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# Factors Associated with Breast Cancer Risk According to Tumor Subtypes: Triple Negative Breast Cancer vs. NON-Triple Negative Breast Cancer Among Women in Karachi, Pakistan

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## ABSTARCT

***Objective:*** To evaluate risk factors associated with triple negative breast cancer among women in Karachi Pakistan
***Study Design:*** Multicenter hospital-based case control study
***Place and Duration of Study:*** Aga Khan University Hospital and Karachi Institute of Radiation & Nuclear Medicine Karachi, Pakistan from February 2015 to July 2018.
***Methodology:*** There were three hundred and eighty cases of breast cancer patients who had complete molecular profiling and who were compared with 798 controls and in person interviews were conducted.
***Results:*** The multivariable multinomial logistic regression analyses showed that both triple negative breast cancer and non-triple negative breast cancer subtypes were associated with poor socioeconomic status and low Vitamin D concentrations with triple negative breast cancer risk much higher among women of low socioeconomic status (OR=8.76, 95% CI= 2.45, 31.32) and women with vitamin D deficiency (OR=3.11, 95%CI= 1.17. 8.29).
***Conclusions:*** Correction of Vitamin D deficiency in women maybe a possible cost-effective strategy to prevent triple negative breast cancer like aggressive breast cancer. It should be further tested though cohort studies or clinical trials in our population.

**Key words:** Breast cancer, socioeconomic status, triple negative, vitamin D deficiency

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## INTRODUCTION

Research shows that breast cancer is a heterogeneous disease. Estrogen and progesterone hormone receptor (ER/PR) protein expression status and human epidermal growth factor (HER2) protein expression or gene amplification are important biomarkers with variable risk factors, clinical & pathologic outcomes.[1-3] On the basis of hormone receptor status and gene expression pattern, breast cancer can be classified into four major intrinsic subtypes: luminal A, luminal B, human epidermal growth factor receptor 2 (HER2) enriched, and triple negative breast cancer (TNBC). Expression of estrogen receptor (ER), progesterone receptor (PR), and HER2-neu (HER2) alone are usually used to differentiate between these subtypes in clinical settings. It is also observed that there are possible drifts in molecular subtype throughout breast cancer progression.[4] TNBC has no hormonal markers, and is usually high grade of poorly differentiated type. It has usually poor prognosis with higher risk of recurrence and high five-year mortality rates.[5]

All these breast cancer subtypes are associated with different risk factor due to different etiologies. Older age at first pregnancy is found to be positively associated with HER2 positive subtype in research literature.[6] Analysis in the Nurses' Health Study (NHS) with 2,022 cases reported that the luminal A subtype is associated with reproductive risk factors like age at menarche, and age at first birth.[7] Family history of breast cancer is differentially associated with breast cancer subtypes. In a study in Spain, family history of breast cancer was related to an increased risk of ER-&PR- breast cancer among younger Spanish women.However, studies of breast cancer subtypes conducted among Asian populations' especially Pakistani women are extremely limited. Moreover, there is a lack of information on how Vitamin D deficiency influences the risk of different molecular subtypes of breast cancer. There are contradictory findings of relationship between Vitamin D and breast cancer could be related to tumor heterogeneity, which suggests that effects of Vitamin D may only be exhibited in specific subtypes of breast cancer. Therefore, additional analysis with Vitamin D was conducted on specific breast cancer subtypes. The

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objective of the study was to evaluate the association of breast cancer risk with molecular subtypes of breast cancer (TNBC vs non TNBC).

## METHODOLOGY

The study population for this study was from the multi-center case–control study of breast cancer and Vitamin D study among women visiting two hospitals of Karachi Pakistan.

**Inclusion Criteria:** Breast cancer patients who had complete molecular profiling were included along with controls.

**Exclusion Criteria:** Patients who had a 2+ HER2 immunohistochemistry result without a FISH result were considered to have an inconclusive and, thus, unknown HER2 status.

The ethical approval was obtained by the Human Research Ethics Committee of the University of Adelaide (H-2014-111) and the Ethical Review Committees (ERC) of two hospitals in Karachi Pakistan: Aga Khan University Hospital AKUH(3074 CHS-ERC) & Karachi Institute of Radiation and Nuclear Medicine Hospital KIRAN(Kiran -2(22) All participants provided written informed consent while informed consent was obtained verbally from those who could not read or write as approved by the ERC.Briefly, there were 380 patients who had complete IHC staining for ER, PR, and HER2 and were compared with 798 controls. ER, PR, and HER2 status of participants were recorded as cases were retrieved from medical records of the medical files. Cases with HER2 results of 0, 1+, or 2+ from IHC testing and/or negative results on FISH testing <2 were considered HER2 negative (HER2−); conversely, HER2 results of 3+ on IHC testing were considered HER2 positive (HER2+). Breast cancer subtyping was based on immunohistochemical (IHC) staining which was part of routine diagnostics and performed according to the College of American pathologists (CAP) Clinical Practice Guidelines.[8] On the basis of these receptors, breast cancer subtypes were classified into four groups: ER+ and/or PR+/HER2−; ER+ and/or PR+/ HER2+; ER−/PR−/HER2+; and ER−/PR−/HER2−. Missing values for ER and PR status were minimized by accessing the patients' records and getting their information from labs outside AKUH and KIRAN. Finally, breast cancer subtypes were broadly divided as TNBC (ER−/PR−/HER2− ) and non TNBC subtypes (ER+ and/or PR+/HER2−; ER+ and/or PR+/ HER2+; ER−/PR−/HER2+ subtypes were merged). In addition to basic information on breast cancer diagnosis, information on tumor histology was extracted.All analyses were conducted using SPSS package for Windows 21.0 (SPSS, IBM, Armonk, NY, USA.[9] Descriptive statistics were computed for all variables. Frequencies, mean and standard deviations were obtained for continuous variables, while categorical variables were assessed by percentages. To facilitate analysis, variables with multiple categories were collapsed to fewer categories in a meaningful way. Chi square and Fischer exact tests were used to assess categorical variables. To identify the factors associated with breast cancer subtype, univariable analysis of each variable of interest, crude odds ratio and their 95% confidence intervals, along with *p*-values, were calculated. The reference group for each risk factor was generally determined by the category with the minimal level of risk for breast cancer. Risk factors were included in the multivariable analysis if they were significant at *p*-value <0.25 10 or had biological significance. All statistical tests were two-sided, with *p*-value of < 0.05 used as the cut off for statistical significance. In multivariable analysis, multinomial logistic regression was performed to identify factors associated with breast cancer subtypes, while adjusting for other variables. All independent variables with univariate analyses *p*-values less or equal to 0.25 were included in the model. Analysis was done by the purposeful selection method and all the variables that were selected from the univariable analyses were entered in the model simultaneously to adjust for confounding and to identify interactions between the independent variables. Confounders were identified as any variable that changed the OR of the exposure variable by more than 10% when added to the model. Finally, any variable with a *p*-value >0.05 that was not a confounder or did not interact with other variables was removed from the model to obtain a parsimonious and biologically meaningful model that best explains factors associated with breast cancer subtype. It is important to mention that the statistical power of our analyses is limited by the inclusion of only 73 triple negative breast cancer and 28 HER-2-overexpressing breast cancer cases. We also had to exclude192 potentially eligible cases whose reports for molecular subtypes were not available to be classified into any subtype.

## RESULTS

The main focus of the study was comparing non TNBC (n=307) cases and TNBC (n=73) cases versus controls. We analyzed 73 cases of triple negative breast

cancer (TNBC) vs 307 cases of non-TNBC subtypes.

**Table-I: Sociodemographic, Reproductive and Clinical Characteristics of Breast Cancer cases according to molecular subtypes of Breast Cancer Among women in two Major cancer Hospitals of Karachi, Pakistan.**

| Variables | Categories | Control n=798 (%) | Non- triple Negative Breast Cancer, n=307(%) | Triple Negative Breast Cancer n=73 (%) | *p*-value* |
|---|---|---|---|---|---|
| Age at diagnosis (years) | < 35 | 132(16.5) | 37(12.1) | 11(15.1) | 0.001 |
| | 35-44 | 245(30.7) | 79(25.7) | 22(30.1) | |
| | 45-54 | 231(28.9) | 75(24.4) | 22(30.1) | |
| | 55 & above | 190(23.8) | 116(37.8) | 18(24.7) | |
| Education level | < grade 8 | 180(22.6) | 91(29.7) | 30(41.7) | <0.001 |
| | 8-12 grade | 250(31.3) | 107(35) | 24(33.3) | |
| | > grade 12 | 368(46.1) | 108(35.3) | 18(25) | |
| Marital status | Single/widow/divorced | 151(18.9) | 65(21.2) | 12(16.4) | 0.57 |
| | Married | 647(81.1) | 242(78.8) | 61(83.6) | |
| Employment status | Yes | 184(23.1) | 51(16.6) | 8(11) | 0.005 |
| | No | 614(76.9) | 256(83.4) | 65(89) | |
| Socioeconomic status (SES) | Upper | 131(16.5) | 25(8.3) | 5(7) | <0.001 |
| | Middle | 463(58.4) | 188(62) | 34(47.9) | |
| | Lower | 199(25.1) | 90(29.7) | 32(45.1) | |
| Consangiuos marriage | | 191(24.3) | 82(27.9) | 18(25.4) | 0.49 |
| | No | 594(75.7) | 212(72.1) | 53(74.6) | |
| | Nullipara | 121(15.2) | 38(12.4) | 8(11) | |
| Parity | 1-3 children | 376(47.1) | 138(45) | 38(52.1) | 0.47 |
| | > 3 children | 301(37.7) | 131(42.7) | 27(37) | |
| Abortion | No abortion | 450(56.4) | 164(53.4) | 45(61.6) | 0.2 |
| | < 3 abortions | 277(34.7) | 126(41) | 24(32.9) | |
| | > 3 abortions | 71(8.9) | 17(5.5) | 4(5.5) | |
| Breast feeding history | No | 145(18.5) | 51(16.9) | 9(12.3) | 0.38 |
| | Yes | 640(81.5) | 251(83.1) | 64(87.7) | |
| Lifetime months of breast feeding | No breast feeding | 145(18.5) | 51(16.9) | 9(12.5) | 0.75 |
| | < 12 months | 95(12.1) | 39(12.9) | 10(13.9) | |
| | > 12 months | 545(69.4) | 212(70.2) | 53(73.6) | |
| Family planning (FP) | No FP | 535(78.9) | 190(85.6) | 47(81) | 0.13 |
| | < 24 months | 103(15.2) | 20(9) | 6(10.3) | |
| | > 24 months | 40(5.9) | 12(5.4) | 5(8.6) | |
| Age of mother at first live birth (years)** | | 609(91.6) | 240(90.9) | 60(92.3) | 0.92 |
| | >30 | 56(8.4) | 24(9.1) | 5(7.7) | |
| Menopausal Status | Premenopause | 440(55.7) | 131(43.2) | 35(50) | 0.001 |
| | Post menopause | 350(44.3) | 172(56.8) | 35(50) | |
| Age at menarche (years) | < 12 | 92(12.2) | 27(9.7) | 5(7.7) | 0.23 |
| | 13-14 | 410(54.2) | 152(54.5) | 44(67.7) | |
| | > 14 | 255(33.7) | 100(35.8) | 16(24.6) | |
| Family history of breast cancer | Yes | 212(26.7) | 68(22.2) | 13(17.8) | 0.1 |
| | No | 581(73.3) | 238(77.8) | 60(82.2) | |
| Family history of any cancer | Yes | 312(39.3) | 107(35.3) | 30(41.1) | 0.41 |
| | No | 481(60.7) | 196(64.7) | 43(58.9) | |
| Serum Vitamin D level (ng/dl) | > 30 | 163(25.4) | 50(23.4) | 5(10) | 0.039 |
| | 20-30 | 115(17.9) | 29(13.6) | 11(22) | |
| | < 20 | 364(56.7) | 135(63.1) | 34(68) | |
| Body mass index *** | < 23 | 115(15.6) | 55(19.5) | 11(16.9) | 0.074 |
| | 23-25 | 144(19.6) | 67(23.8) | 19(29.2) | |
| | > 26 | 476(64.8) | 160(56.7) | 35(53.8) | |

**p values generated from Chi-square or Fisher's exact tests*

***Restricted to women who ever had a full-term pregnancy (a pregnancy was considered as full-term if it resulted in a live birth or lasted 7 or more months).*

****BMI, body mass index; BMI was categorized according to the WHO classification for Asian as underweight/normal weight (<23 kg/m$^2$), overweight (23-25 kg/m$^2$) or obese (>26 kg/m$^2$).*

**Table-II: Distribution of histopathology, grade, TNM stages and Tumor Characteristics of Molecular Subtypes of Breast Cancer Among Women in two Major Cancer Hospitals of Karachi, Pakistan**

| Variable | Category | Non- Triple Negative Breast Cancer, (n=307) (%) | Triple Negative Breast Cancer, (n=73) (%) | *p*-value |
|---|---|---|---|---|
| Side of tumor | Right | 157(52) | 32(44.4) | 0.27 |
| | Left | 142(47) | 38(52.8) | |
| | Both | 3(1) | 2(2.8) | |
| Tumor type | Invasive Ductal carcinoma (IDC) | 272(90.1) | 68(94.4) | 0.69 |
| | Invasive lobular carcinoma (ILC) | 12(4) | 2(2.8) | |
| | Others | 18(6) | 2(2.8) | |
| Grade of tumor | III | 96(32.8) | 44(64.7) | <0.001 |
| | I/II | 197(67.2) | 24(35.3) | |
| Tumor size | T1 (T < 2.0 cm) | 62(22.9) | 11(17.2) | 0.05 |
| | T2 (2.0 - 5.0 cm) | 106(39.1) | 20(31.3) | |
| | T3 (T > 5.0 cm) | 47(17.3) | 21(32.8) | |
| | T4 (Extension to the chest wall) | 56(20.7) | 12(18.8) | |
| Nodal involvement | N0/N1 | 198(73.6) | 43(69.4) | 0.68 |
| | N2 | 39(14.5) | 13(21) | |
| | N3 | 32(11.9) | 6(9.7) | |
| Metastasis | No metastasis | 207(72.9) | 48(71.6) | 0.85 |
| | Metastasis | 42(14.8) | 9(13.4) | |
| | Unknown | 35(12.3) | 10(14.9) | |
| TNM Stage | Stage 1 | 41(16.8) | 6(11.3) | 0.77 |
| | Stage 2 | 88(36.1) | 20(37.7) | |
| | Stage 3 | 73(29.9) | 18(34) | |
| | Stage 4 | 42(17.2) | 9(17) | |

We evaluated association of sociodemographic and reproductive and other factors with TNBC and non TNBC subtypes (Table-I). Compared with non-TNBC, TNBC cases tended to be younger less than 35 yrs. (15.1%), less educated with 41.7% having studied less than grade[8]. More TNBC cases belonged to the lower SES group (45.1 %) compared with Non-TNBC case group (29.7 %) and controls (25.1%).Vitamin D concentrations were more likely to be deficient (<20 ug/dl) in TNBC cases (68%) and non-TNBC cases (63.1%) as compared to controls (56.7%). Women with sufficient concentrations of Vitamin D (> 30ug/dl) had the least number of TNBC cases (10%).Socioeconomic status showed least number of TNBC cases in upper SES (7%) and higher numbers in lower SES group (45.1%) when compared to non TNBC cases.Table-II represents the distribution of histopathology, grade, TNM stages and tumor characteristics among TNBC and non TNBC subtypes of breast cancer patients showing Univariate multinomial logistic regression analyses were used to calculate odds ratios (ORs) and their associated 95% confidence intervals (CIs), to compare different subtypes of breast cancer with a common control group. This approach is helpful in performing a series of simple binary logistic regression models for different tumor subtypes-control comparisons.Odds ratios for non TNBC cases versus controls, and TNBC cases versus controls, through univariate multinomial logistic regression analyses are presented in Table-III. Age of 55 yrs and above was positively associated with risk of non TNBC (OR=2.18, 95% CI=1.41, 3.35). Premenopausal status had a protective effect only among women with non TNBC subtype (OR= 0.61, 95% CI= 0.46, 0.79). Most of the women were unemployed being housewives and this was also associated with risk of both TNBC and non TNBC with higher OR among TNBC (OR= 2.43. 95%CI=1.15, 5.17). TNBC was higher among women with education less than grade 8 (OR=3.41, 95%=1.85, 6.28). Less than grade 8 concentrations of schooling was also associated with non TNBC (OR=1.72, 95%=1.24, 2.40). Poor SES was associated with both TNBC and non TNBC with stronger association with TNBC (OR= 4.21 95% CI=1.60, 11.09) and less strong with non TNBC (OR= 2.37, 95% CI=1.44, 3.89). Vitamin D deficiency (VDD) was associated with TNBC (OR= 3.05. 95% CI= 1.17, 7.93). Vitamin D insufficiency (VDI) was also associated with TNBC (OR= 3.12, 95%CI= 1.05, 9.22).

The multivariable multinomial logistic regression analyses showed that both TNBC and non-TNBC subtypes were associated with poor socioeconomic status and low Vitamin D concentrations with TNBC risk much higher among women of low SES (OR=8.76,

95% CI= 2.45, 31.32) and women with vitamin D deficiency (OR=3.11, 95%CI= 1.17. 8.29) (Table-IV).

**Table-III: Univariate Multinomial Logistic Regression analyses of the Association Between Risk Factors and Breast Cancer Subtypes Among Women in Two Major Cancer Hospitals of Karachi, Pakistan**

| Variables | Non- Triple Negative Breast Cancer | 95% CI | *p*-value | Triple Negative Breast Cancer | 95% CI | *p*-value |
|---|---|---|---|---|---|---|
| | OR | | | OR | | |
| **Age** | | | | | | |
| 55 & above | 2.18 | 1.41, 3.35 | <0.001 | 1.14 | 0.52, 2.49 | 0.74 |
| 45-54 | 1.16 | 0.74, 1.81 | | 1.14 | 0.54, 2.43 | |
| 35-44 | 1.15 | 0.74, 1.79 | | 1.08 | 0.51, 2.29 | |
| <35 | 1(Ref) | | | 1(Ref) | | |
| **Education level** | | | | | | |
| < grade 8 | 1.72 | 1.24, 2.4 | <0.001 | 3.41 | 1.85, 6.28 | <0.001 |
| 8-12 grade | 1.46 | 1.07, 1.99 | | 1.96 | 1.04, 3.69 | |
| > grade 12 | 1(Ref) | . | | 1(Ref) | . | |
| **Marital status** | | | | | | |
| Single/widow/divorced | 1.15 | 0.83, 1.6 | 0.6 | 0.84 | 0.44, 1.61 | 0.4 |
| Married | 1(Ref) | . | | 1(Ref) | . | |
| **Socioeconomic Status** | | | | | | |
| Lower | 2.37 | 1.44, 3.89 | 0.01 | 4.21 | 1.60, 11.09 | 0.04 |
| Middle | 2.13 | 1.34, 3.37 | | 1.92 | 0.74, 5.02 | |
| Upper | 1(Ref) | . | | 1(Ref) | | |
| **Consanguineous marriage** | | | | | | |
| Yes | 1.2 | 0.89, 1.63 | 0.23 | 1.06 | 0.60, 1.85 | 0.84 |
| No | 1(Ref) | . | | 1(Ref) | . | |
| **Parity** | | | | | | |
| Nullipara | 0.72 | 0.48, 1.10 | 0.12 | 0.74 | 0.33, 1.67 | 0.46 |
| 1-3 children | 0.84 | 0.64, 1.12 | | 1.13 | 0.67, 1.89 | |
| > 3 children | 1(Ref) | . | | 1(Ref) | . | |
| **History of abortion** | | | | | | |
| No abortion | 1.44 | 0.82, 2.52 | 0.2 | | 0.58,4.81 | 0.33 |
| 1- 3 abortion | 1.77 | 1.00, 3.13 | | 1.43 | 0.48, 4.26 | |
| > 3 abortion | 1(Ref) | . | | 1(Ref) | . | |
| **Age at first live birth (years)** | | | | | | |
| < 30 | 0.92 | 0.56, 1.52 | 0.74 | 1.1 | 0.43, 2.86 | 0.83 |
| > 30 | 1(Ref) | . | | 1(Ref) | . | |
| **Family planning** | | | | | | |
| no FP | 1.18 | 0.61, 2.30 | 0.58 | 0.7 | 0.27, 1.87 | 0.22 |
| < 24 months | 0.65 | 0.29, 1.45 | | 0.47 | 0.14, 1.61 | |
| > 24 months | 1(Ref) | . | | 1(Ref) | . | |
| **History of breast feeding** | | | | | | |
| No | 0.9 | 0.63, 1.27 | 0.54 | 0.62 | 0.30, 1.28 | 0.19 |
| Yes | 1(Ref) | . | | 1(Ref) | . | |
| **Menopausal status** | | | | | | |
| Premenopause | 0.61 | 0.46, 0.79 | 0.001 | 0.8 | 0.49, 1.30 | 0.35 |
| Postmenopause b | 1(Ref) | . | | 1(Ref) | . | |
| **Age at menarche (years)** | | | | | | |
| < 12 | 0.75 | 0.46, 1.22 | 0.24 | 0.87 | 0.31, 2.43 | 0.78 |
| 12 to 14 | 0.95 | 0.70, 1.27 | | 1.71 | 0.95, 3.10 | |
| > 14 | 1(Ref) | . | | 1(Ref) | . | |
| **Family history of breast cancer** | | | | | | |
| Yes | 0.78 | 0.57, 1.07 | 0.12 | 0.59 | 0.32, 1.10 | 0.09 |
| No | 1(Ref) | . | | 1(Ref) | . | |
| **Serum Vitamin D level (ng/dl)** | | | | | | |
| < 20 | 1.21 | 0.83, 1.76 | 0.31 | 3.05 | 1.17, 7.93 | 0.02 |
| 20-30 | 0.82 | 0.49, 1.38 | | 3.12 | 1.05, 9.22 | |
| > 30 | 1(Ref) | | | 1(Ref) | | |
| **Body mass index c** | | | | | | |
| < 23 | 1.42 | 0.99, 2.06 | 0.06 | 1.3 | 0.64,2.33 | 0.4 |
| 23-25 | 1.38 | 0.99, 1.95 | | 1.79 | 1.00, 3.23 | |
| > 26 | 1(Ref) | . | | 1(Ref) | . | |

*a Restricted to women who ever had a full-term pregnancy (a pregnancy was considered as full-term if it resulted in a live birth or lasted 7 or more months) b Restricted to postmenopausal women cBMI, body mass index; BMI was categorized according to the WHO classification for Asian OR are compared to controls Bold values indicate statistical significance (p<0.05)*

**Table-IV: Adjusted Odds Ratio of Association of Vitamin D with triple Negative Breast Cancer (TNBC ) and Non TNBC Subtypes of Breast Cancer Using Multinomial Logistic Regression Analyses**

| Variable | Triple Negative Breast Cancer | 95%CI | *p*-value | Non Triple Negative Breast Cancer | 95%CI | *p*-value |
|---|---|---|---|---|---|---|
| | OR | | | OR | | |
| **Socioeconomic status** | | | | | | |
| Lower | 8.76 | 2.45, 31.32 | <0.001 | 4.08 | 2.06, 8.10 | <0.001 |
| middle | 2.39 | 0.80, 7.15 | | 3.21 | 1.85, 5.57 | |
| upper | 1(Ref) | | | 1(Ref) | | |
| **Serum Vitamin D (ng/ml)** | | | | | | |
| < 20 | 3.11 | 1.17, 8.29 | 0.02 | 1.41 | 0.95, 2.09 | 0.09 |
| 20-30 | 3.45 | 1.13, 10.56 | | 0.92 | 0.54, 1.57 | |
| > 30 | 1(Ref) | | | 1(Ref) | | |

*Abbreviations: OR, odds ratio CI, confidence interval, OR are compared to controls, adjusted for hospital and menopausal status*

## DISCUSSION

This study allowed us to investigate the associations between well-known risk factors among non TNBC and TNBC subtypes of breast cancer among Pakistani women. Overall TNBC typically constitutes 10-20 % of all breast cancer subtypes 11 but in our study sample it was high and constituted 22.7 % of all subtypes. The frequency of TNBC is reported to vary between different ethnic groups. In a pooled data from three population-based studies and consisting of 558 TNBC and 5,111 controls, TNBC accounted for 12% of newly diagnosed breast cancers.[12] In a retrospective study of White patients in West Virginia, Hospital, TNBC occurred in 18.9% of the 620 patients being diagnosed at age <50 years 13 In another study, TNBC comprised 17.28% of the breast cancers in Pakistani women diagnosed at the Armed forces Institute of Pakistan Rawalpindi.[14] Variation in incidence of TNBC could be due to multiple factors including differences in environmental exposures or behaviors and genetic factors.The mean age of TNBC cases was younger (46.1 SD 11.7 years) than mean age of non TNBC cases (49.4 SD 12.5 years ) which is consistent with other studies.[15] Among non- TNBC cases, there was a lower risk of breast cancer in premenopausal women while among TNBC cases there was no association with menopausal status. Our result of protective effect of premenopause with non TNBC and not with non TNBC is consistent with similar results in theWomen's Health Initiative study.[16] The significant association of TNBC with poor SES, as shown in this study, is similar to a study in West Virginia Hospital, where TNBC was high among socioeconomically deprived population.[13] California Cancer Registry also reported poor SES as a risk factor for TNBC among white women [17]. In a study by Banegas *et al*., women living in a low socioeconomic status (SES) neighborhood had an increased risk of TNBC diagnosis and higher mortality due to breast cancer.[18] This points towards the potential impact of SES, an important social determinant of health, on risk factors that may be etiologically important in increasing the risk of developing TNBC. It may be due to poverty related lifestyles choices of these women like eating lesser healthy foods, lack of healthy physical activity, and having exposure to higher concentrations of environmental carcinogens. Additionally, low SES may also be related to reproductive factors like younger age at first pregnancy and lack or shorter duration of breastfeeding, both of which are risk factors for TNBC. It is important to identify lifestyle choices which are modifiable, and may help decrease TNBC among poor women. Conversely TNBC was more common among high SES in The San Francisco Bay Area Breast Cancer Study.12 However, like our study, there were no associations of TNBC risk with reproductive factors like age at menarche or parity (12).Triple-negative breast tumors are shown to be associated with a younger age, high tumor size, higher-grade tumors , and a higher rate of node positivity.[19] Another study from Karachi from (2013-2020), showed late age at first birth and lower parity were associated with triple negative breast cancer compared to triple positive breast cancer.[20]

In the Carolina Breast Cancer Study, among premenopausal patients, TNBC was found to be more common among women with a younger age at menarche, higher parity, younger age at full-term pregnancy, shorter duration of breast-feeding, and higher body mass index (BMI).[21] However, no remarkable associations of reproductive factors with TNBC or non TNBC were observed in this study. As established for all breast cancers, the risk of TNBC was

increased in women of low SES and those with lesser education indicating again the important role of environmental related factors. These findings emphasize the importance of the contribution of poverty to the etiopathogenesis of this aggressive subtype. This also implies that women living in conditions of poverty are exposed to unidentified carcinogenic factors in the environment that are responsible for the increased risk of TNBC. These factors were, however, not to be identified in the scope of the study objectives. Our results confirmed the findings of previous studies that showed TNBC was associated with Vitamin D deficiency.[22-23] Based on these findings, correction of Vitamin D deficiency in women is a reasonable and cost-effective strategy to reduce the incidence of all subtypes of breast cancer, and in particular the aggressive TNBC. Larger prospective studies or clinical trials are needed to further confirm these findings.Limitation of the study was we did not have complete molecular profile of all cases enrolled in study. Though missing values for receptor status were minimized by accessing the patients and accessing their outside AKUH and KIRAN lab's results but still we had missing data on HER-2/ neu and ER/PR status on 192 breast cancer cases. Therefore, we could not analyse all four tumour subtypes separately but had to merge different subtypes as non TNBC group. The relatively low number of HER 2 enriched breast cancer cases in our study limited the power of some variables in this subtype with inconsistencies in results limiting our understanding of reproductive risk factors relationship to risk for the non-luminal breast cancer subtypes.More epidemiological studies of the highest quality and collaborative research between epidemiologists, pathologists and clinicians are needed to understand the different etiology of this aggressive disease. In this study we were unable to identify distinct molecular subtypes of TNBC. A major deficiency is the availability of breast cancer registry and population-based data on the incidence of the sub-types of breast cancer.

## CONCLUSION

Correction of Vitamin D deficiency in women maybe a reasonable and cost-effective strategy to prevent TNBC like aggressive breast cancer. It should be further tested though large cohort studies or clinical trials in our population.

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**Authors' Contribution**

Following authors have made substantial contributions to the manuscript as under:

US & AS: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

SU: Data acquisition, data analysis, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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