a more significant role in the prognosis and diagnosis of breast cancer, so by proper screening at the genetic level as early, can predict the disease in females which will help the clinicians in better management of the disease. Hence the burden of disease and its ill effects can be reduced in the patient care system. Thus the Vitamin D levels in the serum and the vitamin D receptors (VDR) transcriptionally controls its target genes in the cell proliferation, differentiation, and death pathways in a ligand-dependent manner, providing protection against cancer growth and progression. Also, there are strong relationships between VDR polymorphisms and steroid hormone (estrogen, progesterone, and androgen) receptors, which will help in the prognosis and diagnosis of breast cancer disease. We recommend early screening of these receptors using advanced molecular biology techniques like Real-time Polymerase chain reaction, Chemiluminescence, Western blotting which will help to detect the genotyping of these genes at the earlier stages and are non-invasive, patient-friendly, reliable, and accurate. Vitamin D receptor gene polymorphism and steroid receptors themselves can act as early predictive biomarkers for many studies that are to be warranted further in the different ethnic populations with large sample sizes.

Keywords- ***Breast cancer, steroid receptors, prognosis, Vitamin D Receptor (VDR), polymorphisms.***

***Corresponding Author**

Ashok Kumar Dogra , Department of Biochemistry, Swami Rama Himalayan University, Dehradun, Uttarakhand, India

Received On 10 January, 2022
Revised On 23 March, 2022
Accepted On 17 February, 2022
Published On

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors

Citation Ashok Kumar Dogra, Pranav Prakash, Sanjay Gupta, Meenu Gupta, Archana Prakash, Haamid Bashir , Role of Vitamin D Receptor Gene Polymorphism with Steroid Receptors in Breast Cancer: an Update.(2022).Int. J. Life Sci. Pharma Res.12(3), 1-6 http://dx.doi.org/10.22376/ijpbs/lpr.2022.12.2.L1-6

1. INTRODUCTION

Breast cancer is the most frequent cancer in women, accounting for more than one out of every ten new cancer diagnoses each year. It is also the second most common cancer among females [1,2]. Despite remarkable advancements in cancer therapy, the heterogeneity of breast cancer and the development of anticancer drug resistance remain key hurdles for effective treatment [3]. The majority of breast cancers are caused by the uncontrolled multiplication of epithelial cells in the mammary gland's ducts and lobules [4]. Having a first-degree relative with the disease, extremely thick breasts, past benign breast biopsy results, being on oral contraceptives, and having the first child at 30 years or older are all risk factors for breast cancer [5]. Furthermore, modifiable risk factors such as alcohol consumption and a high-fat diet contribute to the disease incidence [6]. The vitamin D receptor (VDR) is a mediator for the cellular effects of vitamin D and interacts with other cell signalling pathways that influences the cancer development. The biologically most active form of vitamin D is 1,25(OH)2D, which mainly exerts its anti-proliferative effects by binding to the vitamin D receptor (VDR) and acting in the complex as a transcriptional factor for a variety of genes, including those involved in cell differentiation and cell growth [7,8]. Higher vitamin D levels are thought to protect against a variety of malignancies, including breast cancer, through genomic effects controlled by the vitamin D receptor (VDR) [9]. There is mounting evidence that vitamin D is linked to a lower risk of breast cancer [10]. Apart from calcium homeostasis, vitamin D and its receptor (VDR) promote cell differentiation and suppress the proliferation of cells that have cancer-preventive capabilities [11]. The human VDR gene, which is located on chromosome 12q13-14, has about 470 single-nucleotide polymorphisms (SNPs). Fok1 (rs2228570), Bsm1 (rs1544410), Poly A (rs17878969), Apa1 (rs7975232), and Taq1 (rs7975232) are the most investigated SNPs [12]. Breast cancer risk is controlled by the prognosis of VDR gene polymorphism and steroid receptor status [13,14]. The polymorphism of the vitamin D receptor (VDR) is based on single nucleotide polymorphisms that might interfere with the activity of vitamin D which affects the prognosis of tumor [15]. This review touches on the role of Vitamin D receptor gene polymorphisms and steroid receptors status in prognosis and diagnosis of breast cancer as early biomarkers that can help clinicians to manage the disease better. Previously, many literature reviews on breast cancers touched on the epidemiology and prevalence, but no such study has been directly found which has been done on the plausible role of vitamin D receptor gene polymorphisms and on the steroid receptors status, if any are less and being done on single genes. For that, a comprehensive literature review has been done systematically to assemble all the latest material in all ethnic populations and prepare the same for the better management and prognosis of the disease. The aim and objective of this review are to offer a complete assessment of the role of VDR polymorphism and steroid receptor status in terms of modifying impacts on breast cancer risk, severity, progression rate, and disease prognosis, which will help the clinicians in improving the health index of patients.

2. METHODOLOGY

Using the National Library of Medicine's (NLM) PubMed, Medline and Scopus database search of previous years, we conducted an updated comprehensive evaluation of published research. Keywords including 'VDR gene polymorphisms,' 'Steroid receptors status,' and 'breast cancer' were utilized in the search. Articles on the role of VDR gene polymorphisms and steroid receptor status in relation to breast cancer risk and incidence were included in our analysis. Animal studies and research on breast cancer in men or teenagers were omitted from our investigation. The quality of the evidence for each part of the statement was rated as high (randomised controlled trial (RCT) evidence – level 1), moderate (intervention short of RCT or large observational studies – level 2), or low (case series, case reports, expert opinion – level 3). The clinical significance and weight of opinion favouring each statement were used to determine whether it was strong (S – recommendation) or weak (W – suggestion). Strong suggestions are clinically relevant best practices that will be applied to the majority of patients in the majority of circumstances. In contrast, weak statements should be examined by the clinician and will be applicable best practices only to specific patients or in certain circumstances.

3. Vitamin D receptors and breast cancer prognosis

Vitamin D interact with VDR in the breast epithelium in the same cell or in nearby cells in order to keep the cell differentiated and quiescent [16]. By studying case-control samples from breast cancer samples, Hemida et al., showed that the expression of VDR in breast cancer tissues was elevated, and an association exists with the expression of estrogen receptor alpha [17]. Heublein et al., and Huss et al., conducted retrospective studies which indicated that low VDR expression is a poor prognostic indicator for breast cancer[18,19]. Currently, the number of tumor node metastasis (TNM) is the most significant factor in determining how breast cancer patients will respond to treatment. However, individual differences reduces the accuracy of prediction. It is therefore imperative that breast cancer patients have a reliable biological indicator that can help predict their prognosis [20]. Despite the fact that vitamin D levels and VDR expression are associated with breast cancer prognosis [21,22], it seems that the expression of VDR is more reliable for considering the prognosis of breast cancer since vitamin D levels changes that are highly dependent on dietary exposure to sunlight. Furthermore, VDR protein expression has prognostic significance, but it has also been reported that expression of VDR mRNA has prognostic value in breast cancer [23,24]. Further studies are needed to elucidate the roles of expression and polymorphism of VDR mRNA in breast cancer.

4. Steroid receptor status concerning Breast cancer

Estrogen, Progesterone, and Androgen receptors (ER, PR, and AR), like VDR, are members of the nuclear receptor superfamily [25].

4.1 Estrogen Receptor

The estrogen Receptor (ER) is widely acknowledged as a significant predictor of the start of breast cancer in women. According to a survey done by the Department of Surgical Oncology and the Division of Molecular Medicine in India, 50.8 percent of patients were found to be ER-negative, whereas 49.2 percent were found to be ER-positive [26]. Surveillance, Epidemiology, and End Results (SEER) studies, on the other hand, showed that ER is negative in more than double the proportion of patients with breast cancer. It was discovered that 50.8 percent of breast cancer patients had negative ER, compared to 23.4 percent who had positive ER. The SEER reports also revealed that 24.8 percent of patients had Triple-

negative (TPN) tumors, compared to 15 percent of patients with TPN tumors, and that 76.4 percent of patients had a median age of 53 years. The study found that ER-negative increased among young women, with 63.5 percent of those under 50 years old, and dropped as women's ages grew [27]. In the primary tumor stage, ER staining was found in 74.2 percent of Whites, 13.7 percent of Blacks, 8.5 percent of Hispanics, 3.0 percent of Asians, and 0.6 percent of Others, respectively. It was discovered that patients with ER-positive rates ranging from 1% to 9% are more likely to get chemotherapy than patients with ER-positive rates greater than 10% who are treated with endocrine treatment. The presence of a positive estrogen receptor in women indicates an increased risk of breast and ovarian cancer. As a result, positive estrogen receptor cells boost surrounding cell proliferation, release paracrine hormones, and promote epithelial cell development[28]. Negative ER cells, on the other hand, aid mammary gland growth and restore the proliferation process. Positive estrogen receptor cells are identified in 20% of sporadic breast cancer cases, while negative estrogen receptor cells are seen in 70% to 80% of instances. When the size of the tumor grows large enough to reduce the estrogen effect on the patients, the ER-positive cells undergo metamorphosis and eventually perish. As a result, the negative estrogen receptor cell, like the positive estrogen receptor cell, undergoes neoplastic change as it grows older. As the tumor increases in size, positive estrogen receptors become differentiated, while negative estrogen receptors become stimulated by mitogenic signaling [29].

4.2 Progesterone Receptor

Progesterone receptors are members of the nuclear subfamily 3 (NR3C3) and belong to the protein group C. Positive progesterone receptors are malignant cells in the breast that are extremely sensitive to progesterone and have receptors that help hormones proliferate. Hormone therapy is used to inhibit estrogen receptors in those who have positive progesterone receptor cancer. Patients with negative progesterone receptor cancer, on the other hand, are not suggested to receive hormone therapy because it was ineffective for them [30]. When looking at the positive and bad aspects of progesterone receptors in India, it was discovered that they were present in 49.8% of patients suffering from breast cancer. There are 204 cases of lobular carcinoma and 50 cases of invasive lobular carcinoma. Invasive ductal carcinoma was found in 817 Indian women, and breast cancer was found in 16 of every 1087 Indian women. The Indians had a more significant percentage of progesterone receptors, which exhibited negative breast cancer proportions [31]. According to a survey conducted by the National Institutes of Health in the United States, 67.2 percent of women with breast cancer in the United States have positive progesterone receptors as well as positive estrogen receptors, while 19.0 percent of women with breast cancer have negative progesterone receptors and negative estrogen receptors. It was also shown that 12.2 percent of breast cancer patients had negative progesterone-receptors with positive estrogen-receptors, whereas 1.6 percent had positive progesterone-receptors with negative estrogen-receptors. It was also discovered that women with breast cancer who had positive progesterone-receptor and positive estrogen-receptor symptoms have a 30%-60% decreased likelihood of dying from the disease [32].

4.3 Androgen Receptor

The nuclear receptor known as the androgen receptor is strongly linked to the development of the prostate. It's a breast cancer expression that also recognizes luminal genes and the luminal-androgen receptor (LAR) subtype. The expression of numerous luminal genes is linked to positive androgen receptors, which are also linked to the HER2 pathway. Furthermore, when compared to positive androgen receptors, androgen receptors were negatively related with grade I/II vs III malignancies in high proportions [33,34].

5. VDR polymorphism and Steroid receptor status concerning Breast Cancer prognosis.

The steroid hormone receptor status has an impact on the link between vitamin D and breast cancer risk. Reduced 25(OH)D has been linked to an increased incidence of advanced breast cancer and, to a lesser extent, ER+ PR+ breast cancer, which has a better prognosis [35]. Due to its lack of relationship with numerous determinants or markers of breast cancer, such as tumour type, lymph node status, hormone receptors, Ki-67 expression, and p53 levels, the VDR polymorphism was previously not considered a predictive factor for breast cancer [36]. Hormone receptor status, Ki-67, triple-negative status, and tumour size are all linked to lower VDR expression, according to a recent study[37]. Because of the lack of conclusive research in this area, more research is needed. VDR expression varies greatly between different types of breast cancers, with studies reporting almost 90% expression in ER+ tumours compared to only 27% in basal/triple-negative tumours, implying an inverse association between VDR expression and cancer severity [38]. Abbas et al., found a link between the TaqI VDR polymorphism (containing at least one copy of the t allele) and ER-positive postmenopausal breast cancer [39]. A recent investigation found that FokI polymorphisms have a similar favorable effect on the development of ER+ cancer in Saudi women patients [14]. Premenopausal Chinese women with both the aa genotype of the ApaI polymorphism and the ER haplotype experienced a delayed onset of menarche. This finding is substantial as the age of menarche is a significant risk factor for breast cancer [40]. The steroid hormone receptor status is known to influence the relationship between vitamin D and breast cancer risk [41]. Various processes such as frequency identification of FFLL and FfLL genotypes against FokI and poly(adenylate) grouping, comparing tumor grade, lymph node involvement, and estrogen receptor (ER) status among cancer patients, and VDR genotype are some of the methods that aid in determining the levels, stages, and progression of cancer in patients. For example, adjusted odds ratios (OR) for age at sampling, HRT use, and menopausal state at diagnosis were observed to be 1.12 (0.62–2.04) in the identification of total tumor grade in tumor grade I, FFLL or FfLL. As a result, it can be concluded that the use of FokI, poly (adenylate) grouping, and other techniques aided in gaining a thorough understanding of the tumor grade present in breast cancer patients [42]. VDR polymorphisms, which include the bb genotype, are directly linked to the spread of breast cancer. The bb genotype, which was derived from VDR BsmI polymorphisms, was found to have four times the chance of developing metastases as the BB genotype [43]. Furthermore, the TT genotype derived from the VDR TaqI polymorphism is strongly linked to an elevated risk of 1.8 lymph node metastases. Females that have more of the haplotype baTL have a higher chance of acquiring metastatic illness, especially in Caucasian female communities [44]. As a result, VDR polymorphisms are connected to the development of breast cancer risks in individuals.

6. *Heterogeneity in VDR expression concerning Steroid receptors status*

The effects of vitamin D endocrine signaling on distinct cell types in mammary tissues are poorly understood. Breast cancer is a diverse illness that develops from a variety of mammary epithelial cell types [45]. The inner luminal layer and outer basal layer of myoepithelial cells make up the mammary epithelium. A recent genome-wide transcriptome research in human tissues found that CYP24A1 is expressed differently in luminal progenitor cells, suggesting that the vitamin D pathway may play a role in mammary cell lineage development [46]. In normal human breast tissues, major steroid receptors VDR, ER, and AR revealed differential expression between luminal and basal cell types. VDR expression, which is commonly coupled with ER and/or AR, was observed during particular stages of luminal cell differentiation and suppression during other stages, according to Santagata et al.[47]. In some cases of breast cancer, variations in VDR expression may lead to unresponsiveness or resistance to vitamin D supplementation. Additionally, epigenetic alterations in VDR and CYP24A1 have been linked to vitamin D resistance [48]. Individuals with malignancies that are positive for ER, VDR, and AR all have a better prognosis [49]. These findings highlight the need of assessing VDR polymorphisms and steroid receptor status in breast cancer samples at the same time [50,51].

7. CONCLUSIONS

VDR gene polymorphism is a highly effective indicator for predicting and assessing the beginning and progression of breast cancer when taken collectively. Further research on the link between VDR genetic polymorphisms and breast cancer incidence in patients who are classified based on their steroid receptor (ER, PR, and AR) status is required. This will allow researchers to explore the relationship between VDR and the molecular subtypes of breast cancer, which will aid in the development of more tailored therapies for patients. In this review article more has been added related to breast cancer, prognosis, and diagnosis which will help the clinicians to establish new treatment protocols in systematically managing the diseases. Further studies are needed for eradicating the diseases by targeting the gene at the protein level in the human body system, in order to improve the health index of our woman gender. Therapeutic approaches are needed for best treatment protocols and will curtain fewer surgical procedures.

8. AUTHOR'S CONTRIBUTION STATEMENT

Ashok Kumar Dogra, conceptualized, prepared the original draft, reviewed, edit the draft, and designed the study; Dr. Pranav Prakash curated data; Dr. Sanjay Gupta, discussed methodology; Dr. Meenu Gupta, review the draft; Dr. Archana Prakash, analyzed and revised the draft; Haamid Bashir, provided valuable inputs towards designing the manuscript. All authors approved, read, and approved the final version of the manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none

10. REFERENCES

1. Alkabban FM, Ferguson T. Breast cancer. StatPearls [Internet]. Updated 2021 Aug 7:2021 Jan-.
2. Singh PG, Basalingappa KM, Ts G, Mp NR, Rajashekara S P, Bv S. Awareness of Breast cancer and current perspectives: an overview. Int J Pharma Bio Sci. 2021;12(2) (Apr–Jun):54-9. doi: 10.22376/ijpbs.2021.12.2.b54-59.
3. Liu Z, Delavan B, Roberts R, Tong W. Lessons learned from two decades of anticancer drugs. Trends Pharmacol Sci. 2017;38(10):852-72. doi: 10.1016/j.tips.2017.06.005, PMID 28709554.
4. Brunetti A, Manfioletti G. Editorial: Hormone receptors and breast cancer. Front Endocrinol. 2019;10:205. doi: 10.3389/fendo.2019.00205, PMID 31001204.
5. Nelson HD, Zakher B, Cantor A, Fu R, Griffin J, O'Meara ES, Buist DS, Kerlikowske K, van Ravesteyn NT, Trentham-Dietz A, Mandelblatt JS, Miglioretti DL. Risk factors for breast cancer for women aged 40 to 49 years: a systematic review and meta-analysis. Ann Intern Med. 2012;156(9):635-48. doi: 10.7326/0003-4819-156-9-201205010-00006, PMID 22547473.
6. Bauer SR, Hankinson SE, Bertone-Johnson ER, Ding EL. Plasma vitamin D levels, menopause, and risk of breast cancer: dose–response meta-analysis of prospective studies. Medicine. 2013;92(3):123-31. doi: 10.1097/MD.0b013e3182943bc2, PMID 23625163.
7. Goodwin PJ, Ennis M, Pritchard KI, Koo J, Hood N. Prognostic effects of 25- hydroxyvitamin D levels in early breast cancer. J Clin Oncol. 2009;27(23):3757-63. doi: 10.1200/JCO.2008.20.0725, PMID 19451439.
8. de La Puente-Yagüe M, Cuadrado-Cenzual MA, Ciudad-Cabañas MJ, Hernández-Cabria M, Collado-Yurrita L. Vitamin D: And its role in breast cancer. Kaohsiung J Med Sci. 2018;34(8):423-7. doi: 10.1016/j.kjms.2018.03.004, PMID 30041759.
9. Wang Y, Zhu J, DeLuca HF. Where is the vitamin D receptor? Arch Biochem Biophys. 2012;523(1):123-33. doi: 10.1016/j.abb.2012.04.001, PMID 22503810.
10. Anderson LN, Cotterchio M, Cole DE, Knight JA. Vitamin D-related genetic variants, interactions with vitamin D exposure, and breast cancer risk among Caucasian women in Ontario. Cancer Epidemiol Biomarkers Prev. 2011;20(8):1708-17. doi: 10.1158/1055-9965.EPI-11-0300, PMID 21693626.
11. McCullough ML, Bostick RM, Mayo TL. Vitamin D gene pathway polymorphisms and risk of colorectal, breast, and prostate cancer. Annu Rev Nutr. 2009;29:111-32. doi: 10.1146/annurev-nutr-080508-141248, PMID 19400699.
12. Köstner K, Denzer N, Müller CS, Klein R, Tilgen W, Reichrath J. The relevance of vitamin D receptor (VDR) gene polymorphisms for cancer: a review of the literature. Anticancer Res. 2009;29(9):3511-36. PMID 19667145.
13. Yao S, Zirpoli G, Bovbjerg DH, Jandorf L, Hong CC, Zhao H, Sucheston LE, Tang L, Roberts M, Ciupak G, Davis W, Hwang H, Johnson CS, Trump DL, McCann SE, Ademuyiwa F, Pawlish KS, Bandera EV, Ambrosone CB. Variants in the vitamin D pathway,

serum levels of vitamin D, and estrogen receptor negative breast cancer among African-American women: a case-control study. Breast Cancer Res. 2012 Apr 4;14(2):R58. doi: 10.1186/bcr3162, PMID 22480149, PMCID PMC3446393.

14. Nemenqani DM, Karam RA, Amer MG, Abd El Rahman TM. Vitamin D receptor gene polymorphisms and steroid receptor status among Saudi women with breast cancer. Gene. 2015 Mar 10;558(2):215-9. doi: 10.1016/j.gene.2014.12.065, PMID 25560187.
15. Aristarco V, Johansson H, Gandini S, Macis D, Zanzottera C, Tolva G, Feroce I, Accornero C, Bonanni B, Guerrieri-Gonzaga A, Serrano D. Association of Vitamin D Receptor and Vitamin D-Binding Protein Polymorphisms with Familial Breast cancer prognosis in a mono-institutional cohort. Nutrients. 2021 Apr 6;13(4):1208. doi: 10.3390/nu13041208, PMID 33917614, PMCID PMC8067530.
16. Welsh J. Vitamin D metabolism in mammary gland and breast cancer. Mol Cell Endocrinol. 2011;347(1-2):55-60. doi: 10.1016/j.mce.2011.05.020, PMID 21669251.
17. Hemida MA, AbdElmoneim NA, Hewala TI, Rashad MM, Abdaallah S. Vitamin D Receptor in Breast Cancer Tissues and Its Relation to Estrogen Receptor Alpha (ER-α) Gene Expression and Serum 25-hydroxyvitamin D Levels in Egyptian Breast Cancer Patients: A Case-control Study. Clin Breast Cancer. 2019;19(3):e407-14. doi: 10.1016/j.clbc.2018.12.019, PMID 30833174.
18. Heublein S, Mayr D, Meindl A, Kircher A, Jeschke U, Ditsch N. Vitamin D receptor, retinoid X receptor and peroxisome proliferator-activated receptor gamma are overexpressed in BRCA1 mutated breast cancer and predict prognosis. J Exp Clin Cancer Res. 2017;36(1):57. doi: 10.1186/s13046-017-0517-1, PMID 28427429.
19. Huss L, Butt ST, Borgquist S, Elebro K, Sandsveden M, Rosendahl A, Manjer J. Vitamin D receptor expression in invasive breast tumors and breast cancer survival. Breast Cancer Res. 2019;21(1):84. doi: 10.1186/s13058-019-1169-1, PMID 31358030.
20. Soljic M, Mrklic I, Tomic S, Omrcen T, Sutalo N, Bevanda M, Vrdoljak E. Prognostic value of vitamin D receptor and insulin-like growth factor receptor 1 expression in triple-negative breast cancer. J Clin Pathol. 2018;71(1):34-9. doi: 10.1136/jclinpath-2016-204222, PMID 28663327.
21. Rose AA, Elser C, Ennis M, Goodwin PJ. Blood levels of vitamin D and early stage breast cancer prognosis: systematic review and meta-analysis. Breast Cancer Res Treat. 2013;141(3):331-9. doi: 10.1007/s10549-013-2713-9, PMID 24104883.
22. Dizdar O, Harputluoglu H, Altundag K. Vitamin D intake and breast cancer risk in postmenopausal women. Arch Intern Med. 2007;167(22):2532; author reply 2532. doi: 10.1001/archinte.167.22.2532-a. PMID 18071186.
23. Murray A, Madden SF, Synnott NC, Klinger R, O'Connor D, O'Donovan N, Gallagher W, Crown J, Duffy MJ. Vitamin D receptor as a target for breast cancer therapy. Endocr Relat Cancer. 2017;24(4):181-95. doi: 10.1530/ERC-16-0463, PMID 28213567.
24. West DC, Pan D, Tonsing-Carter EY, Hernandez KM, Pierce CF, Styke SC, Bowie KR, Garcia TI, Kocherginsky M, Conzen SD. GR and ER Coactivation alters the expression of differentiation genes and associates with improved ER+ breast Cancer outcome. Mol Cancer Res. 2016;14(8):707-19. doi: 10.1158/1541-7786.MCR-15-0433, PMID 27141101.
25. Piperigkou Z, Karamanos NK. Estrogen receptor-mediated targeting of the extracellular matrix network in cancer. Semin Cancer Biol. 2020;62:116-24. doi: 10.1016/j.semcancer.2019.07.006, PMID 31310807.
26. Manjunath S, Prabhu JS, Kaluve R, Correa M, Sridhar TS. Estrogen receptor negative breast cancer in India: do we really have higher burden of this subtype? Indian J Surg Oncol. 2011 Jun 1;2(2):122-5. doi: 10.1007/s13193-011-0072-8, PMID 22693404.
27. McKay JD, McCullough ML, Ziegler RG, Kraft P, Saltzman BS, Riboli E, Barricarte A, Berg CD, Bergland G, Bingham S, Brustad M, Bueno-de-Mesquita HB, Burdette L, Buring J, Calle EE, Chanock SJ, Clavel-Chapelon F, Cox DG, Dossus L, Feigelson HS, Haiman CA, Hankinson SE, Hoover RN, Hunter DJ, Husing A, Kaaks R, Kolonel LN, Le Marchand L, Linseisen J, McCarty CA, Overvad K, Panico S, Purdue MP, Stram DO, Stevens VL, Trichopoulos D, Willett WC, Yuenger J, Thun MJ. Vitamin D receptor polymorphisms and breast cancer risk: results from the National Cancer Institute Breast and Prostate Cancer Cohort Consortium. Cancer Epidemiol Biomarkers Prev. 2009 Jan 1;18(1):297-305. doi: 10.1158/1055-9965.EPI-08-0539, PMID 19124512.
28. 28. Hodgkinson K, Forrest LA, Vuong N, Garson K, Djordjevic B, Vanderhyden BC. GREB1 is an estrogen receptor-regulated tumour promoter that is frequently expressed in ovarian cancer. Oncogene. 2018;37(44):5873-86. doi: 10.1038/s41388-018-0377-y, PMID 29973689.
29. Miedel MT, Gavlock DC, Jia S, Gough A, Taylor DL, Stern AM. Modeling the Effect of the Metastatic Microenvironment on Phenotypes Conferred by Estrogen Receptor Mutations Using a Human Liver Microphysiological System. Sci Rep. 2019;9(1):8341. doi: 10.1038/s41598-019-44756-5. PMID 31171849.
30. Beato M, Klug J. Steroid hormone receptors: an update. Hum Reprod Update. 2000 May 1;6(3):225-36. doi: 10.1093/humupd/6.3.225, PMID 10874567.
31. Chatterjee K, Bhaumik G, Chattopadhyay B. Estrogen receptor and progesterone receptor status of breast cancer patients of eastern India: A multi-institutional study. South Asian J Cancer. 2018;7(1):5-6. doi: 10.4103/sajc.sajc_60_17, PMID 29600223.
32. Linden HM, Peterson LM, Fowler AM. Clinical potential of estrogen and progesterone receptor imaging. PET Clin. 2018;13(3):415-22. doi: 10.1016/j.cpet.2018.02.005, PMID 30100079.
33. Vidula N, Yau C, Wolf D, Rugo HS. Androgen receptor gene expression in primary breast cancer. npj Breast Cancer. 2019;5(1):47. doi: 10.1038/s41523-019-0142-6, PMID 31840050.
34. Giovannelli P, Di Donato M, Galasso G, Di Zazzo E, Bilancio A, Migliaccio A. The androgen receptor in breast cancer. Front Endocrinol (Lausanne). 2018;9:492. doi: 10.3389/fendo.2018.00492, PMID 30210453.

35. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, Pietenpol JA. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest. 2011;121(7):2750-67. doi: 10.1172/JCI45014, PMID 21633166.
36. de Sousa Almeida-Filho B, De Luca Vespoli HDL, Pessoa EC, Machado M, Nahas-Neto J, Nahas EAP. Vitamin D deficiency is associated with poor breast cancer prognostic features in postmenopausal women. J Steroid Biochem Mol Biol. 2017;174:284-9. doi: 10.1016/j.jsbmb.2017.10.009, PMID 29031688.
37. Friedrich M, Villena-Heinsen C, Tilgen W, Schmidt W, Reichrat J, Axt-Fliedner R. Vitamin D receptor (VDR) expression is not a prognostic factor in breast cancer. Anticancer Res. 2002;22(3):1919-24. PMID 12168894.
38. Al-Azhri J, Zhang Y, Bshara W, Zirpoli G, McCann SE, Khoury T, Morrison CD, Edge SB, Ambrosone CB, Yao S. Tumor expression of vitamin D receptor and breast cancer histopathological characteristics and prognosis. Clin Cancer Res. 2017;23(1):97-103. doi: 10.1158/1078-0432.CCR-16-0075, PMID 27407090.
39. Welsh J, Wietzke JA, Zinser GM, Byrne B, Smith K, Narvaez CJ. Vitamin D-3 receptor as a target for breast cancer prevention. J Nutr. 2003;133(7);Suppl:2425S-33S. doi: 10.1093/jn/133.7.2425S, PMID 12840219.
40. Abbas S, Nieters A, Linseisen J, Slanger T, Kropp S, Mutschelknauss EJ, Flesch-Janys D, Chang-Claude J. Vitamin D receptor gene polymorphisms and haplotypes and postmenopausal breast cancer risk. Breast Cancer Res. 2008;10(2):R31. doi: 10.1186/bcr1994, PMID 18419802.
41. Dogra AK, Prakash A, Gupta S, Gupta M, Bhat SA. Genetic variations of vitamin D receptor gene and steroid receptors status in breast cancer risk: an updated review. Advances in Biomarker Sciences and Technology. 2022;4:1-11. ISSN 2543-1064. doi: 10.1016/j.abst.2022.01.001.
42. Xu H, Long JR, Li MX, Deng HW. Interaction effects between estrogen receptor alpha and vitamin D receptor genes on age at menarche in Chinese women. Acta Pharmacol Sin. 2005;26(7):860-4. doi: 10.1111/j.1745-7254.2005.00122.x, PMID 15960894.
43. Guy M, Lowe LC, Bretherton-Watt D, Mansi JL, Peckitt C, Bliss J, Wilson RG, Thomas V, Colston KW. Vitamin D receptor gene polymorphisms and breast cancer risk. Clin Cancer Res. 2004 Aug 15;10(16):5472-81. doi: 10.1158/1078-0432.CCR-04-0206, PMID 15328186.
44. Ruggiero M, Pacini S, Aterini S, Fallai C, Ruggiero C, Pacini P. Vitamin D receptor gene polymorphism is associated with metastatic breast cancer. Oncol Res. 1998 Jan 1;10(1):43-6. PMID 9613456.
45. Lundin AC, Söderkvist P, Eriksson B, Bergman-Jungeström M, Wingren S. Association of breast cancer progression with a vitamin D receptor gene polymorphism. South-East Sweden Breast Cancer Group. Cancer Res. 1999 May 15;59(10):2332-4. PMID 10344739.
46. Lim E, Wu D, Pal B, Bouras T, Asselin-Labat ML, Vaillant F, Yagita H, Lindeman GJ, Smyth GK, Visvader JE. Transcriptome analyses of mouse and human mammary cell subpopulations reveal multiple conserved genes and pathways. Breast Cancer Res. 2010;12(2):R21. doi: 10.1186/bcr2560, PMID 20346151.
47. Santagata S, Thakkar A, Ergonul A, Wang B, Woo T, Hu R, Harrell JC, McNamara G, Schwede M, Culhane AC, Kindelberger D, Rodig S, Richardson A, Schnitt SJ, Tamimi RM, Ince TA. Taxonomy of breast cancer based on normal cell phenotype predicts outcome. J Clin Invest. 2014;124(2):859-70. doi: 10.1172/JCI70941, PMID 24463450.
48. Albertson DG, Ylstra B, Segraves R, Collins C, Dairkee SH, Kowbel D, Kuo WL, Gray JW, Pinkel D. Quantitative mapping of amplicon structure by array CGH identifies CYP24 as a candidate oncogene. Nat Genet. 2000;25(2):144-6. doi: 10.1038/75985, PMID 10835626.
49. Lovelace DL, McDaniel LR, Golden D. Long-Term Effects of Breast Cancer Surgery, Treatment, and Survivor Care. J Midwifery Womens Health. 2019;64(6):713-24. doi: 10.1111/jmwh.13012, PMID 31322834.
50. Sellami M, Bragazzi NL. Nutrigenomics and breast cancer: state-of-art, future perspectives and insights for prevention. Nutrients. 2020;12(2):512. doi: 10.3390/nu12020512, PMID 32085420.
51. Davis CD, Milner JA. Nutrigenomics, vitamin D and cancer prevention. J Nutrigenet Nutrigenomics. 2011;4(1):1-11. doi: 10.1159/000324175, PMID 21430387

Original Article

DOI: 10.7860/JCDR/2024/69296.19241

Biochemistry Section

Vitamin D and Vitamin D Receptor FokI, ApaI, and BsmI Gene Polymorphisms and their Relation with the Risk of Breast Carcinoma: A Case-control Study

ASHOK KUMAR DOGRA[1], ARCHANA PRAKASH[2], SANJAY GUPTA[3], MEENU GUPTA[4]

ABSTRACT

Introduction: Breast cancer stands as the leading cause of mortality among women in developing nations. The potential role of Vitamin D in mitigating the incidence of breast cancer is thought to stem from its ability to impede cell proliferation by interacting with the Vitamin D Receptor (VDR). The VDR gene is responsible for encoding the VDR, which plays a pivotal role in mediating the effects of vitamin D.

Aim: To analyse vitamin D levels and the association of VDR FokI, ApaI, and BsmI genotypic distribution frequency with the risk of breast cancer.

Materials and Methods: The case-control study included 220 samples, including 110 breast cancer patients and 110 age-matched control women aged 30-70 years. The Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) genotyping was performed using Deoxyribonucleic acid (DNA) extracted from blood, and the circulating levels of 25-hydroxyvitamin D by case/control were estimated by chemiluminescence immunoassay.

Results: The 3' VDR polymorphism BsmI sequence showed minimal association with breast cancer risk. The bb genotype had a significantly lower odds ratio of 0.056 (p-value <0.05). Conversely, the BB and Bb genotypes exhibited no statistically significant associations with odds ratios of 1.76 (95% CI: 0.36-8.54; p-value >0.05) and 1.30 (95% CI: 0.27-6.25; p-value >0.05), respectively. Isolated analysis of the FokI variant revealed a significant association with increased breast cancer risk, with odds ratios of 5.49 (FF) and 6.00 (Ff), both demonstrating statistical significance (p-value <0.05), and a Chi-square value of 0.006. Additionally, the p-value for serum Vitamin D levels was found to be highly significant at p-value <0.001, indicating that the levels were significantly lower in individuals newly diagnosed with breast cancer compared to those in the healthy control group.

Conclusion: The study found a significant link between breast cancer susceptibility and VDR (FokI) polymorphism FF and Ff genotypes, with minimal impact observed for (BsmI) polymorphism bb genotype. This implies that certain genetic variations, especially in the FokI polymorphism of the VDR gene, are associated with an elevated risk of breast cancer.

Keywords: ApaI single nucleotide polymorphisms, Breast cancer, BsmI single nucleotide polymorphisms, FokI single nucleotide polymorphisms

INTRODUCTION

Every year, breast cancer leads the world in morbidity and mortality rates for women [1]. In India, this disease accounts for 30.1% of all female cancer cases (Globocan 2020; http://globocan.iarc.fr/). The Indian subcontinent has seen an increase in cancer incidence, mortality, and morbidity [2-5]. The increasing mortality is likely due to ineffective screening strategies, advanced-stage diagnosis, and inadequate medical facilities. Recent studies have indicated that vitamin D may play a role in the development of breast cancer. In several studies, there is evidence that low levels of 25-hydroxyvitamin D {25(OH)D} increases the risk of breast cancer and have also shown an association between dietary intakes, dietary supplements, and skin production of vitamin D and breast cancer risk [6-8]. Aside from the classical role of vitamin D in calcium and phosphorus homeostasis, calcitriol exerts anticancer properties through transcriptional and/or non genetic mechanisms [9]. The active vitamin D3 (1α,25-dihydroxy vitamin D3) exerts its biological effects via the VDR. It belongs to the family of nuclear receptors and is a ligand-dependent transcription factor [10]. Cell cycle arrest, senescence, differentiation, and apoptosis are induced by the Vitamin D-ligand VDR in a variety of tumour types [11].

There are several polymorphisms in the coding and non coding regions of VDR on 12q13.11. Numerous Single Nucleotide Polymorphisms (SNPs) have been identified in and around exons 2-9 as well as in the 3' UTR region of the VDR gene [12]. The most commonly studied SNPs are those containing Restriction Fragment Length polymorphisms (RFLPs) rs1544410, rs2228570, and rs7975232, determined by restriction endonucleases BsmI, FokI, and ApaI [13-15]. The VDR contains the BsmI SNP (A/G) in intron eight near the 3' end, and its effect on VDR protein expression and activity is unclear. However, in Caucasians, Chinese, and Japanese Americans, it is in strong linkage disequilibrium with a polyadenosine microsatellite repeat, which may affect mRNA stability or translation activity [16]. A FokI site present in the 5' promoter region substitution results in thymine (T) to cytosine (C) that changes the first of two possible translation initiation sites, resulting in different-sized VDR proteins. An f allele is three amino acids longer than an F allele and transcriptionally less active [17]. An ApaI SNP (C/A) variable site is located in intron 8 of the VDR gene. VDR polymorphisms may alter expression and function in breast cells, thereby modulating breast cancer risk [18].

Several studies performed on Caucasian populations have given inconsistent results regarding FokI, BsmI, and ApaI SNPs and breast cancer risk [19-22]. There have been very few studies on Asian populations. An association was found between the FokI SNP and Japanese-American women in the Hawaii-Los Angeles

Multiethnic Cohort (MEC), but not in large Chinese studies or small Iranian studies [23-25]. A BsmI SNP was associated with Iranian women and Japanese-American women from the Multiethnic Cohort (MEC) study, but not with two Chinese studies [26]. The ApaI SNP showed mixed results in different populations and was primarily studied in African Americans, Caucasians, and Chinese [27-29]. In the present study, three polymorphisms, one from the 5' region (FokI) and two from the 3' region of the VDR gene (ApaI and BsmI), and vitamin D, were investigated to assess the association with the risk of breast cancer. The study aims to offer insights into genetic factors impacting breast cancer susceptibility for personalised risk assessment and prevention.

MATERIALS AND METHODS

The present study was a hospital-based age-matched case-control study conducted at the Cancer Research Institute, a tertiary care centre located at Jolly Grant, Dehradun, Uttarakhand, India during the period from the year 2020 to 2023. This study included 110 freshly diagnosed breast cancer patients and age-matched 110 healthy controls aged 30 to 70 years. The institute's ethics committee approved the study, which was conducted in accordance with all the provisions of the Declaration of Helsinki (Letter No. SRHU/HIMS/ETHICS/2020/193). A written informed consent was obtained from all study participants.

Inclusion and Exclusion criteria: The inclusion and exclusion criteria were primarily used to select patients. The inclusion criteria included breast cancer patients selected based on histopathological confirmation, both pre- and postmenopausal women, and excluding those on hormonal therapy, with other cancers, recent Vitamin D supplementation, or pregnant or lactating. Age-matched healthy female volunteers were included as controls.

Sample size estimation: The sample size was estimated using the n-Master software for a matched case-control study (1:1) matching. Assuming that the proportion of exposed controls is 50% and the level of significance is 5% with a power of 90% to detect a two-fold increase in risk. The minimum number of required discordant pairs is 110.

Data collection: A comprehensive proforma was used to capture demographics, co-morbidities, family history, and anthropometric information, as well as pertinent clinical information from our online hospital database. The tumour morphology was classified according to criteria; Elston and Ellis used architectural aspects, nuclear differentiation levels, and mitotic index based on the 8th edition of the TNM staging system for breast cancer developed by the American Joint Committee on Cancer (AJCC) [30]. The quality of genomic DNA was assessed through agarose gel electrophoresis, and VDR gene polymorphisms were genotyped using PCR-RFLP analysis.

The Vitamin D levels in serum were determined for all freshly diagnosed cases and controls using the Chemiluminescent Immunoassay (CIA) method by trained laboratory technicians. Serum 25(OH)D levels were classified based on our institution's laboratory reference standards as sufficient/normal (75-250 nmol/L), insufficient (50-<75 nmol/L), and deficient (<50 nmol/L) [31]. Results were expressed in nmol/L.

Genotyping analysis: FokI, BsmI, and ApaI genotyping utilised PCR-RFLP analysis, employing agarose gels for DNA quality confirmation. The FokI polymorphism was detected using the following primers:

Forward: 5'GAT GCC AGC TGG CCC TGG CAC TG 3' and Reverse: 5'ATG GAA ACA CCT TGC TTC TTC TCC CTC 3', yielding a 272 bp fragment spanning the FokI site (Raza S et al., 2019) [32]. The BsmI polymorphism was detected using the following primers: Forward: 5'CAACAAGACTACAAGTACCGC GTCAGTGA3' and Reverse: 5'AACCAGCGGGAAGAGGTCAAG GGG 3', generating an 825 bp fragment surrounding the BsmI site (Raza S et al., 2017) [33]. The ApaI-RFLP was detected by the following primers: Forward: 5' CAG AGC ATG GAC AGG GAG CAA G 3' and Reverse: 5' CGG CAG CGG ATG TAC GTC TGC AG 3', yielding a 352 bp fragment spanning the ApaI site (El-Shorbagy HM et al., 2017) [29]. The following conditions were used for the PCR: initial denaturation at 94°C for three minutes, followed by 34 cycles of cyclic denaturation at 94°C for one minute, annealing 50 seconds at 71°C for FokI, 71°C for ApaI, and 58°C for BsmI, then extension at 72°C for one minute and one final cycle of final extension at 72°C for eight minutes, and final hold at 4°C. After PCR, the amplified PCR products were digested according to the manufacturer's instructions with FokI, BsmI (New England Biolabs, USA), and ApaI (Promega). In 2% agarose, fragments were stained with ethidium bromide to determine whether the enzyme recognition site was present (lowercase) or absent (uppercase). The genotypes for VDR-FokI (FF, Ff, ff), VDR-BsmI (BB, Bb, bb), and VDR-ApaI (AA, Aa, aa) polymorphisms were assigned. Randomly selected samples of three genotypes including Homozygous dominant, recessive, and heterozygous were confirmed by SNP sequencing, and the results were 100% concordant.

STATISTICAL ANALYSIS

Statistical analysis, including odds ratios and Chi-square tests, were used to evaluate associations between specific VDR gene polymorphisms and breast carcinoma risk. Data entered into Microsoft Excel 2010 were analysed using statistical software version Statistical Package for Social Sciences (SPSS) 20.0. Normality was assessed by the Kolmogorov-Smirnov test. An Independent t-test was used for two groups, and Analysis of Variance (ANOVA) for more than two groups to compare mean differences. The deviation from Hardy-Weinberg Equilibrium (HWE) was tested for polymorphisms by examining the differences between genotype frequencies observed and those expected, utilising the χ^2 test. Descriptive statistics and graphical representations were used to enhance the result interpretation. It is considered statistically significant when the p-value <0.05, and statistically insignificant if the p-value >0.05.

RESULTS

In this hospital-based case-control study, 110 patients and 110 healthy controls were compared. Among the study participants, demographic characteristics and risk factors were analysed, with predominantly 37 (33.6%) cases and 40 (36.4%) controls falling within the 40-49 age range. Urban residency accounted for 70% of cases and 86.4% of controls, while rural and Semiurban areas had lower frequencies. Premenopausal status was balanced, with 58.2% of cases and 61.8% of controls. Normal BMI was observed in 58.2% of cases and a higher percentage in controls (85.5%). Notably, 12.7% of cases had a positive first-degree family history, contrasting with the absence of such history in controls [Table/Fig-1].

Based on TNM staging, tumour morphology among 110 breast cancer patients showed Grade-I tumours with one case having vitamin D <50 nmol/L, two cases with 50 to <75 nmol/L, and

Characteristics		Cases (%)	Controls (%)
Age (years)	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
Areas	Rural	22 (20)	6 (5.5)
	Urban	77 (70)	95 (86.4)
	Semiurban	11 (10)	9 (8.2)
Menopausal	Premenopausal	64 (58.2)	68 (61.8)
	Postmenopausal	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
First degree family history	Yes	14 (12.7)	0

[Table/Fig-1]: Demographic characteristics of breast cancer patients and their matched controls.

none with 75-250 nmol/L (p-value=0.433). For Grade-II tumours, 51 (67.1%) had <50 nmol/L, 20 (26.3%) had 50 to <75 nmol/L, and 5 (6.6%) had 75-250 nmol/L (p-value 0.433). Grade-III tumours had 21 (67.7%) with <50 nmol/L, 8 (25.8%) with 50 to <75 nmol/L, and 2 (6.5%) with 75-250 nmol/L (p-value 0.433). No significant associations between tumour grades and vitamin D levels were found, contributing to understanding the tumour grade-vitamin D status relationship in this patient population.

The genotypes and allele frequencies of FokI, ApaI, and BsmI were illustrated in [Table/Fig-2]. In both cases and controls, the HWE of 0.15 indicates equilibrium. In accordance with HWE, genotypic data were found to be reliable, indicating that selection or genetic drift has little influence on the genotypic distribution.

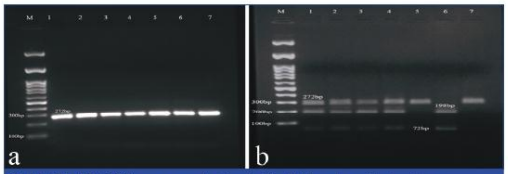
The examination of the ApaI (rs 7975232) VDR polymorphisms revealed an amplification product with a size of 352 bp [Table/Fig-4a]. On agarose gels, 352 bp bands were genotyped as AA homozygotes. The homozygote (AA) produces 216 bp and 135 bp fragments, while heterozygotes (AA) display three fragments, 352 bp, 216 bp, and 135 bp [Table/Fig-4b]. 40.9% were heterozygous Aa, 49.1% were homozygous AA, and 10% were homozygous aa with respect to the Apa1 polymorphism among 110 cases and 110 controls. The ApaI genotypes showed no association with breast cancer risk.

The analysis of BsmI (rs1544410) polymorphisms showed an amplified product size of 825bp [Table/Fig-5a]. Two or three fragments showing the Bsm1 restriction site (b) indicated intact amplification (B) reveals the absence. The undigested bands of 825 bp indicated a homozygous BB genotype. bb homozygotes produced two fragments (650 bp and 175 bp), and Bb heterozygotes produced three fragments (825 bp, 650 bp, and 175 bp) on agarose gel [Table/Fig-5b]. Bsm1 polymorphism cases comprised 53.6% heterozygous Bb, 42.7% homozygous BB, and 3.7% homozygous bb. Additionally, 47.3%, 50%, and 2.7% of the control groups were genotyped.

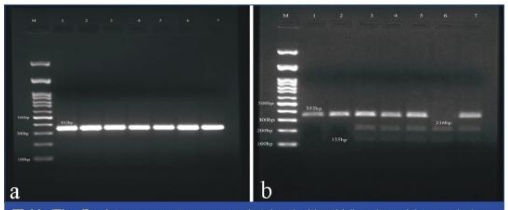
Genotypes		Cases	Allele probabilities (p-value HWE)	Control	Allele probabilities (p-value HWE*)	OR (95% CI)	p-value*	χ^2 value
Genotypes (FokI)	FF	60	F=0.69, f=0.30 (0.15)	64	F=0.77, f=0.22 (0.15)	5.49 (1.72-17.64)	0.004	0.006
	Ff	32		42		6.00 (1.83-19.67)	0.003	
	ff	18		4		-	0.181	
Genotypes (ApaI)	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				

[Table/Fig-2]: An association of the genotypes of breast cancer patients and controls was made in the study.
*Hardy Weinberg equilibrium, *p<0.05, Significant

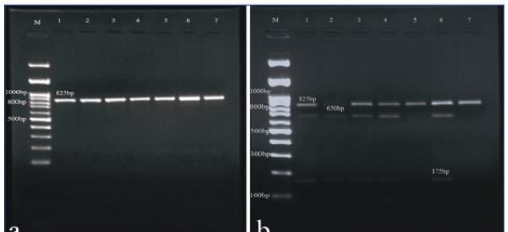
An analysis of FokI VDR polymorphisms yielded the amplification product with a size of 272bp [Table/Fig-3a]. An amplification product without FokI restriction site (F), while present in two or three fragments, indicates FokI restriction site (f). A non digested, single 272bp band genotype FF as homozygous, while Homozygotes (ff) showed two fragments of 198 and 72 bp, and heterozygotes (Ff) showed three fragments of 272, 198, and 72 bp [Table/Fig-3b]. The distribution of polymorphism in VDR FokI showed that 29.1% constituted the Heterozygous Ff, 54.5% were Homozygous FF, and 16.4% presented as homozygous ff cases, whereas the corresponding Control group genotype frequencies were 58.2%, 38.2%, and 3.6%, respectively. There was a significant association between the FokI genotypes FF and Ff and breast cancer risk. With a 95% Confidence Interval (CI), the odds ratios were 5.49 (1.72, 17.64) and 6.00 (1.83, 19.67) with p-values of 0.004 and 0.003.



[Table/Fig-3]: A 2% agarose gel stained with ethidium bromide depicts an amplification product of 272bp was obtained for Fok1. Ladder 100bp is Lane M. Lanes 1-7 show 272bp FokI amplified PCR products (a). F (T allele) is in upper band, C (C allele) is in lower bands. 100bps ladder on lane M. Lanes, 1-3, represent Ff heterozygotes, Lanes 4-6, represents FF homozygotes and Lane 7 represent ff homozygotes (b).



[Table/Fig-4]: A two percent agarose gel stained with ethidium bromide revealed an amplification product of size 352 bp for ApaI polymorphism. Lane M shows a 100 bp ladder and lanes 2-7 show a 352 bp product (a); The upper band indicates A (C allele), lower band indicates a (A allele). 100bp ladder in lane M. There are Aa homozygotes in Lanes 1,2 and Aa heterozygotes in Lanes 3,4,5,7 and aa homoygote in Lane 6 (b).



[Table/Fig-5]: A two percent agarose gel stained with ethidium bromide revealed an amplification product of size 825 bp for BsmI polymorphism. Lane M shows a 100 bp ladder and lanes 2-7 show a 825 bp product (a). The upper band indicates B (A allele), lower band indicates b (G allele). 100 bp ladder in lane M. There are BB homozygotes in Lanes 5,7 and Bb heterozygotes in Lanes 1,3,4,6 and bb homoygote in Lane 2 (b).

A comparative analysis of vitamin D levels in breast cancer patients and the control group indicated mean values and standard deviations. Breast cancer patients had a mean vitamin D level of 43.54±19.58 nmol/L, while the control group had a higher mean of 89.89±26.13 nmol/L. The independent t-test showed a highly significant p-value of <0.001, signifying a substantial difference.

These findings highlight potential biomarkers for breast cancer, suggesting that low levels of vitamin D are related to increased breast cancer risk and lead to implications for diagnosis and prognosis in this patient population.

Moreover, a comparative analysis of genotypes and vitamin D levels in breast cancer patients and the control group, focusing on specific genotypes (FF, Ff, ff for FokI; AA, Aa, aa for ApaI; BB, Bb, bb for BsmI) was conducted. Mean vitamin D levels with standard deviations were assessed for both cases and controls. It shows significance in uncovering potential associations between distinct genetic variations and vitamin D levels. Significant differences in mean Vitamin D levels were observed among both cases (p-value=0.006) and controls (p-value=0.001) for different genotypes of BsmI. The data suggested varying degrees of association between genotypes and vitamin D levels [Table/Fig-6].

Genotypes		Cases	Control
		Mean±SD	
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

[Table/Fig-6]: Comparison of genotypes and Vitamin D levels (nmol/L) among Breast cancer patients and control groups.
*One-way ANOVA test, *p<0.05, significant

DISCUSSION

Breast cancer prevails as the predominant cancer in women globally and in India, with an age-adjusted prevalence of 25.8 cases per 100,000 women and a fatality rate of 12.7 per 100,000 women [34]. While developed regions still show higher occurrence rates, emerging countries, including India, face increased death rates from breast cancer [35]. Age is a significant risk factor, and present study indicates a notable prevalence of 33.6% in women aged 40-49. Tumour size and lymph node involvement are pivotal prognostic factors, with a common occurrence of lymph node infiltration at diagnosis (30-50% of cases). Metropolitan regions, age 50-59, and premenopausal status were prominent in these cases.

Present study revealed a significant association between the FokI genotype and the occurrence of breast cancer within the study population. Specifically, the FF genotype showed a substantial increase in breast cancer risk (OR: 5.49, p-value: 0.004), while the Ff genotype was associated with a significant risk reduction (OR: 6.00, p-value: 0.003). Conversely, the ff genotype was less frequent in the control group compared to the case group. These findings are consistent with Mishra DK et al., study on African American and Hispanic populations, as well as Chakraborty M et al., study within the Indian population, highlighting the elevated risk associated with Fok1 FF and Ff genotypes in breast cancer susceptibility [36,37].

In this study, analysis of the ApaI genotype distribution revealed a lower frequency of AA genotypes in cases (OR: 2.87, p-value: 0.069), with Aa genotypes showing a slightly higher frequency (OR: 2.31, p-value: 0.157). However, no significant association with breast cancer risk was observed for ApaI genotypes. This aligns with the ongoing study by Ahmed JH et al., on the African population, where no significant association was found between the Apa1 polymorphism and the condition under study [28].

Examining BsmI genotypes in present study, it was found that there were no significant associations with breast cancer risk. The ApaI genotypes (Aa and aa) revealed notable differences in vitamin D levels between cases and controls (p-values: 0.316 and 0.001), suggesting a potential relationship with breast cancer. These findings were in line with Reimers LL et al., population-based case-control study conducted on Long Island, New York, emphasising the influence of vitamin D-related gene polymorphisms on breast cancer susceptibility [38]. However, BsmI genotypes in present study showed no significant association with vitamin D levels. The p-value for serum vitamin D levels was highly significant at 0.001, signifying a substantial decrease in levels among individuals newly diagnosed with breast cancer compared to those in the healthy control group within our studied population. In a study by Ingles SA et al., African-American women with LS and LL poly(A) variations demonstrated a 50% lower risk of breast cancer than those with the SS genotype, particularly in the presence of the FF (FokI) mutation [39]. Whitfield KG et al., study on human fibroblast cell lines highlighted the statistical significance of VDR activity when both FokI and poly(A) genotypes were considered together [40]. Present study, aligning with previous research, conducted a comparative analysis of FokI, ApaI, and BsmI genotypes along with vitamin D levels (<50, 50 to <75, and 75 to 250 nmol/L) in breast cancer patients and controls [41,42]. Notably, statistical comparison between the groups for vitamin D levels for those carrying ApaI genotypes (Aa and aa) showed significant differences in vitamin D concentrations in cases and controls, and BsmI genotypes (Bb and bb) showed significant differences in vitamin D concentrations in cases and controls, suggesting that vitamin D status may be influenced by these genotypes.

Limitation(s)

As with any research study, the present study has both strengths and limitations. For the studied analysis, only a few factors were taken into account, and quite a few factors were unmatched. Moreover, only a few VDR polymorphisms are considered for the 5' and 3' ends of this gene. Despite these limitations, this study significantly advanced the understanding of VDR polymorphisms (FokI, BsmI, and ApaI genotypes) and breast cancer risk.

CONCLUSION(S)

This study identifies an association between VDR (FokI) polymorphism FF and Ff genotypes and minimal impact for (BsmI) polymorphism bb genotype in breast cancer susceptibility. These findings could be useful in predicting breast cancer risk or whether a woman who has breast cancer will develop metastases. Highly significant serum vitamin D levels between breast cancer and control groups highlight the significant influence of VDR polymorphisms, particularly FokI, stressing the need for comprehensive studies across diverse ethnic populations to understand VDR gene variations' impact on breast cancer development thoroughly. Moreover, consideration of prognostic risk factors is needed for therapeutic applications in the context of breast cancer. Vitamin D's potential preventive role in breast cancer, achievable through safe and affordable supplementation, emphasises its modifiability. The documented link between vitamin D deficiency and increased breast cancer risk

underscores its public health significance, necessitating larger-scale investigations. VDR abundance in breast cancer tissues suggests potential treatment targets. Research on VDR FokI polymorphism gains importance, considering its potential moderation by family history.

Acknowledgement

Authors would like to express their gratitude to all participants for their invaluable assistance and unwavering patience, as well as to the hospital and institution that played a role in the recruitment of both cases and controls.

REFERENCES

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. Available from: https://doi.org/10.3322/caac.21492.

[2] Ali I, Wani WA, Saleem K, Correspondence. Cancer scenario in India with future perspectives. Cancer Ther. 2011;8(8):56-70.

[3] Babu GR, Lakshmi SB, Thiyagarajan JA. Epidemiological correlates of breast cancer in south India. Asian Pac J Cancer Prev. 2013;14(9):5077-83. Available from: https://doi.org/10.7314/APJCP.2013.14.9.5077.

[4] Balasubramaniam S, Rotti SB, Vivekanandam S. Risk factors of female breast carcinoma: A case control study at Puducherry. Indian J Cancer. 2013;50(1):65. Available from: https://doi.org/10.4103/0019-509X.112307.

[5] Srinath Reddy K, Shah B, Varghese C, Ramadoss A. Responding to the threat of chronic diseases in India. Lancet. 2005;366(9498):1744-49. Available from: https://doi.org/10.1016/S0140-6736(05)67343-6.

[6] Abbas S, Linseisen J, Slanger T, Kropp S, Mutschelknauss EJ, Flesch-Janys D, et al. Serum 25-hydroxyvitamin D and risk of post-menopausal breast cancer--Results of a large case-control study. Carcinogenesis. 2008;29(1):93-99. Available from: https://doi.org/10.1093/carcin/bgm240.

[7] Crew KD, Gammon MD, Steck SE, Hershman DL, Cremers S, Dworakowski E, et al. Association between plasma 25-Hydroxyvitamin D and breast cancer risk. Cancer Prev Res (Phila). 2009;2(6):598-604. Available from: https://doi.org/10.1158/1940-6207.CAPR-08-0138.

[8] Lowe LC, Guy M, Mansi JL, Peckitt C, Bliss J, Wilson RG, et al. Plasma 25-hydroxy vitamin D concentrations, vitamin D receptor genotype and breast cancer risk in a UK Caucasian population. Eur J Cancer. 2005;41(8):1164-69. Available from: https://doi.org/10.1016/j.ejca.2005.01.017.

[9] Haussler MR, Jurutka PW, Mizwicki M, Norman AW. Vitamin D Receptor (VDR)-mediated actions of $1\alpha,25(OH)_2$ vitamin D_3: Genomic and non-genomic mechanisms. Best Pract Res Clin Endocrinol Metab. 2011;25(4):543-59. Available from: https://doi.org/10.1016/j.beem.2011.05.010.

[10] Faiz M, Younus A, Yasmin A. Serum Vitamin D status and VDR (FokI) polymorphism association study in Pakistani females with breast cancer. J Cancer Res Ther. 2023;19(Supplement):S87-S92. Doi: 10.4103/jcrt.JCRT_1364_20. PMID: 37147988.

[11] Jones G, Strugnell SA, DeLuca HF. Current understanding of the molecular actions of vitamin D. Physiol Rev. 1998;78(4):1193-231. Available from: https://doi.org/10.1152/physrev.1998.78.4.1193.

[12] Dogra A, Prakash A, Gupta S, Gupta M, Bhat SA. Genetic variations of vitamin D receptor gene and steroid receptors status in breast cancer risk: An updated review. Adv Biomark Sci Technol. 2022;4(8). Available from: https://doi.org/10.1016/j.abst.2022.01.001.

[13] Morrison NA, Yeoman R, Kelly PJ, Eisman JA. Contribution of trans-acting factor alleles to normal physiological variability: Vitamin D receptor gene polymorphism and circulating osteocalcin. Proc Natl Acad Sci USA. 1992;89(15):6665-69. Available from: https://doi.org/10.1073/pnas.89.15.6665.

[14] Gross C, Eccleshall TR, Malloy PJ, Villa ML, Marcus R, Feldman D. The presence of a polymorphism at the translation initiation site of the vitamin D receptor gene is associated with low bone mineral density in postmenopausal Mexican-American women. J Bone Miner Res. 1996;11(12):1850-55. Available from: https://doi.org/10.1002/jbmr.5650111204.

[15] Yballe CM, Vu TH, Hoffman AR. Imprinting and expression of insulin-like growth factor-II and H19 in normal breast tissue and breast tumour. J Clin Endocrinol Metab. 1996;81(4):1607-12. Available from: https://doi.org/10.1210/jcem.81.4.8636375.

[16] Engel LS, Orlow I, Sima CS, Satagopan J, Mujumdar U, Roy P, et al. Vitamin D receptor gene haplotypes and polymorphisms and risk of breast cancer: A nested case-control study. Cancer Epidemiol Biomarkers Prev. 2012;21(10):1856-67. Available from: doi.org/10.1158/1055-9965.EPI-12-0551.

[17] Arai H, Miyamoto K, Taketani Y, Yamamoto H, Iemori Y, Morita K, et al. A Vitamin D receptor gene polymorphism in the translation initiation codon: Effect on protein activity and relation to bone mineral density in Japanese women. J Bone Miner Res. 1997;12(6):915-21. Available from: https://doi.org/10.1359/jbmr.1997.12.6.915.

[18] Dogra AK. An updated insights into Vitamin D receptor gene variation and steroid receptor status in breast cancer risk. New Advances in Medicine and Medical Science B P International (a part of SCIENCEDOMAIN International). 2023;2:64-89. Available from: https://doi.org/10.9734/bpi/namms/v2/5569C.

[19] Gnagnarella P, Pasquali E, Serrano D, Raimondi S, Disalvatore D, Gandini S. Vitamin D receptor polymorphism FokI and cancer risk: A comprehensive meta-analysis. Carcinogenesis. 2014;35(9):1913-19. Available from: doi.org/10.1093/carcin/bgu150.

[20] Raimondi S, Pasquali E, Gnagnarella P, Serrano D, Disalvatore D, Johansson HA, et al. BsmI polymorphism of vitamin D receptor gene and cancer risk: A comprehensive meta-analysis. Mutat Res. 2014;769:17-34. Available from: https://doi.org/10.1016/j.mrfmmm.2014.06.001.

[21] Tang C, Chen N, Wu M, Yuan H, Du Y. Fok1 polymorphism of vitamin D receptor gene contributes to breast cancer susceptibility: A meta-analysis. Breast Cancer Res Treat. 2009;117(2):391-99. Available from: https://doi.org/10.1007/s10549-008-0262-4.

[22] Wang J, He Q, Shao Y, Ji M, Bao W. Associations between vitamin D receptor polymorphisms and breast cancer risk. Tumour Biol. 2013;34(6):3823-30. Available from: https://doi.org/10.1007/s13277-013-0967-9.

[23] McKay JD, McCullough ML, Ziegler RG, Kraft P, Saltzman BS, Riboli E, et al. Vitamin D receptor polymorphisms and breast cancer risk: Results from the National Cancer Institute Breast and Prostate Cancer Cohort Consortium. Cancer Epidemiol Biomarkers Prev. 2009;18(1):297-305. Available from: https://doi.org/10.1158/1055-9965.EPI-08-0539.

[24] Dorjgochoo T, Delahanty R, Lu W, Long J, Cai Q, Zheng Y, et al. Common genetic variants in the Vitamin D pathway including genome-wide associated variants are not associated with breast cancer risk among Chinese women. Cancer Epidemiol Biomarkers Prev. 2011;20(10):2313-16. Available from: https://doi.org/10.1158/1055-9965.EPI-11-0704.

[25] Shahbazi S, Alavi S, Majidzadeh-AK, Ghaffarpour M, Soleimani A, Mahdian R. BsmI but not FokI polymorphism of VDR gene is contributed in breast cancer. Med Oncol. 2013;30(1):393. Available from: https://doi.org/10.1007/s12032-012-0393-7.

[26] Guo B, Jiang X, Hu X, Li F, Chen X. Association between vitamin D receptor gene polymorphisms and breast cancer in a Chinese population. Int J Clin Exp Med. 2015;8(5):8020-24.

[27] Abd-Elsalam EA, Ismaeil NA, Abd-Alsalam HS. Vitamin D receptor gene polymorphisms and breast cancer risk among postmenopausal Egyptian women. Tumour Biol. 2015;36(8):6425-31. Available from: https://doi.org/10.1007/s13277-015-3332-3.

[28] Ahmed JH, Makonnen E, Fotoohi A, Yimer G, Seifu D, Assefa M, et al. Vitamin D status and association of VDR genetic polymorphism to risk of breast cancer in Ethiopia. Nutrients. 2019;11(2):289. Available from: https://doi.org/10.3390/nu11020289.

[29] El-Shorbagy HM, Mahmoud NH, Sabet S. Association of vitamin D receptor gene polymorphisms with breast cancer risk in an Egyptian population. Tumour Biol. 2017;39(10):101042831772773. Available from: https://doi.org/10.1177/1010428317727738.

[30] Edge SB, Compton CC. The American joint committee on cancer: The 8th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol. 2010;17(6):1471-74.

[31] Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911-30. Doi: 10.1210/jc.2011-0385. Epub 2011 Jun 6. Erratum in: J Clin Endocrinol Metab. 2011;96(12):3908. PMID: 21646368.

[32] Raza S, Dhasmana A, Bhatt MLB, Lohani M, Arif JM. Molecular mechanism of cancer susceptibility associated with Fok1 single nucleotide polymorphism of VDR in relation to breast cancer. Asian Pac J Cancer Prev. 2019;20(1):199-206. Available from: https://doi.org/10.31557/APJCP.2019.20.1.199.

[33] Raza, S, Jahan R, Dhasmana A, Brahma Bhatt ML, Lohani M, Arif JM. Vitamin D receptor gene Bsm1 polymorphism and risk of breast cancer. Biochemical and Cellular Archives. 2017;17(2):657-63.

[34] Malvia S, Bagadi SA, Dubey US, Saxena S. Epidemiology of breast cancer in Indian women. Asia Pac J Clin Oncol. 2017;13(4):289-95.

[35] Ghoncheh M, Pournamdar Z, Salehiniya H. Incidence and mortality and epidemiology of breast cancer in the world. Asian Pac J Cancer Prev. 2016;17(3):43-46.

[36] Mishra DK, Wu Y, Sarkissyan M, Sarkissyan S, Chen Z, Shang X, et al. Vitamin D receptor gene polymorphisms and prognosis of breast cancer among African-American and Hispanic Women. PLoS One. 2013;8(3):e57967.

[37] Chakraborty M, Arora M, Ramteke A, Yadav V, Naaz H, Muntakhab M, et al. FokI polymorphism of Vitamin D receptor gene and deficiency of serum Vitamin D increases the risk of breast cancer in North Indian women. Endocrine. 2023;81(1):168-74.

[38] Reimers LL, Crew KD, Bradshaw PT, Santella RM, Steck SE, Sirosh I, et al. Vitamin D-related gene polymorphisms, plasma 25-hydroxyvitamin D, and breast cancer risk. Cancer Causes Control. 2015;26(2):187-203. Doi: 10.1007/s10552-014-0497-9. Epub 2014 Nov 25. PMID: 25421379; PMCID: PMC4302042.

[39] Ingles SA, Haile R, Henderson B, Kolonel L, Coetzee G. Association of vitamin D receptor genetic polymorphism with breast cancer risk in African-American and Hispanic women. In: Norman AW, Bouillon R, Thomasset M, editors. Vitamin D: Chemistry, biology and clinical applications of the steroid hormone. Riverside, CA: University of California; 1997:813-14.

[40] Whitfield GK, Remus LS, Jurutka PW, Zitzer H, Oza AK, Dang HT, et al. Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. Mol Cell Endocrinol. 2001;177(1-2):145-59.

[41] Yao S, Zirpoli G, Bovbjerg DH, Jandorf L, Hong CC, Zhao H, et al. Variants in the vitamin D pathway, serum levels of vitamin D, and estrogen receptor negative breast cancer among African-American women: A case-control study. Breast Cancer Res. 2012;14(2):R58. Available from: https://doi.org/10.1186/bcr3162.

[42] Gohar SF, Al-Hassanin S, Montaser B, Soliman S. Association of serum vitamin D Level and vitamin D receptor polymorphism (Apa 1) and breast cancer. Annals Ann Oncol. 2018;29(9):ix7. Available from: https://doi.org/10.1093/annonc/mdy426.020.

PARTICULARS OF CONTRIBUTORS:
1. PhD Scholar, Department of Biochemistry, Swami Rama Himalayan University, Jolly Grant, Dehradun, Uttarakhand, India.
2. Professor, Department of Biochemistry, Swami Rama Himalayan University, Jolly Grant, Dehradun, Uttarakhand, India.
3. Professor, Department of Biosciences, Swami Rama Himalayan University, Jolly Grant, Dehradun, Uttarakhand, India.
4. Professor, Department of Radiation Oncology, Swami Rama Himalayan University, Jolly Grant, Dehradun, Uttarakhand, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Ashok Kumar Dogra,
PhD Scholar, Department of Biochemistry, Swami Rama Himalayan University,
Jolly Grant, Dehradun-248140, Uttarakhand, India.
E-mail: akbhagat.pu@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jan 01, 2024
- Manual Googling: Feb 02, 2024
- iThenticate Software: Feb 27, 2024 (12%)

ETYMOLOGY: Author Origin

EMENDATIONS: 9

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jan 01, 2024**
Date of Peer Review: **Jan 30, 2024**
Date of Acceptance: **Feb 29, 2024**
Date of Publishing: **Apr 01, 2024**

Sr.No.	Name	UHID	Name of Participating Centre	Name of the Department	Date of first Diagnosis	Date of informed consent/Sample collection	Address	Rural=1/Urban=2/Semi-Urban=3	Mobile no.
1	Sumitra Devi	2976691	Cancer Research Institute, SRHU, Del	Radiation Oncology	5/19/2020	6/4/2020	Pauri Garhwal, Uttarakhand	3	7310977483
2	Kamla Semwal	2944878	Cancer Research Institute, SRHU, Del	Radiation Oncology	2/14/2020	6/27/2020	Mahadev Puran, Dehradun	2	9627491853
3	Madhu Rawat	2993004	Cancer Research Institute, SRHU, Del	Radiation Oncology	6/29/2020	8/5/2020	Pauri Garhwal, Uttarakhand	3	8279952456
4	Krishna Devi	2990272	Cancer Research Institute, SRHU, Del	Radiation Oncology	6/26/2020	8/5/2020	Doiwala, Dehradun	2	9910206149
5	Pushpa Devi	3008937	Cancer Research Institute, SRHU, Del	Radiation Oncology	7/20/2020	8/13/2020	Village Sarp,PO kyari	1	8037916907
6	Hemo(Hemi Devi)	2994001	Cancer Research Institute, SRHU, Del	Radiation Oncology	7/6/2020	8/24/2020	Ward no. 10 Jaspur U.P.	2	9917879867
7	Kunti Devi	3015757	Cancer Research Institute, SRHU, Del	Radiation Oncology	8/21/2020	10/31/2020	Uttarkashi, Uttarakhand	1	8755314251
8	Salochana Devi	3029024	Cancer Research Institute, SRHU, Del	Radiation Oncology	10/3/2020	11/6/2020	Kaulagarh, Dehradun	2	9897907121
9	Vijay Laxmi	3007983	Cancer Research Institute, SRHU, Del	Radiation Oncology	7/29/2020	11/11/2020	Tehri, Garhwal, Uttarakhand	3	7249997761
10	Shansha Devi	3028783	Cancer Research Institute, SRHU, Del	Radiation Oncology	9/24/2020	11/12/2020	26/21, East Canal Road, Dehradun	2	9634658793
11	Rameshwari Devi	3040514	Cancer Research Institute, SRHU, Del	Radiation Oncology	11/2/2020	12/9/2020	Rani Pokhri, Dehradun	2	9808120555
12	Amara Devi	3039888	Cancer Research Institute, SRHU, Del	Radiation Oncology	11/6/2020	12/28/2020	Rudarprayag, Uttarakhand	3	7535800900
13	Nahid Jahan	3057077	Cancer Research Institute, SRHU, Del	Radiation Oncology	11/9/2020	1/29/2021	Jaspur, US Nagar, Uttarakhand	2	7017378318
14	Sushma	3065546	Cancer Research Institute, SRHU, Del	Radiation Oncology	12/25/2020	2/9/2021	Village Tashia, Uttarakhand	1	9412145838
15	Soniya	3078681	Cancer Research Institute, SRHU, Del	Radiation Oncology	2/5/2021	3/6/2021	Gumaniwala, Dehradun	2	7668033749
16	Reena Walia	3077041	Cancer Research Institute, SRHU, Del	Radiation Oncology	2/15/2021	3/12/2021	Jogiwala, Dehradun	2	9837645030
17	Rekha	3254182	Cancer Research Institute, SRHU, Del	Radiation Oncology	12/29/2021	3/14/2021	Village Fatehpur, Herbertpur, Deh	1	9805754705
18	Sushma Bhatia	3029804	Cancer Research Institute, SRHU, Del	Radiation Oncology	12/18/2020	3/15/2021	Doiwala, Dehradun	2	8006666419
19	Sunita Jakhmola	2993114	Cancer Research Institute, SRHU, Del	Radiation Oncology	7/4/2020	3/16/2021	Haripur, Dehradun	2	7830737010
20	Shameena Khatun	3000187	Cancer Research Institute, SRHU, Del	Radiation Oncology	7/20/2020	3/20/2021	Bijnor, U.P.	2	7303104393
21	Mamta	3086927	Cancer Research Institute, SRHU, Del	Radiation Oncology	2/24/2021	3/24/2021	Amroha, U.P.	2	8941974243
22	Anju Singla	3104077	Cancer Research Institute, SRHU, Del	Radiation Oncology	2/27/2021	4/1/2021	9-Old Survey Road, Dehradun	2	8445109529
23	Shivani Bhandari	3083480	Cancer Research Institute, SRHU, Del	Radiation Oncology	2/8/2021	4/6/2021	Block D lane no. 9, Aman Vihar, De	2	9897295059
24	Prabha	3112014	Cancer Research Institute, SRHU, Del	Radiation Oncology	4/8/2021	4/21/2021	Village Rankhandi, Saharanpur, U.I	1	9084566563
25	Resha	3098350	Cancer Research Institute, SRHU, Del	Radiation Oncology	3/1/2021	4/22/2021	Village Bhera Bharatpur, Uttarakh	1	9917523584
26	Rachana Devi	3082263	Cancer Research Institute, SRHU, Del	Radiation Oncology	2/12/2021	5/4/2021	Karanpur, Bijnor, U.P.	2	6397703886
27	Siya Devi	3108677	Cancer Research Institute, SRHU, Del	Radiation Oncology	4/2/2021	5/6/2021	Piran Kaliyar Majri, Dehradun	2	9927082357
28	Shikha Singh	3115478	Cancer Research Institute, SRHU, Del	Radiation Oncology	2/19/2021	5/6/2021	Vikas Colony, Rishikesh, Uttarakha	2	8869007290
29	Swati Verma	3119530	Cancer Research Institute, SRHU, Del	Radiation Oncology	3/23/2021	5/12/2021	147-Lane5B, Tarun vihar, Dehradu	2	9412198589
30	Vimla Saini	3078918	Cancer Research Institute, SRHU, Del	Radiation Oncology	2/5/2021	5/13/2021	Village Tankan, Saharanpur, U.P.	1	9627689298
31	Shabnam	3124780	Cancer Research Institute, SRHU, Del	Radiation Oncology	5/11/2021	5/13/2021	Village-Fholdapatti, Moradabad, U	1	9837628428
32	Ragini	3109727	Cancer Research Institute, SRHU, Del	Radiation Oncology	3/17/2021	5/27/2021	Panchayat ghar, Paonta Sahib, H.P	2	9816149044
33	Bhawna Sharma	3121274	Cancer Research Institute, SRHU, Del	Radiation Oncology	4/10/2021	6/10/2021	39/5, Guru Road, Patel Nagar, Deh	2	8410081777
34	Rita	3113985	Cancer Research Institute, SRHU, Del	Radiation Oncology	4/15/2021	6/18/2021	Fatehpur Tanda, Dehradun	2	959516491
35	Mohini Negi	3093675	Cancer Research Institute, SRHU, Del	Radiation Oncology	3/8/2021	7/9/2021	Rishikesh, Uttarakhand	2	9760040668
36	Vira(Veera) Devi	3136143	Cancer Research Institute, SRHU, Del	Radiation Oncology	6/14/2021	7/23/2021	Village Kimkhet, Uttarakhand	1	9412165728
37	Sarveen	3140228	Cancer Research Institute, SRHU, Del	Medical Oncology	6/25/2021	8/4/2021	Village Ishaqpur, Saharanpur U.P.	1	9756857806
38	Rakesh	3160838	Cancer Research Institute, SRHU, Del	Radiation Oncology	8/4/2021	8/4/2021	Village Mullapur Kareen U.P.	1	9758027016
39	Prabha Devi	3136927	Cancer Research Institute, SRHU, Del	Radiation Oncology	6/10/2021	8/5/2021	110- Chander Nagar Dehradun	2	8307695679
40	Imrana Parveen	3152181	Cancer Research Institute, SRHU, Del	Radiation Oncology	7/19/2021	8/5/2021	Ambedkar Colony, Raipur Road, De	2	9897923307
41	Sukarma	3145694	Cancer Research Institute, SRHU, Del	Radiation Oncology	7/6/2021	8/9/2021	Village Kalyanpur, Haridwar, Uttar	1	9557157314
42	Shraddha Agarwal	3127716	Cancer Research Institute, SRHU, Del	Radiation Oncology	5/24/2021	8/11/2021	49-Bileshwar colony, Dehradun, U	2	8279886384
43	Shakuntala	3148388	Cancer Research Institute, SRHU, Del	Radiation Oncology	7/8/2021	8/12/2021	Village Gundiyat ghanv Purola, Utt	1	9458144079
44	Jarina	3170423	Cancer Research Institute, SRHU, Del	Radiation Oncology	8/23/2021	8/16/2021	Islampur. Bijnor, U.P.	2	9719511027
45	Rosa (Rusa) Devi	3159834	Cancer Research Institute, SRHU, Del	Radiation Oncology	8/3/2021	8/18/2021	Village- Dharkuri, Uttarakhand	1	9265225724
46	Suman	3151481	Cancer Research Institute, SRHU, Del	Radiation Oncology	7/17/2021	8/24/2021	Saharanpur. U.P.	2	9012973000
47	Leela Devi	3148933	Cancer Research Institute, SRHU, Del	Radiation Oncology	7/12/2021	8/24/2021	Lakshr, Haridwar, Uttarakhand	2	9410529376
48	Nida Hasmat	3170463	Cancer Research Institute, SRHU, Del	Medical Oncology	8/24/2021	8/27/2021	Lane 2, Clementown, Dehradun, U	2	9548457528
49	Sangeeta Sharma	3161738	Cancer Research Institute, SRHU, Del	Radiation Oncology	8/9/2021	8/31/2021	Village Nagager, RaniPokhri, Dehra	1	9411261607
50	Raj Bala	3176999	Cancer Research Institute, SRHU, Del	Radiation Oncology	7/7/2021	9/6/2021	Nand Vihar, Saharanpur, U.P.	2	8171284148
51	Salama Begum	3146803	Cancer Research Institute, SRHU, Del	Radiation Oncology	7/12/2021	9/8/2021	Patti Chauhan, US Nagar, Dehradu	2	9917442660
52	Rekha Upadhyay	3061280	Cancer Research Institute, SRHU, Del	Radiation Oncology	1/4/2021	9/15/2021	Ward no. 4, Dehradun	2	9410334615
53	Saroj Verma	3157583	Cancer Research Institute, SRHU, Del	Radiation Oncology	7/29/2021	9/24/2021	Haridwar, Uttarakhand	2	9808013276
54	Asha Devi	3113706	Cancer Research Institute, SRHU, Del	Radiation Oncology	5/2/2021	10/1/2021	Village Ropa, Chamoli, Uttarakhan	3	9627448160
55	Mahendri	3191641	Cancer Research Institute, SRHU, Del	Radiation Oncology	10/5/2021	10/5/2021	Village Mehrana Sambhal U.P.	1	9870903297
56	Reshma	3164469	Cancer Research Institute, SRHU, Del	Radiation Oncology	8/7/2021	10/6/2021	Akka Dilari, Moradabad, U.P.	2	9756510206
57	Mala Sharma	3170393	Cancer Research Institute, SRHU, Del	Radiation Oncology	8/11/2021	10/6/2021	63- Peerwali Colony, Haridwar, Utt	2	8393089940
58	Jareena Parveen	3165199	Cancer Research Institute, SRHU, Del	Radiation Oncology	8/13/2021	10/7/2021	Lane no. 14, Azad Nagar, Haldwan	2	9837724687
59	Paramjeet Kaur	3169548	Cancer Research Institute, SRHU, Del	Radiation Oncology	5/17/2021	10/16/2021	Tehri, Garhwal, Uttarakhand	3	9412027784
60	Anita Chug	3172139	Cancer Research Institute, SRHU, Del	Radiation Oncology	8/17/2021	10/20/2021	Vikas Nagar, Dehradun, Uttarakha	2	9634607985
61	Pushpa Taneja	3195194	Cancer Research Institute, SRHU, Del	Medical Oncology	8/13/2021	10/22/2021	Clementown, Dehradun	2	9837259372
62	Sarla Devi	3202061	Cancer Research Institute, SRHU, Del	Radiation Oncology	10/19/2021	10/29/2021	Village Mohmmadpur, Saharanpur	1	9149237310
63	Lata Chauhan	3172259	Cancer Research Institute, SRHU, Del	Radiation Oncology	8/28/2021	10/30/2021	H.No. 138, Kanwali Balliwala, Dehı	2	7989329742
64	Sudha	3200041	Cancer Research Institute, SRHU, Del	Radiation Oncology	10/14/2021	11/1/2021	Hidayatpur, Bijnor, U.P.	2	9564465093
65	Shikha	3212439	Cancer Research Institute, SRHU, Del	Radiation Oncology	9/2/2021	11/11/2021	Lakshr, Haridwar, Uttarakhand	2	9528973075
66	Siddhi Bhat	3157223	Cancer Research Institute, SRHU, Del	Radiation Oncology	7/30/2021	11/12/2021	Clementown, Dehradun	2	9412057546
67	Mamta Rawat	3202324	Cancer Research Institute, SRHU, Del	Radiation Oncology	10/20/2021	11/12/2021	Bhunar, Dehradun	2	6395448686
68	Geeta Sharma	3217964	Cancer Research Institute, SRHU, Del	Radiation Oncology	6/2/2021	11/22/2021	Village Kharson, Tehri, Uttarakhan	3	7248058110
69	Shakina	3222070	Cancer Research Institute, SRHU, Del	Medical Oncology	11/20/2021	11/30/2021	Bijnor, U.P.	2	8859089606
70	Uma Sharma	3222680	Cancer Research Institute, SRHU, Del	Radiation Oncology	6/4/2021	12/1/2021	Clementown, Dehradun	2	8533082960
71	Anuiya Devi Kanthwl	3223745	Cancer Research Institute, SRHU, Del	Radiation Oncology	12/3/2021	12/3/2021	Village Babina Pauri Garhwal	3	9412940915
72	Ranjit Kaur	3163121	Cancer Research Institute, SRHU, Del	Radiation Oncology	5/19/2021	12/6/2021	Haridwar, Uttarakhand	2	7018599451
73	Anandi Chauhan	3183089	Cancer Research Institute, SRHU, Del	Radiation Oncology	9/14/2021	12/14/2021	Near OBC bank Balawala, Dehradu	2	7251021233
74	Neelu Sharma	3228660	Cancer Research Institute, SRHU, Del	Radiation Oncology	11/27/2021	12/14/2021	Defence Colony, Uttarakhand	2	9719263398
75	Rihana	3229520	Cancer Research Institute, SRHU, Del	Radiation Oncology	12/15/2021	12/16/2021	Saharanpur, U.P.	2	7906920930
76	Santosh Devi	3225261	Cancer Research Institute, SRHU, Del	Radiation Oncology	12/7/2021	2/3/2022	Gaon-Thana Mohalla, Dehradun, U	2	7895662402
77	Akila Khan	3229757	Cancer Research Institute, SRHU, Del	Radiation Oncology	10/9/2021	2/17/2022	Village- Sirsal, Saharanpur, U.P.	1	9997777745
78	Mamta	3246346	Cancer Research Institute, SRHU, Del	Radiation Oncology	1/11/2022	2/17/2022	Chandrabani, Dehradun, Uttarakha	2	8755958325
79	Darshani Devi	3234837	Cancer Research Institute, SRHU, Del	Radiation Oncology	12/22/2021	2/18/2022	Dandi Motharowala, Uttarakhand	2	7425902032
80	Geeta Devi	3247576	Cancer Research Institute, SRHU, Del	Radiation Oncology	1/28/2022	3/15/2022	New Nahar Colony, Bahadrabad, H	1	9719314839
81	Israna	3263025	Cancer Research Institute, SRHU, Del	Medical Oncology	3/5/2022	3/17/2022	Dehradun, Uttarakhand	2	8923440311
82	Savita Sharma	3307335	Cancer Research Institute, SRHU, Del	Radiation Oncology	5/16/2022	5/20/2022	Muzaffarnagar, U.P.	2	8057032846
83	Swati Sharma	3302697	Cancer Research Institute, SRHU, Del	Surgical Oncology	5/21/2022	5/21/2022	Haridwar, Uttarakhand	2	9997005385
84	Rina	3307155	Cancer Research Institute, SRHU, Del	Radiation Oncology	5/14/2022	5/21/2022	Moradabad, U.P.	2	7300768877
85	Tarannum Bee	3308426	Cancer Research Institute, SRHU, Del	Radiation Oncology	5/21/2022	5/21/2022	US Nagar, Dehradun	2	9756610755
86	Saroj	3317214	Cancer Research Institute, SRHU, Del	Radiation Oncology	3/11/2022	6/3/2022	Bijnor, U.P.	2	8006584159
87	Shanti Ray	3321223	Cancer Research Institute, SRHU, Del	Medical Oncology	5/10/2022	6/10/2022	US Nagar, Dehradun	2	8126138372
88	Meena Bisht	3321300	Cancer Research Institute, SRHU, Del	Medical Oncology	6/1/2022	6/10/2022	Lane 7, E-Block, Dehradun	2	9410317791
89	Tabassum	3321396	Cancer Research Institute, SRHU, Del	Radiation Oncology	5/17/2022	6/10/2022	Mohalla Neel Khudan, Haridwar	2	7037850320
90	Laxmi Devi	3324685	Cancer Research Institute, SRHU, Del	Radiation Oncology	6/10/2022	6/16/2022	Village, Sambhal, U.P.	1	8941080715
91	Urmila Rawat	3296137	Cancer Research Institute, SRHU, Del	Radiation Oncology	5/4/2022	6/17/2022	Village Dungri, Tehri-Garhwal	3	9520606317
92	Nardei	3360721	Cancer Research Institute, SRHU, Del	Radiation Oncology	8/20/2022	8/30/2022	Uttarkashi, Uttarakhand	2	9084366689
93	Rihana Parveen	3360354	Cancer Research Institute, SRHU, Del	Radiation Oncology	8/18/2022	9/29/2022	Sharanpur U.P.	2	9058106263
94	Arti Devi	3362592	Cancer Research Institute, SRHU, Del	Radiation Oncology	7/12/2022	11/22/2022	104 Chhabra Ward No. 2, Dehradu	2	9719019316
95	Suman Chaudhary	3404129	Cancer Research Institute, SRHU, Del	Radiation Oncology	11/28/2022	11/28/2022	Sahabpur, Bijnor, U.P.	2	9847590342
96	Kusumlata	3377974	Cancer Research Institute, SRHU, Del	Radiation Oncology	9/27/2022	11/30/2022	Shekhpur, Bijnor, U.P.	2	9837589045
97	Sureshna Devi	3357571	Cancer Research Institute, SRHU, Del	Radiation Oncology	8/20/2022	11/30/2022	96- Subhash Nagar, Dehradun, Utt	2	7906610175
98	Urmila Negi	3389695	Cancer Research Institute, SRHU, Del	Medical Oncology	10/28/2022	12/2/2022	Village Tuntowala,Mehuwala, Uttz	1	7535908143
99	Neha Nautiyal	3403883	Cancer Research Institute, SRHU, Del	Radiation Oncology	6/25/2022	12/5/2022	Raipur, Dehradun, Uttarkhand	2	7895531917
100	Poonam	3385899	Cancer Research Institute, SRHU, Del	Radiation Oncology	9/15/2022	12/7/2022	Gram Majra, Malakpur, Haridwar,	2	7409663675
101	Laxmi Devi	3400862	Cancer Research Institute, SRHU, Del	Radiation Oncology	11/28/2022	12/16/2022	Village Chaudel, Pauri, Uttarakhan	3	9548769730
102	Santosh Devi	3394775	Cancer Research Institute, SRHU, Del	Radiation Oncology	11/9/2022	12/23/2022	Village Asmauli, U.P.	1	8923375043
103	Sushma Kalra	3400711	Cancer Research Institute, SRHU, Del	Radiation Oncology	8/31/2022	12/24/2022	Teachers Colony, Udham Singh Na	2	9368466587
104	Bharti Chand	3383213	Cancer Research Institute, SRHU, Del	Radiation Oncology	8/31/2022	12/27/2022	Shiv Mandir, Saspur, Dehradun, Ut	2	7906076547
105	Indu Vaish	3408003	Cancer Research Institute, SRHU, Del	Radiation Oncology	12/8/2022	1/3/2023	Prem Nagar, Dehradun, Uttarakha	2	8864823232
106	Shivani	3408114	Cancer Research Institute, SRHU, Del	Radiation Oncology	12/6/2022	1/3/2023	Nathuwala, Dehradun, Uttarakhan	2	9897383045
107	Anjana Devi	3410629	Cancer Research Institute, SRHU, Del	Surgical Oncology	12/31/2022	1/20/2023	Aamwala Nalapani, Tapowan, Deh	2	9761852539
108	Neema Bora	3420610	Cancer Research Institute, SRHU, Del	Radiation Oncology	1/10/2023	1/20/2023	208- Siv Nagar, Dehradun, Uttarak	2	7989415106
109	Farjana	3406601	Cancer Research Institute, SRHU, Del	Radiation Oncology	11/19/2022	1/24/2023	Mawakot, Pauri, Uttarakahand	3	7055245983
110	Savitri Devi	3422664	Cancer Research Institute, SRHU, Del	Radiation Oncology	1/13/2023	1/25/2023	Dehradun, Uttarakhand	2	9084925659

Age	Sex	Menopausal status(Pre=1, post=2)	Age at Diagnosis	First Degree Family history (Yes=1, None=2)	History of any other Cancer	Weight(kg)	Height(cm)	BMI(kg/m^2)	BMI Category (Normal=1, Underweight=2, Overweight=3, Obese class I=4)
46	Female	1	46	2	None	52	159	20.6	1
50	Female	2	50	1	None	54	154	22.8	1
47	Female	1	47	2	None	70	162	26.7	3
39	Female	1	39	1	None	50	157	20.3	1
51	Female	2	51	1	None	54	157	21.9	1
46	Female	1	46	2	None	65	157	26.4	3
54	Female	2	54	2	None	58	152	25.1	3
54	Female	2	54	1	None	62	157	25.2	3
51	Female	2	51	2	None	53	162	20.2	1
42	Female	1	42	2	None	40	152	17.3	2
63	Female	2	63	1	None	38	142	18.8	1
67	Female	2	67	1	None	66	157	26.8	3
45	Female	1	45	2	None	49	164	18.2	2
43	Female	1	43	2	None	61	159	24.1	1
39	Female	1	39	1	None	63	157	25.6	3
57	Female	2	57	2	None	65	144	31.3	4
48	Female	1	48	2	None	51	152	22.1	1
51	Female	2	51	2	None	66	160	25.8	3
43	Female	1	43	2	None	80	162	30.5	4
59	Female	2	59	2	None	64	157	26	3
44	Female	1	44	2	None	65	161	25.1	3
50	Female	2	50	2	None	60	154	25.3	3
35	Female	1	35	2	None	55	158	22	1
38	Female	1	38	2	None	62	158	24.8	1
40	Female	1	40	2	None	67	160	26.2	3
30	Female	1	30	2	None	64	164	23.8	1
57	Female	2	57	1	None	53	155	22.1	1
30	Female	1	30	2	None	58	165	21.3	1
34	Female	1	34	2	None	56	157	22.7	1
68	Female	2	68	1	None	52	168	18.4	2
37	Female	1	37	2	None	62	155	25.8	3
38	Female	1	38	2	None	60	158	24	1
42	Female	1	42	2	None	65	158	26	3
34	Female	1	34	2	None	53	156	21.8	1
65	Female	2	65	2	None	51	155	21.2	1
44	Female	1	44	2	None	63	166	22.9	1
33	Female	1	33	2	None	69	150	30.7	4
44	Female	1	41	2	None	59	161	22.8	1
56	Female	2	56	2	None	64	158	25.6	3
37	Female	1	37	2	None	72	160	28.1	3
52	Female	2	52	2	None	56	165	20.6	1
30	Female	1	30	2	None	51	159	20.2	1
44	Female	1	44	2	None	58	156	23.8	1
55	Female	2	55	2	None	60	165	22	1
47	Female	1	47	1	None	50	164	18.6	1
46	Female	1	46	2	None	57	153	24.3	3
51	Female	2	51	2	None	64	166	23.2	1
37	Female	1	37	2	None	59	153	25.2	3
52	Female	2	52	2	None	53	165	19.5	1
62	Female	2	62	2	None	50	162	19.1	1
47	Female	2	47	1	None	49	162	18.7	1
36	Female	1	36	2	None	68	162	25.6	3
56	Female	2	56	2	None	70	157	28.4	3
52	Female	2	52	2	None	61	160	23.8	1
58	Female	2	58	2	None	66	165	24.2	1
45	Female	1	45	2	None	53	152	22.9	1
33	Female	1	33	2	None	58	156	23.8	1
31	Female	1	31	2	None	62	154	26.1	3
55	Female	2	55	2	None	79	179	24.7	1
37	Female	1	37	2	None	62	164	23.1	1
65	Female	2	65	2	None	58	144	28	3
42	Female	1	42	2	None	60	161	23.1	1
67	Female	2	67	2	None	60	168	21.3	1
41	Female	1	41	2	None	58	157	23.5	1
30	Female	1	30	2	None	58	162	22.1	1
49	Female	2	49	1	None	67	163	25.2	3
33	Female	1	33	2	None	65	165	23.9	1
57	Female	2	57	2	None	50	163	18.8	1
40	Female	1	40	1	None	55	164	20.4	1
43	Female	2	43	2	None	81	160	31.6	4
52	Female	2	52	2	None	58	165	21.3	1
55	Female	2	55	1	None	65	162	24.8	1
46	Female	1	46	2	None	60	154	25.3	3
43	Female	1	43	2	None	66	161	25.5	3
45	Female	1	45	2	None	68	162	25.9	3
36	Female	1	36	2	None	54	164	20.1	1
62	Female	2	62	2	None	60	154	25.3	3
45	Female	1	45	2	None	59	154	24.9	1
51	Female	2	51	2	None	62	156	25.5	3
70	Female	2	70	2	None	50	163	18.8	1
38	Female	1	38	2	None	41	150	18.2	2
47	Female	1	47	2	None	55	154	23.2	1
38	Female	1	38	2	None	65	155	27.1	3
32	Female	1	32	2	None	60	152	26	3
38	Female	1	38	2	None	68	154	28.7	3
45	Female	1	45	2	None	57	165	20.9	1
32	Female	1	32	2	None	55	155	22.9	1
57	Female	2	57	2	None	58	152	25.1	3
41	Female	1	41	2	None	68	158	27.2	3
48	Female	1	48	2	None	50	159	19.8	1
52	Female	2	52	2	None	68	166	24.7	1
60	Female	2	60	2	None	52	165	19.1	1
40	Female	1	40	2	None	59	151	25.9	3
40	Female	1	40	2	None	58	150	25.8	3
62	Female	2	62	2	None	59	155	24.6	1
61	Female	2	61	2	None	65	158	26	3
51	Female	2	51	2	None	48	149	21.6	1
32	Female	1	32	2	None	58	156	23.8	1
33	Female	1	33	2	None	53	153	22.6	1
39	Female	1	39	2	None	58	159	22.9	1
68	Female	2	68	2	None	58	154	24.5	1
48	Female	2	48	2	None	55	150	24.4	1
50	Female	2	50	2	None	40	150	17.8	2
42	Female	1	42	2	None	47	152	20.3	1
44	Female	1	44	2	None	61	156	25.1	3
41	Female	1	41	2	None	59	157	23.9	1
54	Female	2	54	2	None	67	168	23.1	1
36	Female	1	36	2	None	60	149	27	3
32	Female	1	32	2	None	61	159	24.1	1
69	Female	2	69	2	None	50	155	20.8	1

Co-Morbid conditions(None=1, Type-2 Diabetes=2, Hypertension=3, Hypothyroidism=4)	Method of Diagnosis	Pathological Diagnosis	Breast Cancer laterality (Left=1, Right=2)	Benign/Malignant	TNM Staging (	Stage Groupings
3	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2NoMx	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT3NoMo	IIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2N1aMx	IIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT3NoMx	IIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2N3aMo	IIIC
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT3NoMo	IIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2N2Mx	IIIA
3	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2NoMo	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2N1aMx	IIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2N1Mx	IIB
2	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2N2aMx	IIIA
2	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT4N1Mx	IIIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2N3aMo	IIIC
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2NoMo	IIA
2	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	cT4bN1Mx	IIIB
3	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT3N1aMo	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT1NoMx	I
3	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT4aN1Mo	IIIB
3	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2NoMx	IIA
3	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT1cN1aMo	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT3N1Mo	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2N2aMx	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT3NxMx	II B
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	cT2N1Mo	II B
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT3N3aMx	IIIC
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2N1aMx	IIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2N2aMx	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2N1Mo	IIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2N2aMx	IIIA
3	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT3N1aMx	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	cT2N2bMx	III A
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2NoMx	II A
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT3NoMo	II B
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT1NoMx	I
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2NoMo	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	PT3NoMx	IIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2N2aMx	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	cT2N1Mo	IIB
2	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT3N2aMx	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	cT2NoMo	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2NoMo	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2N2bMx	III A
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2NoMx	II A
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2N3aMx	IIIC
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	cT4N1Mo	IIIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2NoMo	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2NoMo	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2NoMx	II A
3	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2N1bMo	IIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	Stage IV	IV
2	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT3NoMo	IIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2NoMo	IIA
2	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2N3Mo	IIIC
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2N1aMo	IIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	cT2N1Mo	IIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2N2aMo	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2N1aMx	II B
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2N2aMx	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2N3aMx	IIIC
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2NoMx	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2N2Mo	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	cT2N1Mo	II B
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT1NoMx	I
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	cT1N1Mo	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2NoMx	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT3N2aMx	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT1cN1Mo	IIA
3	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2N2Mo	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2NoMo	IIA
2	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	cT2N1Mo	IIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	cT2NoMo	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT1cN1aMx	IIA
3	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2NoMx	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2NoMo	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	cT2N1Mo	IIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2NoMo	IIA
3	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT3N2Mo	IIIA
3	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	cT2N1Mo	IIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2N2aMo	IIIA
3	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2N1aMx	IIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2N2aMx	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	cT2NoMo	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT4N1aMx	IIIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	cT2NoMo	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT1cNoMo	I
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	cT3N1Mo	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT1cN2aMx	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	cT2N3Mo	IIIC
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	cT1NoMo	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	cT2N2Mo	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2N1aMo	IIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	cT3N1Mo	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2NoMo	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT3N2aMx	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	cT4bN1Mo	IIIB
3	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2NoMx	IIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT1NoMx	I
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT1cNoMx	I
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	cT4bN2Mo	IIIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2N2aMx	III A
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	cT2N1Mo	IIB
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT3N1aMx	IIIA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2N1aMx	II B
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT2NoMx	II A
2	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	cT2N1Mo	II B
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	pT3N3aMx	III C
1	Clinical; Microsopic; Imaging techniques	Ductal Carcinoma in situ	1	Malignant	pTisNxMx	NA
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	cT2N1Mo	II B
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	1	Malignant	pT2NoMx	II A
1	Clinical; Microsopic; Imaging techniques	Infiltrating Ductal Carcinoma	2	Malignant	cT1NoMo	I

Pathological Primary Tumor Size(cm)	Tumor size (upto 2cm=1, >2 and upto 5cm=2, >5cm=3)	Histologic Sub type(TumorGrade)	Tumor Grade (Well differentiated=1, Moderately differentiated=2, Poorly differentiated=3)
3.4×2.4×2.4 cm	2	Grade II	2
8.2×7.0×4.4 cm	3	Grade III	3
3.0×2.5×2.3 cm	2	Grade II	2
5.9×4.6×3.0 cm	3	Grade II	2
3.5×3.0×0.5 cm	2	Grade II	2
6.5×3.5×3.0 cm	3	GradeII	2
4.0×3.2×3.0 cm	2	Grade II	2
4.0×3.0×3.0 cm	2	Grade II	2
4.4×3.5×2.6 cm	2	Grade II	2
3.4×3.2×1.4 cm	2	Grade II	2
4.0×2.0×1.8 cm	2	Grade II	2
4.0×3.5×2.2 cm	2	Grade III	3
3.0×1.5×0.8 cm	2	Grade II	2
2.8×2.4×2.2cm	2	Grade III	3
4.8×1.8×2.0 cm	2	Grade II	2
5.1×4.2×4.0 cm	3	Grade II	2
1.2×1.0×0.5 cm	1	GradeI	1
3.1×2.0 cm	2	Grade III	3
4.5×3.5×3.0 cm	2	Grade III	3
1.2×1.0×0.8 cm	1	Grade II	2
5.8×4.0×3.5cm	3	Grade II	2
4.4×2.8×2.5 cm	2	Grade III	3
5.5×5.0×5.0cm	3	Grade II	2
3.8×3.1×2.2cm	2	Grade II	2
4.8×2.0×2.0cm	2	Grade II	2
3.5×3.0×2.5cm	2	Grade II	2
3.5×3.0×3.0 cm	2	Grade II	2
2.3×1.2×0.6 cm	2	Grade III	3
2.2×1.8×1.2cm	2	Grade III	3
5.5×4.5×3.5 cm	3	Grade II	2
4.1×3.5×2.8 cm	2	Grade III	3
2.1×1.8×1.0 cm	2	Grade II	2
5.5×3.5×3.0cm	3	Grade II	2
1.5×1.0×1.0 cm	1	Grade II	2
4.0×3.0×2.8 cm	2	Grade II	2
5.1×5.0×1.8cm	3	Grade III	3
2.2×1.0×0.8 cm	2	Grade III	3
3.1×2.3×1.8cm	2	Grade II	2
5.6×3.0×2.0 cm	3	Grade II	2
6.5×3.5×4.8 cm	3	Grade III	3
2.0×1.5×1.1 cm	1	Grade III	3
4.0×3.8×2.6 cm	2	Grade III	3
2.2×1.8×1.2cm	2	Grade II	2
2.8×2.7×1.8cm	2	Grade II	2
4.5×3.3×7.7 cm	2	Grade II	2
2.1×1.4×1.0cm	2	Grade II	2
4.0×3.5×3.0cm	2	Grade II	2
3.8×3.2×2.4 cm	2	Grade II	2
4.5×4.0×1.5 cm	2	Grade II	2
5.3×2.0×5.3 cm	3	Grade II	2
6.0×5.0×4.0 cm	3	Grade III	3
2.2×1.2×1.2 cm	2	Grade II	2
2.9×2.0×1.2 cm	2	Grade III	3
4.0×3.6×2.8 cm	2	Grade III	3
3.4×2.3×2.0cm	2	Grade II	2
4.2×2.2×1.2 cm	2	Grade II	2
2.8×2.5×1.8 cm	2	Grade II	2
3.8×2.0×1.5cm	2	Grade II	2
3.0×2.5×1.5cm	2	Grade II	2
4.8×4.0×3.0cm	2	Grade III	3
3.4×3.0×2.0 cm	2	Grade II	2
3.0×2.0×1.5 cm	2	Grade II	2
1.7×1.0×0.8cm	1	Grade II	2
2.0×1.4×1.2 cm	1	Grade II	2
2.8×1.8×1.5cm	2	Grade III	3
5.5×3.0×2.8 cm	3	Grade II	2
2.8×1.8×1.6 cm	2	Grade II	2
3.5×3.2×3.5 cm	2	Grade III	3
1.7×1.2×1.1 cm	1	Grade II	2
3.5×2.9	2	Grade III	3
2.2×1.8×1.3cm	2	Grade II	2
1.8×1.5×0.8 cm	1	Grade II	2
3.7×2.8×1.7 cm	2	Grade II	2
3.0×2.5×2.1cm	2	Grade II	2
2.7×2.3cm	2	Grade II	2
3.4×2.9×1.5 cm	2	Grade III	3
4.5×3.5×2.5 cm	2	Grade II	2
1.9×1.8×1.6 cm	1	Grade II	2
4.0×2.5×2.0 cm	2	Grade II	2
2.5×1.5×1.3 cm	2	Grade II	2
2.5×2.5×1.0 cm	2	Grade III	3
1.9×1.6×1.4 cm	1	Grade II	2
2.6×2.4×2.2 cm	2	Grade II	2
1.9×1.8×1.6 cm	1	Grade II	2
1.5×1.2×0.8 cm	1	Grade II	2
6.4×5.2×2.6 cm	3	Grade II	2
1.2×1.0×0.8 cm	1	Grade II	2
3.8×3.2×3.2 cm	2	Grade III	3
1.5×1.8×0.2 cm	1	Grade I	1
4.0×4.0×3.0 cm	2	Grade II	2
2.4×2.0×1.8 cm	2	Grade II	2
5.1×5.3cm	3	Grade II	2
0.8×0.5×0.3 cm	1	Grade II	2
6.0×4.0×3.0 cm	3	Grade II	2
5.3×3.0×2.2 cm	3	Grade III	3
2.5×2.5×2.0 cm	2	Grade II	2
1.8×1.6×1.3 cm	1	Grade II	1
1.3×1.2×1.0cm	1	Grade II	2
5.3×5.0×2.8 cm	3	Grade II	2
3.0×1.8×1.5 cm	2	Grade III	3
2.9×2.3×1.8 cm	2	Grade II	2
6.5×5.0×2.5 cm	3	Grade III	3
3.8×3.5×2.0 cm	2	Grade III	3
4.0×2.5×1.5 cm	2	Grade II	2
3.1×3.0×2.7 cm	2	Grade II	2
7.0×6.0×2.6 cm	3	Grade III	3
NA	NA	NA	
2.4×1.5×1.9 cm	2	Grade II	2
3.0×2.0×2.5 cm	2	Grade III	3
2.0×1.0×0.2 cm	1	Grade II	2

Lymph node status (Present=1, Absent=2, Unknown=3)	No. of lymph nodes involved /showing tumor	Number of Axillary lymph nodes removed	Modified Richardson Bloom Score	Total MRB Score	Residual Tumor	Angiolymphatic Invasion (Positive=1, Negative=2)
2	0	8	2+3+2=7	7	Ro	1
2	0	14	3+3+3=9	9	Ro	1
1	1	28	2+2+2=6	6	Ro	1
2	0	13	2+2+2=6	6	Ro	1
1	5	6	2+3+2=7	7	Ro	1
2	0	17	2+2+2=6	6	Ro	1
1	4	18	2+2+2=6	6	Ro	1
2	0	19	2+3+3=7	7	Ro	1
1	2	25	2+2+2=6	6	Ro	1
1	1	12	3+2+2=7	7	Ro	1
1	9	12	2+2+2=6	6	Ro	1
1	1	12	3+2+3=8	8	Ro	1
1	20	23	2+2+2=6	6	Ro	1
2	0	10	3+3+2=8	8	Ro	1
2	NA	NA	2+2+2=6	6	Unknown	1
1	3	12	3+2+2=7	7	Unknown	1
2	0	18	1+2+2=5	5	Ro	2
1	8	15	3+3+2=8	8	Ro	1
2	0	10	3+3+2=7	7	Ro	1
1	2	4	2+2+2=6	6	Ro	1
1	2	17	2+2+2=6	6	Ro	1
1	4	19	3+2+3=8	8	Ro	1
2	NA	NA	2+2+2=6	6	Ro	1
1	NA	NA	2+2+3=7	7	NA	1
1	15	22	2+2+3=7	7	Ro	1
1	2	7	2+2+2=6	6	Ro	1
1	4	23	2+2+3=7	7	Ro	1
1	1	11	3+3+3=9	9	Ro	1
1	4	9	2+3+3=8	8	Ro	1
1	3	15	3+2+2=7	7	Ro	1
1	NA	NA	2+3+3=8	8	NA	1
2	0	27	2+2+2=6	6	Ro	1
2	0	10	2+2+2=6	6	Ro	1
2	0	14	2+2+2=6	6	Ro	2
2	0	18	2+2+2=6	6	Ro	1
2	0	18	3+3+3=9	9	Ro	1
1	7	26	3+3+3=9	9	Ro	1
1	NA	NA	2+2+2=6	6	Unknown	1
1	4	15	2+2+2=6	6	Ro	1
2	0	19	3+3+3=9	9	Ro	1
2	0	15	3+3+3=9	9	Ro	1
1	5	26	3+3+3=9	9	Ro	1
2	0	10	2+2+3=7	7	Ro	1
1	23	29	2+2+2=6	6	Ro	1
2	0	9	2+2+2=6	6	Ro	1
2	0	10	2+2+2=6	6	Ro	1
2	0	33	2+2+3=7	7	Ro	1
2	0	13	2+2+2=6	6	Ro	2
1	4	22	2+3+1=6	6	Ro	1
1	NA	NA	2+2+2=6	6	Unknown	1
2	0	27	3+2+3=8	8	Ro	1
2	0	11	3+2+2=7	7	Ro	1
1	31	31	2+3+3=8	8	Ro	1
1	1	15	2+3+3=8	8	Ro	1
2	NA	NA	2+3+2=7	7	Unknown	1
1	5	21	3+2+2=7	7	Ro	2
1	1	15	2+2+2=6	6	Ro	1
1	4	35	2+2+3=7	7	Ro	1
1	16	28	3+2+2=7	7	Ro	1
2	0	15	3+3+2=8	8	Ro	1
1	9	16	2+2+3=7	7	Ro	1
1	NA	NA	3+2+2=7	7	NA	2
2	0	10	2+2+2=6	6	Ro	2
1	NA	NA	2+3+2=7	7	NA	1
2	0	21	3+3+3=9	9	Ro	1
1	7	7	3+3+1=7	7	Ro	1
1	1	13	2+2+2=6	6	Ro	1
1	7	23	3+2+3=8	8	Ro	1
2	0	25	2+2+2=6	6	Ro	1
1	NA	NA	2+3+3=8	8	Unknown	1
2	NA	NA	2+2+2=6	6	Unknown	1
1	1	23	3+2+2=7	7	Ro	1
2	0	2	3+2+2=7	7	Ro	1
2	0	13	3+2+2=7	7	Ro	2
1	NA	NA	3+2+2=7	7	Unknown	1
2	0	6	3+3+3=9	9	Ro	1
1	4	15	2+2+2=6	6	Ro	1
2	0	10	2+2+2=6	6	Ro	1
1	7	18	2+2+2=6	6	Ro	1
1	3	11	2+2+2=6	6	Ro	1
1	10	14	3+3+2=8	8	Ro	1
2	NA	NA	2+2+2=6	6	NA	2
1	12	13	3+2+2=7	7	Ro	1
2	NA	NA	2+2+2=6	6	NA	2
2	0	19	2+2+2=6	6	Ro	1
1	5	NA	2+2+2=6	6	NA	1
1	4	21	3+2+2=7	7	Ro	1
1	6	9	3+2+2=8	8	Ro	1
2	NA	NA	1+2+2=5	5	NA	2
1	4	NA	2+2+2=6	6	NA	1
1	2	12	2+2+2=6	6	Ro	1
1	NA	NA	2+2+2=6	6	Unknown	1
2	0	4	2+3+3=7	7	Ro	1
1	7	7	2+2+2=6	6	Ro	1
1	NA	NA	3+3+3=9	9	NA	1
2	0	10	3+2+2=7	7	Ro	1
2	0	10	3+2+2=7	7	Ro	1
2	0	8	2+2+2=6	6	Ro	1
1	NA	NA	2+2+2=6	6	NA	1
1	6	15	3+3+3=9	9	Ro	1
1	NA	NA	2+2+2=6	6	NA	1
1	3	13	3+3+2=8	8	Ro	1
1	1	23	3+3+3=9	9	Ro	1
2	0	10	2+2+2=6	6	Ro	1
1	NA	NA	3+2+2=7	7	NA	1
1	16	23	2+3+3=8	8	Ro	1
2	NA	NA	NA		Ro	2
1	NA	NA	2+2+2=6	6	NA	2
2	NA	NA	3+2+2=8	8	NA	1
2			2+2+2=6	6	NA	1

Nipple/Skin Involment(Positive=1, Negative=2)	ER Status(Positive=1, Negative=2)	PR Status(Positive=1, Negative=2)	Her-2 Status(Positive=1, Negative=2)	ki-67 expression(%)	Molecular Phenotypes	Vitamin D level(nmol/L)
2	2	2	2	70	Triple Negative	25.1
2	2	1	2	60	Luminal B	14.4
2	1	1	2	40	Her2- Luminal B	69.9
2	1	1	1	60	Her2+ Luminal B	42.8
2	1	1	2	NA	Her2- Luminal B	76.1
2	2	2	2	80	Triple Negative	48.2
2	2	2	2	30	Triple Negative	39.3
2	2	2	2	55	Triple Negative	39.6
2	1	1	1	60	Her2+ Luminal B	35.6
2	2	2	1	50	Her2 High	50.3
2	2	2	1	80	Her2 High	51.5
1	1	2	2	60	Her2- Luminal B	63.6
2	1	1	2	50	Her2- Luminal B	10.7
2	1	1	2	30	Her2- Luminal B	33.6
1	2	1	1	60	Luminal B	29.2
2	1	1	1	70	Her2+ Luminal B	50.4
2	2	2	2	40	Triple Negative	66.38
1	2	2	2	80	Triple Negative	31.8
2	2	2	2	80	Triple Negative	28.7
2	1	1	2	60	Her2- Luminal B	49.32
2	2	2	2	60	Triple Negative	23.21
2	1	1	2	60	Her2- Luminal B	56.9
2	1	1	2	70	Her2- Luminal B	48.2
2	2	1	1	35	Her2 High	29.6
2	1	1	1	80	Her2+ Luminal B	53.12
1	2	1	1	70	Her2 High	41.2
2	1	1	2	40	Her2- Luminal B	24.8
1	2	2	2	70	Triple Negative	18.8
2	2	2	2	65	Triple Negative	21.3
2	2	2	1	40	Her2 High	53.2
2	2	1	2	70	Her2- Luminal B	32.6
2	1	1	2	40	Her2- Luminal B	42.2
2	1	1	2	30	Her2- Luminal B	22.6
2	1	1	2	60	Her2- Luminal B	36.1
2	1	1	2	60	Her2- Luminal B	38.5
2	2	1	1	50	Her2 High	39.1
2	1	1	2	40	Her2- Luminal B	58.4
2	2	2	1	60	Her2 High	39.1
2	1	1	2	10	Luminal A	55
2	2	2	2	50	Triple Negative	21.32
2	2	2	2	80	Triple Negative	23.2
2	2	1	2	80	Her2- Luminal B	38.4
2	2	2	2	NA	Triple Negative	24.3
2	1	1	2	80	Her2- Luminal B	64.09
2	1	1	1	70	Her2+ Luminal B	57.34
2	1	1	2	25	Her2- Luminal B	36.42
2	1	1	2	30	Her2+ Luminal B	48.34
2	1	1	2	70	Her2- Luminal B	53.3
2	1	1	1	80	Her2+ Luminal B	7.5
1	1	1	2	NA	Her2- Luminal B	12.8
1	2	2	2	70	Triple Negative	17.6
2	1	1	2	70	Her2- Luminal B	22.2
2	1	1	2	60	Her2- Luminal B	39.71
2	1	1	1	60	Her2+ Luminal B	26.1
2	2	2	1	60	Her2 High	34.67
2	2	2	1	20	Her2 High	49
2	2	2	2	70	Triple Negative	32.3
2	1	1	1	80	Her2+ Luminal B	54.12
2	1	1	2	40	Her2 High	44.31
2	2	1	1	60	Her2+ Luminal B	51.23
2	1	1	2	80	Her2- Luminal B	45.18
2	1	1	2	50	Her2- Luminal B	50.4
2	1	1	2	10	Luminal A	74.1
2	2	1	2	50	Her2- Luminal B	51.2
2	2	2	2	67	Triple Negative	23.6
2	1	1	2	60	Her2- Luminal B	39.8
2	1	1	2	20	Her2- Luminal B	59.9
1	1	1	2	15	Her2- Luminal B	133.4
2	1	1	1	NA	Her2- Luminal B	31.1
2	1	1	2	10	Her2- Luminal B	43.1
2	2	2	1	20	Her2 High	40.23
2	1	1	2	70	Her2- Luminal B	25.5
2	1	1	2	40	Her2- Luminal B	93.45
2	2	2	1	NA	Her2 High	38.12
2	2	2	2	90	Triple Negative	21.1
2	2	2	2	80	Triple Negative	27.43
2	1	2	2	25	Her2- Luminal B	44.36
2	1	1	2	40	Her2- Luminal B	29.34
2	1	1	2	30	Her2- Luminal B	55.1
2	1	2	2	87	Her2- Luminal B	23.12
2	1	1	2	50	Her2- Luminal B	51.2
2	1	1	2	10	Luminal A	56.44
1	1	2	1	70	Her2+ Luminal B	17
2	1	1	2	10	Luminal A	59.31
2	1	2	2	80	Her2- Luminal B	70.1
2	2	2	1	40	Her2 High	30.7
2	2	1	2	50	Her2- Luminal B	42.7
1	2	2	2	80	Triple Negative	89.5
2	1	1	2	NA	Her2- Luminal B	65.3
2	1	1	2	NA	Her2- Luminal B	54.9
2	2	2	1	60	Her2 High	28.3
2	1	1	2	NA	Her2- Luminal B	48.37
2	1	1	2	40	Her2- Luminal B	40.5
2	1	1	2	30	Her2- Luminal B	31.46
1	1	1	2	30	Her2- Luminal B	48.78
2	1	1	2	70	Her2- Luminal B	40.3
2	2	2	1	50	Her2 High	23.89
2	1	2	2	60	Her2- Luminal B	46.32
1	2	2	2	NA	Triple Negative	37.7
2	1	1	2	60	Her2- Luminal B	62.2
2	2	2	1	50	Her2 High	83.69
2	2	2	1	50	Her2 High	22.04
2	2	1	1	70	Her2- Luminal B	52.4
2	2	2	2	80	Triple Negative	65.27
2	1	1	2	60	Her2- Luminal B	81.37
2	1	1	2	80	Her2- Luminal B	26.45
2	1	1	2	8	Luminal A	48.54
2	2	1	1	30	Her2+ Luminal B	44.7
2	1	1	2	50	Her2- Luminal B	39.46
2	1	1	1	75	Her2+ Luminal B	82.43

Vitamin D level nmol/L (<50 as Deficient=1, 50 to <75 as Insufficient=2, 75-250 as Normal=3, >250 as Intoxication=4)	DNA A260	DNA A280	Ratio A260/A280	Genotypes FokI FF=1, Ff=2, ff=3	Genotypes ApaI AA=1, Aa=2, aa=3	Genotypes BsmI BB=1, Bb=2
1	0.079	0.044	1.79	3	1	2
1	0.141	0.08	1.76	1	1	2
2	0.091	0.05	1.82	1	1	2
1	0.062	0.034	1.82	3	2	2
3	0.109	0.064	1.7	2	1	1
1	0.109	0.064	1.7	3	1	2
1	0.14	0.079	1.77	3	1	2
1	0.151	0.086	1.75	3	1	1
1	0.121	0.071	1.7	1	1	2
2	0.112	0.065	1.72	3	2	1
2	0.102	0.06	1.7	2	1	2
2	0.132	0.073	1.8	1	1	2
1	0.132	0.075	1.76	1	2	2
1	0.177	0.098	1.8	1	2	1
1	0.126	0.074	1.7	2	1	1
2	0.143	0.089	1.76	1	1	2
2	0.127	0.07	1.81	3	2	2
1	0.119	0.069	1.72	2	3	1
1	0.11	0.064	1.71	1	3	2
1	0.186	0.101	1.84	1	1	2
1	0.134	0.074	1.81	1	2	2
2	0.111	0.064	1.73	3	1	1
1	0.161	0.089	1.8	1	1	2
1	0.131	0.072	1.81	3	2	1
2	0.164	0.091	1.8	1	2	1
1	0.176	0.097	1.81	3	1	2
1	0.108	0.06	1.8	1	2	2
1	0.139	0.077	1.8	1	3	1
1	0.175	0.097	1.8	2	3	2
2	0.169	0.094	1.79	3	1	1
1	0.125	0.069	1.81	2	3	1
1	0.103	0.057	1.8	1	2	2
1	0.174	0.096	1.81	1	1	1
1	0.102	0.057	1.78	3	1	2
1	0.144	0.08	1.8	1	2	2
1	0.175	0.097	1.8	1	1	2
2	0.138	0.076	1.81	1	3	2
1	0.179	0.099	1.8	3	2	1
2	0.159	0.087	1.82	2	1	2
1	0.121	0.066	1.83	1	3	2
1	0.15	0.083	1.8	3	2	2
1	0.141	0.078	1.8	1	3	2
1	0.143	0.08	1.79	3	1	2
2	0.119	0.066	1.8	2	2	1
2	0.133	0.073	1.82	1	1	2
1	0.124	0.068	1.82	3	1	2
1	0.121	0.067	1.8	1	1	1
2	0.155	0.086	1.8	2	1	1
1	0.128	0.071	1.8	1	1	1
1	0.115	0.067	1.71	1	2	1
1	0.117	0.068	1.72	2	1	2
1	0.126	0.072	1.75	1	2	2
1	0.101	0.055	1.83	2	1	2
1	0.154	0.09	1.71	3	3	1
1	0.181	0.101	1.79	1	2	1
1	0.152	0.089	1.7	1	2	2
1	0.154	0.085	1.81	1	1	1
2	0.168	0.093	1.8	1	2	2
1	0.165	0.091	1.81	2	2	2
2	0.031	0.018	1.72	1	2	2
1	0.109	0.061	1.78	2	1	1
2	0.127	0.07	1.81	1	1	2
2	0.182	0.101	1.8	3	2	1
2	0.131	0.072	1.81	2	2	2
1	0.173	0.096	1.8	2	1	1
1	0.146	0.081	1.8	1	2	2
2	0.103	0.057	1.8	2	2	1
3	0.141	0.078	1.8	1	2	1
1	0.122	0.067	1.82	1	1	2
1	0.127	0.073	1.73	1	2	2
1	0.126	0.07	1.8	2	2	1
1	0.12	0.069	1.73	1	2	1
3	0.131	0.072	1.81	2	2	2
1	0.129	0.071	1.81	1	1	1
1	0.111	0.062	1.79	2	2	1
1	0.145	0.081	1.79	2	1	1
1	0.147	0.081	1.81	2	2	1
1	0.101	0.058	1.74	1	1	2
2	0.124	0.069	1.79	2	2	1
1	0.109	0.06	1.81	1	1	2
2	0.117	0.065	1.8	1	2	2
2	0.132	0.073	1.8	1	1	3
1	0.145	0.082	1.79	1	2	2
2	0.149	0.082	1.81	1	1	3
2	0.148	0.083	1.78	2	1	1
1	0.147	0.081	1.81	1	2	2
1	0.121	0.068	1.77	1	1	1
3	0.124	0.069	1.79	1	3	3
2	0.102	0.056	1.8	2	2	1
2	0.128	0.071	1.8	1	2	1
1	0.123	0.068	1.8	2	1	1
1	0.191	0.106	1.8	2	2	2
1	0.021	0.012	1.75	1	1	2
1	0.098	0.054	1.81	1	1	1
1	0.122	0.067	1.82	1	2	2
1	0.119	0.066	1.8	2	1	1
1	0.124	0.068	1.82	2	1	2
1	0.181	0.1	1.8	1	2	2
1	0.132	0.073	1.8	1	1	1
2	0.126	0.07	1.8	1	1	2
3	0.101	0.056	1.8	1	1	3
1	0.085	0.047	1.8	2	3	1
2	0.13	0.072	1.8	2	2	2
2	0.125	0.069	1.81	1	2	1
3	0.133	0.074	1.79	2	1	1
1	0.135	0.075	1.8	1	2	2
1	0.099	0.055	1.8	2	1	1
1	0.146	0.081	1.8	1	2	2
1	0.103	0.057	1.8	1	1	2
3	0.125	0.069	1.81	1	1	1

, bb=3

S.No.	Name	UHID	Name of Participating Centre	Name of the Department	Date of first attendance to hospital for Routine Checkup/ Sample collection	Address
1	Anupa	2981750	Swami Rama Himalayan University, Dehradun, Uttarakhand	Dermatology	6/3/2020	Athoorwala, Dehradun
2	Payal Chauhan	2840224	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	6/3/2020	Jolly grant, Dehradun
3	Savita Pande	2407251	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	7/22/2020	Pauri, Garhwal, Uttarakhand
4	Sunita	3001398	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	7/23/2020	Bhaniyawala, Dehradun
5	Anita Dabral	3007900	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	8/10/2020	Rishikesh, Uttarakhand
6	Rani	3008725	Swami Rama Himalayan University, Dehradun, Uttarakhand	Ortho Unit-I	8/13/2020	Bijnor, U.P.
7	Bahdi Devi	3009920	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	8/17/2020	Village Sunargaon, Uttarakhand
8	Tabasum	3371134	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	9/10/2020	Haridwar, Uttarakhand
9	Mamta Aswal	2693764	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	9/17/2020	4/1, Teg Bhadur Road, Dehradun
10	Prakash Devi	3024273	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	9/21/2020	Bapu gram, Rishikesh, Uttarakhand
11	Raj Kumari	3030282	Swami Rama Himalayan University, Dehradun, Uttarakhand	Ortho Unit-II	10/6/2020	Bijnor, U.P.
12	Vibha Ghildiyal	3032080	Swami Rama Himalayan University, Dehradun, Uttarakhand	Dermatology	10/10/2020	Staff, Jolly grant, Dehradun
13	Neha Sharma	3044254	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	11/13/2020	Bijnor, U.P.
14	Sushila	3047617	Swami Rama Himalayan University, Dehradun, Uttarakhand	Dermatology	11/23/2020	Peeth Bazar, Haridwar, Uttarakhand
15	Hajra	3059574	Swami Rama Himalayan University, Dehradun, Uttarakhand	Ortho Unit-II	12/22/2020	Bijnor, U.P.
16	Mohini Sethi	2318370	Swami Rama Himalayan University, Dehradun, Uttarakhand	Ortho Unit-I	1/1/2021	Staff, Jolly grant, Dehradun
17	Vandana Kukreti	3065767	Swami Rama Himalayan University, Dehradun, Uttarakhand	Ortho Unit-II	1/6/2021	Staff, Jolly grant, Dehradun
18	Kamlesh	3062434	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-II	1/7/2021	Village Satpura, U.P.
19	Prem Lata	3066932	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	1/9/2021	Sai Dham, Phase 4, Haridwar, Uttarakand
20	Amita	3067923	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	1/11/2021	228, Salawala, Dehradun
21	Sunita	3068184	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	1/12/2021	Staff, Jolly grant, Dehradun
22	Manju Rastogi	3071701	Swami Rama Himalayan University, Dehradun, Uttarakhand	General Medicine Unit-III	1/21/2021	Kila Afjalgarh,Bijnor, U.P.
23	Santosh Devi	3079723	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	2/8/2021	Amroha, U.P.
24	Alma Panwar	3080918	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-II	2/11/2021	Village Chidderwala, Uttarakhand
25	Meenu Pradhan	3082546	Swami Rama Himalayan University, Dehradun, Uttarakhand	General Medicine Unit-III	2/13/2021	Solani puram, Roorkee, Uttarakhand
26	Kamla Sharma	3083879	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	2/17/2021	Bhaniyawala, Dehradun
27	Adesh Devi	3084158	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	2/18/2021	Surjannagar, Moradabad, U.P.
28	Manju Panday	3084311	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	2/18/2021	Awas vikas Modal, Haridwar, Uttarakhand
29	Sushma Devi	3077931	Swami Rama Himalayan University, Dehradun, Uttarakhand	Ortho Unit-II	2/26/2021	Rani Pokhri, Dehradun
30	Jahana	3096776	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	3/13/2021	Village Mebla, U.P.
31	Gunjan Tomar	2915835	Swami Rama Himalayan University, Dehradun, Uttarakhand	General Medicine Unit-III	3/24/2021	Jolly grant, Dehradun
32	Raija Devi	3110128	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	4/5/2021	Dehradun
33	Meenu Majumdar	3313819	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	4/10/2021	Bijnor, U.P.
34	Priyanka Gupta	2819744	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	5/24/2021	Dehradun
35	Munni Devi	3130745	Swami Rama Himalayan University, Dehradun, Uttarakhand	Dermatology	6/1/2021	Jolly grant, Dehradun
36	Neeru goyal	3137308	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	6/17/2021	Haridwar, Uttarakhand
37	Reena	3141515	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	6/26/2021	Village Sherpur, Uttarakhand
38	Vinita Devi	3142924	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	6/30/2021	Bhaniyawala, Dehradun
39	Anita Rani	3071908	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	7/17/2021	Haridwar, Uttarakhand
40	Savita Devi	3154522	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	7/23/2021	Dehradun
41	Sarojni Kukreti	3154924	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	7/24/2021	Pauri, Garhwal, Uttarakhand
42	Rajni	3160572	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	8/4/2021	Saharanpur, U.P.
43	Kavita Bisht	3160977	Swami Rama Himalayan University, Dehradun, Uttarakhand	E.N.T	8/4/2021	Tehri Garhwal, Uttarakhand
44	Sarla	3161642	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	8/6/2021	Saharanpur, U.P.
45	Kiran Rawat	3156226	Swami Rama Himalayan University, Dehradun, Uttarakhand	Dermatology	8/7/2021	Doiwala, Dehradun
46	Amrit Kaur	3164327	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	8/11/2021	Kheri Doiwala, Dehradun
47	Usha Devi	3173356	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	8/27/2021	Moradabad, U.P.
48	Namita Nautiyal	3084192	Swami Rama Himalayan University, Dehradun, Uttarakhand	E.N.T	8/31/2021	Shaheed Dwar, Athoorwala, Dehradun
49	Babita Rani	3187724	Swami Rama Himalayan University, Dehradun, Uttarakhand	Ortho Unit-III	9/22/2021	Haridwar, Uttarakhand
50	Baljeet Kaur	3198598	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	10/12/2021	Doiwala, Dehradun
51	Urmila Devi	3200366	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	10/16/2021	Sivalik nagar, Haridwar, Uttarakhand
52	Chanchal Rani	3200509	Swami Rama Himalayan University, Dehradun, Uttarakhand	Ortho Unit-III	10/16/2021	83-Shivalik nagar, Haridwar, Uttarakhand
53	Anisa	3204201	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	10/23/2021	Village Sambhal, U.P.
54	Munni Devi	3214524	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	11/13/2021	Tehri Garhwal, Uttarakhand
55	Mirdula Rani	3215148	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	11/15/2021	Bijnor, U.P.
56	Shanti Devi	3223017	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	12/2/2021	Dehradun
57	Geetanjali	3228007	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	12/13/2021	Chamoli, Uttarakahand
58	Anita Kapurwan	3097471	Swami Rama Himalayan University, Dehradun, Uttarakhand	Ortho Unit- II	1/4/2022	Rani Pokhri, Dehradun
59	Sundra Devi	3244960	Swami Rama Himalayan University, Dehradun, Uttarakhand	Ortho Unit- III	1/19/2022	Bhaniyawala, Dehradun
60	Renu gaur	3258468	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	2/25/2022	H.No. 14-Jeewani mai wali gali, Rishikesh, Utt
61	Sunita Devi	3256103	Swami Rama Himalayan University, Dehradun, Uttarakhand	Ortho Unit-II	3/4/2022	62-Shiv nagar, Dehradun
62	Charanjeet Kaur	3262763	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	3/5/2022	U S nagar, Uttarakhand
63	Savita	3265129	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	3/9/2022	Bijnor, U.P.
64	Deepa Devi	3271294	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	3/21/2022	Rishikesh, Uttarakhand
65	Manju	3274617	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-II	3/25/2022	322, Gurukul Kangari, Haridwar, Uttarakhand
66	Lalita Nayal	2639625	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	3/28/2022	Dehradun
67	Raksha Chauhan	3283227	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-II	4/8/2022	Bijnor, U.P.
68	Sunita Rani	3284753	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	4/11/2022	Bijnor, U.P.
69	Jonny Devi	3306496	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	5/18/2022	Saharanpur, U.P.
70	Rima Gupta	3320277	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	6/8/2022	Moradabad, U.P.
71	Abha Gupta	3318958	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	6/9/2022	Dehradun
72	Anusuiya Bartwal	3323904	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	6/15/2022	Athoorwala, Dehradun
73	Savita Singh	3327137	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	6/20/2022	Haridwar, Uttarakhand
74	Urmila Devi	3352464	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	8/1/2022	Vinayal hills, Shastradhara road, Dehradun
75	Seema Tyagi	3358196	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	8/13/2022	Bijnor, U.P.
76	Rambatauri	3358731	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	8/17/2022	Dehradun
77	Nirdesh	3362728	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	8/24/2022	Bijnor, U.P.
78	Sushma Devi	3364622	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	8/27/2022	Saharanpur, U.P.
79	Vinita Kandwal	3367562	Swami Rama Himalayan University, Dehradun, Uttarakhand	E.N.T	9/2/2022	Amit gram gumaniwala, Dehradun
80	Shabana Khan	3368228	Swami Rama Himalayan University, Dehradun, Uttarakhand	Ortho Unit-I	9/5/2022	Haridwar, Uttarakhand
81	Asha Kunj	2242429	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	9/17/2022	Staff, Jolly grant, Dehradun
82	Sunita Sharma	2665780	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	9/26/2022	Staff, Jolly grant, Dehradun
83	Rajeshwari Devi	3378874	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	9/29/2022	Pauri, Garhwal, Uttarakhand
84	Yukta Rajput	3378874	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	10/10/2022	Bijnor, U.P.
85	Saraswati	3255718	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	10/13/2022	U S nagar, Uttarakhand
86	Anuradha	2723712	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	10/15/2022	Bijnor, U.P.
87	Nazneen Bano	3388635	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	10/22/2022	Roorkee, Uttarakhand
88	Alka Devi	3158771	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	10/28/2022	Saharanpur, U.P.
89	Gauri	3392386	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	11/1/2022	WNo. 13, Ranipokhri, Dehradun
90	Manju Kaushal	3096906	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-II	11/11/2022	Vikas nagar, Dehradun
91	Shivani Sharma	3396723	Swami Rama Himalayan University, Dehradun, Uttarakhand	Ortho Unit-II	11/11/2022	Haridwar, Uttarakhand
92	Sutlesh	3396862	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	11/14/2022	WNo. 5, Ranjhawala, Dehradun
93	Pooja	3398920	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	11/16/2022	Haridwar, Uttarakhand
94	Sunita Devi	3400332	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	11/19/2022	Tehri Garhwal, Uttarakhand
95	Rupa Devi	3397731	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	11/21/2022	Haridwar, Uttarakhand
96	Kamlesh	3410905	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	11/23/2022	U S nagar, Uttarakhand
97	Usha Devi	3404810	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	11/30/2022	Athoorwala, Dehradun
98	Ritika agarwal	2763389	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	12/1/2022	Staff, Jolly grant, Dehradun
99	Ganeshi Devi	3407259	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	12/6/2022	Pauri, Garhwal, Uttarakhand
100	Geeta rana	3257903	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	12/8/2022	Staff, Jolly grant, Dehradun
101	Anjali arora	3414951	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	12/26/2022	U S nagar, Uttarakhand
102	Manju Kothari	3415459	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-II	12/27/2022	Doiwala, Dehradun
103	Anita Bisht	2916705	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	1/7/2023	Bhaniyawala, Dehradun
104	Sulochana	3420749	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	1/9/2023	Tehri Garhwal, Uttarakhand
105	Sheetal	3421600	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	1/11/2023	U S nagar, Uttarakhand
106	Snehlata sharma	3307451	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-I	1/27/2023	Staff, Jolly grant, Dehradun
107	Ashiya	3430666	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	2/4/2023	Haridwar, Uttarakhand
108	Monika Biswas	3431731	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	2/7/2023	Staff, Jolly grant, Dehradun
109	Surama Devi	3434736	Swami Rama Himalayan University, Dehradun, Uttarakhand	HIMS	2/14/2023	Tehri Garhwal, Uttarakhand
110	Parvati Devi	3362964	Swami Rama Himalayan University, Dehradun, Uttarakhand	Medicine Unit-III	8/24/2023	Athoorwala, Dehradun

Rural=1/Urban=2/Semi-Urban=3	Mobile no.	Age	Sex	Menopausal status (Pre=1, post=2)	Weight (kg)	Height (cm)	BMI(kg/m²)	BMI category (Normal=1, Underweight=2, Overweight=3, Obese class I=4)
2	9411142880	38	Female	1	56	159	22.2	1
2	7017960496	30	Female	1	51	160	19.9	1
3	9557877664	47	Female	1	53	161	20.4	1
2	7414598764	55	Female	2	50	154	21.1	1
2	8126202021	48	Female	2	54	154	22.8	1
2	6397129708	32	Female	1	49	161	18.9	1
1	7302717417	50	Female	2	53	167	19	1
2	7906942233	30	Female	1	49	162	18.7	1
2	9410314098	53	Female	2	58	152	25.1	3
2	958249226	65	Female	2	52	159	20.6	1
2	9368263627	40	Female	1	51	161	19.7	1
2	9411156293	57	Female	2	58	165	21.3	1
2	8941014997	36	Female	1	60	163	22.6	1
2	9897750170	44	Female	1	51	165	18.7	1
2	8057306401	46	Female	1	61	161	23.2	1
2	9438567623	66	Female	2	64	158	25.6	3
2	7351116177	41	Female	1	48	149	21.6	1
1	7253050830	41	Female	1	48	166	17.4	2
2	9958383086	55	Female	2	39	150	17.3	2
2	9456590746	36	Female	1	55	160	21.5	1
2	8218539943	49	Female	2	57	165	20.9	1
2	9410600001	57	Female	2	62	159	24.5	1
2	8532870716	51	Female	2	51	155	21.2	1
1	8859449223	60	Female	2	49	162	18.7	1
2	7895298039	59	Female	2	65	159	25.7	3
2	9719298959	46	Female	1	51	161	19.7	1
2	7017556475	45	Female	1	57	165	20.9	1
2	9897425471	42	Female	1	60	154	25.3	3
2	9412985064	51	Female	2	50	162	19.1	1
1	7417248559	35	Female	1	59	165	21.7	1
2	9412158901	40	Female	1	56	162	21.3	1
2	7248121568	45	Female	1	52	161	20.1	1
2	8449955170	55	Female	2	48	162	18.3	2
2	6261883112	40	Female	1	53	160	20.7	1
2	9534493059	62	Female	2	45	158	18	2
2	9837326188	42	Female	1	59	153	25.2	3
1	8865894326	32	Female	1	54	164	20.1	1
2	7415639874	36	Female	1	47	159	18.6	1
2	9760325992	50	Female	2	54	165	19.8	1
2	9411771547	46	Female	1	57	163	21.5	1
3	9410744733	51	Female	2	49	152	21.2	1
2	9719678085	33	Female	1	47	159	18.6	1
3	8968876612	30	Female	1	54	162	20.6	1
2	8859018204	57	Female	2	54	163	20.3	1
2	7895483129	37	Female	1	51	160	19.9	1
2	9837154452	32	Female	1	57	163	21.5	1
2	9649479679	55	Female	2	57	163	21.5	1
2	8449891263	35	Female	1	49	158	19.6	1
2	830754546	45	Female	1	52	160	20.3	1
2	7983488117	34	Female	1	55	163	20.7	1
2	7705042267	40	Female	1	48	159	19	1
2	9911046969	60	Female	2	48	162	18.3	2
1	6398692576	44	Female	1	61	165	22.4	1
3	9647862391	40	Female	1	56	159	22.2	1
2	9410263125	45	Female	1	62	164	23.1	1
2	8057688048	35	Female	1	56	166	20.3	1
3	8979566361	43	Female	1	55	159	21.8	1
2	9756612755	40	Female	1	52	163	19.6	1
2	9084083818	65	Female	2	61	155	25.4	3
2	9867563522	49	Female	2	51	167	18.3	2
2	877434947	46	Female	1	57	164	21.2	1
2	8006000254	43	Female	1	58	159	22.9	1
2	9760905160	38	Female	1	58	164	21.6	1
2	8861899450	38	Female	1	49	161	18.9	1
2	7456961833	45	Female	1	58	158	23.2	1
2	9410170728	42	Female	1	61	164	22.7	1
2	8057923429	63	Female	2	54	161	20.8	1
2	9568464890	41	Female	1	56	163	21.1	1
2	9761422683	32	Female	1	57	162	21.7	1
2	9058298310	49	Female	2	56	149	25.6	3
2	9897256189	50	Female	2	51	157	20.7	1
2	7078287118	32	Female	1	47	155	19.6	1
2	9045775183	46	Female	1	42	161	16.2	2
2	7070834349	58	Female	2	62	164	23.1	1
2	9675063599	38	Female	1	56	163	21.1	1
2	9528178050	39	Female	1	48	160	18.7	1
2	9759012549	41	Female	1	58	159	22.9	1
2	9837013422	48	Female	2	57	165	20.9	1
2	9997952238	34	Female	1	49	160	19.1	1
2	9997211116	33	Female	1	54	159	21.4	1
2	8650820711	50	Female	1	56	164	20.8	1
2	8650384890	36	Female	1	58	159	22.9	1
3	9675656504	56	Female	2	49	160	19.1	1
2	9634810273	31	Female	1	56	164	20.8	1
2	7300740113	30	Female	1	58	160	22.7	1
2	9927560126	33	Female	1	60	164	22.3	1
2	9319540500	48	Female	2	57	161	22	1
2	9756692464	51	Female	2	63	162	24	1
2	6397698163	49	Female	2	58	159	22.9	1
2	8273719044	51	Female	2	63	164	23.4	1
2	9690310698	30	Female	1	55	162	21	1
2	9837520465	62	Female	2	51	163	19.2	1
2	9927931446	35	Female	1	48	162	18.3	2
3	9557934352	30	Female	1	54	164	20.1	1
2	9149105759	33	Female	1	57	161	22	1
2	8534849970	46	Female	1	59	165	21.7	1
2	8527375553	66	Female	2	61	162	23.2	1
2	9411714685	37	Female	1	45	159	17.8	2
3	8126138832	56	Female	2	59	162	22.5	1
2	9411642829	36	Female	1	48	158	19.2	1
2	7302041166	45	Female	1	53	165	19.5	1
2	7895242122	43	Female	1	56	164	20.8	1
2	9418751796	52	Female	2	49	161	18.9	1
3	9557681443	50	Female	2	59	164	21.9	1
2	7017195433	52	Female	2	54	161	20.8	1
2	8449741172	46	Female	1	59	164	21.9	1
2	9012647754	54	Female	2	48	161	18.5	1
2	8979151623	49	Female	2	56	160	21.9	1
2	8979423039	53	Female	2	61	164	22.7	1
2	9675318190	54	Female	1	61	164	22.7	1

History of any cancer	Co-Morbid conditions	Vitamin D level(nmol/L)	Vitamin D level nmol/L (<50 as Deficient=1, 50 to <75 as Insufficient=2, 75-250 as Normal=3, >250 as Intoxication=4)	DNA A260	DNA A280	Ratio A260/A280
None	None	114.7	3	0.162	0.09	1.8
None	None	68	2	0.171	0.096	1.78
None	None	107.7	3	0.191	0.11	1.73
None	None	99	3	0.108	0.06	1.8
None	None	156	3	0.147	0.081	1.81
None	None	51	2	0.139	0.076	1.82
None	None	79.2	3	0.142	0.081	1.75
None	None	89.32	3	0.122	0.069	1.76
None	None	77.5	3	0.117	0.068	1.72
None	None	102.7	3	0.119	0.07	1.7
None	None	167.7	3	0.181	0.101	1.79
None	None	125.2	3	0.184	0.102	1.8
None	None	99.7	3	0.166	0.093	1.78
None	None	100.5	3	0.129	0.072	1.79
None	None	71.5	2	0.125	0.069	1.81
None	None	27.5	1	0.112	0.065	1.72
None	None	47.2	1	0.132	0.075	1.76
None	None	164.2	3	0.179	0.101	1.77
None	None	43.2	1	0.141	0.081	1.74
None	None	68.5	2	0.178	0.101	1.76
None	None	114	3	0.168	0.093	1.8
None	None	112.2	3	0.129	0.072	1.79
None	None	68.2	2	0.152	0.084	1.8
None	None	46.5	1	0.119	0.069	1.72
None	None	103	3	0.182	0.101	1.8
None	None	48.9	1	0.127	0.071	1.78
None	None	53.2	1	0.152	0.084	1.8
None	None	63.6	2	0.147	0.082	1.79
None	None	51.2	2	0.115	0.067	1.71
None	None	50.8	2	0.121	0.071	1.7
None	None	76.2	3	0.159	0.088	1.8
None	None	59.4	2	0.135	0.075	1.8
None	None	101	3	0.159	0.089	1.78
None	None	88.5	3	0.142	0.079	1.79
None	None	94.1	3	0.172	0.095	1.81
None	None	72.4	2	0.156	0.087	1.79
None	None	71.4	2	0.151	0.083	1.81
None	None	59.3	2	0.163	0.092	1.77
None	None	121.7	3	0.153	0.09	1.7
None	None	101	3	0.155	0.086	1.8
None	None	90.9	3	0.161	0.089	1.8
None	None	98.2	3	0.108	0.06	1.8
None	None	94.5	3	0.131	0.075	1.74
None	None	89.2	3	0.105	0.061	1.72
None	None	101.4	3	0.145	0.08	1.81
None	None	39.2	1	0.136	0.08	1.7
None	None	106.3	3	0.154	0.09	1.71
None	None	89.2	3	0.166	0.092	1.8
None	None	89.1	3	0.141	0.081	1.74
None	None	88.7	3	0.106	0.061	1.73
None	None	66.7	2	0.119	0.066	1.8
None	None	106.2	3	0.178	0.101	1.76
None	None	66.5	2	0.101	0.057	1.77
None	None	115	3	0.175	0.102	1.71
None	None	152	3	0.184	0.104	1.76
None	None	71.5	2	0.111	0.061	1.81
None	None	78.7	3	0.181	0.101	1.79
None	None	87.8	3	0.191	0.106	1.81
None	None	108.7	3	0.177	0.102	1.73
None	None	79.6	3	0.17	0.1	1.7
None	None	106	3	0.169	0.094	1.79
None	None	75.8	3	0.173	0.096	1.8
None	None	71.3	2	0.115	0.064	1.79
None	None	81.3	3	0.11	0.061	1.8
None	None	99.6	3	0.115	0.063	1.82
None	None	95	3	0.119	0.066	1.8
None	None	89	3	0.138	0.077	1.79
None	None	62.4	2	0.125	0.071	1.76
None	None	77	3	0.129	0.071	1.81
None	None	89.78	3	0.129	0.072	1.79
None	None	77.46	3	0.158	0.089	1.77
None	None	78.42	3	0.181	0.104	1.74
None	None	81.22	3	0.183	0.107	1.71
None	None	85.3	3	0.141	0.078	1.8
None	None	77.65	3	0.163	0.092	1.77
None	None	80.41	3	0.159	0.091	1.74
None	None	84.7	3	0.116	0.068	1.7
None	None	82.39	3	0.108	0.06	1.8
None	None	100.56	3	0.117	0.068	1.72
None	None	91.01	3	0.133	0.078	1.7
None	None	75.59	3	0.141	0.081	1.74
None	None	77.46	3	0.155	0.091	1.7
None	None	76.67	3	0.166	0.092	1.8
None	None	109.22	3	0.123	0.068	1.8
None	None	110.96	3	0.111	0.065	1.7
None	None	83.26	3	0.151	0.087	1.73
None	None	91.94	3	0.102	0.057	1.78
None	None	155.5	3	0.109	0.06	1.81
None	None	80.82	3	0.177	0.102	1.73
None	None	87.19	3	0.171	0.1	1.71
None	None	84.96	3	0.164	0.091	1.8
None	None	80.37	3	0.116	0.068	1.7
None	None	85.99	3	0.161	0.089	1.8
None	None	108.67	3	0.119	0.07	1.7
None	None	96.99	3	0.183	0.105	1.74
None	None	102.43	3	0.192	0.109	1.76
None	None	130.47	3	0.101	0.056	1.8
None	None	101.42	3	0.133	0.078	1.7
None	None	77.77	3	0.141	0.078	1.8
None	None	125.76	3	0.125	0.069	1.81
None	None	115.74	3	0.117	0.065	1.8
None	None	103.73	3	0.116	0.064	1.81
None	None	81.45	3	0.137	0.08	1.71
None	None	148.14	3	0.139	0.081	1.71
None	None	148.4	3	0.147	0.082	1.79
None	None	99.61	3	0.191	0.109	1.75
None	None	75.33	3	0.196	0.109	1.79
None	None	75.37	3	0.181	0.105	1.72
None	None	83.6	3	0.187	0.106	1.76
None	None	91.2	3	0.144	0.08	1.8

Genotypes FokI FF=1, Ff=2, ff=3	Genotypes ApaI AA=1, Aa=2, aa=3	Genotypes BsmI BB=1, Bb=2, bb=3
3	1	1
2	1	2
1	2	1
1	1	2
2	1	3
1	2	1
2	1	2
2	1	1
1	2	2
1	1	1
1	2	3
3	1	2
2	1	1
1	2	2
2	1	1
1	3	1
1	2	2
2	2	3
1	2	1
2	3	1
1	1	1
1	2	1
2	1	1
1	3	2
1	1	1
1	3	1
1	2	2
2	1	1
1	2	2
1	1	1
1	2	2
2	2	2
1	2	2
3	1	1
1	2	2
1	1	1
1	2	1
1	1	2
2	1	1
1	2	1
1	1	2
1	2	2
1	1	2
1	2	1
2	1	2
1	2	1
1	2	1
2	1	1
1	1	1
1	2	2
2	1	2
2	1	1
1	2	1
1	1	2
1	2	1
1	1	1
2	1	1
1	1	2
1	2	1
2	1	1
2	2	2
1	2	2
1	1	2
2	2	1
1	1	2
2	1	1
2	2	2
2	1	1
1	1	2
2	1	1
1	2	2
2	1	1
1	1	1
1	1	2
2	2	1
1	2	2
1	1	2
1	2	1
1	1	2
1	2	2
2	1	1
1	2	2
2	1	1
2	1	2
1	2	1
1	1	2
2	1	2
1	2	1
2	1	1
2	2	2
3	1	1
1	1	2
2	1	2
1	2	2
2	1	1
2	2	2
1	1	2
2	3	1
1	1	2
2	1	2
1	1	1
1	2	2
2	1	1
2	1	1