ASSOCIATION OF GENETIC AND NON-GENETIC RISK FACTORS WITH SPECIFIC BRCA MUTATION POSITIVE BREAST CANCERS IN SOME PAKISTANI FEMALES

Samina Malik, Atif Hanif*, Samina Khokher, Zafar Iqbal***, Sobia Rana***, Mohammad Imran***, Mohammad Bilal*, Mohammad Arslan*****

Department of Physiology Avicenna Medical College, *University of Veterinary and Animal Sciences, **Breast Clinic, INMOL, ***Department of Physiology & Cell Biology, University of Health Sciences, Lahore, Pakistan

Background: Breast cancer is the most common malignancy among Asian women including Pakistan where recurrent mutations among certain sub-ethnic groups predisposing to breast cancer have recently been established. **Study Design:** The current retrospective study involves identification of genetic and non-genetic risk factors in 27 specific mutation positive females out of a total of 100 females diagnosed with breast cancer, representing a sample from the Punjabi ethnic population of the city of Lahore. The study has been carried out by telephonic communication with the mutation positive patients or their relatives. **Results:** Out of the total 27% patients positive for specific BRCA mutations, 23% were positive for *BRCA1* mutations and 4% for *BRCA2*. Among a total of 100 breast cancer patients the *BRCA1*-IVS14, 1G>A mutation was identified in 5 Punjabi ethnic females with Rajput subethnicity, *BRCA1*-3889delAG in 10 (8 with Mughal and 2 with Khan subethnicity), *BRCA1*-2080insA in 8 (Rajput subethnics) and *BRCA2*-3337C>T in 4 (Minhas subethnic) subjects. Two *BRCA1* mutations, namely 3889delAG and 2080insA were found to coexist in only one study case (with Mughal subethnicity). All the mutation positive breast cancers had unilateral ductal carcinoma. Of the 23 cases positive for screened *BRCA1* mutations, 17 were diagnosed for breast cancer at a relatively early age (age≤40) and 6 were diagnosed at late age (age≥41) whereas all cases positive for single *BRCA2* mutation under consideration were diagnosed at late age. Furthermore, 24 of 27 patients with specific *BRCA* mutations had a positive family history of breast cancer. **Conclusion:** The high prevalence of the screened *BRCA* mutations in certain Punjabi sub-ethnicities indicates the importance of counseling. It is suggested that consanguinity may be a risk factor for recurrent population specific mutations. Hormonal factors including use of oral contraceptives, polycystic ovaries, central obesity, nulliparity, late age at first pregnancy, lack of breast feeding and activity as well as dietary factors like active or passive smoking seem to have little role in study population. Dietary factors like active or passive smoking were less likely risk factors than dietary deficiency of vitamins, especially in low income group. Anxiety and exposure to traffic pollution were additional risk factors.

Keywords: Breast cancer, Malignancy, Mutations, Risk factors

INTRODUCTION

In Pakistan and India in multiple families certain BRCA1 mutations were detected including: 185delAG,[1,2] 4184delTCAA,[3,4] 4284delAG, 3889delAG, IVS14-1G>A , and 2080insA, and a BRCA2 mutation 3337 C>T[4]. According to a Pakistani study that involved screening of exons 2, 11, 12, 15 (IVS14) and 20 of BRCA1 and exons 10, 11 and 22 of BRCA2, five BRCA1 mutations and one BRCA2 mutation was detected as candidate founder mutations including the last 6 mutations mentioned above.[4] Their distribution was specific to geographically and genetically distinct ethnic groups in Pakistan.[4] According to an estimate this screening detected 70–80% of all germline mutations in these 2 genes. Three of these mutations are located on chromosome 11, including 2080insA, 3889delAG and 3337C>T. These mutations are predominant founder mutations in Pakistani population. 3337C>T mutation in BRCA2 gene, has also been described in families from southern China and Hong Kong—separate origins of the same mutation are more likely than ancestral link.[5,6] Breast cancer is also the most common cancer in women in Pakistan, followed by cancer of the oral cavity and the ovary.[7] Pakistan has one of the highest rates of breast cancer in Asia, and a high proportion of cases occur below the age 40 years.[7,8] Pakistani population is one of the highest incidence population with the BRCA mutation prevalence of 6.7% among breast cancer patients and 15.8% among ovarian cancer patients, which was one of the highest recorded outside of the Ashkenazi Jewish population.[4]

A study on Pakistani female population selected for family history and age from the city of Lahore consisting of 176 affected individuals from families with breast or ovarian cancer. The study revealed 30/176 (17%) mutations, out of which 23/30 (76.6%) were detected in BRCA1 and 7/30 (23.3%) in BRCA2. Median age at diagnosis of breast cancer was 31 years and 29 years respectively in presence and absence of BRCA1 mutations,[9] with no significant difference in the two ages.

The major (non-genetic) risk factors for breast and ovarian cancer are hormonal and dietary factors. Pakistani women, however, are less likely to these risk factors, due to cultural trends like early age at first pregnancy, multiple child births and prolonged breast-feeding. In addition, there is less preference for use of oral contraceptives and consumption of tobacco or alcohol.[10,11] Diet poor in fruits and vegetables (vitamins) is a possible risk factor.[11]

Another major risk factor associated with breast cancer is consanguinity. Consanguineous marriage is practiced in much of the Muslim world including North and Sub-Saharan Africa, the Middle East, the West, and South Asia, and by migrants from these regions. Approximately 40% of the Pakistani population has practiced consanguinity for nearly 300 years.[12]

MATERIAL AND METHODS

Allele specific PCR technique was used to amplify extracted DNA from peripheral blood of 100 Punjabi patients suffering from breast cancer. The primer sets were based on normal and mutant sequences for *BRCA1* and *BRCA2* genes and were synthesized by Invitrogen; Genelink, New York, USA. A detail of life style, sub-ethnicity, dietary and hormonal factors was obtained from the positive cases only by telephonic communication.

RESULTS

Table 3.1: Association of individual BRCA1 and BRCA2 mutations, with genetic and non-genetic factors.

Gene involved	**BRCA1**	**BRCA1**	**BRCA1**	**BRCA1**	**BRCA2**
Mutation name	IVS14, 1G>A	3889delAG	2080insA	Both 3889delAG and 2080insA	3337C>T
BRCA ratio (%)	5/27 (18.5%)	10/27 (37%)	8/27 (30%)	1/27 (4%)	4/27 (15%)
Mean Age of onset	33 years	40 years	44 years	50 years	53 years
No. (Stage 1/2/3/4)	5 (3)	8 (3) 2 (4)	5 (2) 3 (3)	1 (4)	4 (3)
Histology **Ductal: Lobular**	5:0	10:0	8:0	1:0	4:0
Axillary: Cervical lymph node	5:0	10:10	8:0	1:1	4:0
Ethnicity: **No. (Specify sub-ethnicity)**	Punjabi 5 (Rajput)	Punjabi 8 (Mughal) 2 (Khan baradri)	Punjabi 8 (Rajput)	Punjabi 1 (Mughal)	Punjabi 4 (Minhas)
Parity: **(nullipara/multipara)**	8 (multi-parous)	3 (multi-parous)	5 (multi-parous)	10 (multi-parous)	8 (multi-parous)
Age at 1st pregnancy	16 yrs	29 yrs	16 yrs	17 yrs	16 yrs
Gene involved	BRCA1	BRCA1	BRCA1	BRCA1	BRCA2
Mutation name	IVS14, 1G>A	3889delAG	2080insA	Both 3889delAG and 2080insA	3337C>T
Breast feeding **(Max=2 yrs)**	(1.25): (2) years	(0.25-2): (2) years	(2): (2) years	(1-1.25): (2) years	(2-2.5): (2) years
Life style: **Active** **Sedentary** **(central obesity)**	5:0 0/5 (0%)	10:0 0/10 (0%)	8:0 0/8 (0%)	1:0 0/1 (0%)	4:0 0/4 (0%)
Family history of breast cancer Ratio (%)	0/5 (0%)	8/10 (80%)	6/8 (75%)	0/1 (0%)	0/4 (0%)
Family history of any other cancer Ratio (%)	0/5 (0%)	Lung cancer (father) 2/10 (20%)	0/8 (0%)	Cancer Neck and Axilla (son) 1/1 (100%)	0/4 (0%)
Diet : use of pan/huqqa/naswar Ratio (%)	0/5 (0%)	0/10 (0%)	0/8 (0%)	0/1 (0%)	0/4 (0%)
Smoking: Active/Passive (A:P)	0:0	0:3	0:0	0:0	0:1
Intake of fruits/salad:	0/5 (0%)	5/10 (50%)	8/8 (100%)	0/1 (0%)	0/4 (0%)
Consanguinity:	5/5 (100%)	10/10 (100%)	8/8 (100%)	1/1 (100%)	4/4 (100%)

DISCUSSION

The present study provides prevalence of three population specific BRCA1 mutations and one BRCA2 mutation in association with breast cancer in 100 females from the city of Lahore, representative of the Punjab province of Pakistan. Rates generated by the cancer registries of different countries vary, particularly with respect to methods, quality and reporting of cancer incidence data.[13]

The prevalence figure of the present study (27%) is close to European population (Spanish families) touching 26.3% in the year 2003.[14] Current study shows significantly high prevalence of BRCA1 mutations (23%) as compared to BRCA2 mutations (4% only). In the rest of population (73%), there may be some unknown mutations or factors responsible for breast cancer. This BRCA1/BRCA2 ratio is not in line with the study on another European population of Italy where there is low mutation prevalence but a significantly high association with BRCA2 mutations as compared to BRCA1 mutations in hereditary breast cancer families.[15]

The prevalence report from the present study in Pakistani (Punjabi) females with breast cancer, unselected for family history is close to an Indian study. In India, the prevalence figures were 6 out of 24,i.e, 25% in 2004.[16] The two reports were in line but the sample size was small in Indian study and the study included only familial cases (with positive family history) of breast cancer, so the prevalence is not comparable.

According to a study in 2006 on BRCA1 and BRCA2 mutations in breast and /or ovarian cancer patients (selected for family history) reported from Pakistani population, the prevalence was only 17%.[9] The results of this study are not in line with it due to difference in inclusion criteria as that study included only familial cases.

Another Pakistani study was conducted on unselected Pakistani breast and ovarian cancer patients in 2002. It involved more than 33% Mohagir subjects (immigrants from India) and reported further low prevalence of 6.7%,[4] as compared with the current study. The significantly increased prevalence in the current study may be due to the study population that included no Mohajir subethnicity, known for low prevalence.[4,9,17]

Two of the four founder mutations (mutations that are recurrent in a population) identified in the study (2080insA and IVS14-1G>A) were unique to Pakistan.[4] The other two mutations (3889delAG and 3337C>T) were also reported in the populations of Dutch and Chinese respectively along with Pakistani population.[4–6] All the subjects in the present study were Punjabi, based in the Pakistani city of Lahore which along with the city of Karachi has a high reported prevalence of breast cancer (1/3 of all malignant tumours of female patients).[18] More than half of the subjects (15/27) in the current study had mutations previously reported in Punjabi (people belonging to the Punjab Province of Pakistan)[4] population, i.e., (3889delAG in 10 and IVS14, 1G>A in 5 subjects). Although 8 cases among the 100 included in the study consisting of Punjabis (people belonging to the most populated province of Punjab) were found positive for a previously reported[4] Pashtun (people belonging to North West Frontier Province of Pakistan) ethnic mutation (2080insA). This appearance of a known Pashtun mutation in Punjabi subjects might be due to common ancestral roots with Pashtun ethnicity, though no such data was known to the subjects and their relatives. The present study provides the first report of 2080insA mutation in Punjabi population.

The present study revealed a rare finding, i.e., simultaneous occurrence of two founder mutations (2080insA and 3889delAG) in the same individual case. This co-existence of the two mutations may be the result of a cross marriage between Punjabi and Pashtun carriers in the pedigree, although no such history was known. The patient was regarded as the proband (first appearance of cancer in the family) with no family history of breast cancer but consanguinity and 1st cousin marriage in parents were predominant features.[12] This finding also suggests the importance of counselling regarding family planning in the heterozygous mutant and mutation screening in her family. It was suggested that identification of cases with more than one mutation would increase our understanding between various mutations and would focus on genetic counseling.[19] The carrier of coexistent mutation was grand multiparous (parity=10). In a Canadian study multiparity appeared to have a protective role in BRCA1 mutants, but increased risk in BRCA2 mutants.[20] The present study contrarily revealed an association of multiparity with BRCA1 coexistent mutations (2080insA and 3889delAG) and with another BRCA1 mutation (IVS14, 1G>A) screened in this study. The BRCA2 mutation included in the present study was also associated with multiparity, although the number of BRCA2 carriers was small. Another study revealed that there was no statistically significant difference in breast cancer risk between parous (women who has born children) and nulliparous (women who has not born children) women but there was a 14% decreased risk for each additional birth in both carriers of BRCA1 and BRCA2 (in age above 40).[21] This association of multiparity is also not in line with another study that associates parity with lower risk of breast cancer in BRCA1/2 mutation mutants.[22] The age at first pregnancy was 17 years in this unusual carrier of 2

mutations. Another study supports that having an early first child does not confer protection against breast cancer in BRCA mutation carriers.[23]

There was a report of early onset cancer neck and axilla in one of the male outcomes of the carrier with coexistent BRCA1 mutations, resulting into adverse prognosis and death. It was reported in a previous study on Pakistani population that males belonging to BRCA2 positive families developed cancer in neck and breast,[4] but the current study provides the first report of cancer neck and axilla in a male outcome of a mother with two co-existent BRCA1 mutations. In the current study population we found no association with history of male breast cancer in family of either BRCA1 or BRCA2 mutation carriers. Another study on Israeli men shows strong association of BRCA2 mutations with male breast cancer. Ratio of BRCA1 to BRCA2 mutations was 8:21[24] in presence of male breast cancer.

Generally early age at onset is attributed to BRCA mutations.[25] According to the present study, only BRCA1 mutations were associated with early onset of breast cancer at mean age of 39 years. In another study on Pakistani population [9], the median age of onset of breast cancer was 31 years, with BRCA1 mutation. This difference may be due to selection criteria, i.e., 48% of study cases belonged to families with 1 female breast cancer diagnosed at age ≤ 30 years. Furthermore more than 50% breast cancer cases from BRCA1/2-associated families were affected by age 40 and 90% by age 50. In the present study, the BRCA1-mutation-positive breast cancer case subjects had cancer diagnosed, on average, 14 years earlier than did the BRCA2-mutation-positive case subjects (at ages 39 years and 53 years, respectively). In another Pakistani study this difference was seen but the figure was 10 instead of 14, i.e., at ages 36.8 (for BRCA1) and 46.4 years (for BRCA2).[4] This age incidence is quite in line with the present study (39 years with BRCA1 and 53 years with BRCA2), may be due to similar selection criteria (subjects unselected for age and family history).

In case of individual mutations of BRCA1/2, there was no difference in histopathology. All the mutation carriers belonged to ductal histopathology. Collectively 93% of the breast cancer cases, both mutants (with BRCA1/2 mutation) and non-mutants belonged to the same histopathology. The remaining 7% cases belonged to Lobular histopathology and fall in non-carrier group. This high prevalence of ductal variety in heterozygous mutants as well as non-mutants is in line with another study that declares that ductal carcinoma is found in 60% of breast malignancies observed in clinical practice.[26] This increased expression of BRCA1 with ductal carcinoma was found in majority (84%) of cases in another study on pathogenesis of breast cancer.[27] Association of ductal carcinoma with BRCA1 mutations is also seen in another study.[28] Comparing BRCA1 related breast cancer in the present study with, BRCA1 related prostate cancer in Ashkenazi Israelis, no difference was noted among histo-pathologic features of cases with or without founder mutations but no difference was found in mean age at diagnosis as well.[29] According to a study in USA, ductal carcinoma in situ (carcinoma that has not crossed the basement membrane) was considered a part of breast and ovarian cancer syndromes defined by BRCA1 and BRCA2 mutations.[30]

In current study, late onset (age≥41) as well as grade 2 breast cancer was significantly related to absence of the investigated BRCA1/BRCA2/BRCA mutations. This association with age of onset is indirectly supported by another study that shows significant association of early onset of breast cancer with biologically more severe disease.[31] The association with absence/presence of mutation is directly supported by Breast Cancer Linkage Consortium (1997), that states that the histology of breast cancer in women predisposed by reason of carrying BRCA1/2 mutations in comparison with sporadic cases (cases without mutation) of breast cancer usually exhibit adverse histopathology picture.[32–34]

It may have implications for screening and management. A significant number of grade 2 patients had unilateral cancer, which is considered as less aggressive than bilateral cancer. In the present study, axillary node involvement was a common feature in young as well as elderly carriers of BRCA1/2 mutations without an association with disease adversity (grade/stage). Contrarily, another study shows an association of node negative, early stage breast cancer, in young women with more aggressive pathogenesis [35]. In yet another study there is an association of lymph node negative status in young women with worse prognosis.[36]

The present study indicates that family history is not a significant predisposing factor in mutants or non-mutants of BRCA mutations. This is in contrast with another study that shows that among the breast cancer cases, there was a significant association with family history of breast cancer in absence of BRCA1/2 mutations, as compared to the healthy controls.[37]

In this study, as compared to BRCA1 carriers, 1st child birth before age 20 years (average age of 16 years) was associated with higher risk of breast cancer in BRCA2 mutants as compared with BRCA1 mutants. This association is contrary to another study[21] that supports the association of early

(before age of 20 years) 1st child birth with high risk of breast cancer in BRCA1 carriers as compared with BRCA2 mutation carriers.

Among unilateral and bilateral cases of breast cancer, there was a significant relation between unilateral cases and ductal type of breast cancer. This is indirectly supported by another study that associates bilateral cancer more with multi-centric disease (lobular histopathology with many foci in both breasts) in comparison with unilateral.[38]

In current study there was a significant relation between left unilateral breast cancer and positive family history. So far no correlation of right or left side involvement is established with the family history. In another study bilateral cancer is associated with family history.[38] According to two other studies breast cancer in a female first degree relative has been considered a strong risk factor for bilateral cancers.[39,40] In this study on 100 patients unselected for family history, 85% patients gave positive family history of breast cancer in 1–3 first degree relatives and 15% were with negative family history. Our figures were far from the interpretation by the Collaborative Group on Hormonal Factors in Breast Cancer published in Lancet in 2001 which gave a value of 88.8% (8 out of 9) that represents breast cancer patients with negative family history.[41]

In some of the BRCA1/2 mutations (3889delAG and 3337C>T) an associated risk factor was passive smoking. In the commonest mutation of the study (3889delAG), family history of lung cancer was also associated with the risk of passive smoking. Another study indicates that smoking is a risk factor for breast cancer before age 50 in BRCA1/2 mutation carriers.[42] In the present study the age of onset of BRCA1 and BRCA2 mutation in presence of this risk factor was 40 years and 53 years respectively, both these age groups are in line with that study. Studies with thorough exposure of passive smoking indicate passive and active smoking as risk factors for pre menopausal breast cancer [43]. Women by age 50 years were at greatest risk of breast cancer in association with both passive and active smoking according to a German study.[44] Diet poor in fruits and vegetables was a risk factor in 10/27 mutants with BRCA1/2 mutations. Another study supports that vegetables reduce the breast cancer risk.[45] According to American Institute for Cancer research, diet rich in vegetables and fruits reduces the risk of breast cancer.[46] All the mutant cases with BRCA1/2 mutations in the study had active life style and no central obesity so these two risk factors were not contributory towards development of breast cancer but still levels of physical activity were inadequate to meet recommendations for prevention of chronic diseases according to another study.[47] Exposure to traffic pollution was reported by 18/27 mutants, especially by BRCA2 mutation carriers (4/4) and two co-existent BRCA1 mutations carrier. According to a study on exposure to traffic emissions throughout life and breast cancer risk, high exposure at menarche is associated with pre-menopausal breast cancer and high exposure at the time of woman's first birth for postmenopausal breast cancer.[48] In the present study 100% history of exposure was provided by women with average age 53 (those with BRCA2 mutation) and 50years (woman with two BRCA1 mutations). There was no association in the present study with polycystic ovary syndrome or use of oral contraceptive pills with the risk of breast cancer, though these are regarded as important risk factors.[49,50] History of anxiety was present in 15/27 heterozygous mutants of BRCA1 mutations but was absent in those of BRCA2 mutations. Stress was reported as a negative prognostic factor in breast cancer cases and its associated increase in Monoamine oxidase (MAO) activity of platelets was considered a risk factor for survival with breast cancer in an Italian study.[51] Another study shows increased MAO activity levels in parents and their proband children in association with greater rates of 'high MAO related' disorders.[52]

The study concludes that genetic and non-genetic factors have a significant association with breast cancer incidence in population of Punjab (Pakistan). Punjabi females with early onset of breast cancer around 39 years of age are more likely to have a population specific BRCA 1 mutation. Punjabi females with late onset (around 53 years) are more likely to have a population specific BRCA2 mutation. Females with family history of breast cancer are more at risk of developing left unilateral breast cancer. Unilateral breast cancer women are likely to have ductal histopathology and are likely to be diagnosed in grade 2. Elderly females with grade 2 are less likely to be the carriers of BRCA mutation. Association between consanguinity, first cousin marriage of parents and breast cancer risk suggests that recessive genes may play a role in the etiology of breast cancer. Coexistence of two BRCA1 mutations is a single proband is an outcome of dominance of familial recessive genes. Coexistent mutations of BRCA1 genes are also a risk factor for cancer of neck and axilla in the male members of the families. Grand multiparity (even with 1st pregnancy at an early age) is an identified risk factor for coexistent BRCA1 mutations in postmenopausal women (around age 50).

This research provides the data for female population of Punjab (Pakistan) with breast cancer. Population specific BRCA1 and BRCA2 mutation screening around their respective risk ages may

prevent breast cancer by available prophylactic choices. The female family members of the mutants from high risk population may have aggressive screening (genetic and radiological etc) for early detection. Counseling regarding number of child births and consanguineous marriages should be made available at mass level. Self breast examination should be promoted. A broader study can be designed including different provincial populations to represent the overall prevalence in Pakistan. A study for significant comparison of genetic and non-genetic risk factors can be designed. For more elaborative data, DNA sequencing can be carried out.

REFERENCES

1. Risch HA, McLaughlin JR, Cole DEC, Rosen B, Bradley L, Kwan E, *et al*. Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet 2001;68:700–10.
2. Bar-Sade RB, Kruglikova A, Modan B, Gak E. The 185delAG BRCA1 mutation originated before the dispersion of Jews in the Diaspora and is not limited to Ashkenazim. Hum Mol Genet 1998;7:801–5.
3. Neuhausen SL, Mazoyer S, Friedman L, Stratton M. Haplotype and phenotype analysis of six recurrent BRCA1 mutations in 61 families: results of an international study. Am J Hum Genet 1996;58:271–80.
4. Liede A, Malik IA, Aziz Z, Rios Pd Pde L, Kwan E, Narod SA. Contribution of BRCA1 and BRCA2 mutations, to breast and ovarian cancer in Pakistan. Am J Hum Genet 2002;71:595–606.
5. Khoo US, Chan KY, Cheung AN, Xue WC, Shen DH, Fung KY, *et al*. Recurrent BRCA1 & BRCA2 Germline mutations in ovarian cancer: a founder mutation of BRCA1 identified in the Chinese population. Hum Mutat 2002;19:307–8.
6. Khoo US, Ngan HY, Cheung AN, Chan KY, Lu J, Chan VW, *et al*. Mutational analysis of BRCA1 & BRCA2 genes in Chinese ovarian Cancer identifies 6 novel germline mutations. Hum Mutat 2000;16:88–9.
7. Bhurgri Y, Bhurgri A, Hassan SH, Zaidi SH, Rahim A, Sankaranarayanan R, *et al*.Cancer incidence in Karachi, Pakistan: first results from Karachi Cancer Registry. Int J Cancer 2000;85:325–9.
8. Usmani K, Khanum A, Afzal H, Ahmad N. Breast cancer in Pakistani women. J Environ Pathol Toxicol Oncol 1996;15:251–3.
9. Rashid MU, Zaidi A, Torres D, Sultan F, Benner A, Naqvi B, *et al*. Prevalence of BRCA1 & BRCA2 mutations in Pakistani breast & ovarian cancer patients. Int J Cancer 2006;119(12):2832–9.
10. Hussain R, Bittles AH. The prevalence and demographic characteristics of consanguineous marriages in Pakistan. J Biosoc Sci 1998;30:261–75.
11. Sinha R, Anderson DE, McDonald SS, Greenwald P. Cancer risk and Diet in India. JPGM 2003;49:222–8.
12. Tay JS, Lai PS. Consanguinity and health. Lancet 1991;24;338:515.
13. Struewing JP, Hartge P, Wacholder S, Baker SM, Berlin M, McAdams M, *et al*. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med 1997;336:1401–8.
14. Diez O, Osorio A, Duran M. Analysis of BRCA1 & BRCA2 genes in Spanish Breast/ovarian cancer patients; a high proportion of mutations unique to Spain and evidence of founder effects. Hum Mutat 2003;22(4):301–12.
15. Giannini G, Capalbo C, Ristori E, Ricevuto E, Sidoni T, Buffone A, *et al*. Novel BRCA1 and BRCA2 germline mutations and assessment of mutation spectrum and prevalence in Italian breast and/or ovarian cancer families. Breast Cancer Res Treat 2006;100(1):83–91.
16. Suresh Hedau, Neeraj Jain, Syed A. Husain, Ashish K, Mandal Bhudev C. Das. Novel germline mutations in breast cancer susceptibility genes BRCA1, BRCA2 and P53 gene in breast cancer patients from India. Breast Cancer Research and Treatment 2004;88:177–86.
17. Parkin DM, Whelan SL, Ferlay J, Raymond L, Young J. Cancer incidence in five continents, vol.7. Lyon, France: IARC, 1997.
18. Malik IA, Mubrik A, Luqman M, Ullah K, Ahmad M, Alam SM, *et al*. Epidemiological and morphological study of breast cancer in Pakistan. J Environ Pathol Toxicol Oncol 1992;11:353.
19. Lindsay A. Farrer, Kathleen S. Arnos, James H. Asher, Clinton T. Baldwin, Scott R. Diehl, Thomas B. Friedman *et al*. Waardenberg syndrome (WS) type 1 is caused by defected loci on chromosome 2. Am J Hum Genet 1994;55(4):728–37.
20. Narod SA. Modifiers of risk of hereditary breast cancer. Oncogene 2006;25(43):5832–6.
21. Andrieu N, Goldgar DE, Easton DF, Rookus M, Brohet R, Antoniou AC, *et al*. IBCCS Collaborators Group. Pregnancies, breast-feeding, and reast cancer risk in the international BRCA1/2 Carrier Cohort Study (IBCCS). J Natl Cancer Inst 2007;99(14):1130–1.
22. Antoniou AC, Shenton A, Maher ER, Watson E, Woodward E, Lalloo F, Easton DF, Evans DG. Parity and breast cancer risk among BRCA1 and BRCA2 mutation carriers. Breast Cancer Res 2006;8(6):R72.
23. Kotsopoulos J, Lubinski J, Lynch HT, Klijn J, Ghadirian P, Neuhausen SL, *et al*. Age at first birth and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. Breast Cancer Res Treat 2007;105(2):221–8.
24. Chodick G, Struewing JP, Ron E, Rutter JL, Iscovich J. Similar prevalence of founder BRCA1 and BRCA2 mutations among Ashkenazi and non-Ashkenazi men with breast cancer: Evidence from 261 cases in Israel, 1976–1999. Eur J Med Genet 2008;51(2): 141–7.
25. Musolino A, Bella MA, Bortesi B, Michiara M, Naldi N, Zanelli P, *et al*. BRCA mutations, molecular markers, and clinical variables in early-onset breast cancer: A population-based study. Breast. 2007;16(3):280–92. Epub 2007 Jan 25.
26. Ellis IO, Schnitt SJ, Sastre-Garau X et al. Invasive breast carcinoma. In Tavassoli FA, Devilee P eds. World Health Organization classification of tumours: tumours of the breast and female genital organs. Lyon: IARC Press 2003.
27. Ribeiro-Silva A, Ramalho LN, Garcia SB, Brandão DF, Chahud F, Zucoloto S. p63 correlates with both BRCA1 and cytokeratin 5 in invasive breast carcinomas: further evidence for the pathogenesis of the basal phenotype of breast cancer. Histopathology 2005;47:458–66.
28. Laakso M, Loman N, Borg A, Isola J. Cytokeratin 5/14-positive breast cancer: true basal phenotype confined to BRCA1 tumors. Mod Pathol. 2005.
29. Giusti RM, Rutter JL, Duray PH, Freedman LS, Konichezky M, Fisher-Fischbein J, *et al*. A twofold increase in BRCA mutation related prostate cancer among Ashkenazi Israelis is not associated with distinctive histopathology. J Med Genet; 40 (10): 787-92. 2003.
30. Claus EB, Petruzella S, Matloff E, Carter D. Prevalence of BRCA1 and BRCA2 mutations in women diagnosed with ductal carcinoma in situ. JAMA 2005;293(8):964–9.
31. Gajdos C, Tartter PI, Bleiweiss IJ, Bodian C. Brower ST. Stage 0 to stage III breast cancer in young women. J Am Coll Surg 2000;190(5):523–9.
32. Breast Cancer Linkage Consortium. Pathology of familial breast cancer: differences between breast cancers in carriers of BRCA1 & BRCA2 mutations & sporadic cases. Lancet 1997;349:1505–10.

33. Stoppa-Lyonnet D, Ansquer Y, Dreyfus H, Gautier C, Gauthier-Villars M, Bourstyn E, *et al*. Familial invasive breast cancers: worse outcome related to BRCA1 mutations. J Clin Oncol 2000;18:4053.
34. Lakhani SR, van de Vijver MJ, Jacquemier J, Anderson TJ, Osin PP, McGuffog L, *et al.* Breast Cancer Linkage Consortium. The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, & p53 in patients with mutations in BRCA1 & BRCA2. J Clin Oncol 2002;20:2310–8.
35. Fowble BL, Schultz DJ, Overmoyer B, Solin LJ, Fox K, Jardines L, *et al*. The influence of young age on outcome in early stage breast cancer. Int J Radiat Oncol Biol Phys 1994;30(1):23–33.
36. Klauber N. Tumor biology of breast cancer in young women. Breast Dis 2005;23:9–15.
37. Claus EB, Schildkraut J, Iversen ES Jr, Berry D, Parmigiani G. Effect of BRCA1 & BRCA2 on the association between breast cancer risk & family history. J Natl Cancer Inst 1998;90(23):1824–9.
38. Newman LA, Sahin AA, Cunningham JE. A case-control study of unilateral and bilateral breast carcinoma patients. Cancer 2001;91(10):1845–53..
39. Joan Elizabeth Cunningham. A case-control study of bilateral compared with unilateral breast cancer. The University of Texas, School of Public Health.1998.
40. Sunil R Lakhani. The pathology of familial breast cancer: Morphological aspects. Breast Cancer Research 1999;1:31–5.
41. Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. Lancet 2001;358(9291):1389–99.
42. Breast Cancer Family Registry; Kathleen Cuningham Consortium for Research into Familial Breast Cancer (Australasia); Ontario Cancer Genetics Network (Canada). Smoking and risk of breast cancer in carriers of mutations in BRCA1 or BRCA2 aged less than 50 years. Breast Cancer Res Treat. 2008;109(1):67–75. Epub 2007 Oct 31.
43. Johnson KC. Accumulating evidence on passive and active smoking and breast cancer risk. Int J Cancer 2005;117(4):619–28.
44. Kropp S, Chang-Claude J. Active and passive smoking and risk of breast cancer by age 50 years among German women. Am J Epidemiol. 2002;156(7):616–26.
45. Rao DN. Role of vegetarian diet in cancers of the esophagus and female breast in India. Vegetarian Congress Research Presentations, Section 1: Diet and Chronic Disease. Loma Linda University Press, 1997. pp. 4, 2003.
46. World Cancer Research Fund, American Institute for Cancer Research. Food, nutrition, and the prevention of cancer: a global prospective/World Cancer Research Fund, in association with American Institute for Cancer Research. Washington, DC: American Institute for Cancer Research, 1997.
47. Vaz M, Bharathi AV. Practices and perceptions of physical activity in urban, employed, middle class Indians. Indian Heart J 2000;52:301–6.
48. Nie J, Beyea J, Bonner MR. Exposure to traffic emissions throughout life and risk of breast cancer: the Western New York Exposures and Breast Cancer (WEB) study. Cancer Causes Control. 2008;19(4):435.
49. Spritzer PM, Morsch DM, Wiltgen D. Polycystic ovary syndrome associated neoplasms. Arq Bras Endocrinol Metabol 2005;49(5):805–10. Epub 2006 Jan 23.
50. Rohan TE, Miller AB. A cohort study of oral contraceptive use and risk of benign breast disease. Int J Cancer. 1999;82(2):191–6.
51. Giraldi T, De Vanna M. Mental adaptation to cancer: depression and blood platelet monoamine oxidase activity in breast cancer patients. Anticancer Res 2007;27(3B):1715–9.
52. Puchall LB, Coursey RD. Parents of high risk subjects defined by levels of monoamine oxidase activity. Schizophr Bull 1980;6(2):338–46.

Address for Correspondence:

Dr. Samina Malik, Department of Physiology, Avicenna Medical College, Lahore, Pakistan.
Email: drsemymalik@yahoo.com