**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)

"Materials and Methods: This study comprises 206 newly diagnosed BC patients."

Demographic and cancer specific variables were obtained from patients’ records.

Obesity was defined as a body-mass index of 30 kg/m2 or greater according to the recommendation of WHO.

Firstly, patients were grouped by menopausal status and then subdivided by obesity to compare ER, PR, HER2, triple-negative, triple-positive status and 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: Our study results revealed that although menopausal status affects ER, PR, HER2, and triple-negative receptor statuses distinctly, obesity does not appear to have a similar effect on these markers.

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

**Introduction**

Approximately 2 billion adults worldwide are overweight or obese [2].

Breast cancer is the leading cause of cancer-related deaths in women globally, accounting for 25% of all female cancer cases, with a higher prevalence in young women.

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 used to classify BC tumors [9].

This study has focused on the impact 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 who were admitted to our oncology clinic with newly diagnosed BC between January 2012 and July 2013 were consecutively selected, regardless of the type and stage of the disease.

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

Information including patients' history, physical examination results, and clinicopathological features such as 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) were gathered 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 [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 was calculated as weight in kilograms divided by the square of the height in meters.

According to the World Health Organization's recommendation [12], obesity is defined as having a body mass index of 30 kg/m2 or greater.

***Statistical Analysis***

Analyses were conducted using NCSS 11 (Number Cruncher Statistical System, 2017 Statistical Software) and MedCalc Statistical Software version 18 (MedCalc Software bvba, Ostend, Belgium.

org; 2018)

Besides descriptive calculations (mean ± standard deviation, frequency and percentage values), normality test of continuous variables was performed by the Shapiro-Wilk test.

The Chi-Square test and the Mann Whitney U test were utilized for comparisons between two independent groups; for comparisons involving three or more groups, the one-way analysis of variance (ANOVA) and Kruskall-Wallis H tests were employed, based on the distribution of the unpaired samples.

Pearson correlation analysis was used to identify relationships between variables that did not satisfy the normal distribution hypothesis.

Interruption points for BMI levels were calculated by ROC analysis.

A P value of less than 0.05 was deemed significant.

**Results**

The study encompassed 206 newly diagnosed BC patients, comprising of 82 obese (28 pre-menopausal and 54 post-menopausal) and 124 non-obese patients (62 pre-menopausal and 62 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% were at stage I, 43.69% were at stage II, 28.16% were at stage III, and 14.56% were at stage IV.

Table 1 shows the distribution of cancer stages in obese and non-obese patients.

There was no difference in 5-year mortality rate between obese and non-obese patients (p= 0.071)

To compare the status of ER, PR, HER2, triple-negative, and triple-positive between obese and non-obese patients, we categorized patients based on their 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 categorized based on obesity (BMI <30 and ≥30 kg/m2), demonstrating that obesity in breast cancer did not change the ER, PR, HER2, triple-positive, and triple-negative status in both patient groups (for p values see table 2). However, only obese post-menopausal breast cancer patients showed a slight decrease in triple-negative status compared to non-obese patients (p=0.03).

There was no difference 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 comparing ER, PR, HER2, triple-positive, and triple-negative receptor statuses and 5-year mortality rates between pre-menopausal and post-menopausal patients, statistically significant differences were observed 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 many studies, a clear consensus on the correlation between immunohistochemistry (IHC) surrogates of breast cancer (BC) and body mass index (BMI) is yet to be established due to conflicting results.

A significant issue in this discrepancy is that obesity measurement is inconsistent, driven by different anthropometric measurements like BMI and waist-hip ratio.

Moreover, it is important to understand that these measurements do not always accurately predict metabolic health, as obesity does not consistently correlate with metabolic abnormalities.

g.

, insulin resistance) [14–16]

In this study focused on a Turkish population, it is demonstrated that there are significant disparities in ER, PR, HER2, and triple-negative receptor statuses between pre- and post-menopausal groups. However, within these groups, no difference was observed in these markers between obese and non-obese individuals, except for triple-negative receptor status in postmenopausal patients.

Similarly, we found no differences in the 5-year mortality rates between pre- and post-menopausal groups, or 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]

Attempts have been made to explain these inconsistent results with potential explanations such as study design, population type 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.

Similarly, the literature presents conflicting results concerning the impact of obesity on IHC markers, with many studies primarily focusing on 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.

The role of obesity in breast cancer is not clinically significant despite the substantial data from some previous trials.

***Conclusions***

The literature has contradictory results regarding the effect of BMI on either survival/mortality or IHC markers.

Our study conducted in the Turkish population demonstrated that while menopausal status significantly influences ER, PR, HER2, and triple-negative receptor statuses, obesity does not seem to have the same impact on these markers.

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

This study did not receive financial support from any public, commercial, or non-profit funding agencies.

**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.