



# HER2/neu Levels, Premature Termination of Pregnancy and Breast Cancer Risk: A Hospital-Based Study from Lahore, Pakistan

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## ABSTRACT

Breast cancer is a leading cause of death in women. The objective of the present study was to evaluate diagnostic utility of HER2/neu test and the association of premature termination of pregnancy with breast cancer. Two hundred breast cancer patients from INMOL hospital, Lahore (Pakistan) were interviewed on reproductive factors. HER2/neu levels were determined by ELISA from 52 patients and 37 age-matched control women. The results of survey study showed that “premature termination of pregnancy” emerges as a potential risk factor of breast cancer. Number of pregnancies has significant association with premature termination of pregnancies ( $P = 6 \times 10^{-11}$ ). The regression coefficient for number of pregnancies is  $0.220 \pm 0.029 = 1.25 \times$  i.e. a 25% increase for each additional pregnancy. There is a significant positive association between premature termination of pregnancies and number of children ( $P = 0.024$ ): the regression coefficient is  $0.108 \pm 0.048 = 1.11 \times$  i.e. 11% increase for each additional child. The analysis of Her2/neu showed that its levels were higher in younger patients ( $\leq 42$  years) than in older patients ( $0.884$  vs  $0.614$  units,  $P = 0.049$ ). The ROC curve indicated that the optimal test (maximum Se + Sp) occurred at HER-2 Neu values of  $= 0.615$ , where Se = 0.73 and Sp = 0.49. The AUC value for this test was 0.57, with 1 being a perfect test, and 0.5 being equivalent to random allocation. In brief, premature termination of pregnancy/ miscarriage is a potential risk factor and HER2/neu cannot be used as a suitable diagnostic test.

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### Authors' Contribution

MM conceived the idea and supervised the study. SY performed the experimental work. AAL helped in collection of samples and literature review. MW helped in manuscript writing and compiling of results. PCT performed all the statistical tests.

### Key words

Breast cancer, Premature termination of pregnancy, Number of children, HER2/neu, ROC curve

## INTRODUCTION

Breast cancer (BC) is the leading and lethal cause of death among women all over the globe. The rate of BC incidence, survival and mortality varies in various countries, regions and areas of the world. These differences in prevalence of the disease are generally associated with the population structure, life style, genetic makeup, environment and changed risk factors (Hortobagyi *et al.*, 2005). In Pakistan, the breast cancer frequency is higher at young age as compared to the western world where its rate is growing in older women. Moreover, the rate of BC in women is 60 per 100,000 in Pakistan whereas with a similar socio-economic profile in India, the rate is comparatively low, i.e., 21 per 100,000 (Naeem *et al.*, 2008). According to latest statistics of World Health Organization (2019) there are 36.8% new BC cases in females as compared to all

other cancer cases in Pakistan. This might be due to the changed epidemiological and risk factors in Pakistan.

The incidence of BC might be correlated to altered epidemiological and risk factors such as age, menarche and menopause, family history of breast cancer, and nulliparity as reported by many investigators (Kim *et al.*, 2015; Mansha *et al.*, 2016). Similarly, socio-demographic factors, genetic predisposition and environmental factors have been found to be associated with incidence of BC (Thakur *et al.*, 2017). Some reproductive factors such as termination of pregnancy (TOP), premature births, recurrence of fetus removal and oral contraceptives have been reported as potential risk factors of cancer in women (Sathwara *et al.*, 2017). In a case control study, older age at the first birth has emerged as risk factor which escalates the occurrence of BC by six times (OR, 6.34; 95% CI, 2.04–27) (Kim *et al.*, 2015). Similarly, older age at the first full term pregnancy and abortions increased the risk of developing BC in women (Karim *et al.*, 2015).

BC is classified in to three categories on the basis of estrogen receptor, progesterone receptor and

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HER2 receptors, an approach being used to have better therapeutic and clinical outcome (Li *et al.*, 2017). HER2/neu is well known biomarker used for this purpose and it can be detected in the patient blood (Slamon *et al.*, 1987). It can be assessed in 15-40% of patients having primary BC using various techniques including enzyme-linked immunosorbent assay, ELISA (Wang *et al.*, 2000; Krainer *et al.*, 1997). Numerous scientific reports indicate that serum levels of HER2/neu are correlated with tumor size, tumor burden (Kang *et al.*, 2010), prognosis of disease (Carney *et al.*, 2003; Bramwell *et al.*, 2009) and response to prediction of neo-adjuvant chemotherapy (Lee *et al.*, 2012).

The aim of present study was to demonstrate the frequency and distribution of epidemiological and potential risk factors like premature termination of pregnancy, number of pregnancies, age at first pregnancy and number of children, among BC patients. Miscarriages and premature termination of pregnancy (TOP) is an important factor which will be emphasized in the current study to get insight in the development of breast cancer due to onset of hormonal cycles in women body. Very few scientific reports are available about these factors. The second objective of this study was to determine the clinical utility of HER2/neu biomarker by using ELISA in case and control subjects. The present study will articulate the role of various epidemiological and potential risk factors and also explores the sensitivity and efficiency of HER2/neu test in diagnosis of BC.

## MATERIALS AND METHODS

The study was approved by the Ethical Review Committee of University of Education. Written informed consent of the patients was taken from case and control women. This study was conducted in Institute of Nuclear and Molecular Oncology (INMOL) Hospital, Lahore and University of Education, Lahore. Two hundred case patients were selected from those already on treatment or were newly diagnosed with BC between Oct. 2016 and Nov. 2017. They were interviewed at a separate place in the hospital to answer the questions in private. A well-structured questionnaire about major reproductive factors such as prior termination of pregnancy (TOP), age of first pregnancy, number of pregnancies, number of children, number of miscarriages/abortions was administered to the BC patients. The patient information was recorded with great care and kept confidential and anonymous. The results about the age at menarche, menopause, marital status, pregnancy, parity and diagnosis of BC have already been published by us.

Out of above mentioned 200 patients only 52 gave

their consent to provide their blood for assessment of HER2/neu. Thirty-seven age-matched healthy women (controls) attending the hospital for other issues gave their blood for comparison of HER2/neu levels. Blood samples (5 ml) were collected in plain vacutainer tubes under sterile conditions from 52 case and 37 control women. Samples were allowed to clot and centrifuged at 1500 rpm for 15 minutes. Blood serum was separated and stored at -70°C. A human HER2/neu ELISA kit of 96 strip wells (Glory Science Co. Ltd., USA) was used to measure the levels of HER2/neu protein as per the instructions of the manufacturer. According to Hercep test, scores 0, 1+, 2+, 3+ were given to HER2/neu levels. Patients having HER2/neu levels from 0 to 1+ are negative, 3+ are positive and 2+ are at borderline.

### Statistical analysis

R statistical package version 3.4.2 was used for all analyses (R Core Team, 2017). Values for continuous variables were summarized as means±SD median (range). Values for categorical variable were represented as frequency (percentage). Univariable and multivariable Poisson regression models were used to assess factors associated with the number of miscarriages in breast cancer patients. The distribution of patients with respect to miscarriages, pregnancies, no. of children and age at first pregnancy was determined by statistical R.

The distributions of HER2/neu values in case ( $n=52$ ) and control ( $n=37$ ) patients was compared by two-sample *t*-test on log-transformed values. To further evaluate the potential of HER2/neu as a diagnostic test for BC, the sensitivity (Se) and specificity (Sp) of the test at different cut-off values of HER2/neu was used, and from these a receiver operator characteristic (ROC) curve was constructed (Hanley and McNeil, 1982). In addition, the area under the curve (AUC) was calculated as a diagnostic measure of the test, and optimal cut-point of HER2/neu determined, and the values which maximized the Youden index  $J=Se+Sp-1$ . The ROC analysis was undertaken using the AUC package in R (Ballings and Van den Poel, 2013).

## RESULTS

### Breast cancer and number of miscarriages

Frequency of reproductive parameters, namely number of pregnancies, age at first pregnancy and number of children in breast cancer patients are shown in Figure 1. Most of the breast cancer patients experienced four to five pregnancies. Maximum number of pregnancies experienced by a patient was 10 but these patients were

fewer in number. Further, most of the patients were 20-25 years age at their first pregnancy. Few patients, at their first pregnancy were in a range of 30-35 years of age. Most of the patients had up to two children but some patients had five or more children, a factor associated with miscarriages.

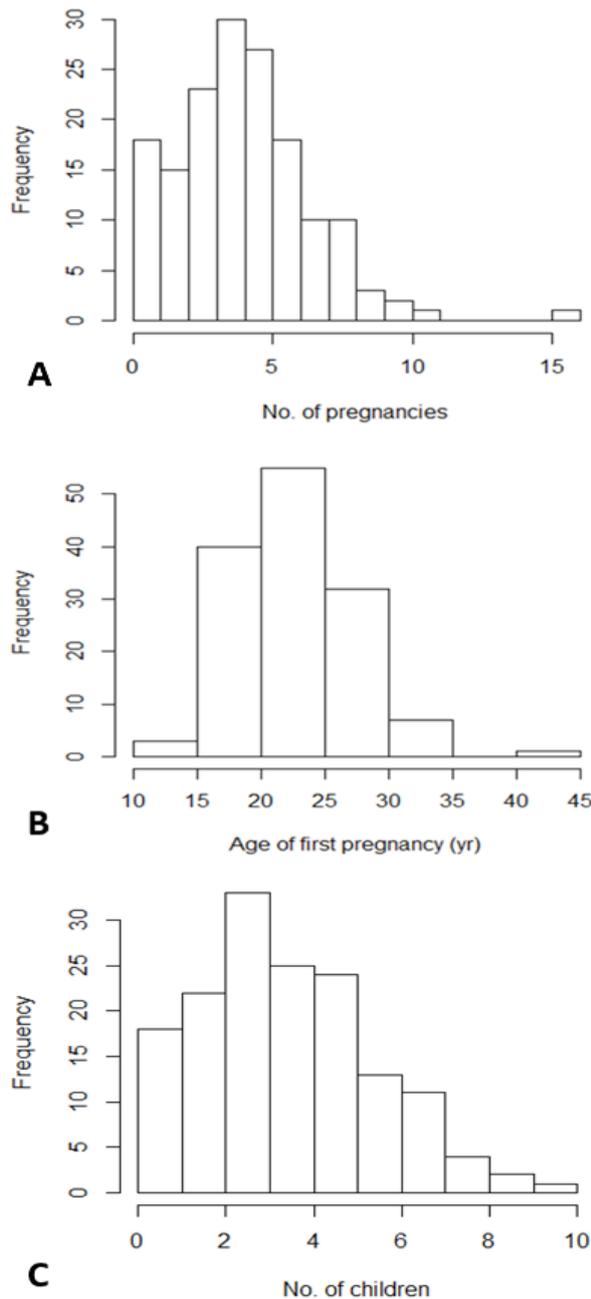


Fig. 1. Number of pregnancies (A), age of first pregnancy (B) and Number of children (C) of breast cancer patients, who were admitted to INMOL Hospital.

Miscarriages and premature termination of pregnancy (TOP) is an important factor which may be involved in the development of breast cancer due to onset of hormonal cycles in woman body.

Assessment of significance of reproductive factors associated with the number of miscarriages is shown in Table I based on the results of univariable Poisson regression models. Age is not a significant predictor ( $P= 0.11$ ) of any association. Two of the potential risk factors such as number of pregnancies and number of children show significant associations with the number of miscarriages/ premature termination of pregnancy. Number of pregnancies has (unsurprisingly) a highly significant association with the number of premature termination of pregnancies/miscarriages ( $P= 6 \times 10^{-11}$ ). The regression coefficient for number of pregnancies is  $0.220 \pm 0.029$ , indicating an increase in the mean number of miscarriages by a factor of  $1.25 \times$  i.e. a 25% increase for each additional pregnancy. There is a significant positive association between number of premature termination of pregnancies and number of children ( $P= 0.024$ ): the regression coefficient is  $0.108 \pm 0.048$ , so there is an increase of  $1.11 \times$  for each additional child i.e. 11% increase.

**Table I. Associations between potential risk factors and number of miscarriages in breast cancer patients based on univariable Poisson regression models.**

Predictor	P-value
Age (yr)	0.109
Age at diagnosis (yr)	0.216
No. of pregnancies	$6 \times 10^{-11}$
Age of first pregnancy (yr)	0.408
No. of children	0.026
Age of menarche (yr)	0.766
Age of menopause (yr)	0.732
Diabetes (Y/N)	0.834
Exercise (Y/N)	0.720
Any education (Y/N)	0.683

According to multivariable model, the regression coefficient and its standard errors confirmed the direction of association ( $0.220 \pm 0.029$ ) i.e. increasing number of miscarriages with increasing number of pregnancies. Conventionally these associations are denoted in terms of risk ratio and 95% confidence interval. Here the risk ratio (RR) was obtained by exponentiating the regression coefficient, i.e. for number of pregnancies,  $RR = \exp(0.220) = 1.25$ . The 95% CI was obtained as

$\exp(b \pm 1.96 \times \text{se}(b))$ , where  $b$  was the estimated regression coefficient, and  $\text{se}(b)$  was its standard error. So, the risk ratio for the number of pregnancies was  $1.25\times$  and (95% CI is 1.17-1.32). This was significantly greater than 1 (which would indicate risk increased by 25%).

#### Serum HER2/neu levels

The levels of HER2/neu expression in breast cancer patients were determined at different age groups based on whether they were above or below the median age (42 years) as shown in Table II. For the younger patients ( $\leq 42$  years), the median HER2/neu value was 0.884 units, compared with 0.614 units in older ( $> 42$  years) patients. The difference for the two age groups was marginally significant, based on a Wilcoxon rank sum test ( $P=0.049$ ). The maximum value showed by the patients was 4.52 units. Our test for HER2/neu revealed that a small number of patients (3.8%) were HER2/neu-positive and 5.8% patients were at borderline while 90.4% patients were HER2/neu-negative which showed positive outcome of the tumor treatment.

**Table II. Levels of Serum HER-2/neu in different age groups in breast cancer patients.**

Range	Age $\leq 42$ years	Age $> 42$ years
Min.	0.06	0.12
Q1	0.35	0.28
Median	0.54	0.39
Mean	0.88	0.61
Q3	0.89	0.59
Max	4.52	3.05

Figure 2 shows the distribution of HER2/neu expression for case and control groups, and it shows that levels of HER2/neu were slightly higher in control group than the case group. Both distributions were highly positively skewed. After log-transformation for normality, the difference in means was found to be non-significant based on a two-sample  $t$ -test ( $P=0.28$ ), with the back-transformed means being  $0.473 \pm 0.052$  units for cases and  $0.575 \pm 0.085$  units for control. This indicates that the serum HER2/neu would not be useful as an indicator of breast cancer.

#### ROC analysis

Figure 3 shows the ROC curve for using the HER2/neu value as a diagnostic test for BC. The curve was defined at every possible HER2/neu value. (Note that nearer the curve comes to the top left hand corner ( $\text{Se}=1$ ,  $\text{Sp}=1$ , i.e. a perfect test), the better is the test).

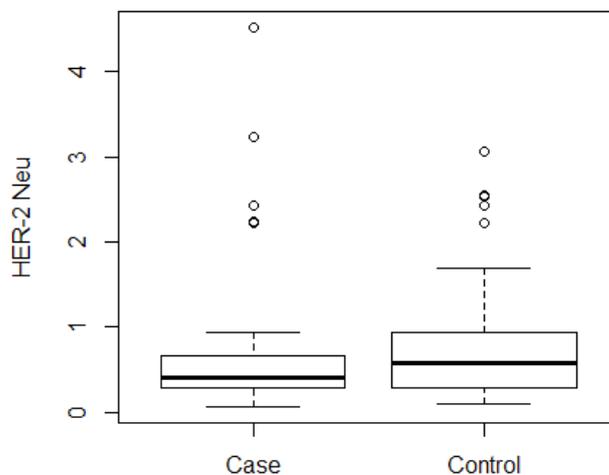


Fig. 2. Distribution of HER2/neu for case and control groups.

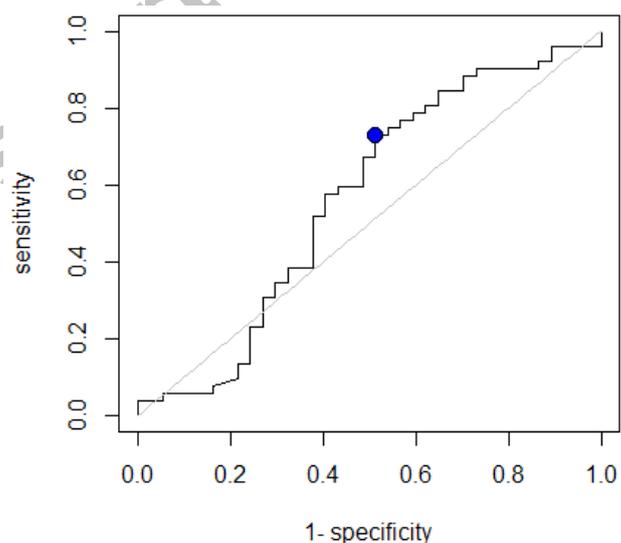


Fig. 3. ROC curve for HER2/neu expression as a diagnostic test for breast cancer (BC). Values below a HER2/neu threshold are indicative of BC. The blue dot on the curve indicates the optimal test (maximum Youden index,  $J = \text{Se} + \text{Sp} - 1$ ).

The dot on the curve on the RHS of Figure 3 indicated the optimal test (maximum Youden index), and this occurred at a value of HER2/neu = 0.615, where  $\text{Se} = 0.73$  and  $\text{Sp} = 0.49$ . That is using this threshold, 73% of patients with BC would be correctly diagnosed as having BC, while 49% of those without BC would correctly be diagnosed (with 27% of BC patients and 51% of non-BC patients incorrectly diagnosed). Further, the AUC value for this test was 0.57, with 1 being a perfect test, and 0.5 being

equivalent to random allocation. Based on this, the test would be considered to be poor as a diagnostic tool. (This was also consistent with the results comparing groups via the *t*-test).

## DISCUSSION

We found that the number of pregnancies had a significant positive association with the number of miscarriages with a 25% increase per pregnancy. Similarly, there was an 11% increase in the number of miscarriages for each additional child. It shows that women who suffered from TOPs or miscarriages had high risk of evolving breast cancer. Further, it is reported that induced premature birth might be a causal hazard for the development of breast disease. Induced abortion may add to the risk of breast disease by postponing the planning of a full-term pregnancy which is a defensive factor (Cathcart-Rake *et al.*, 2018).

The present study showed that number of children was associated with number of miscarriages. Here the regression coefficient of positive 0.1 with P value ( $p=0.024$ ) indicates the number of children associated with number of miscarriages. Further, the regression coefficient and its standard errors confirmed the direction of association ( $0.220\pm 0.029$ ) i.e. increasing number of miscarriages with increasing number of children. There was a high correlation ( $r=0.88$ ) between the number of children and the number of pregnancies, the difference largely being the number of miscarriages. However, it should be noted that these findings of association of number of pregnancies with number of children was not surprising, since increase in each of these variables, there necessarily were more opportunities for a miscarriage. Our study is supported by Brind *et al.* (2018) who showed that there was a strong association between abortion and breast cancer in South Asia. The association between breast cancer and induced abortion was complex. Birth control methods and abortion were the critical risk factor of breast cancer (Brind *et al.*, 2018). Further, it is reported that after diagnosis and treatment of breast cancer, the chemotherapy would have adverse effects on survival and full term pregnancy, if she conceived (Yuan *et al.*, 2019). Hence it was concluded that women who suffered from TOP's had high risk of evolving breast cancer. Such risk factors may induce danger of breast cancer by postponing the planning of a full-term pregnancy which is a defensive factor (Ramírez-Torres *et al.*, 2010).

The levels of HER2/neu in most of the BC patients were low than control group. The low levels of HER2/neu showed that the patients had undergone various management approaches like chemotherapy, radiotherapy,

lumpectomy and quadrantectomy. Our findings are supported by Lee *et al.* (2012) who showed that high sHER2 levels were associated with advanced clinical stage mastectomy and neoadjuvant chemotherapy. Our results are further supported by Garoufali *et al.* (2008) who showed that mean sHER2/neu levels are significantly high in advanced stage as compared to early stage of breast cancer. Moreno-Aspitia *et al.* (2013) reported that high levels of sHER2/Neu were correlated with poor prognosis in BC patients. To assess the efficiency of HER2/neu test, our results showed that 52% of patients were correctly diagnosed. On the basis of AUC (0.57) test the HER2/neu test appeared to be poor as a diagnostic tool. Therefore, additional markers required to diagnose the cancer effectively.

The present small survey hospital based study has certain limitations. Two hundred BC patients gave their consent to be included in the survey study. However, a very small number of patients (mostly well aware about the disease and at advanced stage,  $n=52$ ) provided blood for analysis of HER2/neu levels. Among them, most of the patients were subjected to various management strategies such as (chemotherapy, radiotherapy, lumpectomy and quadrantectomy) and very few patients were at an early stage of diagnosis and had not undergone any treatment. In addition, only 37 age matched caretakers/ relatives of these patients provided blood for comparison (control) but they refused to participate in survey study. Therefore, the survey results include only the BC patients.

## CONCLUSION

Miscarriages/premature termination of pregnancy (TOP) has emerged as a risk factor which may be involved in the development of breast cancer due to onset of hormonal cycles in woman body. We found that the number of pregnancies had a significant positive association with the number of miscarriages, with a 25% increase per pregnancy. Similarly, there was an 11% increase in the number of premature termination of pregnancy for each additional child. So, the women who experience premature termination of pregnancy evolve high risk of breast cancer by postponing the planning of a full term pregnancy. HER2/neu levels in most of the BC patients were low than control group reflecting that these patients had undergone various interventions like chemotherapy, radiotherapy, lumpectomy, and quadrantectomy. The efficiency of HER2/neu test showed that 52% of patients were correctly diagnosed. On the basis of AUC (0.57) test the HER2/neu test appeared to be poor as a diagnostic tool.

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### Statement of conflict of interest

The authors have declared no conflict of interest.

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