

# MOLECULAR SUBTYPES OF BREAST CARCINOMA AND ITS ASSOCIATED RISK FACTORS AT TERTIARY CARE HOSPITAL, PESHAWAR, KHYBER PAKHTUNKHWA

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## KEYWORDS

molecular subtypes, breast cancer, associated factors

## ABSTRACT

**Introduction:** Breast cancer is the most commonly diagnosed cancer in women worldwide and is characterized by molecular and clinical heterogeneity. In gene expression profiling studies, breast cancer is classified into five subtypes: luminal A, luminal B, HER-2 over expression, basal-like, and normal breast-like. Although clinical differences between subtypes are well described in the literature, etiological heterogeneity is poorly investigated.

**Objective:** To determine the molecular subtypes of breast carcinoma and its associated factors in tertiary care hospital Peshawar, Khyber Pakhtunkhwa, Pakistan. **Material and Methods:** The study was executed in cross sectional design, which included 221 diagnosed breast cancer patients. **Variables:** family history, age at first full term pregnancy, number of children, duration of lactation, menstrual history, menopausal status, blood type, smoking, obesity, use of oral contraceptives, hormone replacement therapy and in vitro fertilization were investigated. **Results:** Reproductive and hormonal characteristics (breastfeeding, pregnancy, age at first birth, hormone replacement therapy) were associated with luminal subtype compared with non luminal breast cancer with p values less than 0.05 which are statistically significant. Obesity and overweight increased the risk of triple negative subtype, especially in premenopausal women. Older age and use of hormone replacement therapy were associated with the risk of HER-2-overexpressing breast cancer. **Conclusion:** Our data demonstrate significant heterogeneity associated with conventional breast cancer risk factors and tumor subtypes.

## INTRODUCTION

Breast cancer is one of the most common malignancies and the leading cause of cancer-related deaths in women worldwide. Its incidence is increasing rapidly in both developing and developed countries, with approximately 2 million new cases worldwide in 2018, accounting for 11.6% of all cancers in women. Breast cancer morbidity and mortality in developed countries are declining due to increased early detection through mass screening and improved targeted therapy (Bray et al., 2008)<sup>1</sup>.

Breast cancers are heterogeneous with respect to histopathological features, metastatic patterns, molecular features, outcome, and response to treatment. Heterogeneity has a corresponding impact on clinical outcome. Prognosis depends on tumor size, axillary lymph node status, histologic grade, histology, and biological markers such as estrogen receptor (ER), progesterone receptor (PR), and HER2/neu expression profile (Opdahl et al., 2013)<sup>2</sup>.

Recent classifications attempt to classify the disease at the molecular level provide important predictive information about the potential responsiveness of tumors to different therapeutic modalities. Microarray-based breast cancer gene expression indicates that there are several molecular subtypes of breast cancer (eg, luminal A, luminal B, HER2-positive and basal) (Perou et al., 2000)<sup>3</sup>. Presently IHC is accepted as an adequate surrogate marker for molecular subtypes. This surrogate IHC method for the determination of molecular

subtypes uses ER, progesterone receptor (PR), HER2 and Ki67 antibodies. It is better than molecular testing as it is more economical and technically simpler (Nielsen et al., 2004)<sup>4</sup>.

The luminal molecular subtypes are characterized by estrogen receptor (ER) and progesterone receptor (PR) positivity and further subdivided into Luminal A and Luminal B, based on proliferative index. Luminal A subtype is low grade tumors and includes 40-55% of all breast carcinoma. These do not express HER 2/neu and have low proliferative index. Luminal B cancers are approximately 15%–20%, may or may not express HER 2 and have a worse prognosis than luminal A cancers. These often have lower expression levels of HRs, higher Nottingham grade, and higher proliferative rates. A 14% cut-off for Ki-67 was endorsed in St. Gallen 2011 to separate Luminal B from Luminal A tumors (Goldhirsch et al., 2011)<sup>5</sup>. Triple negative breast cancer represents 10 to 17% of all breast cancers (Podo et al., 2010)<sup>6</sup>.

The clinical significance of molecular classification of breast cancer has not yet been established. Molecular subtyping using immune histochemistry provides additional prognostic and predictive information so the present was conducted with the following objectives.

### **OBJECTIVES**

- 1) To determine molecular subtypes of breast carcinoma in Khyber Pakhtunkhwa
- 2) To determine the factors associated with molecular subtypes on the basis of ER, PR & HER 2 status of breast carcinoma in Khyber Pakhtunkhwa

### **Material and Methods:**

The study was conducted at Surgical-B ward, Khyber Teaching Hospital, Peshawar, Pakistan by using Cross Sectional Study Design with non probability consecutive sampling technique in duration of 6 months. Sample size (n= 221) was determined by using open epi software with 7.61% anticipated frequency of the most common molecular subtype luminal A breast carcinoma with error margin 3.5% and confidence level of 95%.

### **Sample Selection:**

**Inclusion criteria:** Confirm breast carcinoma patients having age between 18 to 70 years belonging to the Khyber Pakhtunkhwa province. Patients with multiple lesions or admissions were considered once.

**Exclusion criteria:** Benign tumors like fibro adenoma and inflammatory conditions involving skin. Accessory auxiliary breast tissue cases were excluded from the study.

### **Data Collection Procedure:**

The study was conducted after approval from hospital's research committee and ethical approval was taken from ethical review committee of Khyber Teaching Hospital (MTI) Peshawar (MTI: Medical Training Institute). After informed consent that ensures confidentiality, patients meeting the inclusion criteria were included in the study. The purpose and benefit of the study was explained to the sample patients. All the respondents were subjected to detailed history, followed by complete routine examination and baseline investigations. All the above mentioned information including demographic features was recorded in a pre-designed questionnaire. A strict exclusion criterion was followed to control confounders and bias in the study results.

### **Data Analysis Procedure:**

Data were analyzed with SPSS version 27. Frequencies and percentages were calculated for categorical variables such as age, family history, age of menarche, age at first full term pregnancy, molecular subtypes, number of children, breast feeding, use of oral contraceptives, hormone replacement therapy (HRT), in vitro fertilization, obesity and smoking such as molecular subtype, oral contraceptive use, HRT use, in vitro fertilization, family history, breastfeeding, and smoking. Means and standard deviations were calculated for quantitative variables such as age, age at menarche, age at menopause, age at first full-term pregnancy, number of children, and body mass index. Molecular subtypes were divided into age, family history of molecular subtype, oral contraceptives, HRT use, in vitro fertilization, family history, breastfeeding, smoking, age, age at menarche, age at menopause, age at first trimester pregnancy, childbirth number, body mass index.

A post-stratified chi-square test is applied and P-values are held to have significance less

than 0.05. A multinomial logistic regression was applied to adjust for the effects of confounding variables and the results are shown in adjusted and unadjusted results.

### **Operational Definitions**

#### **Breast Carcinoma:**

- Breast cancer is a disease in which cells in a breast tissue grow and multiply out of control.

#### **Molecular subtypes:**

- Luminal A subtype: ER +ive and/or PR +ive, Her 2 -ive
- Luminal B subtype: ER +ive and/or PR +ive, Her 2 +ive
- Her 2 over expressing subtype: ER -ive and/or PR -ive, Her 2 +ive
- Basal like: ER -ive and/or PR -ive, Her 2 -ive, cytokeratin 5/6 +ive and or Her 1 +ive
- Unclassified: Negative for all 5 markers i.e. ER, PR, Her 2, Her 1, cytokeratin 5/6

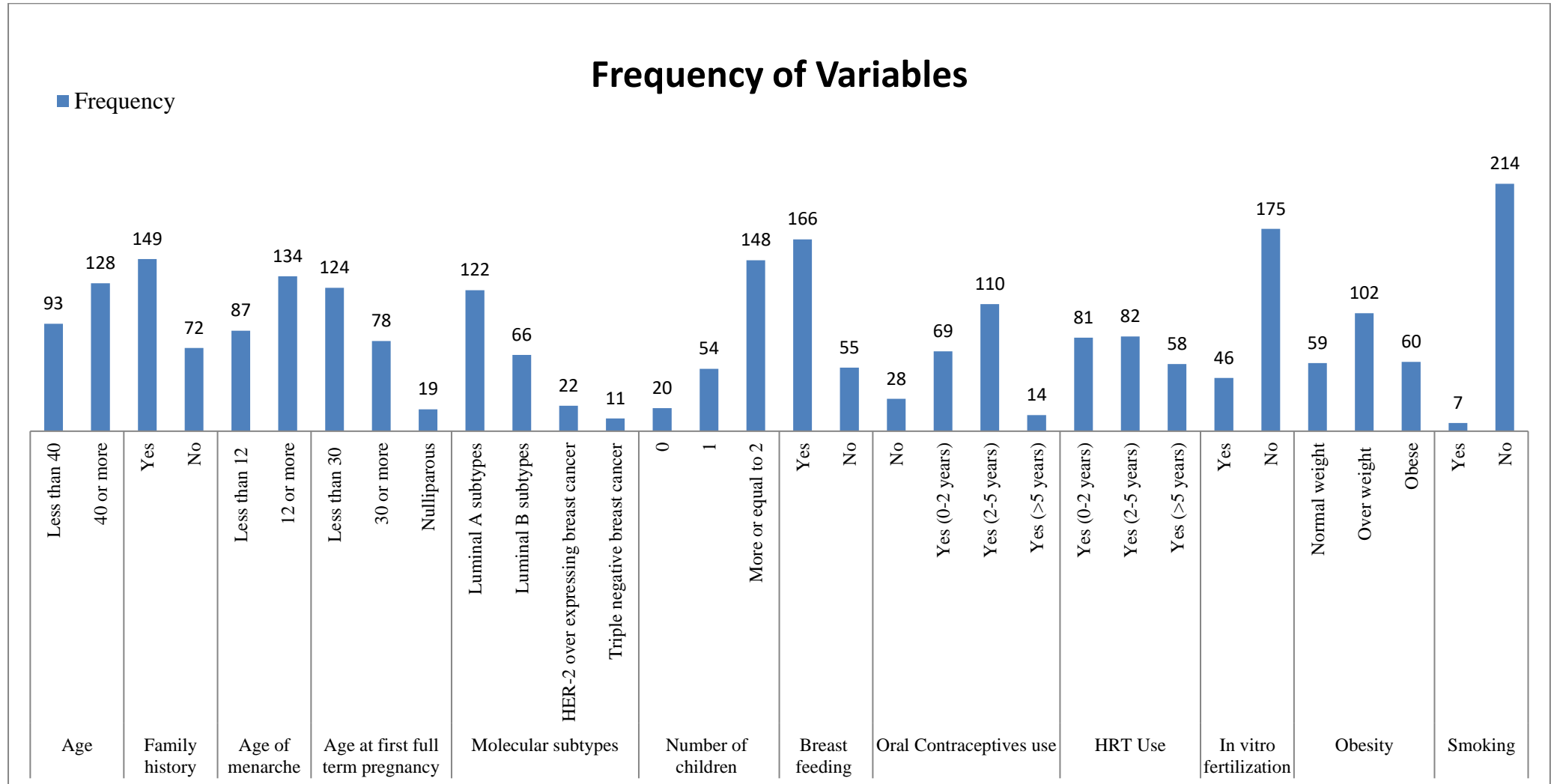
**Modifiable risk factors:** Obesity or overweight, menopausal hormone therapy, alcohol use, sedentary life style.

**Non modifiable risk factors:** Personal or family history of breast or ovarian cancer, benign breast disease like ductal carcinoma insita or atypical hyperplasia, inherited genetic changes in breast cancer susceptibility genes (BRCA 1 or BRCA 2), dense breast tissue, high dose radiation to chest

**Reproductive factors:** Oral contraceptives use, high natural level of sex hormone, prolong menstrual period, not having children or children after 30 years of age.

### **Results:**

The figure below demonstrates frequency of all the variables examined of the patients under the study. Out of 221, 93 patients were having age less than 40 years while the rest were of 40 or more than 40 years. 149 respondents were having family history of the disease while 72 have not. Age of menarche was less than 12 years for 87 and of 12 or more years for 124 patients. The age at first full term pregnancy was less than 30 years for 124 patients and 30 or more years for 78 patients while 19 patients were in subcategory: nulliparous. Among 221 participants 122 have luminal A subtype, 66 have luminal B subtype, 22 have HER-2 over expressing breast cancer and 11 have triple negative breast cancer of molecular subtypes. The figure further reveals that 20 responders have no child, 54 have only one child while 148 responders were having 2 or more children. Frequency of the patients who did breast feeding was 166 while the rest didn't feed through breast. 28 responders didn't used oral contraceptives, while 69 have used less than or up to 2 years, 110 have between 2-5 years and 14 have more than 5 years. The figure further shows that 81 participants under goes through Hormone Replacement Therapy (HRT) for less than or up to 2 years, 82 between 2 to 5 years and 58 for more than 5 years. 46 patients have passed through in vitro fertilization while 175 have not. The number among 221 patients was 59 for normal weight, 102 for overweight and 60 for obese, while only 07 responders were found to be smokers as shown in the figure.



**Figure:** Frequency of variables studied of the sample (n=221) patient

The relation between age (less than 40 and equal to or above 40 years of the sample patients) with molecular subtypes of breast carcinoma is shown in the Table 1 by applying a  $\chi^2$  test. Among the total 93 participants below the age of 40 years 52 were having luminal A, 25 have luminal B and 8 each have HER-2 over expression and triple negative breast cancer. 128 respondents have equal or above the age of 40 years, in whom 70, 41, 14 and 3 were having luminal A, luminal B, HER-2 over expressing and triple negative breast cancer respectively. The table further shows that P value 0.07 is  $> \alpha = 0.05$  and thus null hypothesis is accepted, however the luminal A breast cancer in both the sub categories (age) was dominant insignificantly.

Patients with positive family history of luminal A breast cancer type were maximum among all the patients having different molecular subtypes with a value of 81. They also has a significant relation with each other (P value =  $0.02 < \alpha = 0.05$ ) and thus rejecting null hypothesis (Table 2). Table 3 reveals significantly maximum number (73) of patients having luminal A breast cancer with menarche age of or more than 12 years. The same type is also dominant in the patients (49) with age of menarche less than 12 years. The alternative hypothesis of dependency of molecular subtype of breast cancer with age of menarche of patients is accepted as P value (0.001) is less than  $\alpha$  value (0.01 or 0.05).

Table 4 manifests that luminal A breast cancer is dominant among all the sub categories of age at 1<sup>st</sup> full term pregnancy of the studied patients. The table also shows dependency of both the variables as the mentioned molecular subtype occurs maximally among the patients with age of less than 30 years. P value which is 0.01 is  $< \alpha = 0.05$  and hence null hypothesis is rejected. All the studied molecular subtypes were predominant in the patients (147 out of 221) having 2 or more children in whom Luminal A breast cancer type occurs significantly (P =  $0.001 < \alpha = 0.01$ ) more than the rest molecular subtypes with patient number of 83 as shown in Table 5.

Table 6 shows that molecular subtypes of cancer are dependent on the breast feeding. Significantly more patients were diagnosed with luminal A breast cancer who have done breast feeding and thus alternative hypothesis is accepted (P =  $0.04 < \alpha = 0.05$ ). Table 7 chalked out that patients who have used oral contraceptives for 2-5 years are more likely to have luminal A breast cancer. The table also shows that use of the above does not have any significant impact on the disease prevalence and overallly independent of each other as P  $> \alpha$  ( $0.34 > 0.05$ ) so accepting null and rejecting alternative hypothesis. The luminal A breast cancer has equal probability to prevail in all the subcategories of oral contraceptive use.

Table 8 demonstrates that prevalence of luminal A breast cancer was significant in the patients who used HRT up to 2 years. P = 0.001 is  $< \alpha = 0.01$ , showing that null hypothesis is rejected. Table 9 indicates that the hypothesis of relationship between the disease subtypes and in vitro fertilization is accepted (alternative hypothesis). P = 0.004 means that luminal A breast cancer type occurred predominantly in the patients who didn't undergoes the said process. Occurrence of the above mentioned disease has an equal chance in normal weight, over weight and obese patients as P value is  $> \alpha$  as shown in Table 10. The critical value of  $\chi^2$  test (P = 0.001) in Table 11 shows that luminal A breast cancer prevailed significantly more in the patients with no smoking history.

**Table 1 : Association between molecular sub types and age**

Variables	Sub categories	Molecular Subtypes				P-Value
		Luminal A breast cancer	Luminal B breast cancer	HER-2 Over expressing breast cancer	Triple negative breast cancer	
Age	< 40 years	52	25	8	8	0.07
	≥ 40 years	70	41	14	3	

**Table 2: Association between molecular sub types and family history**

Variable	Sub categories	Molecular Subtypes				P-Value
		Luminal A breast cancer	Luminal B breast cancer	HER-2 Over expressing breast cancer	Triple negative breast cancer	
Family history	Yes	81	50	13	5	0.02
	No	41	16	9	7	

**Table 3: Association between molecular sub types and age of menarche**

Variable	Sub categories	Molecular Subtypes				P-Value
		Luminal A breast cancer	Luminal B breast cancer	HER-2 Over expressing breast cancer	Triple negative breast cancer	
Age of Menarche	< 12 years	49	22	8	8	0.001
	≥ 12 years	73	44	14	3	

**Table 4: Association between molecular sub types and age at 1<sup>st</sup> full term pregnancy**

Variable	Sub categories	Molecular Subtypes				P-Value
		Luminal A breast cancer	Luminal B breast cancer	HER-2 Over expressing breast cancer	Triple negative breast cancer	
Age at 1 <sup>st</sup> full term pregnancy	< 30 years	66	41	14	3	0.01
	≥ 30 years	44	19	8	7	
	Nulliparous	11	6	0	2	

**Table 5: Association between molecular sub types and no. of children**

Variable	Sub categories	Molecular Subtypes				P-Value
		Luminal A breast cancer	Luminal B breast cancer	HER-2 Over expressing breast cancer	Triple negative breast cancer	
No. of Children	No child	7	4	4	0	0.001
	1 child	32	19	8	0	
	≥ 2 children	83	43	10	11	

**Table 6: Association between molecular sub types and breast feeding**

Variable	Sub categories	Molecular Subtypes				P-Value
		Luminal A breast cancer	Luminal B breast cancer	HER-2 Over expressing breast cancer	Triple negative breast cancer	
Breast feeding	Yes	111	44	11	0	0.001
	No	11	22	9	13	

**Table 7: Association between molecular sub types and oral contraceptive use**

Variable	Sub categories	Molecular Subtypes				P-Value
		Luminal A breast cancer	Luminal B breast cancer	HER-2 Over expressing breast cancer	Triple negative breast cancer	
Oral contraceptive use	No	17	11	0	0	0.34
	0-2 years	40	19	5	5	
	2-5 years	54	33	17	6	
	> 5 years	11	3	0	0	

**Table 8: Association between molecular sub types and HRT use**

Variable	Sub categories	Molecular Subtypes				P-Value
		Luminal A breast cancer	Luminal B breast cancer	HER-2 Over expressing breast cancer	Triple negative breast cancer	
HRT use	0-2	63	19	7	5	0.001
	2-5 years	30	27	7	5	
	> 5 years	29	20	8	1	

**Table 9: Association between molecular sub types and In vitro fertilization**

Variables	Sub categories	Molecular Subtypes				P-Value
		Luminal A breast cancer	Luminal B breast cancer	HER-2 Over expressing breast cancer	Triple negative breast cancer	
In vitro fertilization	Yes	23	11	7	5	0.004
	No	87	58	20	10	

**Table 10: Association between molecular sub types and obesity**

Variable	Sub categories	Molecular Subtypes				P-Value
		Luminal A breast cancer	Luminal B breast cancer	HER-2 Over expressing breast cancer	Triple negative breast cancer	
Obesity	Normal weight	34	16	6	3	0.32
	Over weight	55	32	10	5	
	Obese	33	18	6	3	

**Table 11: Association between molecular sub types and smoking**

Variable	Sub categories	Molecular Subtypes				P-Value
		Luminal A breast cancer	Luminal B breast cancer	HER-2 Over expressing breast cancer	Triple negative breast cancer	
Smoking	Yes	4	1	1	1	0.001
	No	102	51	47	14	

**Discussion:**

Opdahl et al., 2013<sup>2</sup> found that luminal A breast cancer type occurs more in older patients than young, they also stated that breast cancer incidence increases with age until menopause which is in agreement with our study findings. Fulford et al. (2006)<sup>7</sup> reported that majority of the studied patients have also one of molecular subtypes of breast carcinoma in their family and thus concurs with our conclusion. Bray et al.<sup>8</sup> and Edge et al. in 2018<sup>9</sup> stated that responders with history of age at menarche more than 12 years are more likely to have luminal A breast cancer which concurs our results. Pegram et al., (2012)<sup>10</sup> from their research concluded that women with 1<sup>st</sup> pregnancy below the age of 30 years and more than 2 children were victim of one of molecular subtypes of breast carcinoma, which coincides our findings.

From the present study it was determined that breast feeding reduces the risk of breast carcinoma which is confirmed by Ferlay et al., 2009<sup>11</sup>, Huang et al., 2000<sup>12</sup> and Jensen and Jordan (2003)<sup>13</sup> who found more cases of breast cancer in the patients whom didn't feed their children through breast. Curtis et al. (2012)<sup>14</sup> studied the molecular subtypes of the disease among the patients who undergoes oral contraceptives for different periods and found no connection of it with disease incidence. Parker et al., 2009<sup>15</sup> observed no significant difference in the number of patients having each separately molecular subtype of breast carcinoma, who took oral contraceptives for different periods and hence confirms our results.

Harris et al. (2016)<sup>16</sup> diagnosed that hormone replacement therapies (HRT) has high impact on breast cancer, they reported a large number of cases in the patients who under goes HRT and thus meets our results. Coates et al., 2015<sup>17</sup> and Hoadley et al., 2014<sup>18</sup> from their studies found almost equal cases of molecular subtypes of carcinoma in differently weighted patients, our study also reported no considerable effect of weight of participants on the disease occurrence and thus their findings are in agreement with ours. Phipps et al., 2008<sup>19</sup> and Ma et al., 2006<sup>20</sup> etc observed that majority of cancer patients are addictive smokers.

**Conclusions**

Luminal A breast cancer type occurred dominantly as compared with the rest of the types. Moreover, the said type is also dominant for all the categorical variables. All the variables except age (<40 and ≥40 years), duration for use of oral contraceptives (No use, usage for 0-2, 2-5 and > 5 years) and obesity (normal weight, over weight and obese) are significantly associated with molecular subtypes of breast cancer especially with luminal A breast cancer.

**Recommendations**

Girls and women must immediately concern specialists if they feel something abnormal related to their breast and strictly follow the advises given. The complainers must honestly answer all the questions of the consultant. The disease can be occurred at any age and those who have family history must be vigilant, furthermore, women with or without children, both can be victim of the disease. Breast feeding, which reduce chances of the disease is recommended.

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