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Analysis of Risk Factors Related to Expression of Basement Membrane Protein NID1 in Females with Breast Cancer

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ABSTRACT

Among Asian population at present, we comparatively acquire less information associated with breast cancer risk factors. This study has analyzed the data gathered from two-renowned tertiary health care centers targeting a large subpopulation of Karachi. This will greatly help in determining the most prevalent risk factors as well as the clinical findings of breast cancer lung metastatic females among the target population. This comparative cross-sectional study which is aimed to determine the risk factors associated with breast cancer such as patient's demographic profile as well as reproductive risk factors. Moreover, we have also analyzed the clinical data of our study subjects based on which they were diagnosed as well as treated. Total n = 175 breast cancer cases were recruited in this study, out of which 121 (69.2%) patients were untreated (Group A). The remaining 54 (30.8%) patients, who received cancer treatment as adjuvant therapy, were included as treated (Group B) cases. Regarding patient's demographic data, mean age of enrolled patients was found to be 48.26±10.74 years. The mean age of menarche was found to be 13.39±0.89 years. The calculated BMI (body mass index) was found to be 20.4. Majority of the enrolled subjects have acquired primary or basic education and most of them were married and housewives. For the reproductive risk factors of breast cancer, majority gave no history of contraceptive usage. Also, most of the females have three children or more with the history of breast feeding. It was concluded that, lack of education combine with low socioeconomic status, which further aggravates the health outcomes especially by neglecting the early diagnosis of the disease. Also, higher risk was observed for married women, housewives with extended family that is 3 or more children. Breast feeding for extended duration (at least 2 years) and less age at first childbirth were observed as protective factors for this cancer. Also, majority of our study subjects were diagnosed at late stages of cancer (stage III and stage IV). Therefore, early detection will not only cure the breast cancer patients but also prevent them undergoing painful circumstances.

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TU designed the study, analysed the data and wrote the manuscript. BW, SM and SNNS review the data and manuscript. MA helped in the experiment. NR, LF and SFAR helped in manuscript writing.

Key words
Breast cancer risk factors, Clinical data, Demographics, Diagnostic profile, Reproductive risk factors.

INTRODUCTION

Worldwide, breast cancer is considered as one of the predominant malignancies in women. The cancer originated from the breast tissue, has the highest potential to invade the adjacent tissues as well as to the distant body organs secondary to uncontrolled proliferation of cancer cells (Khan *et al*., 2018). With an estimated deaths of 40,450 in the year 2016, breast cancer is considered as the second leading cause of mortality worldwide (Wei and Siegal, 2018). Over the past three decades, gradual increase in breast cancer incidence has also been observed in developing Asian countries (Coleman *et al*., 1993). Currently, Pakistan has been nominated on 9th position in the world ranking by WHO, owing to a very high mortality rate of nearly 17,000 deaths per year (Khan *et al*., 2017; Bhurgri *et al*., 2000). The cancer is known to metastasize to distant body organs. Lungs, liver, bone and brain are the four most common targeted organs (Lyden *et al*., 2016; Mills III, 2017). Fortunately, cancer has an excellent prognosis, if duly diagnosed and treated in its early stages (Taylor, 2001).

Several studies have been implicated to the risk factors associated with breast cancer, especially in the western world. These studies have revealed that some populations are exposed to relatively higher risks. However, among Asian population at present, we comparatively acquire less information associated to breast cancer risk factors (Masood and Kamal, 2004). The major assertion that faces challenges nowadays, is promoting the widespread concept that majority of women who are diagnosed with breast cancer, have no established clinical risk factors

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(Engmann *et al*., 2017). Till date, various studies have been conducted in which stratification of risks factors have been employed. This valued information can therefore be used in future for the primary prevention as well as for the improvement of public health interventions related to breast cancer (Trentham-Dietz *et al*., 2016).

In Pakistan, an alarming increase in breast cancer incidence has been reported in young females by numerous epidemiological studies (Shaukat *et al*., 2013). Literature search gave us insight that increasing age is one of the most important risk factors for breast cancer (Bernstein, 2002). Also, major inheritance susceptibility including germline mutation of *BRCA1* and *BRCA2* genes which were also found to be involved (Goodwin *et al*., 2012; Mavaddat *et al*., 2012). In addition, increase BMI in postmenopausal women, positive family history along with reproductive risk factors which also imposes greater risk to the disease (Oh *et al*., 2017). Increase sex hormones (estrogen and progesterone) exposure related to reproductive risk factors, which is mainly responsible to an increase susceptibility of cancer. The underlying mechanism is that hormones like estrogen and progesterone through their respective receptors in breast tissue, mediates their cell-proliferative effects as well as the process of apoptosis. Hence, receptor expression levels of these hormones as well as cell-proliferation marker Ki67, in breast tissue can therefore serve both as predictive as well as prognostic markers in breast cancer (Hormones and Group, 2013). Also, their expression levels attributed to a greater risk of cancer in women who have previous diagnosis of benign breast disease (BBD) (Oh *et al*., 2016). Hence, early menarche, late menopause, infertility, first pregnancy over the age of 30 and hormonal therapy (estrogen with or without progesterone) are also considered as the most prevalent breast cancer risk factors (Ferlay *et al*., 2015).

This comparative cross-sectional study which is aimed to determine the expression levels of a normal basement membrane protein Nidogen-1(NID1) in the blood samples, considering it as a potential biomarker of lung metastasis in breast cancer females (Urooj *et al*., 2020). We recruited both untreated and treated cases. In addition based on questionnaire, we also took history related to the risk factors associated with breast cancer such as patient's demographic profile including age, menarche age, height, and weight (BMI) as well as marital status, occupation, qualification, family history as well as reproductive risk factors. Moreover, we have also analyzed the clinical data of our study subjects based on which they were diagnosed. This includes mammogram findings, receptor status (ER, PR and HER-2/neu), cancer staging, histological type, and grading of breast cancer. The data was analyzed after it was gathered from two-renowned tertiary health care centers that targets a large subpopulation of Karachi. This study will greatly help us in determining the most prevalent risk factors as well as the clinical findings of breast cancer patients among the target population.

SUBJECTS AND METHODS

Study design and patient's selection

This is a comparative cross-sectional study which lasted for 10 months from January 2019 to October 2019. Consecutive sampling technique was adopted. Total 175 breast cancer cases were recruited, out of which 121 (69.2%) patients were untreated (Group A). The remaining 54 (30.8%) patients, who received cancer treatment as adjuvant therapy, were included as treated (Group B) cases in this study. This study was approved by the Ethics Review Committee (ERC) of Ziauddin University, Karachi, Pakistan (Reference No: 0661118TUANA).

Patient's stage classification and mode of treatment of group B breast cancer patients

Staging was done as per AJCC TNM (American Joint Committee on Cancer) classification. Among untreated cases (Group A), majority of patients (55.4%) were diagnosed with stage II whereas 38.8% with stage III and 5.8% with stage IV (advanced stage with lung metastasis). However, among treated cases (Group B), 37% females were diagnosed with stage II, 44.4% with stage III and 18.5% with stage IV (advanced stage with lung metastasis). Related to the mode of treatment acquired by Group B cases, majority of the patients (83.3%) received chemotherapy, followed by breast surgery (72.2%). The less reported was the modality of radiotherapy (20.8%) by our study subjects.

Methodology

All the patients recruited in this study were registered from the oncology department of Jinnah Postgraduate Medical Center and Jamal Noor Hospital, Karachi. Once enrolled and after the informed consent, we took history related to breast cancer risk factors as mentioned in the questionnaire. Patient's clinical data was also analyzed based on which they were diagnosed, and treatment was planned. Various investigations such as mammogram, trucut biopsy, CT scan imaging, receptor's status and histopathological reports were evaluated for this purpose. Patients having breast pathologies other than breast cancer were excluded from this study.

Statistical analysis

The data was entered and analyzed by using SPSS version 20. The descriptive analysis along with the tabular

representation of data were used to report the history related to breast cancer risk factors including demographic profile, reproductive risk factors, patient's clinical findings (diagnostic profile) as well as the mode of treatment of (Group B) cancer patients. Frequency and percentage were reported for study's categorical variables. While, mean and standard deviation were used for numeric study variables, respectively. In addition, few demographic indicators, mode of treatment and disease characteristics of the patients were also displayed by bar and pie chart. The normality of numeric variables (age, menarche age, weight, and height) was assessed by using Shapiro-Wilk test. The age of the patients was found to be normally distributed among both the study groups (Group A and B), while menarche age, weight and height of the patients were found to be skewed. Pooled-t/Mann-Whitney U Tests were applied for parametric and non-parametric numeric variables. Whereas, Chi-Square/Fisher-Exact Tests were used for parametric and non-parametric categorical variables of this study. P value of <0.05 was considered as statistically significant.

RESULTS

Patients demographics

The mean age of enrolled breast cancer patients was found to be 46.65 ± 10.74 years among Group B and 48.98 ± 10.72 years among Group A cases. The overall mean age of subjects from both groups was 48.26±10.74 years. Age of menarche was found to be 13.39±0.89 years whereas subject's weight was of 54.76±5.18 kg and height of 5.39±0.16 feet, respectively as shown in Table I of quantitative demographic indicators.

Table II shows qualitative demographic indicators of patients enrolled in this study. It is evident that majority of patients including 38 cases (69.1%) in Group A and 17 cases (30.9%) in Group B, have acquired primary or basic education. Also, majority of the breast cancer females which constituted 118 (69.8%) cases in Group A and 51 (30.2%) cases in Group B were married. Whereas, under the heading of occupation 104 (69.3%) cases in Group A and 46 (30.7%) cases in Group B were housewives.

Table I.- Quantitative demographic indicators of breast cancer patients.

Demographic indicators	Treated			Untreated			p-value
	Mean ± SD	Median	IQR	Mean ± SD	Median	IQR	
Age[a] (year)	46.65 ± 10.74	-	-	48.98 ± 10.72	-	-	0.187
Age of menarche (year)	13.26 ± 0.83	13	1	13.45 ± 0.91	13	1	0.228
Weight (kg)	54.37 ± 6.05	53	3	54.93 ± 4.76	55	5	0.111
Height (foot)	5.39 ± 0.14	5.4	0.2	5.39 ± 0.17	5.4	0.3	0.972

[a]Pooled t-test / Mann-Whitney U test applied.

Table II.- Qualitative demographic indicators of breast cancer patients.

Demographic indicators		Treated		Untreated		p-value
		n	%	n	%	
Qualification[a]	Illiterate	15	35.7	27	64.3	0.853
	Primary / Basic	17	30.9	38	69.1	
	Matriculation	12	27.3	32	72.7	
	Intermediate	8	33.3	16	66.7	
	Graduation	2	20.0	8	80.0	
	Subtotal	54	30.9	121	69.1	
Marital status[b]	Married	51	30.2	118	69.8	0.374
	Unmarried	3	50.0	3	50.0	
	Subtotal	54	30.9	121	69.1	
Occupation[c]	Housewife	46	30.7	104	69.3	0.793
	Working woman	8	33.3	16	66.7	
	Subtotal	54	31.0	120*	69.0	
Breast cancer family history[d]	Yes	18	30.0	42	70.0	0.866
	No	35	31.3	77	68.8	
	Subtotal	53**	100.0	119**	100.0	

[a,c,d]Chi-Square Test / [b]Fisher-Exact Test applied. * Missing occupation history in the case. ** Missing breast Cancer family history in the cases.

In addition, we also asked about the family history of breast cancer, for which majority cases in Group A that is 77 (68.8%) and 35 (31.3%) cases in Group B, gave negative response. However, remaining cases that is 40% in each group, reported positive family history. P-value, however, was found to be statistically insignificant when two groups were compared with respect to demographic indicators by applying the relevant tests.

History related to reproductive factors associated with breast cancer risk

The history related to reproductive risk factors of breast cancer such as contraceptive usage, history of breast feeding, number of children as well as age at first childbirth were also asked and are shown in Table III. Regarding the history of contraception, majority gave negative response. However, among remaining cases, IUCD was the most common method employed by 20 (80.0%) cases of Group A and 5 (20.0%) cases of Group B. Also, four out of every five patients breast fed their children whereas, enrolled subjects among both the groups reported three children or more during their reproductive life.

Moreover, for age at first childbirth, majority of Group A cases were of 20 years whereas, among Group B, 22 years, and above age. P-value was also found to be statistically insignificant when the two study groups were compared with respect to reproductive risk factors by applying the relevant tests.

Table III.- Reproductive risk factors of breast cancer patients.

History of patients		Treated		Untreated		p-value
		n	%	n	%	
Contraception use	None	38	40.4	56	59.6	0.062
	Oral	2	16.7	10	83.3	
	IUCD	5	20.0	20	80.0	
	Tubal ligation	1	100	0	0.0	
	Subtotal	46*	100	86*	100	
Breast feeding	Yes	45	31.3	99	68.8	0.146
	No	4	16.7	20	83.3	
	Subtotal	49**	100	119**	100	
Number of children	0	7	41.2	10	58.8	0.578
	1	4	44.4	5	55.6	
	2	9	33.3	18	66.7	
	3	15	35.7	27	64.3	
	4	5	20.0	20	80.0	
	5	6	21.4	22	78.6	
	6 and above	8	29.6	19	70.4	
	Subtotal	54	100	121	100	
Age at first childbirth in years	<= 18	3	33.3	6	66.7	0.326
	19	10	32.3	21	67.7	
	20	12	20.7	46	79.3	
	21	9	30.0	21	70.0	
	22 =<	13	41.9	18	58.1	
	Subtotal	47***	100	112***	100	

Chi-square test of independence was applied. * Missing Contraception Usage History in the cases. ** Missing breast-feeding history in the cases. *** Missing age at first childbirth history in the cases.

Diagnostic profile (clinical data) of breast cancer females

Table IV shows the parameters of diagnostic profile or clinical data, based on which breast cancer females in our study were diagnosed as well as treated. Based on mammographic findings, majority of females among both groups, reported BIRAD category 5. Second, for biological subtype of breast cancer, when receptor status was analyzed, ER +ve, PR +ve, HER 2neu –ve and Triple –ve breast cancer was most reported by the subjects of both study groups. This evaluation of biological markers (ER, PR, HER2neu) is important in determining the disease prognosis as well as for predicting response to hormonal and HER2-directed therapy. The American Society of Clinical Oncology/College of American Pathologists consensus panel has published guidelines to help standardize the performance, interpretation and reporting of assays, used to assess the ER-PR status by immunohistochemistry and HER2 status by immunohistochemistry and *in situ* hybridization (Hammond *et al*., 2010; Wolff *et al*., 2018). For the diagnosis, based on histopathological type of breast cancer, majority subjects reported invasive ductal carcinoma (IDC), which was reported by 114 (95.8%) cases in Group A and 53 cases (98.1%) in Group B.

Moreover, when the patients were tested for histological grading of tumor; almost all patients reported grade II and grade III neoplasia. Lastly for cancer staging, majority among Group A were diagnosed with stage II (55.4%), followed by stage III (38.8%) and stage IV (5.8%) with lung metastasis. However, among Group B cases, majority were diagnosed with stage III (44.4%), followed by stage II (37.0%) and stage IV (18.5%) with lung metastasis. P-value was found to be statistically insignificant when the two study groups (untreated and treated) were compared with respect to clinical data by applying the relevant tests.

Mode of treatment of group B breast cancer patients

Among Group B patients, majority (83.3%) received chemotherapy followed by breast surgery (72.2%). However, radiotherapy (20.8%) was the least reported treatment modality reported by the cases enrolled in this study (Fig. 1).

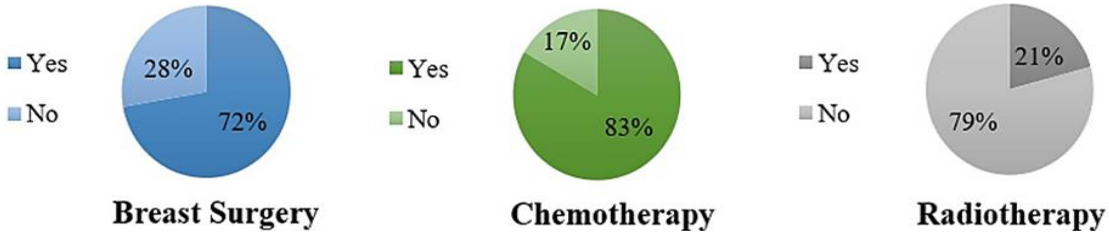

Fig. 1. Mode of treatment among the group B patients.

Table IV.- Diagnostic profile (clinical data) of breast cancer patients.

Diagnosis of breast cancer		Treated		Untreated		p-value
		n	%	n	%	
Mammo-gram diagnosis[a]	BIRAD 2	1	100	0	0.0	0.15
	BIRAD 3	1	20.0	4	80.0	
	BIRAD 4	9	20.5	35	79.5	
	BIRAD 5	36	33.3	72	66.7	
	BIRAD 6	7	43.8	9	56.2	
	Subtotal	54	31.0	120*	69.0	
Biomarker – ER and PR[b]	ER +ve & PR +ve	28	28.9	69	71.1	0.46
	ER -ve & PR -ve	23	39.7	35	60.3	
	ER -ve & PR +ve	0	0.0	1	100	
	ER +ve & PR -ve	3	25.0	10	75.0	
	Subtotal	54	32.1	114**	67.9	
Biomarker – ER, PR and HER 2[c]	Triple positive	10	37.0	17	63.0	0.41
	Triple negative	18	47.4	20	52.6	
	Subtotal	28	43.1	37	56.9	
Biomarker - HER 2[d]	Positive	17	30.4	39	69.6	0.73
	Negative	37	33.0	75	67.0	
	Subtotal	54	32.1	114***	67.9	
Histological type[e]	IDC	53	98.1	114	95.8	
	ILC	1	1.9	4	3.4	
	Metaplastic carcinoma	0	0.0	1	0.8	
	Subtotal	54	100	119[€]	100	
Histological grading of tumor[f]	Grade l	0	0.0	3	2.5	
	Grade ll	28	52.8	59	48.8	
	Grade lll	24	45.3	59	48.8	
	Grade lV	1	1.9	0	0.0	
	Subtotal	53	100	121[†]	100	

[c,d,e,f]Chi-Square Test / [a,b]Fisher-Exact Test applied . * Missing mammogram diagnosis data in the cases. ** Missing Biomarker – ER and PR data in the cases. *** Missing Biomarker - HER 2 data in the cases. [€]Missing histopathological type of breast cancer data in the cases. [†]Missing grading of tumor data in the cases.

DISCUSSION

This study which has determined the expression levels of a normal basement membrane protein Nidogen-1 in the blood samples, considering it as a potential biomarker of lung metastasis in breast cancer females. In addition, we have also evaluated the risk factors related to breast cancer by a questionnaire-based information. Moreover, the clinical data of breast cancer females was also analyzed in this study.

Regarding patient's demographic data, mean age of enrolled patients was found to be 48.26±10.74 years. A study conducted in Iranian women has reported that patients diagnosed with breast cancer were mostly above 44 years (Bidgoli *et al*., 2010). Also, worldwide, majority of females were diagnosed with breast cancer during fourth decade whereas, mortality secondary to cancer was reported mostly among females aged 50 and above (Angahar, 2017). The mean age of menarche was found to be 13.39±0.89 years. A study has declared that early menarche results in molecular alterations within breast tissue leading to an increased risk of breast carcinoma (Johnson *et al*., 2017). On the contrary, late menarche (> 12 years) showed significant reduction in the risk of carcinoma (Toss *et al*., 2017). A meta-analysis related to early menarche and its association with breast cancer has revealed that nulliparity and early menarche when combined with late menopause, will substantially increases the risk due to prolong exposure to estrogen hormone (Khalis *et al*., 2018).

The calculated body mass index (BMI) was found to be 20.4. This showed that majority of subjects were non-obese. A research acclaimed 1.5 times greater risk of breast cancer in overweight (BMI≥25) and obese (BMI≥30) females. Also, for every 5 kg/m2 BMI increase, potential to breast cancer risk increases to 2%. Hence, obesity is considered as one of the major risk factor (Liu *et al*., 2018). On the contrary, another research has revealed that among premenopausal women, higher BMI could serve as a protective factor for breast cancer (Schoemaker *et al*., 2018).

For the association between education status and risk to breast cancer, majority of enrolled subjects have acquired primary education. Lack of education results in decrease health awareness issues which when combined with poverty and low-income status will result in adverse health outcomes (Coughlin, 2019). On the contrary, a

meta-analysis has identified 18 cohort studies in which a total of 10 million women were recruited. An interesting finding in women with higher education status showed to have increase cancer risk as compare to women with lower education level (Dong and Qin, 2020). Whereas, no association was declared between women-education status and breast cancer risk by another research conducted in European Prospective Investigation in Cancer and Nutrition (Menvielle *et al*., 2011).

For the reproductive risk factors of breast cancer, majority of subjects did not give history of contraceptive usage. Increase risk has been associated with the use of oral contraceptives (OCs), when used for longer duration, especially in young women ≤35 years of age (Brinton *et al*., 2018). However, another study has declared that combined hormonal contraceptives demonstrated no contribution in increasing breast cancer risk (Toss *et al*., 2017). Also, when history of breast feeding was acquired, majority females in this study gave positive history. We did not ask about the duration of breast feeding. Various studies declared significant risk with the duration of breast feeding that is for ≤ 6 month duration, for which the calculated risk of breast cancer was around 2 times (Pramanick *et al*., 2020). Third, most of our subjects reported 3 children or more. A study has inferred that greater number of children decreases the risk of breast cancer (Al Qadire, 2017). Lastly, older age at marriage and increase age at first birth are also found to be associated with increased cancer risk (Galukande *et al*., 2016). In our study, majority of females reported age of 20-22 years at first childbirth.

A population-based study has evaluated the characteristics of reproductive life of 2,522 women who are at familial risk of breast cancer. The study declared that at least one full-term pregnancy, breastfeeding, and late age (> 50 years) at menopause, represent the key protective factors in BRCA mutation carriers. While in women, who were at increased familial risk, early age at first full-term pregnancy found to be one of the main protective factors for breast cancer. Nulliparous women had a significant higher risk as compared to parous women. Also, no significant association was observed between breast cancer risk and menstrual irregularities, history of miscarriages and breastfeeding per child (Khalis *et al*., 2018). In this study, majority of females did not give a positive family history. However, 40% of overall cases of this study gave positive family history of breast cancer. Numerous studies have revealed that subjects with a family history of breast cancer have a significantly higher risk than those who did not have a family history (Nindrea *et al*., 2017). The possible reason might be due to the presence of similar genes and lifestyles, especially with regard to first degree relatives (Yarbro *et al*., 2010).

For the diagnostic profile of subjects, based on mammogram findings, majority cases were diagnosed with BIRAD category 5 and 4. Breast imaging reporting and data system (BI-RADS) categorizes breast imaging findings into seven categories, ranging from 0 to 6. BI-RADS categories 4 and 5 denotes high suspicion of malignancy whereas, its advanced level, BI-RADS category 6 denotes confirmed malignancy, proven by biopsy (Pesce *et al*., 2019). Mammography is the most widely used screening method worldwide for early detection of breast cancer (Hu *et al*., 2018). It has an accuracy rate of 85%-90% if certified equipment with highly skilled radiologists are employed for its interpretation. This screening method due to its high sensitivity has the power to identify non-palpable tumors having a size of ≤ 15 mm. Hence, it can reduce mortality up to 30-50% (Coleman, 2017). The results of reducing mortality by mammography were also confirmed by evaluation studies, which were organized in all European regions, where the method is implemented and monitored as a screening tool (Zielonke *et al*., 2020).

Breast cancer is categorized into 3 major subtypes, based on the presence or absence of molecular markers. It includes estrogen or progesterone receptors and human epidermal growth factor 2 (ERBB2; formerly HER2). The percentage of occurrence of these markers in breast cancer patients are: hormone receptor positive/ERBB2 negative -70% of patients, ERBB2 positive- 15%-20%, and triple-negative breast cancer (TNBC) which is 15% of the overall cases (Waks and Winer, 2019). In this study, majority of subjects reported ER +ve, PR +ve, Her-2/ neu -ve and triple -ve breast carcinoma. The AJCC (American Joint Committee on Cancer Estimation) staging system also incorporated the estimation of biological marker statuses with anatomical staging (TNM) as mandatory, for choosing treatment options and evaluation of prognosis of breast cancer patients (Begum *et al*., 2018). The receptor status estimation serves as highly predictive, prognostic, and therapeutic indicators. Hence, they should be advised on routine basis. For ER, PR positive (As in 70% invasive cancer) patients, most likely endocrine therapy like tamoxifen is recommended. Patients reporting HER-2/ neu +ve are usually associated with bad prognosis, due to higher risk of recurrence and mortality however, they will more likely benefit from targeted therapy. Whereas patients diagnosed with TNBC represents overall worst prognosis and shorter disease-free survival. In South Asians women (19%), the prevalence of TNBC was found to be the second highest after Black women (25%) (Ahmed and Azad, 2017).

Breast cancer is also classified based on histological type and grading into biologically and clinically meaningful subgroups. Histological type refers to the

characterization of tumor's growth pattern. In this study, majority of subjects among both groups reported invasive ductal carcinoma (IDC). It is also known as, not otherwise specified (IDC-NOS) or of no special type (IDC-NST) breast carcinoma. It accounts for 50-80% and considered as a type of adenocarcinoma and a diagnosis of exclusion that fail to exhibit enough characteristics to warrant their classification in one of the special types. Whereas, breast cancer special histological types account for up to 25% of all breast cancers (Böcker, 2002; Ellis *et al*., 1992). However, histological grading of breast cancer is an assessment of tumor's degree of differentiation, proliferative activity, and its level of aggressiveness. Majority cases in this study, reported grade II and grade III neoplasia. Also, grading has been associated with the genetic and transcriptomic features of cancer (Elso, 1991; Weigelt *et al*., 2010).

For breast cancer staging, we employed AJCC TNM (American Joint Committee on Cancer) classification (Cserni *et al*., 2018). Each group (Group A and B) constitutes cancer patients from early and advanced stages. The TNM (primary tumor [T], regional lymph nodes [N], distant metastases [M]) staging system began in 1959, as a product of American Joint Committee for Cancer (AJCC) staging (Giuliano *et al*., 2017). The eighth edition of the AJCC staging for breast cancer has modified the traditional anatomic staging system by incorporating the biological factors (receptor status, grade, and multigene assays) to define prognosis and to determine the type of therapy thus, creating a Clinical Prognostic Staging of breast cancer (Giuliano *et al*., 2017). It is based on initial evaluation before starting any systemic therapy. After the resection of primary tumor, one can determined pathological staging. It includes information from clinical staging along with T (tumor size) and N (lymph node) status analysis from surgical resection. The post-resection anatomic information coupled with the pretreatment biomarker findings results in the final Pathological and Prognostic Staging of breast cancer (Chavez-MacGregor *et al*., 2017).

Regarding the mode of treatment acquired by the group B cases of our study, majority of the cases reported chemotherapy (83%), followed by breast surgery (72%). Radiotherapy was the least reported modality (21%) reported by the patients. Breast cancer is treated by various combinations of surgery, radiation therapy, chemotherapy, and hormone therapy. Prognosis and selection of therapy is said to be influenced by the clinical and pathological features (based on conventional histology and immunohistochemistry) of cancer (Asselain *et al*., 2018). Other influential factors includes patient's menopausal status, disease stage, grading, status of biological markers and cancer's histological type (Harris *et al*., 2016). For non-metastatic disease, the recommended systemic therapy is determined by cancer's subtype. Majority of patients with hormone receptor–positive tumors receive hormonal therapy, minority receive chemotherapy as well (Ruddy and Ganz, 2019). Patients with ERBB2-positive tumors receive ERBB2-targeted antibody, other receive small-molecule inhibitor therapy combined with chemotherapy (Group, 2011). Whereas, patients with triple negative tumors receive chemotherapy alone (Bardia *et al*., 2017). Local therapy for all patients with non-metastatic breast cancer consists of surgical resection, with consideration of postoperative radiation if lumpectomy is performed. Also, some systemic therapy is delivered before surgery. However, metastatic cancer is treated according to subtype, with aim is to prolong life and to relieve palliating symptoms (Sparano *et al*., 2018).

CONCLUSION

This study has evaluated the breast cancer risk factors associated with the demographic data and reproductive factors in females from Karachi sub-population. In general, the risks identified in this study has confirmed the established risk factors of breast cancer such as increasing age, early menarche, late menopause, and family history. Lack of education combine with low socioeconomic status, which further aggravates the health outcomes especially by neglecting the early diagnosis of the disease. Also, higher risk was observed for married women, housewives with extended family that is 3 or more children. Breast feeding for extended duration (at least 2 years) and less age at first childbirth were observed as protective factors for this cancer. With respect to clinical data or diagnostic profile, majority of our study subject were diagnosed at late stages of cancer (stage III and stage IV) also depicted by BIRAD cat 5 and grade 2 and grade 3 of carcinoma. As we all know that breast cancer is completely a curable disease and the disease cure lies in its early detection. This evaluation study in a target population has highlighted the risk factors associated with breast cancer and will substantially play role in disease prevention as well as in its early detection. As early detection will not only cure the breast cancer patients but also prevent them undergoing painful turmoil secondary to procedures like mastectomies leaving them with a lifelong psychosocial disturbance.

Statement of conflict of interest

The authors have declared no conflict of interests.

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