REVIEW

Breast Cancer in Pakistan - a Critical Appraisal of the Situation Regarding Female Health and Where the Nation Stands?

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Abstract

Breast cancer (BC) is the most common malignancy of women worldwide. In the past it was considered as disease of older middle aged women, but the incidence of BC in young females is growing in recent years concordant with studies in Pakistan. In this paper, we reviewed the mutant functions of tumor suppressor genes (BRCA1, BRCA2, p53, ATM and PTEN), epigenetic transformation and involvement of estrogen receptors in development of breast cancer. We further reviewed the current situation of BC in Pakistan that depicts a higher incidence in young females. According to SKMCH and RC data, age group 45-49 years is more prone to BC with high rate of incidence 45.42%. A few studies explored the high expression of ER, PR and HER-2/neu in Pakistani females. Moreover, presence of BRCA1 (c.1961dupA) mutation in Pakistani shows concordance with data in different areas of world. But we are unable to find an authentic study that can explore epigenetic based transformation of breast tumors in Pakistan. This area of research needs more attention to explore the complete picture of BC in Pakistan.

Keywords: Breast cancer - p53 - BRCA1 - BRCA2 - epigenetic transformation - estrogen receptor

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Breast Physiology

Breast development starts at the age of puberty and completes its growth during the periods of gestation (Geddes, 2007). Breast has unique internal structure called the mammary glands, is responsible of providing nutrition and immunity to the infant during the period of lactation (Vorbach et al., 2006). Mammary glands are the distinctive organ of body that keeps on developing during different stages of female life. The end buds of primitive ductal system of an infant breast gradually develop into branched ductal system and decreasing the fatty tissue at the age of puberty. Further development remains in cessation until stimulated by pregnancy hormones (Macias and Hinck). The fundamental ductal system grow by the differentiation of two kind of cells; luminal epithelial and myoepithelia cells, which is associated with milk producing cavities called alveoli. These microstructure cavities are surrounded by luminal epithelial cells and myoepithelial cells. Arteries around the alveolus supply antibodies, nutrients and certain toxins to breast milk (Vorbach et al., 2006). Lobule is the cluster of alveoli, finally connected to nipples via lactiferous ducts. Milk ejection is initiated by nipples stimulation which results in triggering nerve impulse to release oxytocin via systematic stimulation of hypothalamus and posterior pituitary gland. The myoepithelial cells contract under the influence of oxytocin to eject milk, by establishing intraductal pressure (Newton and Newton, 1948; Martin et al., 2001; Abuidhail et al., 2014).

Anatomy of Breast

Breast is made up of adipose and glandular tissues, which are supported by Cooper's ligaments. Glandular tissue comprise of 15 - 20 lobes which further consist of 10–100 alveoli (diameter 0.12 nm). Each alveoli is associated with 15-25 ducts that eventually drain into main duct, forming lactiferous sinus and open through nipple surface. There is a conflict between two group of studies regarding average number of ducts linked to nipple (Moffat and Going, 1996; Taneri et al., 2006). Each nipple opening (0.4- 0.7 nm in diameter) is encircled by circular muscles (Geddes, 2007). Internal mammary artery and lateral mammary branches of lateral thoracic artery are the major source of blood to the breast while pectoral branch of the thoracoacromial artery and posterior intercostal arteries serve as miner source of arterial blood supply (Cunningham, 1977). Deep and superficial systems act as efficient venous drainage system that effluent blood

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into internal thoracic, axillary, and cephalic veins. The deep veins develop at another end of mammary arteries for the collection of blood. While sub areolar veins that appear from nipple, flow blood into perareolar vein. These veins act as junction between superficial and deep plexus (Cunningham, 1977). The nerve supply provided by 2nd and 6th intercostal nerves to the breast. The penetration and pattern of distribution of these nerves are different. Subcutaneous tissues are entertained by superficial course of anterior nerves, while the lateral nerves make deep connection in breast. Lateral and anterior cutaneous branches of the 3rd to 5th intercostal nerves are supplied to nipples and areola (Schlenz et al., 2000). Lymphatic study of breast reveals two pathways for lymph drainage including via axillary nodes and internal mammary nodes. Axillary nodes receive majority of lymph (75%) from medial and lateral portions, while internal mammary nodes receive lymph only from deeper portion of breast (Bannister, 1995).

Breast Cancer

Cancer is a disease in which cells have excessive abnormal proliferation. These unwanted cell division result in the formation of mass or lump called tumor and sub type of cancer is named on the type of cells it affects. Breast cancer (BC) is one of the most frequently reported types of cancer world widely, (Mousavi-Jarrrahi et al.) which can be invasive and non-invasive. BC arises due to mutation in lobules and connecting ducts cells, while rest of the organ is made up of fatty connective and lymphatic tissues (American Cancer Society, 2009-2010). Ductal carcinoma in situ (DCIS) also known as duct cell lining cancer which develops mutation in duct cells. It is considered as non-invasive kind of cancer but can be invasive if left untreated (Allred).

Classification Systems of Breast Cancer

The invasive BC breaks and all cell boundaries and surrounds the nearby normal tissue and its prognosis depends on the diagnosis and spread of metastasis of main tumor. Normally, there are two cancer staging system, which are extensively deployed for staging the disease. TNM system utilize the information of tumor size and its size within breast (T), its spread to nearest lymph node(N), presence or absence of metastasis at distant place (M) (Edge and Compton). After the determination of these parameters, different stages like 0, I, II, III, IV are assigned to the BC, where 0 represents in situ stage, I early stage of invasive cancer and IV represent most advance stage. This system of classification is being deployed only for clinical use, whereas Surveillance, Epidemiology, and End Results (SEER) system is used for public health research, planning and cancer registry data. SEER use terms local stage, regional stage and distant stage to represent different stages of cancer, where local stage represents confined BC correspond to stage I and somehow stage II of TNM staging system Cancelled set by Asim Basra. Regional stage corresponds to stage II and III of TNM where cancer spread to nearby lymph nodes or tissue. Distant stage represents the metastasis of cancer to distant organs and lymph nodes corresponding to stage IIIc and IV of TNM. BC is also classified on the basis of gene expressions (Perou et al., 2000). Presence or absence of specific genes like estrogen receptors (ER+/ER-), progesterone receptors (PR+/PR-), and human epidermal growth factor receptor 2 (HER2+/HER2-) are being in use as biomarkers in the diagnosis of BC sub type and for treatment purposes (Goldhirsch et al.; Reis-Filho and Pusztai)

Epigenetic Transformations

BC is induced by the accumulation of altered gene regulations which cause abnormal cell growth and expansion. In addition to genetic mutations, epigenetics also have a role in BC tumorigenesis. According to increasing number of researches, which include focus on initiating molecular mechanisms in cancer development and identification of new biomarkers to predict BC aggressiveness and the potential of epigenetic therapy (Esteller et al., 2001b; Suzuki et al., 2009; Dagdemir et al., 2013). Epigenetic transformations play an important role in cancer progression and prognosis. Epigenetic modifications include DNA methylation and histone modifications. In DNA methylation, CpG dinucleotide undergoes covalent modification at 5' position of cytosine rings. Histone modifications are post-translational covalent modifications on N-terminal tails of four core histones. Gene expressions are also amended by certain non- coding RNA molecules, either by degrading target RNAs or by arresting their translation (Cortez and Jones, 2008; Khan et al., 2012).

Genes that regulate cell- cycle, angiogenesis, apoptosis, tissue invasion, hormone signaling and metastasis, hypermethylation of these genes play an important part in development of BC (Esteller et al., 2001a; Costa et al., 2004; Oshiro et al., 2005; Holm et al., 2010). Tumor suppressor genes (BRCA1 and p16) and DNA repair genes (GSTP1 and CHD1) hypermethylated was reported in breast tumors, these genes are associated with metastasis and invasion (Esteller et al., 2001a). ADAM23 gene which is associated with transcription of surface adhesion molecules of cells, hypermethylation of its promoter was reported in advance stage tumor (Costa et al., 2004). Epigenetic gene silencing of DSC3, KIF1 and NDRG1 in BC tumors were also stated in literature (Oshiro et al., 2005; Han et al., 2013; Guerrero et al., 2014). Hypermethylaton of tumor suppressor gene RASSF1A promoter was associated with silencing of gene in BC tumors, which exhibited a positive correlation with ER expressions. Similar results were found in case of hereditary BC (Alvarez et al., 2013). It was further depicted that RASSF1A methylation could be used as potential biomarker of BC (Xu et al., 2012; Kajabova et al., 2013). Post-translational histone modifications is one of the important epigenetic transformation that play a critical role in BC development, aggressiveness and prognosis. Histone protein has the ability to influence different genes expressions in different sub types of BC tumor (Jaenisch and Bird, 2003; Abdel and Horwitz, 2015).

Histone modifications in invasive breast tumors

revealed remarkable lysine acetylation (H3K9ac, H3K18ac, H4K12ac and H4K16ac), arginine methylation (H4R3me2) and lysine methylation (H3K4me2 and H4K20me3). A positive correlation of H4R3me2, H3K9ac and H4K16ac levels with low lymph node stage and negative correlation with tumor size was found. A strong association of high histone modifications with steroid receptor +ve tumor was depicted (Elsheikh et al., 2009). In Pakistan, we are unable to find any authentic study that can predict epigenetic changes in local population BC tumor.

Terminal duct-lobular unit contain stem cells that are sensitive towards estrogen and progesterone, proliferate during menstrual cycle and pregnancy. These cells have the potential to develop lactating lobules. In prepubertal period, terminal ductal-lobular stem cells of mutant person (germline or somatic mutation) remain in quiescent, although predisposed to malignancy. Damaged DNA of these cells replicate under the influence of puberty hormones. Mutation in p53 or other gene involve in control or regulation of replication process, allows cells to proliferate in uncontrolled manner and activation of proto-oncogenes (GJ, 1995).

Oncogenes activation in BC cells cause mutation of one or more tumor suppressor genes. These genes are responsible for maintaining genomic integrity and hamper damaged DNA propagation. Mutation in many tumor suppressor genes results in loss of cellular capacity to check DNA damage and its repair. In normal state, these genes recognize DNA damage and arrest cell cycle until damage is repaired. Tumor suppressor gene also induce apoptotic cellular death (Buchholz et al., 1999). Approximately 80-90% familial BC is caused by BRCA1 and BRCA2 genes. Inactivation of these tumor suppressor genes are not only involve in BC development but also cause ovarian and prostate cancers (Thorlacius et al., 1998). BRCA1 is large gene, located on chromosome 17q21, 100 kb in size and encodes 1863 aminoacids protein. Expressions of BRCA1 are high in thymus, testis, breast and ovary. DNA repair, transcriptional transactivation, apoptosis and cell cycle control are commanded by Products of this gene (Miki et al., 1994; Shao et al., 1996; Fan et al., 1998). BRCA1a and BRCA1b are the two variants of BRCA1. These variants are phosphoprotein in nature having phosphotyrosine (Wang et al., 1997). BRCA1a and BRCA1b act as coactivator of p53 gene, their interaction with p53 is reported both in vitro and in vivo (Chai et al., 1999).

BRCA2 gene is much larger than BRCA1, located on chromosome 13q12-13, have 10254 base pairs, 26 coding exons and encode 3418 amino acids (Wooster et al., 1994; Wooster et al., 1995; Phelan et al., 1996; Gayther and Ponder, 1998). Similarly BRCA2 proteins, also play a regulatory role in transcription and DNA repair. Its variants are moderately expressed in prostate, mammary gland and are highly expressed in thymus and testis, suggesting their role in differentiation and development (Zou et al., 1999). Another tumor suppressor gene p53 is considered as most notorious gene in human cancer, is located on chromosome 17p13.1 and has the ability to act as transcription factor to regulate growth signals in damaged cells. Approximately 20-40% of human BC has mutations in p53 gene (Greenblatt et al., 1994). An individual born with p53 germline mutation (Li-Fraumeni syndrome) is most susceptible to BC and other malignancies, (Malkin et al., 1990) as this gene loose its growth-suppressive properties (Sunahara et al., 1998). p53 genes also has considerable homology with p63 and p73. These genes encode transactivation, DNA binding proteins and tetramerization domains of considerable homology with p53. Ataxia telangiectasia (AT) gene also called ATM, may also contribute in development of BC, involve in DNA repair and cell- cycle check point (White and Prives, 1999). PTEN is another tumor suppressor gene, mutations in it allows inappropriate activation of PIP3 pathway during tumorigenesis, leads to the uncontrolled proliferation of cells which should undergo apoptosis (Li et al., 1997).

Estrogen Sensitive Breast Cancer

The estrogen receptor (ER) is a member of nuclear hormone receptors that acts as ligand-activated transcription factors (Heldring et al., 2007). Conformational changes in receptor are induced by ligand which acts as homodimer, binds to specific sequence of DNA called estrogen response elements (ERE) and regulates the transcription of multiple target genes. The ER domains include N-terminal hormone-independent transactivation domain (AF1), a highly conserved DNA binding domain (DBD) that can recognize specific ERE regions. A hinge domain makes distinction between ligand-binding domain (LBD) and DBD. LBD contain hormone binding pocket, in the C-terminus second transactivation domain (AF2) is activated by ligand binding (Nilsson et al., 2001).

Genes which code for enzymes or receptor are important in this regard, as they control metabolism and intra cellular transport of estrogen. Mutation in such genes; 17b-hydroxysteroid dehydrogenase 2 (EDH17B2) gene, cytochrome p450c17a (CYR17) gene, and estrogen receptor (ER) gene may contribute to BC development. ER, a type of steroid receptors, regulates the transcription of specific genes by binding as a hormone receptor complex to hormone response elements (HREs), a specific DNA sequence. As a result of interaction between receptor and HREs, certain genes expressions are either up regulated or down regulated depending upon binding and action of auxiliary factors specific to the target gene and the tissue. Transcription of target genes may be effected by Polymorphism in ER and inefficient binding of estrogen to that receptor. AIB1 protein act as nuclear receptor coactivator, may contribute to the development of steroid-dependent cancers, as it bind to estrogen receptor in ligand-dependent fashion and promote estrogen-dependent transcription (Anzick et al., 1997).

Breast Cancer in Pakistan

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries (2004 Update. 2008). Breast cancer (BC) is the most frequently diagnosed cancer worldwide accounting for 23% (1.38 million) of the total new cancer

cases and 14% (458,400) of the total cancer deaths in 2008. The burden of cancer is increasing in economically developing countries as a result of population aging and growth as well as, increasingly an adoption of cancer-associated lifestyle choices including smoking, physical inactivity, and "westernized" diets (Jemal et al., 2011). BC is considered as the most common disease of the middle age group (40–59 years) females (Siddiqui et al., 2000; Siddiqui and Rasool, 2001; Baloch T and Iqbal, 2006). The typical age incidence curve of BC shows a rapid rise until the age 40, then the rate of incidence falls; however, continuing to rise with increasing age until around 50, then it starts to decline, specifically in low risk populations (Kelsey and Gammon, 1991). Pakistan shows the highest reported incidence of BC in Asia except Israel, as per the available literature (Bhurgri, 2004; Mamoon et al., 2009a). BC is the most prevalent disease among females of all ethnic groups of Karachi and accounts one third of all cancer type in females. Furthermore, highest incidence rate of BC in Karachi is observed when compare with Asia (Bhurgri, 2004; 2005; Mamoon et al., 2009a). Pakistan is considered as one of the highest risk regions as far as BC is concerned (Jamal et al., 2006). BC accounts 40% of all female malignancies among Pakistani women (Goel et al., 2008). The mean age of BC diagnosis in Pakistan is >49 years, which is less as compared to western society whereas the mean age is 54 years (Siddiqui et al., 2003). According to Shaukat Khanum Memorial Cancer Hospital and Research Center (SKMCH & RC) twenty year data extending from December 29, 1994 to December 31, 2014, BC is most common type of cancer among Pakistani females with 45.42 percentage. Their data also indicates maximum numbers of BC cases fall under the age range of 45-49 years (2014). The trend of BC diagnosed in young patients is more aggressive with worse prognosis than those detected in older women (Yankaskas, 2005). With the passage of time, the incidence of BC is consistently increasing in younger Pakistani females. As study conducted by Bhurgri et al. in 2006 mentioned the shift in mean age of all cancer during last ten years from 50.0 to 45.75 years in female, which shows an alarming situation in Karachi (Khaliq et al., 2013). A study in Karachi revealed the prevalence of BC in female with average 44.07 years belonging to different ethnic groups as follows; Sindhi 9%, immigrants 17%, Balochi 2%, KPK 2%, Minorities 2% and Punjabi 2% (Khaliq et al., 2013).

Another study conducted in Lady Reading hospital Peshawar, suggested that 30.4% females of age group 40-49 years were most affected by BC as compared to other age groups (Naeem et al., 2008). Pakistani females have heterogeneous type of breast cancer (Siddiqui et al., 2000; Malik, 2002; Siddiqui et al., 2003; Naeem et al., 2008; Baloch et al., 2014). Table 1 shows the % age of different sub types of BC in different areas of Pakistan. Mamoon N et al. 2009 reported the sub- types of BC in females of age ≥ 30 years such as, a high incidence of invasive ductal carcinoma (IDC) 88.7%, invasive lobular carcinoma (ILC) 5.4% and lymph node metastasis 80.8%. Tumor grading II (57.1%) and III (29.8%) were frequently diagnosed with tumor size < 5 cm (23.2%) (Mamoon et al., 2009a). In another study the ratio of invasive ductal carcinoma (IDC) was found 82.6% in patients of mean age 56.52 years (Naeem et al., 2008). Baloch S et al. revealed in their findings, the prevalence of IDC up to 78%, with most common tumor grading stages II, III (Baloch et al., 2014). Infiltrating ductal carcinoma (IDCA) is one of the most common sub type of BC in Pakistan (Mamoon et al., 2009b; Doutani et al., 2012). Mamoon N and his co-researchers in their study mentioned the prevalence of IDCA and compare with older studies. They found the prevalence of IDCA was 81% with tumor grading type II and size < 5cm. These results are much higher as compare to previous published date of the hospital (Mamoon et al., 2009b). A retrospective analysis of 3279 BC specimens collected at Aga Khan university hospital, found IDCA 37%, fibro adenoma 16.95%, fibrocystic change 13.96%, mastitis 6.83% and duct ectasia 5.33% with different tumor sizes (Siddiqui et al., 2003).

A clinical survey (1994-1999) study conducted in National Cancer institute Karachi, evaluated different BC types on the base of clinic pathological features and reported 91% IDCA, 6% intraductal carcinoma and 3% lobular carcinoma (Malik, 2002). Invasive intraductal carcinoma was found 94% in the patients of age 41.9± 10.9 year with tumor grading stages III and IV (Afridi and Ahmed, 2012), which is consistent with previous analysis published by Malik AM et al, according to their findings the prevalence of intraductal carcinoma was 90% (Malik et al., 2010). Ductal carcinoma in situ (DCIS) is a malignant epithelial cell proliferation within the duct lobular system of the breast that cannot be detected by light microscopy. In developing countries like Pakistan, DCIS is least diagnosed as compare to developed countries, because of unavailability of advance medical facilities to diagnose it (Kayani and Bhurgri, 2005). DCIS was reported 1% and 1.2% in two different studies conducted in Karachi, (Baloch et al., 2014) while a high frequency of palpable masses 92.1% was also reported in Pakistani females (Kayani and Bhurgri, 2005). Germline mutations in the BRCA1 and BRCA2 genes are known as the life time risk factor of developing BC. A mutation (c.1961dupA) in BRCA1 has been reported in various families having BC worldwide including Oman, China, Western-Europe, Latin-America, and the Caribbean region. And recently same mutation in BRCA1 was also reported in Pakistani families with high consanguineous marriage history (Rashid et al., 2014).

A study conducted in 2005 reported 9.17% estrogen receptor positive (ER+ve) tumors.(Kayani and Bhurgri, 2005) The data presenting the behavior of ER and PR positive tumors in Pakistani female population is comparable with western data (Sharif et al., 2009). The expression of HER-2/neu, ER and PR in 481 cases of IDCA was reported by Sharif MA et al. with mean age of 48 years and tumor size 4.4 cm. Their findings revealed high expressions of ER (72.3%), PR (62.6%) and 31% HER-2/neu expressions. They further explored an inverse association of ER and PR with HER-2/neu, while a positive association was seen with lymph node metastases ($p < 0.05$) (Sharif et al., 2009). In India, to whom we share genetics, a similar picture is being observed regarding to BC. The rate of BC in India is 100,000 cases per year. It is

estimated that it might reach up to 131000 cases by 2020. Indian females younger than 45 years are more prone to develop BC than older which is comparable with Pakistan (Mamoon et al., 2009b; Tfayli et al., 2010).

Reasons for Breast Cancer

Rising trend of western life style including smoking, alcohol consumption, western diet habit might be the reason of BC in developing countries including Pakistan (Jemal et al., 2011). Obesity and physical inactivity are also linked with BC (Porter, 2008). As in obese females excessive production of extra glandular estrogen, its metabolites and hyperinsulinemia are associated with BC (Coughlin and Smith, 2015) (Mauras et al., 2015). Reduced breast feeding habit and hormonal factors like early menarche, delayed parity are also associated with the development of BC. These factors are being observed in low and middle income countries like Pakistan, Bangladesh and India (Porter, 2008). Hormonal exposure and delayed parity associated with use of assisted reproductive technology (ART) are also risk factors of developing BC (Reigstad et al., 2015). ART is involved in the up regulation of vascular endothelial growth factor (VEGF), which is a key factor to enhance angiogenesis in tumors (Reigstad et al., 2015).

Conclusion

Pakistani society is a multicultural society. Being a developing country, health services and literacy rate is not satisfactory. In Pakistan majority of BC cases are presented late with advance stage of disease. Reason of late consultation to medical physician might be due to illiteracy, fear, absence of screening programs cultural and economic hindrance (Mamoon et al., 2009b). In urban areas of Pakistan, use of cheap disposable plastic items to serve and heat the food in microwave is very common. These plastic items contain bisphenol-A (BPA) compounds and have estrogen activity (EA), which can induced early menarche, obesity, cancer and altered function of reproductive organs (vom Saal et al., 2005). Eating habits of people of Pakistan are different, like barbecue is the tradition of KPK, heterocyclic amines and other carcinogens produced during barbecue are associated with BC and other type of cancer (Zheng et al., 1998; Berjia et al., 2014). This alarming situation related to female health need an immediate attention, nationwide screening programs, awareness programs and an easy and cheap access to medical facility is a need of time.

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Allred DC Ductal carcinoma in situ: terminology, classification,

Table 1. Table Showing the Percentages of Subtypes of BC in Different Areas of Pakistan

Breast Cancer (BC) Sub Type	Age %	Area	Age Range	Reference
Infiltrating ductal carcinoma	90%	Hyderabad, Jamshoro, Sindh	33-45	(Malik et al., 2010)
	81%	Karachi	48	(Siddiqui et al., 2000)
	82.60%	Peshawar	40-59	(Naeem et al., 2008)
	78%	Karachi	15-80	(Baloch et al., 2014)
	81%	Rawalpindi, ISB, NWFP, upper Punjab	36-60	(Mamoon et al., 2009a)
	91%	Karachi	30-66	(Malik, 2002)
	37%	Karachi	40-49	(Siddiqui et al., 2003)
Invasive intraductal carcinoma	94%	Karachi	31-53	(Afridi and Ahmed, 2012)
Ductal carcinoma in situ	16.25%	Karachi	48	(Siddiqui et al., 2000)
	2.40%	Karachi	15-80	(Baloch et al., 2014)
	1%	Karachi	48-95	(Bhurgri, 2005)
	90%	Hyderabad, Jamshoro, Sindh	33-45	(Malik et al., 2010)
Mucinous carcinoma	2.17%	Peshawar	40-59	(Naeem et al., 2008)
	12%	Karachi	15-80	(Baloch et al., 2014)
	0.52%	Karachi	48	(Siddiqui et al., 2000)
Infiltering lobular carcinoma	6.50%	Peshawar	40-59	(Naeem et al., 2008)
	0.34%	Karachi	48	(Siddiqui et al., 2000)
	1.20%	Karachi	15-80	(Baloch et al., 2014)
Papillary carcinoma	4.35%	Peshawar	40-59	(Naeem et al., 2008)
	0.17%	Karachi	48	(Siddiqui et al., 2000)
Invasive lobular carcinoma	6.50%	Peshawar	40-59	(Naeem et al., 2008)
Medullary carcinoma	2.17%	Peshawar	40-59	(Naeem et al., 2008)
	6%	Karachi	15-80	(Baloch et al., 2014)
Benin lumps	39.70%	Karachi	15-80	(Baloch et al., 2014)
	30.91%	Rawalpindi, , ISB, NWFP, upper Punjab	36-60	(Mamoon et al., 2009a)
	92.10%	Karachi	48- 95	(Bhurgri, 2005)
Total BC	19%	Baluchistan	31-50	(Doutani H et al., 2012)
	45.41%	Lahore	>18	(2014)

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