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Relationship of Menopausal Status with Molecular Breast Cancer Subtypes

Menopoz Durumunun Moleküler Meme Kanseri Alt Tipleri İle İlişkisi

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ABSTRACT

Objective: Breast cancer is a heterogeneous disease group that exhibits quite different biological behaviors and bear many genomic traces. Its dependence on sex hormones also determines its relationship with menopausal status. It is divided into five molecular subtypes according to receptor analysis and Ki67 level with immunohistochemical markers. This study aimed to examine the relationship between the menopausal status and these molecular subtypes to help predict our treatment strategies.

Material and Method: The database of 250 patients who were operated on for breast cancer in our Oncology Clinic between 2012 and 2020 was retrospectively analyzed. The patients were grouped by their menopausal status and clinicopathological characteristics. Statistical analysis was made at a 95% confidence interval, and a p-value lower than 0.05 was considered statistically significant.

Results: The patients were divided into 2 groups by their menopausal status as 44.8% (n = 112) as premenopausal and 65.2% (n=138) as postmenopausal. In the statistical analysis performed, the level of Ki67 was high in premenopausal women (p=0.015). Also, tumors seen in premenopausal women were associated with ER negativity (p=0.024) and high histological grade (grade3) (p=0.015). It was found that luminal subtype (luminal A, luminal B) breast cancers were observed more frequently in postmenopausal women and non-luminal subtypes (HER2+, TNBC) were observed more frequently in premenopausal women.

Conclusion: Our study confirmed the association of premenopausal patients with subtypes of aggressive nature. Clinicians should anticipate that they may need other treatment options besides hormonal therapy when determining treatment options in young patients.

ÖZET

Amaç: Meme kanseri, oldukça farklı biyolojik davranışlar sergileyen ve birçok genomik iz taşıyan heterojen bir hastalık grubudur. Cinsiyet hormonlarına bağımlılığı da menopoz durumu ile ilişkisini belirler. İmmünohistokimyasal belirteçlerle yapılan reseptör analizine ve Ki67 düzeyine göre beş moleküler alt tipe ayrılır. Bu çalışmada, tedavi stratejilerimizi öngörmemize yardımcı olması için menopoz durumu ile bu moleküler alt tipler arasındaki ilişkiyi incelemeyi amaçladık.

Gereç ve Yöntem: 2012-2020 yılları arasında Onkoloji Kliniğimizde meme kanseri nedeniyle ameliyat edilen 250 hastanın veri tabanı geriye dönük olarak incelendi. Hastalar menopoz durumlarına ve klinikopatolojik özelliklerine göre gruplandırıldı. İstatistiksel analiz %95 güven aralığında yapıldı ve 0,05'ten düşük bir p değeri istatistiksel olarak anlamlı kabul edildi.

Bulgular: Hastalar menopoz durumlarına göre %44.8 (n=112) premenopozal ve %65.2 (n=138) postmenopozal olarak 2 gruba ayrıldı. Yapılan istatistiksel analizde premenopozal kadınlarda Ki67 düzeyi yüksekti (p=0.015). Ayrıca premenopozal kadınlarda görülen tümörler ER negatifliği (p=0.024) ve yüksek histolojik derece (grade3) (p=0.015) ile ilişkiliydi. Postmenopozal kadınlarda luminal alt tip (luminal A, luminal B) meme kanserlerinin, premenopozal kadınlarda ise luminal olmayan alt tiplerin (HER2+, TNBC) daha sık izlendiği bulundu.

Sonuç: Çalışmamız premenapozal hastaların agresif doğaya sahip subtiplerle olan ilişkisini teyit etmiştir. Klinisyenler genç hastalarda tedavi seçenekleri belirlerken hormonal tedavi dışında diğer tedavi seçeneklerinede ihtiyaç duyubileceklerini öngörmelidirler.

Keywords:
Breast cancer
Immunohistochemical analysis
Molecular subtype
Menopausal status

Anahtar Kelimeler:
Meme kanseri
İmmünohistokimyasal analiz
Moleküler alt tip
Menopoz durumu

INTRODUCTION

Breast cancer in younger women has been associated with lower survival and higher recurrence rates than elderly ones. Even though studies describe negatively affecting factors, they have not fully explained the underlying biological nature that drives these aggressive traits (1).

In the present day, the existence of 5 intrinsic breast cancer subtypes has been identified and accepted by gene expression studies and staining techniques based on immunohistochemical (IHC) markers. In clinical practice, IHC staining is grouped according to the expression levels of luminal and non-luminal subtypes, estrogen receptor-α (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER -2), and Ki67. St. The classification into five molecular subtypes has been accepted as per the recommendations of the Gallen consensus (2013) (2).

The relationship between molecular subtypes of breast

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cancer and survival has been investigated in many studies, and it was found that luminal subtypes are associated with better prognosis and less recurrent disease development than others. Recent studies also have evidence of significant differences in treatment strategies for these different subtypes (3).

It was reported that non-luminal subtypes with more aggressive features are seen more in premenopausal women, whereas menopausal women are more associated with luminal subtypes (4). In fact, in a way, there are clues about the treatment of the disease in the genomic sequence. While ER-positive patients are likely to respond to endocrine treatment and HER2-positive patients to trastuzumab treatment, the standard treatment of the Triple-negatie Breast Cancer (TNBC) group remains a mystery.

It is known that breast cancer is dependent on sex hormone levels. This study aimed to investigate the relationship between menopausal status and intrinsic breast cancer subtypes, and clinicopathological characteristics in women with breast cancer. Thereby, we hope to help with the predictability of disease relapse, overall survival, endocrine, and response to chemotherapy regimens.

MATERIAL AND METHOD

Study design

Our study was initiated by obtaining the approval of the ethics committee of Ankara University Faculty of Medicine (Decree no: İ2-119-21).

The database of 277 patients operated on for breast cancer in the Oncology Clinic of our University's Faculty of Medicine Hospital between 2012-2020 was retrospectively analyzed. Patients with surgical and medical menopause for any reason and patients who were not operated due to advanced disease were excluded from the study. İn addition, 27 patients were excluded due to missing data. Demographic and clinicopathological characteristics of the patients were recorded. From histopathological examination results, receptor status (ER, PR, and HER2), Ki67 percentage, tumor-related variables (histological type, size, grade), lymphovascular invasion (LVI) status, axillary lymph node involvement level were recorded retrospectively. The age, menopausal status, the side of the tumor, and the type of surgical procedure performed were recorded from the digital files in the database. Patients were categorized into two classes by their menopausal status.

ER and PR status were determined using immunohistochemical staining (IHC). Positive ER or PR was accepted when ≥1% of invading malignant cells exhibiting nuclear staining or immunoreactivity. Tumors were considered HER2-positive only if they showed HER2 amplification (ratio >2) using IHC staining 3+ (strong, full membrane staining in >30% of cancer cells) or fluorescent in situ hybridization (FISH). ER, PR and HER2 tests were scored as per the American College of Pathologists Guidelines (5). The cutoff rate of KI-67 was accepted as 17%.

The patients were classified according to the recommendations of the St. Gallen International Expert Consensus Report (2013) by molecular breast cancer subtypes. The patients were categorized by the receptor status of their primary tumor as follows: Luminal A (ER + and/or PR + and HER2-); luminal B HER2- (ER + and/or, PR +, HER2- and high Ki-67); luminal B HER2 + (ER +, HER2+, any Ki-67, any PR); HER2 (ER- and PR- and HER2 +) and triple-negative breast cancer (TNBC; ER- and PR- and HER2-) (2).

The status of lymph node metastasis was determined by histopathological evaluation of axillary lymph nodes obtained during mastectomy or axillary dissection. The total number of lymph nodes was determined by summing the number of non-invasive lymph nodes and metastasis-positive lymph nodes.

The patients were staged based on the American Joint Committee on Cancer (AJCC) 8th Edition according to the TNM staging system (stage 1A, 1B, 2A, 2B, 3A, 3B, 3C) (6).

Statistical Analysis

Descriptive statistical analyzes of quantitative variables were made, and all data were expressed as mean±standard deviation (SD), number, percentage, maximum and minimum values. Then, the statistical analysis was performed using SPSS (version 24). Parametric test assumptions were examined before performing the difference analysis. Normality was checked with the Kolmogorov Smirnov test, skewness, and kurtosis. In the case where the assumptions were provided, the difference analysis was performed using the one-way analysis of variance (ANOVA) and the Kruskal Wallis test when it was not provided. Paired comparisons were made using the Mann-Whitney U test. The relationship between categorical variables was analyzed using the chi-square (χ 2 test) test. Statistical analysis was made at a 95% confidence interval. A p-value lower than 0.05 was considered statistically significant.

The clinicopathological characteristics of the patients are summarized in Table 1.

RESULTS

All 250 patients included in the study were women. The patients were divided into 2 groups by their menopausal status as 44.8% (n=112) as premenopausal and 65.2% (n=138) as postmenopausal. The right breast was affected in 52.5% (n=134) of the patients, and the left breast in 48% (n=116). The mean age of the patients was 54.86±13.08 years, the mean age of premenopausal patients was 43.77±5.56 (24-53), and the mean age of postmenopausal patients was 63.86±10.2 (46-93). There was a cumulative accumulation of breast cancer in postmenopausal women between the ages of 51 and 63. The mean percentage of Ki67 was 34.88 ± 24.71 in premenopausal women and 22.02 ± 18.32 in postmenopausal women. According to the histopathological subtypes, the most common type of cases was ductal (premenopausal 33%, postmenopausal 43%), a few of them were lobular, the remaining cases were other histological types such as medullary, tubular, mucinous, metaplastic, adenoid, cystic and papillary carcinoma. In half of the patients (n=125, 50%), axillary nodal involvement was not determined. The mean pathological lymph nodes resected in the remainder was 4.8±4.7 (1-31), and the mean total lymph nodes resected were 11.9±7.2 (1-35). The distribution among the groups was almost equal by the axillary nodal involvement.

Table 1: Distribution of clinicopathological characteristics of 250 patients with breast cancer by menopausal status

Characteristics	Premenopausal (112)		Postmenopausal (138)		Total patients (250)		P-value
	Number (n)	Percentage (%)	Number (n)	Percentage (%)	Number (n)	Percentage (%)	
Type of surgery							0.550
MRM	45	18	61	24.4	106	42.4	
Mastectomy+SLNB	8	3.2	5	2	13	5.2	
BCS+SLNB	32	12.8	43	17.2	75	30	
BCS+AD	27	10.8	29	11.6	56	22.4	
Tumor histology							0.572
Ductal	83	33.2	107	42.8	191	76	
Lobular	10	4	14	5.6	24	9.6	
Other	19	7.6	17	6.8	36	14.4	
T stage							0.825
T1(<2 cm)	59	23.6	71	28.4	130	52	
T2(2-5 cm)	39	15.6	46	18.4	95	34	
T3(> 5 cm)	14	5.6	21	8.4	35	14	
T4	0	0	0	0	0	0	
LVI status							0.674
Negative	59	23.6	69	27.6	128	51.2	
Positive	53	21.2	69	27.6	132	48.8	
Node status							0.349
N0(no)	56	22.4	69	27.6	125	50	
N1(1-3)	31	12.4	49	19.6	80	32	
N2(4-10)	15	6	12	4.8	27	10.8	
N3(> 11)	10	4	8	3.2	18	7.2	

The distribution of clinicopathological characteristics by groups is presented in Table 1.

In the statistical analysis performed, no significant difference was found between the quantitative variables and the groups in terms of tumor size (p=0.609), the number of total lymph nodes resected (p=0.794), and the number of pathological lymph nodes (p=0.690). However, the Ki67 level was significantly higher in premenopausal patients than in postmenopausal patients (p=0.015).

In the chi-square analysis performed between menopausal status and categorical variables, no relationship was found between the type of surgery, histopathological subtype, axillary nodal status, LVI status, TNM stage, PR, and HER2 (p>.05).

However, a significant relationship was found between menopausal status and ER status (p=0.024), histological grade of the tumor (p=0.002), and molecular subtype of the tumor (p=0.032). When the subtypes were examined, we found that premenopausal patients were more associated with luminal A and luminal B, and postmenopausal patients were more associated with other non-luminal subtypes.

In other words, tumors seen in premenopausal women were associated with the presence of advanced histological grade (grade 3), non-luminal subtype (HER2+, TNBC), and ER negativity compared to postmenopausal women. In postmenopausal women, tumors were more closely associated with low histological grade (Grade 2-3), luminal subtype, and ER positivity.

The results are presented in Table 1, together with the distribution of variables by groups.

DISCUSSION

We examined the relationship between women with breast cancer, divided into two groups as premenopausal and postmenopausal, with clinicopathological variables and molecular subtypes. In the statistical analysis performed, the level of Ki67 was high in premenopausal women (p=0.015). Besides, premenopausal tumors were also associated with ER negativity (p=0.024) and high histological grade (grade 3) (p=0.006). We also found that breast cancer in premenopausal women was associated with non-luminal subtypes (HER2+, TNBC). On the other hand, breast cancers diagnosed in the postmenopausal period were mostly associated with luminal subtypes (luminal A, luminal B) (p=0.032).

Anders et al. found a low incidence of ER positivity in young women in their study of 784 breast cancer patients, including large-scale genomic analysis, and that HER-2 had higher expression and tumors had higher histological grade (grade 3) (7). In our study, the relationship between HER-2 expression levels and menopausal groups did not reach statistical significance.

Keegan et al. reported that non-luminal subtypes (HER2+, triple-negative) were found at a higher rate in young women and the tumors were of high histological grade (8). There is a significantly higher rate of luminal subtypes in postmenopausal women in our study. This result is probably due to the significantly low Ki67 and high ER expression in postmenopausal women.

Triple-negative breast cancer has an overall incidence of

Table 1 continuation: Distribution of clinicopathological characteristics of 250 patients with breast cancer by menopausal status

Characteristics	Premenopausal (112)		Postmenopausal (138)		Total patients (250)		P-value
	Number (n)	Percentage (%)	Number (n)	Percentage (%)	Number (n)	Percentage (%)	
Tumor grade							0.006
Grade 1	15	6	27	10.8	42	16.8	
Grade 2	32	12.8	59	23.6	91	36.4	
Grade 3	65	26	52	20.8	117	46.8	
TNM stage							0.279
1A	45	18	48	19.2	93	37.2	
1B	9	3.6	21	8.4	30	12	
2A	9	3.6	19	7.6	28	11.2	
2B	21	8.4	21	8.4	42	16.8	
3A	18	7.2	21	8.4	39	15.6	
3B	0	0	0	0	0	0	
3C	10	4	8	3.2	18	7.2	
4	0	0	0	0	0	0	
Local stage							0.455
Early-stage	84	33.6	109	43.6	193	77.2	
Locally advanced stage	28	11.2	29	11.6	57	22.8	
Receptor status							
ER							0.024
Positive	85	34	120	48	205	82	
Negative	27	10.8	18	7.2	45	18	
PR				0.157			
Positive	78	31.2	107	42.8	185	74	
Negative	34	13.6	31	12.4	65	26	
HER2							0.836
Positive	42	16.8	50	20	92	36.8	
Negative	70	28	88	35.2	158	63.2	
Molecular subtype							0.032
Luminal A	17	6.8	36	14.4	46	21.2	
Lum B (HER2-)	41	16.4	49	19.6	90	36	
Lum B (HER2+)	30	12	47	18.8	77	30.8	
HER2+	12	4.8	3	1.2	15	6	
Triple - (TNBC)	12	4.8	3	1.2	15	6	

11.2% at all ages, with a higher incidence in premenopausal women compared to postmenopausal women, regardless of race (7,9,10). However, in a national comprehensive study conducted by Lin et al. on approximately 15,000 patients, they reported that the probability of having a triple-negative subtype increased in premenopausal women compared to postmenopausal women with a high body mass index(11). Although the rate of triple-negative subtypes was low (6%) in the study, it was more associated with premenopausal women.

Even though HER-2 Neu expression was found to be higher in premenopausal women in the literature, no statistically significant relationship was found in the study(12).

In our study, the tumor size was relatively larger in premenopausal women, and although their tumors were mostly larger than 2 cm, it was not statistically significant (p=0.825). Although half of the patients had no lymphatic metastases, it was observed that most of them were postmenopausal women. On the other hand, among patients with lymphatic metastasis, although the ratio of N2 and N3 was relatively higher in premenopausal women, it was found that similarly, it did not reach statistical significance (0.349).

The majority of cases were ductal carcinomas (76%), and it was observed that these were relatively higher in postmenopausal women. Similarly, about half of the cases showed LVI, and it was observed to be more common in postmenopausal women. However, these variables could not be correlated significantly with the menopausal status in the analysis (p=0.572, p=0674).

On the other hand, approximately half (47%) of the tumors observed had high histological grade (grade 3) and were significantly more common in premenopausal women (p=0.006).

Although the results we obtained show parallelism with the studies in the literature, there are some differences.

Most studies have shown that tumors found in young and premenopausal women have more aggressive phenotypes (TNBC and HER2 positive subtypes). It was also reported that these tumors are associated with higher lymph node metastasis and higher histological grade and size (13,14,15).

Our study showed that the most appropriate clinical classification for the menopausal situation is IHC-based intrinsic subtype classification (p=0.032) rather than the classical TNM staging (p=0.279). This may be due to factors associated with particularly poor genomic character observed in premenopausal women (such as Ki67 and HER-2 elevation, ER negativity). Therefore, all these features are of vital importance in determining treatment strategies. Luminal tumors most likely respond favorably to hormonal therapies such as tamoxifen and trastuzumab treatment for HER2 overexpressing tumors. However, treatment options for TNBC are still limited and non-specific (16).

Our study showed that intrinsic subtype classification based on IHC staining is more helpful in determining treatment strategies. Accordingly, it should be predicted that premenopausal patients will need more chemotherapy due to the high Ki67 proliferation index and trastuzumab therapy due to the high HER-2. On the contrary, it can be predicted that postmenopausal patients will benefit more from hormonal treatments due to ER positivity. Thus, clinicians can predict the menopausal status and possibly the treatment options to be used at the beginning of the treatment. In their meetings with their patients, they can discuss these possible treatment strategies more informatively and openly because of their predictions.

CONCLUSION

As a result, it is known that more difficult treatment processes await these patients due to the aggressive phenotypic features seen in young patients. In this study, it was predicted that premenopausal patients would need other treatment options besides hormonal treatment. We believe this insight will help clinicians prepare their patients for treatment options.

Study Limitations

This study had limitations such as its retrospective nature and symptomatic cases. The distribution of breast cancer molecular subtypes may differ in studies involving symptomatic case series, so population-based randomized studies are needed.

In summary, it was accepted for years that breast cancer occurring in premenopausal women represents an aggressive phenotype. However, the biological parameters that direct the nature of this heterogeneous disease are still largely unknown. More randomized and prospective studies are needed to enlighten this issue.

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