**The Effect of Obesity on Immunohistochemical Surrogate Markers in Pre- and Post-Menopausal Breast Cancer**

**Abstract**

**Aim:** To determine whether obesity has an impact on immunohistochemical surrogates in breast cancer (BC).

**Material and Methods:** This study is consisted of 206 newly diagnosed BC patients. Demographic and cancer specific variables were obtained from patients’ records. Obesity was define as a body-mass index of 30 kg/m2 or greater according to the recommendation of WHO. Firstly, patients were grouped according to menopausal status and each group has been divided according to obesity in order to compare ER, PR, HER2, triple-negative, triple-positive status, 5-year mortality rates.

**Results:** Significant differences were obtained in ER, PR, HER2, and triple-negative receptor statuses between pre-menopausal and post-menopausal patients (p= 0.026, 0.018, 0.036, 0.011, respectively). In pre-menopausal group, there was no difference in ER, PR, HER2, triple-positive, and triple-negative status between obese and non-obese patients (p= 0.696, 0.455, 0.659, 0.662, 0.774, respectively); likewise, in post-menopausal group, there was no difference in ER, PR, HER2, and triple-positive status between obese and non-obese patients (p= 0.786, 0.130, 0.082, 0.437, respectively) except triple-negative status which tended to slightly less in non-obese patients (p=0.03).

**Conclusion:** The results of our study showed that while menopausal status has an impact on ER, PR, HER2, and triple-negative receptor statuses, obesity seems to have not the same impact on these markers.

**Keywords:** obesity, body-mass index, BMI, breast cancer, immunohistochemical marker, estrogen receptor, hormone receptor, HER2, triple-negative breast cancer

**Introduction**

In worldwide, approximately 2 billion adults are overweight or obese [1]. Obesity promotes a number of diseases due to altered body physiology and hormonal environment, also it is associated with an increased risk of developing several cancers and with poorer survival outcomes for patients with those cancers. [2].

Breast cancer (BC) is most common cancer and leading cause of cancer related death among women worldwide, accounting for 25% of female cancer cases at all ages and a greater percentage among young women [3]. Several risk factors for BC has been identified, such as age, genetic mutations, nulliparity, older age at menopause [4], also it has been highlighted that obesity could be associated with BC [5]. Although the studies regarding this association are inconsistent, it has been shown that premenopausal breast cancer risk is reduced by approximately 8% per 5 kg/m2 BMI increase whereas postmenopausal breast cancer risk was positively associated with each 5‐kg/m2 increase in BMI [6].

Immunohistochemistry (IHC) -based surrogate definitions of the molecular subtypes using protein expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) are routinely use to classify BC tumors [7, 8]. Obesity seem to be associated with hormone receptor status in BC patients [9].

This study has focused to analyzes of obesity on IHC-surrogates rather than survival to determine whether obesity has an impact on these markers in BC.

**Materials and Methods**

This descriptive and analytical retrospective study was conducted in July 2018 and included 82 obese and 124 non-obese BC patients older than 18 years. Patients were selected consecutively (selected regardless of type and stage of the disease) who admitted to our oncology clinic with newly diagnosed BC in between January 2012 and July 2013. The diagnosis was established based on clinical, radiological, and histopathological features. This study was approved by our institutional ethical committee.

Exclusion criteria were as follows: Having another tumor at the time of diagnosis, taking systemic chemotherapy for any reason before, surgical intervention for BC before referral to our oncology clinic, male patients with BC, dysregulated diabetes mellitus, kidney disease, cardiovascular disease, rheumatological diseases, pregnancy, and undergoing treatment for BC.

Patients’ history and physical examination results, clinicopathological features including age, weight, height, menopausal status, histopathological type, grade, tumor size, lymph node metastasis, stage, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), lymphovascular invasion (LVI), and perineural invasion (PNI) status were obtained from patients’ records. The information of the mortality was gathered from National Death Certificate System (NDCS).

In order to classify BC, immunohistochemical surrogates (ER, PR, HER2) were used and BC was divided into luminal A, luminal B, HER2, triple-negative and triple-positive subtypes [7]. Luminal A was defined as ER (+), PR (+), HER2 (−); luminal B was defined as ER (+) and/or PR (+), and HER2 (+); HER2 like tumor was defined as ER (−), PR (+), HER2 (+); triple-negative tumor was defined as ER (−), PR (−), HER2 (−); and triple-positive tumor was defined as ER (+), PR (+), HER2 (+). The pathological tumor stage was defined according to the eight edition of the tumor‑node‑metastasis classification of the UICC and WHO [10, 11]. Tumor size was classified as T1 (2 cm), T2 (2–5 cm), or T3 (>5 cm); both tumor size and lymph node metastasis status were evaluated separately.

The BMI calculated as weight in kilograms divided by the square of the height in meters. Obesity was defined as a body-mass index of 30 kg/m2 or greater according to the recommendation of WHO [12].

***Statistical Analysis***

Analyzes were performed with the NCSS 11 (Number Cruncher Statistical System, 2017 Statistical Software) program and MedCalc Statistical Software version 18 (MedCalc Software bvba, Ostend, Belgium; <http://www.medcalc.org>; 2018)

Besides descriptive calculations (mean ± standard deviation, frequency and percentage values), normality test of continuous variables was performed by the Shapiro-Wilk test. Chi-Square and Mann Whitney U test was used in two independent group comparisons and for three or more groups comparisons, one-way analysis of variance (ANOVA) and Kruskall-Wallis H was used according to the distribution of the unpaired samples. Peaerson correlation analysis was used to identify correlations between variables that did not provide a normal distribution hypothesis. Interruption points for BMI levels were calculated by ROC analysis. The value of P < 0.05 was considered as significant.

**Results**

The study included a total number of 206 newly diagnosed BC patients who consisted of 82 obese (28 patients were pre-menopausal whereas 54 patients were post-menopausal) and 124 non-obese patients (62 patients were pre-menopausal whereas 62 patients were post-menopausal). The mean age of obese group was 52.16 ± 13.13 years whereas the mean age of non-obese group was 56 ± 10.37 years. Fifty percent of the non-obese patients were in pre-menopausal status whereas 34.15% of the obese patients were in pre-menopausal status. Descriptive data were shown in table 1.

In overall patients, ER positivity was 68.93% (n=142); PR positivity was 59.70% (n=123); HER-2/neu (C-erb B2) negativity was 69.41% (n=143); triple negativity was 14.07% (n=29); triple positivity was 14.07% (n=29). In obese patients, ER positivity was 69.51% (n=57); PR positivity was 58.54% (n=48); HER-2/neu (C-erb B2) negativity was 71.95% (n=59); triple negativity was 9.68% (n=12); triple positivity was 8.87% (n=11). In non-obese patients, ER positivity was 68.55% (n=85); PR positivity was 60.49% (n=75); HER-2/neu (C-erb B2) negativity was 67.74% (n=84); triple negativity was 13.71% (n=17); triple positivity was 14.52% (n=18).

In all patients, 13.59% patients were stage I; 43.69% patients were stage II; 28.16% patients were stage III and 14.56% patients were stage IV. Distribution of cancer stages in obese and non-obese patients were shown in table 1. There was no difference in 5-year mortality rate between obese and non-obese patients (p= 0.071).

In order to compare ER, PR, HER2, triple-negative, triple-positive status between obese and non-obese patients we have divided patients according to menopausal status. Mean ages of pre-menopausal and post-menopausal BC patients were 43.75 ± 7.1 and 61.40 ± 9.50 years, respectively (p=0.014). There was no difference regarding BMI between groups (p= 0.703).

Pre-menopausal and post-menopausal patients were divided in subgroups according to obesity (BMI <30 and ≥30 kg/m2), and we showed that obesity in breast cancer did not altered the ER, PR, HER2, triple-positive, and triple-negative status in both pre-menopausal and post-menopausal patients (for p values see table 2); but, only post-menopausal, obese breast cancer patients tended to slightly have less triple-negative status than non-obese patients (p=0.03).

There was no differences regarding 5-year mortality rates between obese and non-obese patients in both pre-menopausal and post-menopausal groups (p= 0.907 and 0.570, respectively). We did not find any relationship between BMI values and tumor stages in both pre-menopausal and post-menopausal patients (p= 0.233 and 0.843, respectively). Likewise there was also no relationship between BMI values and 5-year mortality rates in pre-menopausal and post-menopausal patients (p= 0.199 and 0.328, respectively).

When we compare ER, PR, HER2, triple-positive, and triple-negative receptor statuses and 5-year mortality rates between pre-menopausal and post-menopausal patients, statistical significant differences were obtained only in ER, PR, HER2, and triple-negative receptor statuses (for p values see table 2).

**Discussion**

The association between BMI and receptor statuses of BC is complex and inconsistent. Despite numerous studies, a common consensus regarding the relationship between statuses of IHC-surrogates of BC and BMI has not been established, as the results of the studies regarding the effect of BMI on IHC-surrogates of BC are conflicting. One of the most important of this discrepancy is that measurement of the obesity is not consistent and is driven by different anthropomorphic measurements, such as BMI and waist-hip ratio. Also, the reality is that these measurements do not always predict metabolic health due to the fact that is being obese does not consistently mean to have metabolic abnormalities (e.g., insulin resistance) [13]. Also, these studies were conducted in different races, ethnicities, and menopausal statuses which possibly have impacts on these receptors [14–16].

In this Turkish population-based study, we have shown that while there were significant differences in ER, PR, HER2, and triple-negative receptor statuses between pre- and post-menopausal groups, no difference was found regarding to these markers between obese and non-obese patients within each group except in triple-negative receptor status in postmenopausal patients. Likewise, we could not find any difference in 5-year mortality rate between pre- and post-menopausal groups also, between obese and non-obese patients within each group.

A recently published review by Jiralerspong et al. has emphasized the contradictory results of the studies investigating the effect of BMI/obesity on survival and mortality [17]. They also have tried to elucidate these inconsistent results by several potential explanations, such as study design, type of population and population variables. Because there are several factors affecting the survival in BC, this study has focused on the impact of BMI on receptor status rather than survival.

Likewise, there is incompatible results regarding the effect of obesity on IHC markers in literature, also most of them focused on the triple-negative or ER +/- BC patients [18–20].

According to our study results, we agree with the result of a meta-analysis designed by Cheraghi et al., which reported that the role of obesity on BC is not clinically crucial despite the significant statistics of the some previous trials.

***Conclusions***

The literature has contradictory results regarding the effect of BMI on either survival/mortality or IHC markers. The results of our study which is conducted in the Turkish population showed that while menopausal status has an impact on ER, PR, HER2, and triple-negative receptor statuses, obesity seems to have not the same impact on these markers.

***Conflict of Interest:*** None of the authors have any conflicts of interest or financial ties to disclose.

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**References:**

[1] Wang YC, McPherson K, Marsh T, Gortmaker SL, Brown M, Health and economic burden of the projected obesity trends in the USA and the UK. The Lancet. 2011;378:815–25.

[2] Goodwin PJ, Stambolic V, Impact of the obesity epidemic on cancer. Annu. Rev. Med. 2015;66:281–96.

[3] Torre LA, Islami F, Siegel RL, Ward EM, Jemal A, Global Cancer in Women: Burden and Trends. Cancer Epidemiol. Oncol. 2017;26.

[4] Collaborative Group on Hormonal Factors in Breast Cancer. Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. Lancet Oncol. 2012;13:1141–51.

[5] Rice MS, Eliassen AH, Hankinson SE, Lenart EB, Willett WC, Tamimi RM, Breast Cancer Research in the Nurses’ Health Studies: Exposures Across the Life Course. Am. J. Public Health. 2016;106:1592–98.

[5] Picon-Ruiz M, Morata-Tarifa C, Valle-Goffin JJ, Friedman ER, Slingerland JM, Obesity and adverse breast cancer risk and outcome: Mechanistic insights and strategies for intervention: Breast Cancer, Inflammation, and Obesity. CA. Cancer J. Clin. 2017;67:378–97.

[7] Tang P, Tse GM, Immunohistochemical Surrogates for Molecular Classification of Breast Carcinoma: A 2015 Update. Arch. Pathol. Lab. Med. 2016;140:806–14.

[8] Onitilo AA, Engel JM, Greenlee RT, Mukesh BN. Clin. Med. Res. 2009;7:4–13.

[9] Iyengar NM, Hudis CA, Dannenberg AJ, Obesity and inflammation: new insights into breast cancer development and progression. Am. Soc. Clin. Oncol. Educ. Book Am. Soc. Clin. Oncol. Meet. 2013:46–51.

[10] Gospodarowicz MK, John Wiley & Sons. 2017.

[11] Hanby AM, walker C. Tavassoli FA, Devilee P, Pathology and Genetics: Tumours of the Breast and Female Genital Organs. WHO Classification of Tumours series - volume IV. Lyon, France: IARC Press: 2003. 250pp. ISBN 92 832 2412 4. Breast Cancer Res. 2004. doi:10.1186/bcr788.

[12] World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO Consultation presented at the World Health Organization, June 3–5, 1997, Geneva, Switzerland. 1997.

[13] Mathew H, Farr OM, Mantzoros CS, Metabolic health and weight: Understanding metabolically unhealthy normal weight or metabolically healthy obese patients. Metabolism. 2016;65:73–80.

[14] Yersal Ö, Yiğit M, Meydan N, Barutca S, Retrospective evaluation of breast cancer patients with five or more axillary lymph node involvement achieving 5-year overall survival. Eur. Res. J. 2018. doi:10.18621/eurj.368447.

[15] Newman B, Moorman PG, Millikan R, Qaqish BF, Geradts J, Aldrich TE, et al. The Carolina Breast Cancer Study: integrating population-based epidemiology and molecular biology. Breast Cancer Res. Treat. 1995;35:51–60.

[16] Krieger N, Chen JT, Ware JH, Kaddour A, Race/ethnicity and breast cancer estrogen receptor status: impact of class, missing data, and modeling assumptions. Cancer Causes Control CCC. 2008;19:1305–18.

[17] Jiralerspong S, Goodwin PJ, Obesity and Breast Cancer Prognosis: Evidence, Challenges, and Opportunities. J. Clin. Oncol. 2016;34:4203–16. DOI: 10.1200/JCO.2016.68.111

[18] Niraula S, Ocana A, Ennis M, Goodwin PJ, Body size and breast cancer prognosis in relation to hormone receptor and menopausal status: a meta-analysis. Breast Cancer Res. Treat. 2012;134:769–81.

[19] Krieger N, Chen JT, Ware JH, Kaddour A, Race/ethnicity and breast cancer estrogen receptor status: impact of class, missing data, and modeling assumptions. Cancer Causes Control CCC. 2008;19:1305–18.

[20] Hao S, Liu Y, Yu K-D, Chen S, Yang W-T, Shao Z-M, Overweight as a Prognostic Factor for Triple-Negative Breast Cancers in Chinese Women. PloS One. 2015;10:e0129741.

[21] Dignam JJ, Wieand K, Johnson KA, Raich P, Anderson SJ, Somkin C, et al. Effects of obesity and race on prognosis in lymph node-negative, estrogen receptor-negative breast cancer. Breast Cancer Res. Treat. 2006;97:245–54.

[22] Cheraghi Z, Poorolajal J, Hashem T, Esmailnasab N, Doosti Irani A, Effect of Body Mass Index on Breast Cancer during Premenopausal and Postmenopausal Periods: A Meta-Analysis. PLoS ONE. 2012;7:e51446.

Table 1: Overall patients' descriptive data and distribution of these data in accordance with obesity

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Overall (n=206)** | **BMI < 30 (n=124)** | **BMI ≥ 30 (n=82)** |
| Age (years) | 53.69 ± 12.22 | 56 ± 10.37 | 52.16 ± 13.13 |
| ER status [n(%)] |  |  |  |
| positive | 142 (68.93) | 85 (68.55) | 57 (69.51) |
| negative | 64 (31.07) | 39 (31.45) | 25 (30.49) |
| PR status [n(%)] |  |  |  |
| positive | 123 (59.70) | 75 (60.49) | 48 (58.54) |
| negative | 83 (40.30) | 49 (39.51) | 34 (41.46) |
| HER2 status [n(%)] |  |  |  |
| positive | 63 (30.59) | 40 (32.26) | 23 (28.05) |
| negative | 143 (69.41) | 84 (67.74) | 59 (71.95) |
| Luminal A [n(%)] | 117 (59.80) | 69 (55.65) | 48 (58.54) |
| Luminal B [n(%)] | 33 (16.02) | 23 (18.55) | 10 (12.19) |
| Triple-negative [n(%)] | 29 (14.07) | 17 (13.71) | 12 (9.68) |
| Triple-positive [n(%)] | 29 (14.07) | 18 (14.52) | 11 (8.87) |
| LVI [n(%)] |  |  |  |
| present | 89 (50) | 46 (44.23) | 43 (58.1) |
| absent | 89 (50) | 58 (55.77) | 31 (41.9) |
| missing value | 28 | 20 | 8 |
| PNI [n(%)] |  |  |  |
| present | 58 (36.95) | 33 (36.27) | 25 (37.88) |
| absent | 99 (63.05) | 58 (63.73) | 41 (62.12) |
| missing value | 49 | 33 | 16 |
| Menopausal status [n(%)] |  |  |  |
| Pre-menopausal | 80 (38.83) | 62 (50) | 28 (34.15) |
| Post-menopausal | 126 (61.17) | 62 (50) | 54 (65.85) |
| Presence of metastasis [n(%)] | 30 (14.56) | 20 (16.13) | 11 (13.41) |
| Stage [n(%)] |  |  |  |
| 1 (incl. 1a & 1b) | 28 (13.59) | 22 (17.74) | 8 (9.76) |
| 2 (incl. 2a & 2b) | 90 (43.69) | 47 (37.90) | 37 (45.12) |
| 3 (incl. 3a, 3b & 3c) | 58 (28.16) | 35 (28.23) | 26 (31.71) |
| 4 | 30 (14.56) | 20 (16.13) | 11 (13.41) |
| 5-year mortality rate (%) | 15.53 | 16.13 | 14.63 |

BMI: Body-mass index, n: number of patients, ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor receptor 2, LVI: Lymphovascular invasion, PNI: perineural invasion, incl.: Including

Table 2: Comparisons of IHC markers between pre- and post-menopausal groups and between obese and non-obese patients within each group

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Pre-menopausal (n=80)** | | | | | **Post-menopausal (n=126)** | | | | | **P\*** |
| **BMI < 30 (n=62)** | | **BMI ≥ 30 (n=28)** | | **p** | **BMI < 30 (n=62)** | | **BMI ≥ 30 (n=54)** | | **p** |
| **positive** | **negative** | **positive** | **negative** | **positive** | **negative** | **positive** | **negative** |
| ER status (n) | 43 | 19 | 20 | 8 | 0.696 | 42 | 20 | 37 | 17 | 0.786 | ***0.026*** |
| PR status (n) | 44 | 18 | 17 | 11 | 0.455 | 31 | 31 | 31 | 23 | 0.130 | ***0.018*** |
| Triple-positive status (n) | 13 | 49a | 7 | 21a | 0.662 | 5 | 57a | 4 | 50a | 0.437 | 0.348 |
| Triple-negative status (n) | 7b | 55 | 6b | 22 | 0.774 | 10b | 52 | 6b | 48 | ***0.03*** | ***0.011*** |
| HER2 status (n) | 23 | 39 | 9 | 19 | 0.659 | 17 | 45 | 14 | 40 | 0.082 | ***0.036*** |
|  | **% (n)** | | **% (n)** | |  | **% (n)** | | **% (n)** | |  |  |
| 5-year mortality rates  (decease) | 12.90 (8) | | 14.28 (4) | | 0.907 | 19.35 (12) | | 14.81 (8) | | 0.570 | 0.071 |

BMI: Body-mass index, n: number of patients, ER: Estrogen receptor, PR: Progesteron receptor, HER2: Human epidermal growth factor receptor 2, a refers to non-triple positive patients, b refers to non-triple negative patients, \* refers to comparison of IHC-surrogate markers and mortality rates between pre-menopausal and post-menopausal patients.