Original Article

Relationship Between Dynamic MRI Findings and the Prognostic Factors of Breast Cancer

Meme Kanserinin Dinamik MRG Bulguları ile Prognostik Faktörleri Arasındaki İlişki

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ABSTRACT

Introduction: The use of MR imaging in breast cancer is increasing. In recent years, MRI has been gaining use as a modality that can provide important information about breast cancer lesions. Our aim was to determine if any significant relationship existed between dynamic MRI findings and factors which are currently being used to establish prognosis.

Methods: Eighty-one patients with a diagnosis of invasive breast carcinoma and breast MRI were retrospectively reviewed and included in the study. Kinetic MRI features were determined on breast MR images, histopathological results were reviewed and prognostic factors were recorded.

Results: Thirty-five patients' initial enhancement was lower than fifty percent, while 18 patients were between fifty and one hundred percent and 28 patients higher than one hundred percent. Our study is one of the latter, we could not identify any significant relationships between initial enhancement characteristics and dynamic curve types with any of the analyzed prognostic factors.

Dıscussion and Conclusion: Although imaging can provide important data in the identification, diagnosis, and evaluation of many types of disease; imaging findings do not always correlate with patient clinic and prognosis. Up until now, studies on this matter have mostly found nothing of high significance; our study was no exception, however some of the data reported in this study can influence future studies.

Keywords: MRI, Breast imaging, Breast cancer

ÖZET

Giriş ve Amaç: MR görüntülemenin meme kanserinde kullanımı artmaktadır. Son yıllarda MRG, meme kanseri lezyonları hakkında önemli bilgiler sağlayabilen bir yöntem olarak kullanılmaya başlanmıştır. Amacımız, dinamik MRG bulguları ile prognoz belirlemek için kullanılan faktörler arasında önemli bir ilişki olup olmadığını saptamaktı.

Yöntem ve Gereçler: İnvaziv meme kanseri tanısı ve meme MR görüntülemesi olan seksen bir hasta retrospektif olarak tarandı ve çalışmaya dahil edildi. Meme MR görüntülerinde kinetik MR özellikleri belirlendi ve histopatolojik sonuçları yeniden gözden geçirilerek prognostik faktörleri kaydedildi.

Bulgular: Kinetik MR özellikleri ilk faz kontrastlanma düzeyine göre gruplandı ve 35 hastada %50'nin altında, 18 hastada %50 ile %100 arasında ve 28 hastada %100'den fazlaydı. Çalışmamızda, analiz

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edilen prognostik faktörlerin herhangi biri ile erken kontrastlanma özellikleri ve dinamik eğri türleri arasında herhangi bir anlamlı ilişki tespit edemedik.
Tartışma ve Sonuç: Görüntüleme birçok hastalık türünün tanısı, teşhisi ve değerlendirilmesinde önemli veriler sağlasa da görüntüleme bulguları her zaman hasta kliniği ve prognozu ile ilişkili değildir. Şimdiye kadar, bu konudaki çalışmalar çoğunlukla, çalışmamızla benzer şekilde anlamlı bir ilişki bulamadı, ancak çalışmamızda bildirilen bazı veriler gelecekteki çalışmaları etkileyebilir.

Anahtar Kelimeler: MRG, Meme görüntüleme, Meme kanseri

Introduction

The most frequently diagnosed cancer among women worldwide is breast cancer; it is also the leader among deaths caused by cancer in women [1]. Breast cancer awareness and consequently the frequency of early diagnosis through effective screening and self-examination is on the rise [2,3]. Also the mortality rate of breast cancer has reduced in recent years [1]. This progress may be in part due to advancements in imaging modalities which are used in the screening and diagnosis of breast cancer. These imaging methods are: mammography (MMG), ultrasonography (USG), computed tomography (CT), positron emission tomography–CT (PET-CT), and magnetic resonance imaging (MRI) [4]. Other methods used for diagnosing breast cancer or supplementing a diagnosis are: pathologic evaluation of tumor biopsy (presence of malignant cells), breast cancer receptor testing, and genetic marker identification [5]. When breast cancer diagnosis is established, further evaluations are required to understand the extent of the disease and determine treatment path(s).

The determination of prognosis is important for informing the patient, deciding on the options for treatment and other medical/social decisions. Classical guidelines incorporate lymph node status and tumor size together with pathological findings such as histological type and grade of the cancer to determine prognosis [6,7]. The results of this approach are found to be significantly correlated with recurrence-free and overall survival [8]. Molecular biomarkers, from blood or biopsy material, are another approach. The determination of various biomarkers such as Ki-67, cerbB2 (HER2 and sHER2), aromatase, osteopontin, and CEASCAM6 have been found to determine prognosis at different reliability levels [9]. Steroid hormone receptor (estrogen and progesterone receptors) expression measurements are also important for prognosis due to their influence on hormone therapy efficacy [10,11].

Magnetic resonance imaging (MRI), as previously stated, is one of the imaging modalities used in the diagnosis of breast cancer. It is also used as a screening tool in women who are at higher risk for breast cancer [12]. The use of contrast in dynamic MRI, namely dynamic contrast-enhanced MRI, has emerged as a very high sensitivity modality which is effective in the detection and staging of invasive breast cancer [13]. MRI determines the functional characteristics of the tumor, these characteristics were found to be an important addition to the diagnostic approach in breast cancer [14]. A significant relationship between MRI kinetic findings and prognosis may exist, identifying this relationship would improve the approach to breast cancer and may serve as an alternative method in determining prognosis.

We hypothesized that patients with poor prognostic factors will exhibit washout type (type 3) contrast enhancement pattern. The more aggressive malignant lesion will have more cell turnover. In addition, neovas-cularization is increased in the mass. Thus, we would expect the mass to lose contrast in a fast manner in the early phase (consistent with

type 3 curves). Furthermore, various studies associate tumor grade with tumor angiogenesis [15,16]. As tumor grade is one of the classical factors for breast cancer prognosis, determining the level of angiogenesis (via contrast-enhanced dynamic MRI) in the tumor may provide important prognostic data. Several studies have investigated the relationship between classical prognostic factors and kinetic MRI results [17-25], these studies yielded conflicting results.

Our aim was to determine if any relationship exists between kinetic MRI features and classical prognostic factors with a focus on dynamic curve types.

Methods

The study was designed as a retrospective study of invasive breast cancer patients who had been treated at our hospital between 2015 and 2016. Ethical approval was obtained from local ethics committee. Additional approval was obtained from the hospital board as our institution is considered as a reference hospital for breast cancer (Decision number and date: 2010-04/1116 and 07.04.2021).

The MRI values and dynamic results of patients who had MRI indications and had underwent MRI which revealed they had BIRADS-4 or BIRADS-5 lesions were recorded by searching our MRI archive. Inclusion criteria were as follows. (1) Patient had to have a histologically proven invasive cancer of the breast, (2) patient had to have a dynamic MRI conducted for invasive breast cancer. Exclusion criteria were as follows. (1) History of other cancer, (2) having any chronic comorbid condition, (3) missing pathological parameters due to insufficient tissue material. A total of 98 patients were analyzed. Four of these patients who had metastasized stage-4 disease diagnosed with tru-cut biopsy were excluded due to insufficient tissue material for the identification of all pathological parameters. A further 3 were excluded because their pathological examination revealed the tumors to be ductal carcinoma in-situ (DCIS). And 10 were excluded because of benign pathologic results. Thus, a final group of 81 patients were enrolled in the study.

MR Imaging

MR imaging results were acquired with a 1.5 Tesla scanner (Signa HDx, 1.5 T, GE Healthcare) using a dedicated breast coil and evaluated according to breast imaging and reporting data system (BI-RADS) by three breast radiologists with at least 5-year experience in breast MRI evaluation. Images consisted of: axial T1-weighted fast spin echo imaging (TR/TE=400/8.8, a field of view (FOV) of 320 mm, a matrix of 448 x 224, number of excitations (NEX) of 1, and 5 mm slice thickness); axial STIR (TR/TE= 6500/45, TI=150 ms, FOV of 320 mm, a matrix of 416x224, number of excitations (NEX) of 1, and 5 mm slice thickness); axial dynamic images with pre and post-contrast fat saturated gradient T1 sequences (TR/TE= 4/1.5, a flip angle of 10°, FOV of 320 mm, a matrix of 350x350, NEX of 1, and 2.8 mm slice thickness). Images were taken once before contrast and 5 times after contrast injection with 80 second intervals. Contrast material was gadobutrol/gadopentetate dimeglumine with a dose of 0.1 mmol/kg. Re-evaluation of imaging data was performed when needed.

Region of interest (ROI) area was designated between 36-100 mm2 and further calculations were performed automatically by the workstation. Formula used for initial enhancement calculation was: (Signal postcontrast−Signal initial)/ Signal initial × 100%). We divided our cases into three groups in regard to initial enhancement values (<50%, 50-100%, >100%) this grouping was previously performed by [25]. However, a consensus on this matter does not exist.

Determination of kinetic curve type was done according to post-initial enhancement data; type 1 is defined by stable increase of enhancement, type 2 is the formation of a plateau of signal intensity after contrast injection, type 3 is the stable decrease of enhancement in the post-initial phase [26].

Histopathology

Pathological evaluations -according to the tumour, node and metastasis (TNM) classification- and prognosis determinations -according to the classical guidelines via antibody staining- were performed by certified pathologists. Tissue obtained in surgery was fixed in %10 formalin and was processed to paraffin blocks at 5 μm thickness; which was the routine protocol. Tumor type was determined by the WHO classification. Tumor grading (grade 1, 2, and 3) was accomplished by the modified Bloom-Richardson protocol. Lymph node involvement was assessed by the sentinel method. Positive lymph nodes were dissected. Lymphovascular invasion (LVI) was assessed by hematoxylin-eosin staining. Standardized protocols were used in the staining of Ki-67, estrogen and progesterone receptors, and C-erb B2 (Her2/neu) receptor from the invasive part of the tumor; and the assessment was done by calculating the percentage of stained cells.

Statistical Analysis

All analyses were performed on SPSS v21. For the normality test, Shapiro-Wilk test was used. Continuous variables are given as mean ± standard deviation for normally distributed data and median (minimum – maximum) for non-normally distributed data. Comparisons between groups were made with one-way ANOVA for normally distributed data and Kruskal Wallis test for non-normally distributed data. Pairwise comparisons were made with Dunn's test. Categorical variables are given as frequency (percentage). Analysis of categorical variables were made with Chi-square test. $p \leq 0.05$ values were accepted as statistically significant.

Results

We included 81 female patients into our study, median age was 47.27 ± 12.13. Sixty-three (77.8%) patients had invasive ductal carcinoma (IDC) while 9 (11.1%) patients had invasive lobular carcinoma (ILC). In addition, one of the patients were diagnosed with invasive cribriform carcinom, one invasive secretory carcinom, two mixed IDC + ILC, two invasive medullary carcinom, one tubular carcinom and two mucinous carcinom. When we evaluated tumor grades there were 11 (14.9%) patients with Grade 1, 29 (29.2%) patients with Grade 2 and 34 (45.9%) patients with Grade 3 tumor, while grading for 7 patients were missing. Forty-five (55.6%) patients had DCIS component while 10 (12.3%) had lobular carcinoma in situ (LCIS) component. Twenty patients had LVI and 4 (4.9%) patients received neoadjuvant chemotherapy.

When we made comparisons in regard to tumor grades, Ki-67 percentages were significantly higher in patients with Grade 3 tumor ($p<0.001$). There were no significant differences between these regarding age, ER (%) and PR (%). Also we found that 11 of the 15 (73.3%) patients with 3 C-erb B2 score had Grade 3 tumor. This result was also found to be significant ($p=0.011$) (Table 1).

We divided our patients into three groups regarding initial enhancement. Thirty-five patients' initial enhancement was lower than fifty percent, while 18 patients were between fifty and one hundred percent and 28 patients higher than one hundred percent. When we compared Ki-67, ER and PR values we found no significant difference between our groups (Figure 1). When we evaluated tumor types, 31 (49.2%) of the patients with IDC had lower than fifty percent initial enhancement while 5

Table 1. Patients' Characteristics Regarding Tumor Grade

	Grade 1 (n=11)	Grade 2 (n=29)	Grade 3 (n=34)	p
Age, mean ± SD	48.91 ± 10.78	48.66 ± 12.69	44.68 ± 12.25	0.370
Ki-67 (%), median (min - max)	8.00 (2.00 - 21.00)[(a)]	10.00 (2.00 - 80.00) [(a)]	40.00 (5.00 -95.00) [(b)]	<0.001**
ER (%), median (min - max)	90.00 (0.00 - 100.00)	90.00 (0.00 - 100.00)	80.00 (0.00 - 100.00)	0.078
PR (%), median (min - max)	60.00 (0.00 - 100.00)	80.00 (0.00 - 100.00)	65.00 (0.00 - 100.00)	0.396
Type, n (%)				
IDC	7 (12.3)	22 (38.6)	28 (49.1)	
ILC	1 (11.1)	5 (55.6)	3 (33.3)	0.320
Other	3 (37.5)	2 (25.0)	3 (37.5)	
C-erb-B2, n (%)				
0	10 (24.4)	14 (34.1)	17 (41.5)	
1	0 (0.0)	10 (76.9)	3 (23.1)	0.011*
2	0 (0.0)	2 (40.0)	3 (60.0)	
3	1 (6.7)	3 (20)	11 (73.3)	
Lenfovascular Invasion, n (%)				
Negative	6 (16.2)	15 (40.5)	16 (43.2)	0.291
Positive	1 (5.3)	6 (31.6)	12 (63.2)	
Neoadjuvant Chemotherapy, n (%)				
Absent	10 (14.1)	27 (38.0)	34 (47.9)	0.252
Present	1 (33.3)	2 (66.7)	0 (0.0)	

Same letter denotes the lack of statistically significant difference between groups
Ki-67 : Proliferation index ; ER: Estrogen receptor ; PR: Progesterone receptor ; C-erb-B2: Human epidermal growth factor receptor 2 ; IDC: Invasive ductal carcinoma ; ILC: Invasive lobular carcinoma

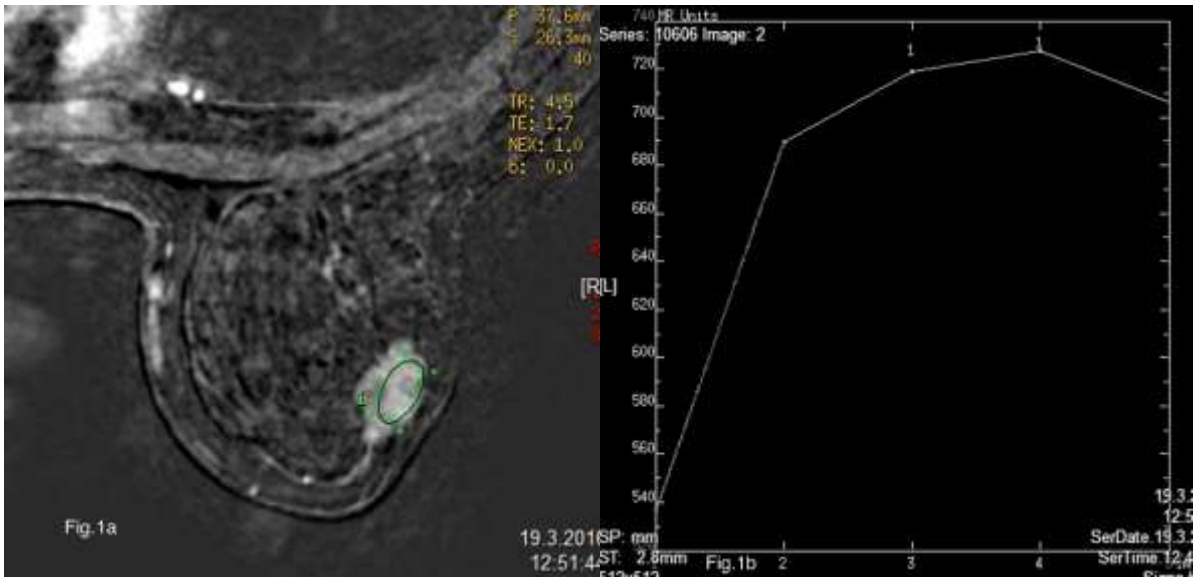

Figure 1a: Dynamic contrast enhanced breast MRI shows a mass with irregular margins in 37-year-old woman. Final pathology showed grade-2 invasive ductal carcinoma. Percentage of Ki-67 were 30%, estrogen and progesterone receptors were both 95%, and c-erbb2 receptor score was 1 in the tumor.

Figure 1b: Kinetic curve analysis shows plateau patterns (type-2) on dynamic CE MR imaging.

Table 2. Patients' Characteristics Regarding Initial Enhancement

	<50% (n=35)	50% - 100% (n=18)	>100% (n=28)	p
Ki-67 (%), median (min - max)	35.00 (3.00 - 95.00)	25.00 (5.00 - 60.00)	20.00 (2.00 - 80.00)	0.069
ER (%), median (min - max)	90.00 (0.00 - 100.00)	90.00 (0.00 - 100.00)	90.00 (0.00 - 100.00)	0.806
PR (%), median (min - max)	70.00 (0.00 - 100.00)	50.00 (0.00 - 90.00)	80.00 (0.00 - 100.00)	0.272
Type, n (%)				
IDC	31 (49.2)	13 (20.6)	19 (30.2)	
ILC	1 (11.1)	5 (55.6)	3 (33.3)	0.014*
Other	3 (33.3)	0 (0.0)	6 (66.7)	
Tumor Grade				
Grade 1	4 (36.4)	1 (9.1)	6 (54.5)	
Grade 2	13 (44.8)	8 (27.6)	8 (27.6)	0.454
Grade 3	17 (50.0)	6 (17.6)	11 (32.4)	
C-erb-B2, n (%)				
0	15 (33.3)	11 (24.4)	19 (42.2)	
1	6 (46.2)	3 (23.1)	4 (30.8)	0.375
2	3 (60.0)	0 (0.0)	2 (40.0)	
3	11 (61.1)	4 (22.2)	3 (16.7)	
Lenfovascular Invasion, n (%)				
Negative	15 (37.5)	10 (25.0)	15 (37.5)	0.230
Positive	12 (57.1)	2 (9.5)	7 (33.3)	
Neoadjuvant Chemotherapy, n (%)				
Absent	32 (41.6)	18 (23.4)	27 (35.1)	0.362
Present	3 (75.0)	0 (0.0)	1 (25.0)	

Ki-67 : Proliferation index ; ER: Estrogen receptor ; PR: Progesterone receptor ; C-erb-B2: Human epidermal growth factor receptor 2 ; IDC: Invasive ductal carcinoma ; ILC: Invasive lobular carcinoma

Table 3. Patients' Characteristics Regarding Dynamic Types

	Type 1 (n=5)	Type 2 (n=40)	Type 3 (n=36)	p
Ki-67 (%), median (min - max)	18.00 (5.00 - 40.00)	21.00 (2.00 - 80.00)	30.00 (3.00 - 95.00)	0.221
ER (%), median (min - max)	90.00 (0.00 - 100.00)	90.00 (0.00 - 100.00)	90.00 (0.00 - 100.00)	0.702
PR (%), median (min - max)	90.00 (0.00 - 90.00)	75.00 (0.00 - 100.00)	50.00 (0.00 - 100.00)	0.356
Initial Enhancement, n (%)				
<50%	0 (0.0)	7 (20.0)	28 (80.0)	
50% - 100%	0 (0.0)	13 (72.2)	5 (27.8)	<0.001**
>100%	5 (17.9)	20 (71.4)	3 (10.7)	
Type, n (%)				
IDC	3 (4.8)	28 (44.4)	32 (50.8)	
ILC	2 (22.2)	6 (66.7)	1 (11.1)	0.061
Other	0 (0.0)	6 (66.7)	3 (33.3)	
Tumor Grade				
Grade 1	2 (18.2)	5 (45.5)	4 (36.4)	
Grade 2	1 (3.4)	14 (48.3)	14 (48.3)	0.570
Grade 3	2 (5.9)	17 (50.0)	15 (44.1)	
C-erb-B2, n (%)				
0	4 (8.9)	26 (57.8)	15 (33.3)	
1	0 (0.0)	6 (46.2)	7 (53.8)	0.318
2	0 (0.0)	1 (20.0)	4 (80.0)	
3	1 (5.6)	7 (38.9)	10 (55.6)	
Lenfovascular Invasion, n (%)				
Negative	3 (7.5)	21 (52.5)	16 (40.0)	0.772
Positive	2 (9.5)	9 (42.9)	10 (47.6)	
Neoadjuvant Chemotherapy, n (%)				
Absent	4 (5.2)	40 (51.9)	33 (42.9)	0.068
Present	1 (25.0)	0 (0.0)	3 (75.0)	

Ki-67 : Proliferation index ; ER: Estrogen receptor ; PR: Progesterone receptor ; C-erb-B2: Human epidermal growth factor receptor 2 ; IDC: Invasive ductal carcinoma ; ILC: Invasive lobular carcinoma

Table 4. Patients' Characteristics when Grouped as Washout and Others

	Type 1 and 2 (n=45)	Washout (n=36)	p
Ki-67 (%), median (min - max)	20.50 (2.00 - 80.00)	30.00 (3.00 - 95.00)	0.113
ER (%), median (min - max)	90.00 (0.00 - 100.00)	90.00 (0.00 - 100.00)	0.466
PR (%), median (min - max)	80.00 (0.00 - 100.00)	50.00 (0.00 - 100.00)	0.184
Initial Enhancement, n (%)			
<50%	7 (20.0)	28 (80.0)	
50% - 100%	13 (72.2)	5 (27.8)	<0.001**
>100%	25 (89.3)	3 (10.7)	
Type, n (%)			
IDC	31 (49.2)	32 (50.8)	
ILC	8 (88.9)	1 (11.1)	0.063
Other	6 (66.7)	3 (33.3)	
Tumor Grade			
Grade 1	7 (63.6)	4 (36.4)	
Grade 2	15 (51.7)	14 (48.3)	0.793
Grade 3	19 (55.9)	15 (44.1)	
C-erb-B2, n (%)			
0	30 (66.7)	15 (33.3)	
1	6 (46.2)	7 (53.8)	0.103
2	1 (20.0)	4 (80.0)	
3	8 (44.4)	10 (55.6)	
Lenfovascular Invasion, n (%)			
Negative	24 (60.00)	16 (40.0)	0.765
Positive	11 (52.4)	10 (47.6)	
Neoadjuvant Chemotherapy, n (%)			
Absent	44 (51.9)	33 (42.9)	0.318
Present	1 (25.0)	3 (75.0)	

Ki-67 : Proliferation index ; ER: Estrogen receptor ; PR: Progesterone receptor ; C-erb-B2: Human epidermal growth factor receptor 2 ; IDC: Invasive ductal carcinoma ; ILC: Invasive lobular carcinoma

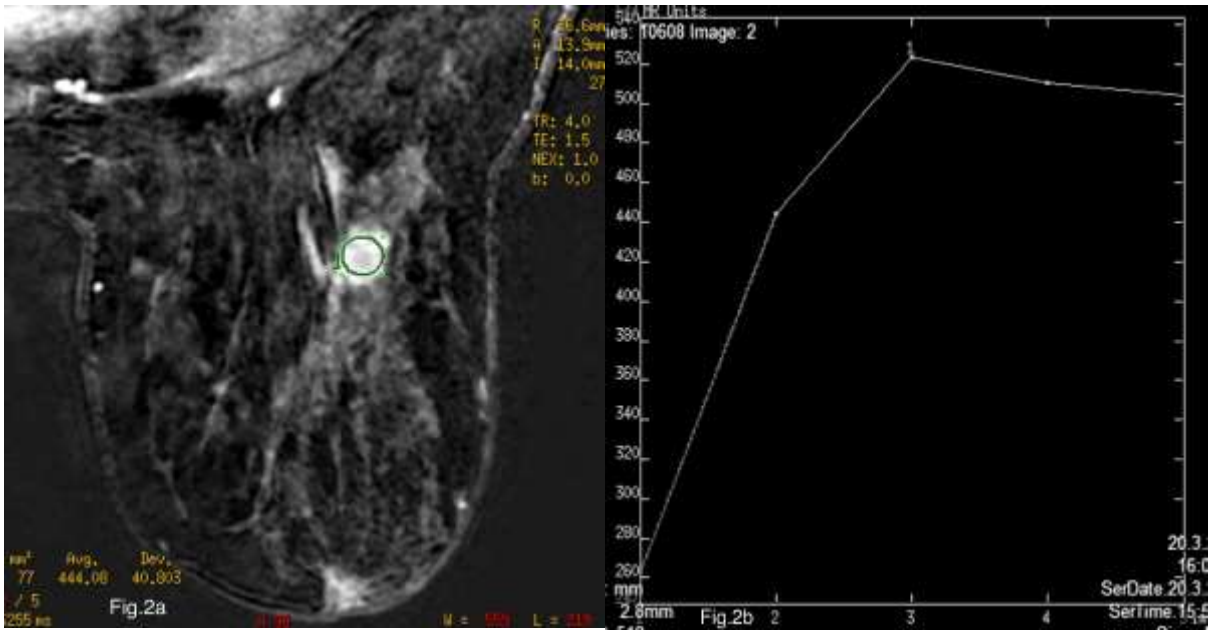

Fig.2a: Dynamic contrast enhanced breast MRI shows a mass with irregular margins in 53-year-old woman. Final pathology showed grade-3 invasive ductal carcinoma. Percentage of Ki-67 were 50%, estrogen and progesterone receptors were 50% and 70% respectively, and c-erbb2 receptor score was 3 in the tumor.
Fig.2b: Kinetic curve analysis shows plateau patterns (type-2) on dynamic CE MR imaging.

(55.6%) of the patients with ILC had between fifty and one hundred percent initial enhancement. This result was also found to be significant (p=0.014) (Table 2).

When we evaluated dynamic types; 5 (6.2%) patients had Type 1 curve, 40 (49.4%) patients had Type 2 curve and 36 (44.4%) patients had Type 3 curve. When we compared our variables between curve type groups we found that all of the patients with Type 1 curve had larger than one hundred percent initial enhancement and 28 of the 36 (77.8%) patients with Type 3 curve had lower than fifty percent initial enhancement (p<0.001). There was no significant difference between our groups regarding other variables (Table 3).

Lastly, we evaluated washout type curves specifically. Which was done by divided the patients into two groups; (1) washout type curve (type 3) and (2) the other curve types (type 1 and 2). This evaluation was performed because a main focus of our hypotheses was that washout type curve (type 3) may have a higher association with prognostic factors. We found initial enhancement was significantly different between groups (as was found previously); however, we again found no significant relationship between any prognostic factors and washout type curve (Figure 2) (Table 4).

Discussion

We aimed to identify any relationship between dynamic MRI findings (with a focus on washout type curve) and factors that affect prognosis in invasive breast cancers. Currently, tumor size and type, histological grade, lymph node status, LVI, steroid receptor (estrogen and progesterone) and human epidermal growth factor receptor expressions, and proliferation markers such as Ki-67 are accepted as important prognosis determining/effecting factors.

In MRI evaluation, tumor margin and its enhancement curve characteristics are accepted to be the most important features in the characterization of breast tumors [27]. Lack of enhancement is reported to have an 88-96% negative predictive value (NPV) for malignancy [28,29]. However, various studies report the enhancement evaluation to be problematic; in a multi-institutional study, 45% of lesions that were found to be malignant were actually reported as having persistent enhancement kinetics [28]. When attention is shifted to curve characteristics, type 2 and 3 curves are reported by some to be associated with malignancy [27]. On the other hand, the ability of diffusion imaging in characterizing microvascular structures has been shown to be important in the evaluation of breast cancer [19,22,30,31]. However, current studies focusing on kinetic evaluation for tumor characterization are rare and present conflicting results. We aimed to identify if any association existed between prognostic factors and enhancement and dynamic MRI findings.

Tumor Grade

As tumor grade is one of the classical prognostic factors, any relationship between histopathological tumor grade and imaging findings would affect the use of imaging modalities in breast cancer diagnosis, screening and evaluation.

When patients were grouped in regard to initial enhancement characteristics (<50%, 50-100%, and >100%), we found no significant difference in tumor grade between groups. Also, no relationship was found when grouping was done in regard to dynamic types (type 1, 2, and 3). Lastly, grouping patients into two (washout type curve, and others) also yielded no significant differences. Only a few studies have reported a minor correlation between grade and enhancement characteristics [22,24,32]. Belli et al. reported

that grade 1 tumors had significantly higher mean apparent diffusion coefficient in comparison to grade 2 and 3 tumors. On the other hand, various studies report a lack of correlation between grade and kinetic MRI findings [33-35], including a 128-patient retrospective study by Baltzer et al. [36]. Our results concur with the latter group of literature.

A variety of explanations may exist for these conflicting results, including a lack of standardization in the grouping and evaluation of enhancement. However, in our study, the low number of grade 1 tumors (11, 13.5%) in comparison to grade 2 and 3 tumors (70, 86.5%) may have affected our results. Also, for dynamic evaluation, type 1 curve group consisted of only five cases which may have had a similar effect in the evaluation of dynamic types.

Ki-67 Index

Nuclear antigen Ki-67 is accepted as an accurate marker for cell proliferation. It is significantly correlated with mitotic activity. Ki-67 is reported as a percentage; called the Ki-67 index. A value above 20% indicates high proliferation and is associated with poor prognosis [37,38].

In our study, we found Ki-67 levels to be significantly higher in grade 3 tumors ($p<0.001$), no such difference was found between grade 1 and 2 tumors. When tumors were grouped according to initial enhancement values and dynamic types, we found no significant difference in Ki-67 values between groups. Also, no significant relationship with Ki-67 values and washout type was found.

Chang et al. also found no significant relationship between curve pattern and Ki-67 level. They reported peak time was the only factor correlated with Ki-67 value [18]. Choi et al. found that Ki-67 index positive invasive ductal cancer cases had a significantly lower apparent diffusion coefficient (ADC) value compared to negative cases [6]. However, this finding may be due to the fact that malignant tumors (which have higher Ki-67 levels) show a significantly lower ADC value in comparison to benign tumors [39]. Another study, by Alduk et al., also reported that they found no relationship between Ki-67 and MRI features [40].

Estrogen and Progesterone Receptor Expression

As previously stated, estrogen and progesterone receptor (ER and PR, respectively) expression is an important factor for breast cancer prognosis; especially when hormone therapy is considered [41]. Patients who present with ER positive tumors are shown to have longer disease-free and overall survival time [42]. The nature and effects of PR and ER expression can shed light on what we can expect in MRI imaging. Some studies report that ER expression is related to angiogenic pathway inhibition which results in diffusion decrease [37,43]. Another effect of ER positivity is reported to be increase in cellularity [44]. When these two factors are taken into account while keeping in mind that ER expression also impacts PR expression, we can come to the conclusion that ER and PR expressions may influence initial enhancement and dynamic MRI results. However, we found no difference between ER or PR expression in regard to initial enhancement groups and dynamic types.

Various studies that aimed to identify a relationship between ER expression and MRI features found conflicting results. Some state that ER negative tumors show more malignant enhancement characteristics [30,36,45]; while others do not support this finding [18,33,40]. When PR expression was assessed, Chang et al. found a lack of relationship between curve type and PR expression [18], as did others [40]. However, Baltzer et al. reported initial

enhancement to be correlated with PR expression [36].

Comparing studies in this regard is difficult as receptor positivity heavily effects treatment paths and the increased use of MRI in higher risk patients may result in bias in patient selection.

C-erb B2 Expression

C-erb B2 (or HER2/neu) overexpression is widely accepted as a factor for poor prognosis [46]. Its expression is inversely correlated with ER expression [46,47]. ER expression is indicative of good prognosis, and C-erb B2 is indicative of poor prognosis. In our study -as stated above- we found no relationship between ER expression and MRI features; thus a lack of relationship between C-erb B2 and the same MRI features was expected. After analysis we found no relationship between C-erb B2 expression and initial enhancement characteristics or dynamic curve types which concurred with our in-study expectation. Chang et al. in their study focusing on curve patterns also found no relationship. However, they found peak time to be correlated with C-erb B2 expression.

Lymphovascular Invasion

There is limited data in literature on the relationship between LVI and MRI features. Alduk et al., in their study of 114 cases of invasive ductal carcinoma, analyzed the relationship between prognostic factors and MRI findings. They reported that a significant relationship between LVI and initial enhancement existed, but failed to find any such relationship in regard to curve type [40]. We found no significant relationship between LVI and either enhancement or curve type.

Strengths and Limitations

Our study has various strengths. Firstly, the number of patients included were 81; a number which matches previous studies. Secondly, most of the major prognostic factors of invasive breast cancers were assessed in our study; thus comparisons with dynamic MRI findings (which were re-evaluated when necessary) were done for all of these factors. Thirdly, we grouped our patients in regard to tumor grade, initial enhancement, and dynamic curve types (also washout vs others) which gave a thorough understanding of the analyses made. Initial enhancement grouping was done according to a previous study (a consensus on this matter does not exist); which makes comparison of studies easier.

There are several limitations to our study. First, this study is based on patients from single center. Thus, the distribution of patients involved in our study group may not be homogeneous. However, our hospital is a regional reference hospital for breast cancer which results in the referring of many cases to us from other hospitals; which would increase homogeneity. Nevertheless, a single-center study may be considered as a limitation. Secondly, the morphological findings of MRI results were not evaluated in our study whereas other studies included morphological findings in their analyses. This may be seen as a limitation; however, our study was focused on the dynamic findings (especially washout curve type) and enhancement characteristics of breast cancer and their comparison with prognostic factors; thus the exclusion of morphological findings did not affect our evaluation.

Although literature on this topic is yet to expand; current studies that report significant relationships can be considered few and far between. Especially curve type and initial-enhancement focused studies point to a lack of association. However, when current literature is reviewed; we see a few important problems on this topic: The lack of consensus for the categorization of dynamic MRI data, differences in operator and/or evaluator evaluations, and variance in study methodologies to the point of incompatibility

between results. If these problems were to be addressed, studies with more data may be performed and comparisons between studies can be made.

Contrast-enhanced dynamic MRI is currently an important imaging tool in the evaluation of invasive breast cancer. The imaging findings can help in identifying important characteristics of the tumor; thus the evaluation of these findings in regard to their relationship with prognostic factors could have yielded important results and changed the way imaging modalities are utilized in breast cancer. However, in our study, neither enhancement characteristics nor dynamic findings were found to be associated with prognostic factors. However, this does not mean that imaging findings are in no way related to patient prognosis; future studies that analyze the relationship between MRI findings and patients' follow-up and survival data are needed to identify direct relationships. Another important matter that needs to be clarified in future studies is the grouping of initial enhancement characteristics. Studies vary in the grouping and utilization of enhancement data; which results in inability to compare study findings with each other. Thus, a first step in enhancement evaluation may be to identify medically relevant enhancement cut-points.

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Original Article

The Expression of HBME1, PAX8, CD56 and CITED1 in Benign Lesions, Benign and Malignant Tumors of Thyroid

Tiroidin Benign Lezyonları, Benign ve Malign Tümörlerinde HBME1, PAX8, CD56 ve CITED1 Ekspresyonun Değerlendirilmesi

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ABSTRACT

Introduction: To evaluate the role of HBME1, PAX8, CD56 and CITED1 in distunguishing benign lesions of the thyroid from malignant tumors and different malignant tumors of the thyroid.

Methods: The patients that underwent thyroidectomy between 2007 and 2013 were included to the study. Patients with Nodular Hyperplasia: 22, Folicular Adenoma: 14, Noninvasive Folicular Thyroid Neoplasm With Papillary-Like Nuclear Features: 3, Well Diferentiated Carcinoma, Not Otherwise Specified: 3, Papillary Carcinoma: 22, Papillary Carcinoma Folicular Variant: 12, Minimally Invasive Folicular Carcinoma: 9, Poorly Diferentiated Carcinoma: 4, Anaplastic Carcinoma: 3 were included to the study. HBME1, CITED1, PAX8, CD56 were applied by immunohistochemical method. In statistical analysis, the sensitivity, specificity, positive predictive and negative predictive values of markers were calculated.

Results: CD56 (loss of expression), HBME1 was significantly higher in Papillary Carcinoma, Papillary Carcinoma Folicular Variant compared to other malignant neoplasms. The most sensitive markers for malignant tumors were PAX8 and CITED1 (90%, 88%). The most specific markers for malignant tumors were HBME1, CITED1 (97%, 92%). The most sensitive and specific marker for Papillary Carcinoma, Papillary Carcinoma Folicular Variant was HBME1 (100%, 80%). CD56 (loss of expression), CITED1 was the most sensitive markers for Papillary Carcinoma Folicular Variant (86%, 86%).

Discussion and conclusion: HBME1 was found an both sensititive and specific marker for Papillary Carcinoma. The cytoplasmic expression of CITED1 was significantly higher in PC compared to other malignant tumors. Nuclear expression of PAX8 was found significantly higher in benign cases compared to malignant tumors. HBME1, CD56 (loss of expression) was found significantly higher in Papillary Carcinoma, Papillary Carcinoma Folicular Variant compared to other malignant tumors.

Keywords: Thyroid, HBME1, CITED1, CD56, PAX8

ÖZET

Giriş ve amaç: HBME1, PAX8, CD56 ve CITED1' in tiroidin benign lezyonlarını, malign tümörlerden ve tiroidin farklı malign tümörlerini birbirinden ayırt etmedeki rolünü değerlendirmektir.

Yöntem ve gereçler: 2007-2013 yılları arasında tiroidektomi yapılan Nodüler Hiperplazi: 22, Foliküler Adenoma: 14, Papiller Benzeri Nükleer Özellikler Gösteren non-İnvaziv Foliküler Tiroid Neoplazmı: 3, İyi Diferansiye Karsinoma, Spesifiye Edilememiş: 3, Papiller Karsinoma: 22, Papiller Karsinoma, Foliküler Varyant: 12, Minimal İnvaziv Foliküler Karsinoma: 9, Az Diferansiye Karsinoma: 4, Anaplastik Karsinoma: 3 çalışmaya dahil edildi. İmmünhistokimyasal yöntem ile HBME1, CITED1, PAX8, CD56 uygulandı. İstatistiksel analizde markerların duyarlılık, özgüllük, pozitif ve negatif prediktif değerleri hesaplandı.

Bulgular: CD56 (ekspresyon kaybı), HBME1, Papiller Karsinoma ve Papiller Karsinoma, Foliküler Varyantta, diğer malign tümörlere göre anlamlı yüksekti. Malign tümörler için en duyarlı markerlar PAX8 ve CITED1 (%90, %88) idi. Malign tümörler için en spesifik markerlar HBME1, CITED1 (%97,

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%92)idi. Papiller Karsinoma, Papiller Karsinoma, Foliküler Varyant için en duyarlı ve spesifik marker HBME1 (%100, %80) idi. Papiller Karsinoma, Folliküler Varyant için en duyarlı markerlar CD56 (ekspresyon kaybı) ve CITED1 (%86, %86)idi.
Tartışma ve sonuç: HBME1, Papiller Karsinoma için duyarlı ve spesifik bulundu. CITED1'in sitoplazmik ekspresyonun Papiller Karsinomada, tiroidin diğer malign tümörlerine göre anlamlı şekilde yüksekti. PAX8'in nükleer ekspresyonu benign olgularda malign olgulara göre anlamlı şekilde yüksek bulundu. HBME1, CD56 (ekspresyon kaybı), Papiller Karsinoma ve Papiller Karsinoma, Foliküler Varyantta diğer malign tümörlere göre anlamlı şekilde yüksek bulundu.

Anahtar Kelimeler: Tiroid, HBME1, CİTED1, CD56, PAX8

Introduction

Palpable thyroid nodules were reported to occur in 5-10% of the population [1]. Most of the thyroid nodules are hyperplastic nodules and folicular adenoma and only 2-5% of them are malignant tumors [1].

The characteristic features for different thyroid neoplasm are specific nuclear pattern for papillary thyroid cancer, invasive pattern for folicular cancer, neuroendocrine features for medullary carcinoma, necrosis and nuclear pleomorphism for poor differentiated carcinoma, high mitosis and anaplastic features for indifferentiated carcinoma. Noninvasive folicular thyroid neoplasm with papillary-like nuclear features (NIFTP) is a new definition of which is suspicious of papillary carcinoma and has no capsule and vascular invasion. Although morphological features are of choice, immunohistochemistry as an ancillary method may be useful in diagnosis of folicular neoplasms.

The fact that the diagnosis will significantly affect the patient's treatment process and prognosis makes this differential diagnosis problem even more critical. Therefore, immunohistochemical markers were searched to contribute to the differential diagnosis of thyroid nodules.

Hector Battifora Mesothelial-1 (HBME1) is a monoclonal antibody for malignant epithelial mesothelioma cells. The expression of HBME1 was shown in papillary and folicular thyroid carcinoma. However expression of HBME1 in normal thyroid tissue was not shown [2]. Cbp/p300 Interacting Transactivator (CITED) proteins are known to regulate nuclear transcription factors. The expression of CITED1 was shown in breast epithelial cells and testicular germ cells. CITED1 can be used in differentiate from papillary thyroid carcinoma and benign thyroid lesions [3]. Paired box-8 (PAX8) is an important gene in thyroid embryogenesis. PAX8 expression has been demonstrated in mullerian, renal and upper urinary tract carcinoma and a sensitive marker for thyroid tumors [4, 5]. CD56 effects migratuar capacity of tumoral cells. Expression of CD56 was reported to be useful in differeanting folicular neoplasms or papillary thyroid carcinoma [4].

Our aim was to evaluate the performance of HBME1, PAX8, CD56, and CITED1 in differantiating benign cases and malignant thyroid tumors and evaluate subtypes of different malignant thyroid tumors.

Materials and Methods

Patient Selection and Design

After approval of instutitional review board (Ethics Comitee 511/2013) the patients that underwent thyroidectomy between 2007 ve 2013 were included to the study. As the study was retrospective no informed consent was obtained. The distrubution of the pathologic diagnosis were as follows; Nodular Hyperplasia (NH): 22, Folicular Adenoma (FA): 14, Noninvasive Folicular Thyroid Neoplasm with Papillary-Like Nuclear Features (NIFTP): 3, Well Diferentiated Carcinoma, Not Otherwise Specified (WDC-NOS): 3, Papillary Carcinoma (PC): 22, Papillary Carcinoma Folicular Variant (PCFV): 12, Minimally Invasive Folicular Carcinoma (MIFC): 9, Poorly Diferentiated Carcinoma (PDC): 4, Anaplastic Carcinoma

(AC): 3 were included to the study. The collected data were histopathologic evaluation, age, gender, thyroid tissue and tumor diameter, presence of multifocality, laterality, microscopic features including vascular invasion, metastasis, lymph node count.

In addition to microscopic features demographic data were also collected for each patient. All parafin blocks were stained with HBME1, CITED1, PAX8, CD56. Table 1. shows the origin of antibodies, dilution, and the company names.

Nicon Eclipse E 600 light microscope including x4, x20, x40, x100 objectives and x10 ocular was used for microscopic evaluation. Level of cytoplasmic and/or cytoplasmic membrane expression for HBME1 and CD56; Level of nuclear and/or cytoplasmic expression for PAX8 and CITED1 was scored between 0-3. Expression with HBME 1 or CD56 <25%, 25-50%, >50% were scored as 1-2-3 respectively. Expression with PAX8 and CITED I <25%, 25-75%, >75% were scored as 1-2-3 respectively.

Statistical Analysis

SPSS software Ver.22 (SPSS Inc. Chicago, USA) was used for statistical analysis. Mean and standart deviation and median (minimum-maximum) was used for continious variables. The normality of data were evaluated with Kolmogorov-Smirnov test and Shapiro wilk test where suitable. Comparison of continious data were performed using Mann-Whitney U test and Independent Sample t test. Kruskall Wallis test was used for comparison of more than 2 groups. Post hoc comparisons were performed using Bonferroni correction. Categoric data were compared using Pearson Chi-square test. Fisher's exact test was used when expected value problem occurred. P<0.05 was regarded as statistical significant.

Results

A total of 92 cases were encountered during the study period. The distrubution of the cases were as follows 22 NH (23%), 14 FA (15%), 3 NIFTP (3%), 3 WDC-NOS (3%), 22 PC (23%), 12 PCFV (13%), 9 MIFC (9%), 4 PDC (4%), 3 AC (3%) . The mean age was 46.7 ± 13.7 years. The mean age was 42.7 in the PCs, which were the majority of our patients, and in the WDC-NOS was the lowest (37.3), while it was significantly higher in the AC (52.7) (p = 0.02).

Seventy-five (78.9%) of the cases were female and 20 (21.1%) were male. When the patients with malignant and benign lesions were compared in terms of gender, there was no significant difference between these values as both benign and malignant cases were higher in women (p = 0.669).

Extrathyroidal spread was noted in all cases of PC with lymph node metastasis. Vascular invasion was observed in 21.4% of malignant cases. Similarly, IHC expression was observed in the primary focus of thyroid and lymph nodes with PC metastasis in 5 patients there was no statistically significant difference (p = 1.0).

Level of expression with HBME1 was significantly higher in malignant cases compared to benign group (61.5% vs 0% for <50% expression, p<0.001). Loss of CD56 expression was significantly higher in malignant group compared to benign cases (78.9% vs 35% for <10% expression p<0.001). CD56 expression in malignant cases was compared, MIFC showed significantly higher expression than other malignant cases (p = 0.00). There was no statistically significant difference between CD56 expression in FA and NH (p = 0.518). Cytoplasmic expression with PAX8 and CITED1 was similar between malignant and benign group. Nuclear expression with CITED1 was similar in benign and malignant cases. Nuclear expression with PAX8 was significantly higher in benign group compared to malignant group (55% vs 14%, p<0.05) (Table 2). When PAX8 cytoplasmic and nuclear expression prevalence of FA and NH were compared, no statistically significant difference was found between the groups (p = 0.213) (p = 0.565).

The cytoplasmic expression of CITED1 was significantly higher in PC compared to other malignant tumors (81.8%, p<0.05). The expression of HBME1 in PC

Table 1. Shows the origin of antibodies, dilution, and the company names.

ANTIBODY	Clon	Dilution	Control	Company
CITED-1	Mouse Monoclonal Antibody	7 ml package	Papillary thyroid carcinoma	Thermo Scientific
HBME-1	Mouse Monoclonal Antibody	7 ml package	Papillary thyroid carcinoma	Thermo Scientific
PAX8	Mouse Monoclonal Antibody	7 ml package	Tonsillary	Thermo Scientific
CD56	Mouse Monoclonal Antibody (MOC-1)	7 ml package	Neuroendocrine carcinoma	Thermo Scientific

HBME1: Hector Battifora Mesothelial-1, CITED1: Cbp/p300 Interacting Transactivator, PAX8: Paired box-8

Table 2. Expression of CITED1, HBME1, PAX8 and CD56 in Malignant, Benign Thyroid Tumors

	Cytoplasmic Staning			Nuclear Staining		
	Benign	Malignant	P	Benign	Malignant	P
CITED-1			0,973			0,889
<10	5 (13,9)	6 (11.5)		37 (92.5)	48 (92.3)	
10-50	9 (22.5)	13 (25)		3 (7.5)	4 (7,7)	
>50	26 (65)	33 (63,5)		0 (0)	1 (1,9)	
HBME-1			<0.001	NA	NA	
<10	39 (97.5)	17 (32.6)				
10-50	1 (2.5)	3 (5.8)				
>50	0 (0)	32 (61.5)				
PAX-8			0,798			0,008
<10	3 (7.5)	5 (9,6)		12(30)	28 (53.9)	
10-50	5 (12.5)	10 (19.2)		6 (15)	10 (19.2)	
>50	32 (80)	37 (71,2)		22 (55)	14 (26.9)	
CD56			<0.001	NA	NA	
<10	14(35)	41(78.9)				
10-50	7 (17.5)	6 (11.5)				
>50	19 (47.5)	5 (9.6)				

HBME1: Hector Battifora Mesothelial-1, CITED1: Cbp/p300 Interacting Transactivator, PAX8: Paired box-8

Table 3. HBME1, CD56, CITED1 (Nuclear and Cytoplasmic Expression) and PAX8 (Nuclear and Cytoplasmic Expression) in all cases

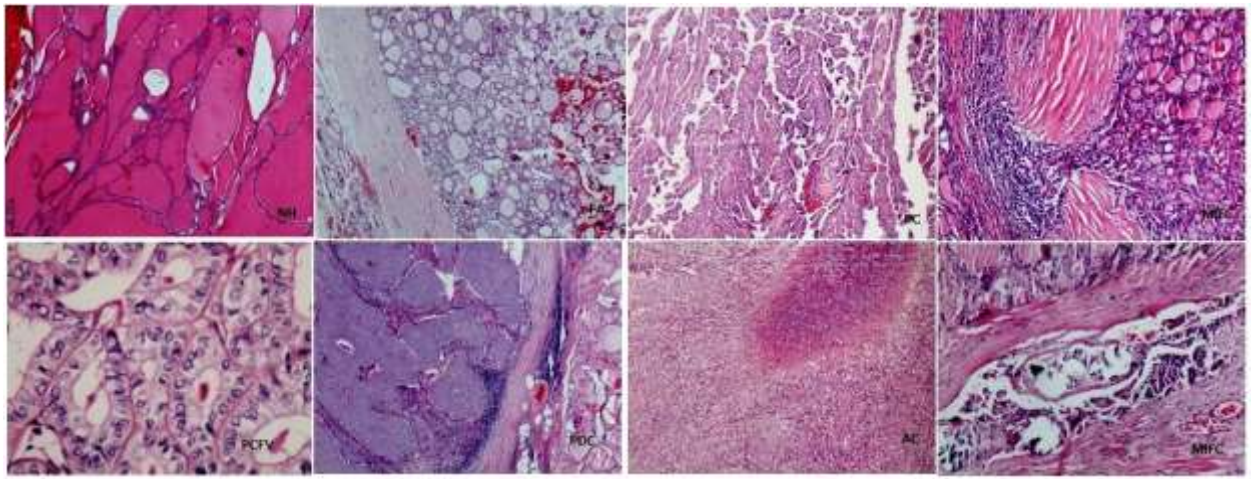
	NH (n%)	FA (n%)	NIFTP (n%)	p	PC (n%)	PCFV (n%)	MIFC (n%)	WDCNOS(n%)	PDC (n%)	AC (n%)	p
CITED1,c				0.201							**0,003**
<10	4(18.2)	1 (7.1)	0 (0)		1(4.5)	2(16,6)	2(22,2)	1 (33,3)	1 (25)	1 (33,3)	
10-50	4(18.2)	5 (35.7)	0 (0)		3(13,6)	4 (33,4)	2(22,2)	1 (33,3)	2 (50)	1 (33,3)	
>50	14(63.6	8 (57.1)	3 (100)		18(81,8	6 (50)	5(55,6)	1 (33,3)	1 (25)	1 (33,3	
CITED1n				0.161							0.188
<10	20(90.9	14 (100)	2 (66.7)		19(85.6	11(90,9)	8(88,9)	3 (100)	4(100	3 (100)	
10-50	2(9.119	0 (0)	1 (33.3)		2 (9,1)	1 (11,1)	1(11,1)	0 (0)	0 (0)	0 (0)	
>50	0 (0)	0 (0)	0 (0)		1 (4,5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
HBME1				NA							**<0.001**
<10	22(100)	14(100)	3(100)		0(0)	0 (0)	8(88,9)	2(66,7)	4(100	3 (100)	
10-50					0(0)	1 (9.1)	1(11,1)	1(33,3)	0 (0)	0 (0)	
>50					22(100)	11 (90.9)	0(0)	0(0)	0 (0)	0 (0)	
PAX8,n				0.146							0,485
<10	7 (31.8)	5 (35.7)	0 (0)		14(63.6	7 (58,4)	8(88,9)	3 (100)	4(100	3 (100)	
10-50	4 (18.2)	2 (14.3)	0 (0)		7 (31,8)	2 (16,6)	0 (0)	0 (0)	0 (0)	0 (0)	
>50	11 (50)	7 (50)	3 (100)		1 (4,5)	3 (25)	1(11,1)	0 (0)	0 (0)	0 (0)	
PAX8,n				0.574							**0,007**
<10	1 (4.5)	2 (14.3)	0 (0)		1 (4,5)	2 (16,7)	0 (0)	1 (33,3)	1 (25)	0 (0)	
10-50	3 (13.6)	2 (14.3)	0 (0)		3 (13,6)	2 (16,7)	2(22,2)	2 (66,7)	1 (25)	1 (33,3)	
>50	18(81.8	10 (71.4)	3 (100)		18(81,8	8 (66,6)	7(77,8)	0 (0)	2 (50)	2 (66,7)	
CD56				0.060							**<0.001**
<10	8 (36.4)	2 (14.3)	3 (100)		21(95.5	12 (100)	1(11,1)	3 (100)	3 (75)	2 (66,7)	
10-50	4 (18.2)	3 (21.4)	0 (0)		1 (4,5)	0 (0)	3(33,3)	0 (0)	1 (25)	1 (33,3)	
>50	10(45.5	9 (64.3)	0 (0)		0 (0)	0 (0)	5(55,6)	0 (0)	0 (0)	0 (0)	

PC: Papillary Carcinoma, PCFV: Papillary Carcinoma Folicular Variant, MIFC: Minimal Invasive Folicular Carcinoma, WDC-NOS: Well Differentiated Carcinoma, not other spesifite, PDC: Poorly Differentiated Carcinoma, AC: Anaplastic Carcinoma, HBME1 Hector Battifora Mesothelial-1, CITED1: Cbp/p300 Interacting Transactivator, PAX8: Paired box-8, c: cytoplasmic, n: nuclear

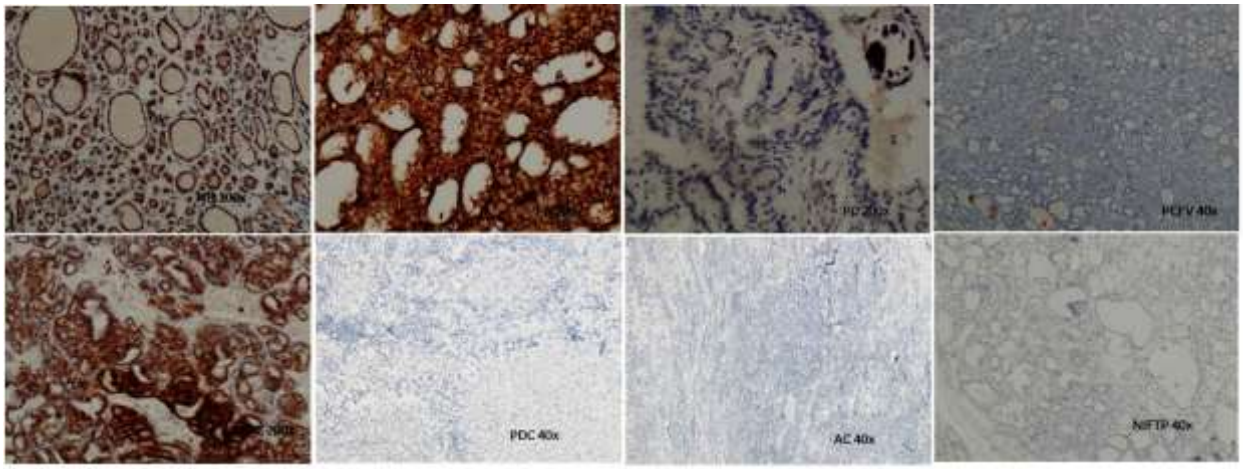
and PCFV was significantly higher compared to other malignant tumors (100% and 90.9). Loss of CD56 expression was significantly higher in PC, WDC-NOS and PCFV compared to other malignant tumors ($p<0.001$). Cytoplasmic expression with PAX8 was higher in PC, PCFV, MIFC and AC compared to WDC-NOS and PDC (Table 3). WDC-NOS, PDC and AC showed nuclear expression <10% with CITED1 and PAX8. The nuclear expression of CITED1 was not different between malignant cases. Only one case (4.5%) showed nuclear expression >50% with CITED1 in PC. Nuclear expression of PAX8 was not different between malignant cases (Table 3). All cases H&E, HBME1, CITED1, PAX8, CD56 expression shows in picture 1, 2, 3, 4, 5.

The sensitivity of CITED1 (cytoplasmic expression), PAX8 (cytoplasmic expression), HBME1 and loss of CD56 was 88%, 90%, 67%, and 78% respectively in discriminating malignant and benign cases. The specificity of CITED1 (cytoplasmic expression), PAX8 (cytoplasmic expression) , HBME1 and loss of CD56 was 12.5%, 7%, 97.5% and 65% respectively (Table 4.) The sensitivity of nuclear expression of CITED1 and PAX8 was 12% and 7% respectively for discriminating benign and malignant thyroid neoplasms. The specificity of nuclear expression CITED1 and PAX8 was 92% and 46% respectively. Positive predictive value of CITED1(cytoplasmic expression), PAX8 (cytoplasmic expression), HBME1 and loss of CD56 were 56%, 56%, 97.2%, 74% respectively. Diagnostic accuracy of HBME1 and loss of CD56 was significantly higher for PC and PCFV (area under curve>0.5) Figure shows sensitivity and specificity of the different markers in diagnosis of malignant thyroid tumors.

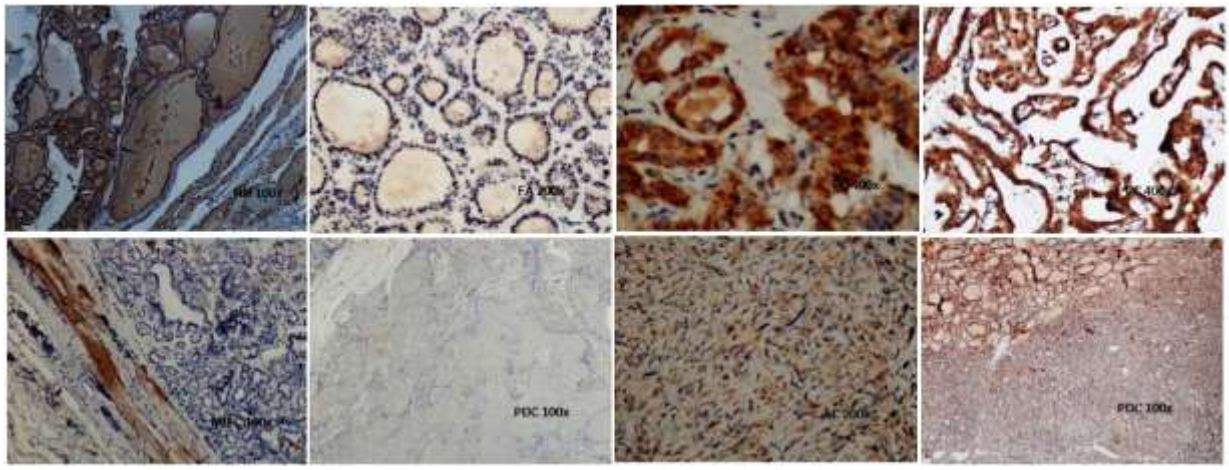
The performance of the various markers in the sensitivity of combination of the markers (HBME1, CITED1, PAX8, loss of CD56) in



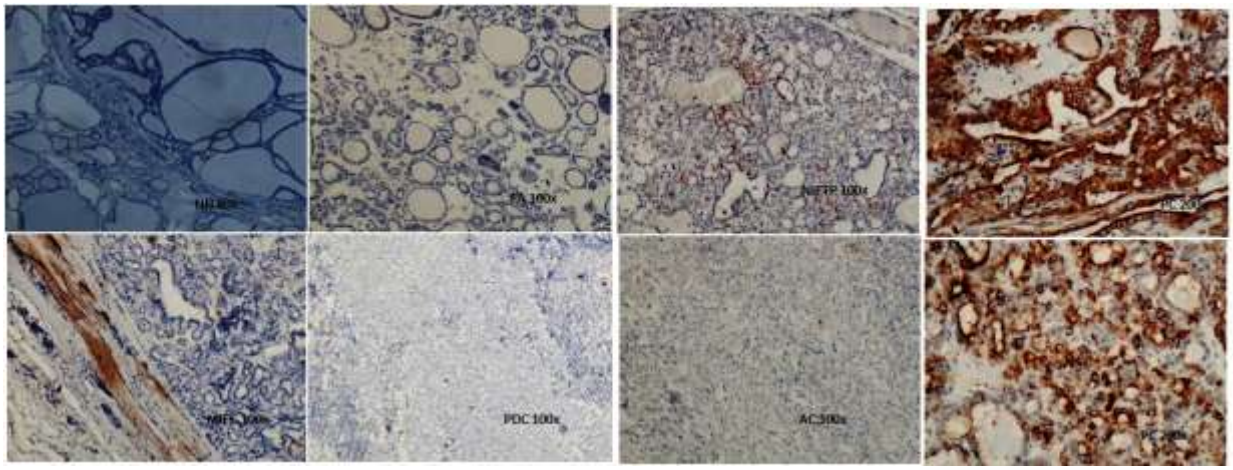
Picture 1: Hematoxylin eosin (H&E) sections of PC (Papillary Carcinoma), PCFV (Papillary Carcinoma, Folicular Variant), MIFC (Minimal Invasive Folicular Carcinoma), PDC (Poorly Differentiated Carcinoma), AC (Anaplastic Carcinoma), FA (Folicular Adenoma), NH (Nodular Hyperplasia)



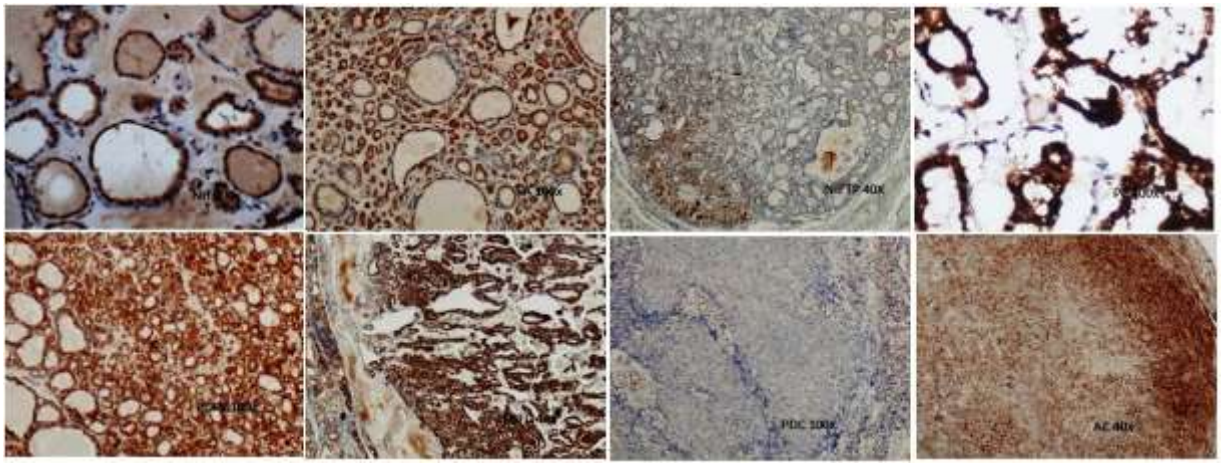
Picture 2: CD56 expression in Noduler Hyperplasia and Folicular Adenoma, MIFC (Minimal Invasive Folicular Carcinoma) loss of expression in the other malignant thyroid tumors



Picture 3: Widespread expression of CITED1 in Papillary Carcinoma and CITED1 expression in other neoplasm and Noduler Hyperplasia



Picture 4: Widespread expression of HBME1 in Papillary Carcinoma, Papillary Carcinoma Folicular varyant and lack of expression in other neoplasm and Noduler Hyperplasia



Picture 5: PAX8 expression in malignant and benign neoplasm and also Nodular Hyperplasia

Table 5. Sensitivity, specificity, positive predictive values, negative predictive values of ımmun markers combination for differantiating benign cases from malignant tumors and sensitivity, specificity, positive predictive values, negative predictive values of ımmun markers combination for differantiating papillary carcinoma from the other malignant thyroid tumors

	Differentiating papillary carcinoma from the other malignant thyroid tumors						Differentiating benign cases from malignant tumors					
	HBME1 CD56(-)	HBME1 CITED1	PAX8 CD56(-)	CITED1 CD56(-)	PAX8 CITED1	HBME1 PAX8	HBME1 CD56(-)	HBME1 CITED1	PAX8 CD56(-)	CITED1 CD56(-)	PAX8 CITED1	HBME1 PAX8
SENSITIVITY (%)	100	94,6	100	100	97,3	100	83,9	69,6	100	100	94,6	98,2
SPECIFICITY (%)	60,3	86,2	5,2	3,4	6,9	5,2	72,2	91,7	8,3	5,6	8,3	8,3
PPV (%)	61,7	81,4	40,2	39,8	40	40,2	82,5	92,9	62,9	62,2	61,6	62,5
NPV (%)	100	96,2	100	100	80	100	74,3	66	100	100	50	75

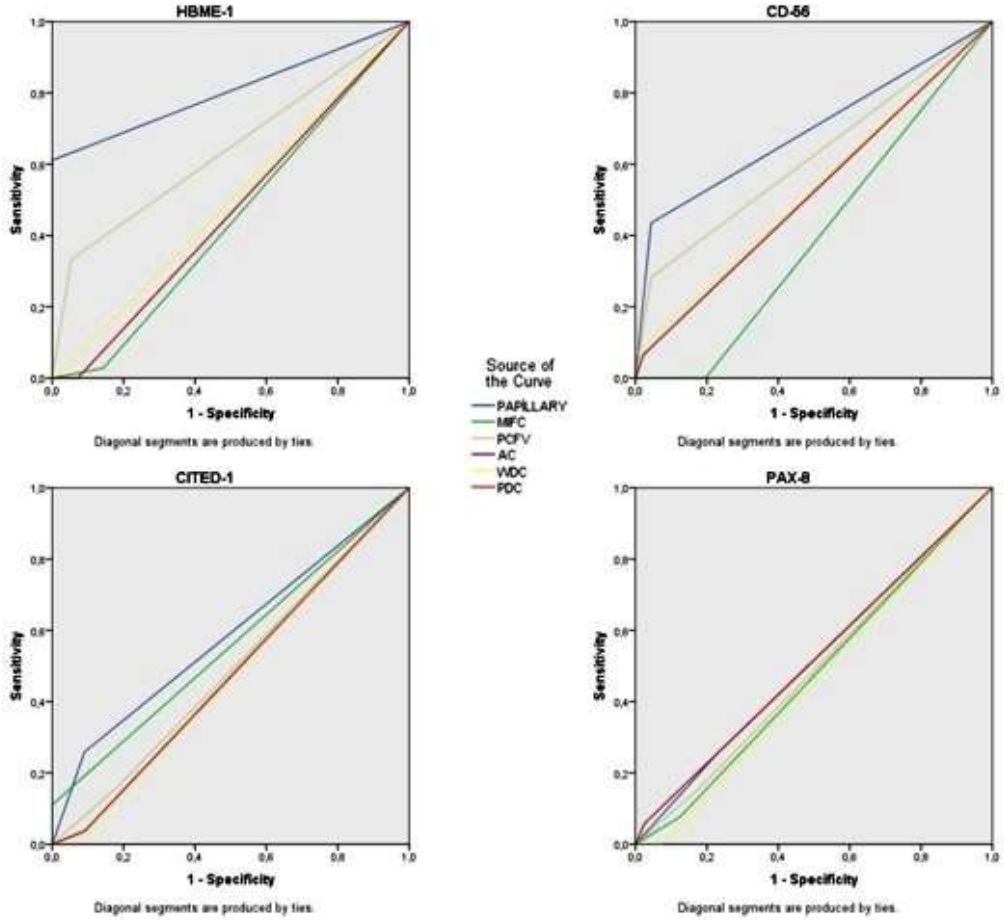


Figure 1. Shows sensitivity and specificity of the different immun markers in diagnosis of malignant thyroid tumors

discriminating benign and malignant cases was 100% however the specificity was low (8.3%). The specificity of PAX8 was extremely low that the combination of markers with PAX8 decreased the specificity (Table 5).

The combination of HBME1 and loss of CD56 showed 100% sensitivity and 60.3% specificity for PC. The combination of HBME1 and CITED1 the sensitivity and specificity were 94,6% and 86,2% respectively for PC (Table 5).

Discussion

Discrimination of benign and malignant thyroid tumors based on only morphologic evaluation may be insufficient. Additional immunhistochemical markers may be needed in cases of similar morphological findings. This study evaluated use of HBME1, PAX8, CD56 and CITED1 in discrimination of benign lesions and malignant thyroid tumors. The most sensitive markers were PAX8 (cytoplasmic expression), and CITED1 (cytoplasmic expression) in discriminating benign lesions and malignant thyroid tumors. However the specificity was lower relatively. The most specific markers were HBME1 expression in discriminating benign lesions and malignant thyroid tumors.

HBME1 was reported to be useful in discrimination of malignant thyroid tumors [6]. The combination of HBME1, GAL-3, CK19 discrimination of FA and PCFV [6]. The sensitivity and specificity of HBME1 in detecting PC was found to be 87% and 96% respectively [7]. In the current study expression with HBME1 in PC and PCFV

patients was significantly higher compared to other malignant thyroid tumors.

HBME1 was also found to be the most sensitive marker for PC [8]. On the other hand the specificity was reported to be low in diagnosis of PC by the study conducted by Cheung et. al [9]. Similarly our findings supported the literature that HBME1 specificity was significantly higher in PC.

The expression of HBME1 in MIFC and AC was relatively low compared to other histologic subtypes. Similarly Atik et. al found that none of the MIFC showed expression with HBME1 [10]. In the current study although sensitivity was low HBME1 positivity was highly specific marker for malignancy. As our study included all types of thyroid malignancies like MIFC and AC the low level of sensitivity may be attributable to low expression of these rare types of malignant thyroid tumors.

Generally cytoplasmic and nuclear expression of CITED1 was regarded as positive in literature [3, 11]. However some of the studies regarded only cytoplasmic positivity for CITED1 [12]. In the current study we evaluated cytoplasmic and nuclear CITED1 expression.

CITED1 was reported to show increased expression in PC [13]. CITED1 expression was observed in 87-100% PC [14]. Since expression of CITED1 was not shown in normal thyroid tissue the marker was proposed to be a useful in discriminitation of benign and malignant thyroid tumors [7]. In the current study cytoplasmic expression of CITED1 was found to be highly specific PC compared to other malignant thyroid tumors.

The expression of CITED1 was not shown in AC [12]. In the current study the expression of CITED1 in AC was relatively high.

PAX8-PPARγ gene fusion was shown in FC [15]. PAX8-PPARγ gene fusion was reported to be specific for FC compared to FA, PC and NH [15]. PAX8-PPARγ gene fusion was shown to be responsible in the patogenesis of FC [16]. In addition up to 70% of FC cases was shown to express PAX8 positivity [17]. Lacroix et al. reported that translocation of PAX8-PPARγ could effect occurence of FC however was not specific for carcinoma and immunhistochemistry was not a reliable marker for detection of translocation of PAX8-PPARγ [18]. We observed high level of expression with PAX8 in FC and the other malignant thyroid tumors. They were also showed similar expression with PAX8 except for WDC-NOS. As a result PAX8 was not a specific marker for FC.

Although nuclear expression of PAX8 is accepted, in some markers for example Ki67 is normally a nuclear staning marker, while cytoplasmic staining in hyalinized trabecular tumor of thyroid [19]. For this reason, we wanted to share our experience on this subject, thinking that it might be meaninful in terms of directing future studies.

Loss of CD56 expression was reported to be a good marker for discriminating benign and malignant cases and decreased expression was shown for PC in the literature [4]. The sensitivity and specificity was reported to be as high as 100% by some authors [20]. Loss of CD56 expression was above 95% in PC, PCFV and MIFC in our study. Ozolins et al. reported the loss of expression CD56 in PC compared to normal thyroid tissue [21].

Since none of the marker has 100% sensitivity and specificity. The combination of the markers may increase sensitivity and specificity. The combination of the markers were shown to be useful by several authors. [22-24].

Nechifor-Boila et al, CD56, CK19, Galektin3 and HBME1 markers which were used together in PC, PCFV, Thyroid Tumors of Uncertain Malignant Potential, found that sensitivity was increased [23]. The combination of HBME1, Galektin3, CK19 and HBME1, CITED1, Galektin3 was shown to be the most efficient combination for discrimination of FC and PCFV [24].

Abd El Atti et al. evaluated the expression of CD56 and Claudin1 in benign, malignant neoplasms and nonneoplastic thyroid tissue of the thyroid. 5 out of 10 Thyroid Tumors Of Uncertain Malignant Potential were diagnosed with PC when combined with loss of CD56 and Claudin1. As

a result, when immunohistochemical markers such as loss of CD56 and Claudin1 were evaluated together, it was concluded that they could be used to separate PCFV from nodules in the folicular patern [22].

In our study, when HBME1, loss of CD56 expression were used together, to discriminating benign and malignant cases the sensitivity was 83.9%, specificity 72.2% and specificity was higher that when HBME1 was used alone. When HBME1, loss of CD56 expression were used together, to discriminating PC and the other malignant tumors the sensitivity was 100%, specificity 60.3% and specificity (91.9) was higher that when HBME1 was used alone but the sensitivity (60.3%) was lower that when HBME1 was used alone. HBME1 and CITED1 expression were used together the sensitivity was 94.6% and the specificity was 86.2%. Although it was observed that sensitivity increased up to 100% in other combinations of markers but specificity was very low. While our results were compatible with some of the results stated in the literature, some of them were not.

In addition that the use of immunohistochemical markers in confirming the diagnosis of tumor in preoperative cytological materials is increasingly common [25]. Indicating that these markers can also be useful in cytological diagnosis.

Conclusions: Cytoplasmic expression of CITED1 was found to be highly specific PC compared to other malignant thyroid tumors. Nuclear expression of PAX8 was significantly higher in benign cases compared to malignant tumors. HBME1 was the most specific marker in discriminating malignant and benign cases. HBME1 and loss of CD56 was an both sensititive and specific markers for PC. The best combination to distinguish PC from the other malignant thyroid tumors was HBME1 and loss of CD56, HBME1 and CITED1 (cytoplasmic expression).

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Original Article

Consolidation with Autologous Stem Cell Transplantation in Patients with Primary Central Nervous System Lymphoma

Primer Santral Sinir Sistem Lenfomalarında Otolog Kök Hücre Nakli ile Konsolidasyon Sonuçları

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ABSTRACT

Introduction: Primary central nervous system lymphomas (PCNSL) are defined as a rare extranodal Non-Hodgkin lymphoma subgroup. The induction regimens involve high dose methotrexate-based chemotherapies mostly for the patients with PCNSL. There is still no standard approach for consolidation therapy. Recently, consolidation with autologous stem cell transplantation (ASCT) after high-dose chemotherapy has been widely used in the treatment of PCNSL. We aim to evaluate the results of PCNS patients who underwent ASCT in our center.

Methods: The data of PCNSL patients diagnosed in Hematology Unit of Dr. Abdurrahman Yurtaslan Ankara Oncology Hospital between 2010 and 2021 were analyzed retrospectively.

Results: Eleven patients were diagnosed with PCNSL diagnosis. The patients' median age included in the study was 53.5 years (range 38-68). Eight patients underwent ASCT for upfront consolidation. Seven patients achieved CR three months after ASCT; one patient was not evaluated due to exitus in the first month of the transplant. Three patients could not achieve ASCT due to transplantation ineligibility patients and mobilization failure. The median follow-up period in the study was 26 months (range 8-82 months). The median overall survival was not reached. Transplant-related mortality was 12.5%, and the mortality rate was 27% in the whole cohort. In patients who received ASCT, of the 62.5% had an almost two-year survival advantage. For the whole cohort, 73% of the patients had change for more prolonged survival among follow-up.

Discussion and conclusion: In our cohort, the PCNSL patients had mostly high-risk disease; however, three-quarters of the patients could receive ASCT, and at the same rate of the patients had advantages for long-term survival.

Keywords: Primary central nervous system lymphoma, consolidation, autologous stem cell transplantation

ÖZET

Giriş ve Amaç: Primer santral sinir sistemi lenfomaları (PSSSL) nadir görülen bir ekstranodal Non-Hodgkin lenfoma alt grubu olarak tanımlanmaktadır. İndüksiyon tedavileri, çoğunlukla PSSSL'li hastalar için yüksek doz metotreksat bazlı kemoterapileri içerir. Konsolidasyon tedavisi için hala standart bir yaklaşım yoktur. Son zamanlarda, yüksek doz kemoterapi sonrası otolog kök hücre transplantasyonu (OKHN) ile konsolidasyon tedavide yaygın olarak kullanılmaktadır. Bu çalışmada, merkezimizde OKHN uygulanan PSSSL hastalarının sonuçlarını değerlendirmeyi hedefliyoruz.

Yöntem ve Gereçler: Dr. Abdurrahman Yurtaslan Ankara Onkoloji Hastanesi Hematoloji Ünitesi'nde 2010-2021 yılları arasında tanı konulan PSSSL hastalarının verileri geriye dönük olarak incelendi.

Bulgular: On bir hastaya PSSSL tanısı konuldu. Çalışmaya dahil edilen hastaların medyan yaşı 53.5 yıl (38-68) idi. Sekiz hastaya indüksiyon tedavisi sonrası upfront konsolidasyon amacıyla OKHN uygulandı. Yedi hasta OKHN'den üç ay sonra tam yanıta ulaştı; bir hasta naklin ilk ayında çıkış nedeniyle değerlendirilmedi. Üç hastaya, transplantasyona uygun olmamaları ve mobilizasyon

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başarısızlığı nedeniyle OKHN yapılamadı. Çalışmadaki ortanca takip süresi 26 aydı (8-82 ay). Median genel sağkalıma ulaşılmadı. Nakille ilişkili mortalite %12.5 ve tüm kohortta mortalite oranı % 27 idi. OKHN olmuş hastaların %62.5'unda iki yıla yakın toplam sağkalım avantajı saptandı. Tüm kohort için hastaların %73'ü takipte daha uzun süreli sağkalım şansı sağlamıştır.

Tartışma ve sonuç: Bizim kohortumuzda, PSSSL hastaları çoğunlukla yüksek riskli hastalığa sahip olmasına rağmen hastaların dörtte üçü OKHN olabildi ve aynı oranda hasta uzun süreli sağkalım avantajı sağladı.

Anahtar Kelimeler: Primer santral sinir sistemi lenfoması, konsolidasyon, otolog kök hücre nakli

Introduction

Primary central nervous system lymphomas (PCNSL) are defined as a rare extranodal Non-Hodgkin lymphoma (NHL) subgroup that is typically localized to the brain, eye, spinal cord, and cerebrospinal fluid (CSF) without a primary tumor in the body [1-3]. Primary central nervous system lymphomas constitute 4% of all brain tumors and 4-6% of extranodal lymphomas [2]. The annual overall incidence rate of PCNSL is up to 0.5 cases per 100,000 [4]. Among immunocompetent individuals, the median age at diagnosis is 60 years[2]. Approximately 90-95% of PCNSL is Diffuse Large B Cell Lymphoma (DLBCL) [2]. The initial presentation usually has an intracranial mass and associated headache, confusion, weakness, and neurological deficits. Tumor infiltration can be observed in the following percentages: brain hemispheres 38%, thalamus 16%, basal ganglion 14%, corpus callosum 14%, periventricular area 12%, cerebellum 12.5%, meninges 20%, cranial nerves 16%, and spinal nerves 1% [5]. Meningeal involvement can be detected by cytological examination of CSF in 16% of the cases with PCNSL. Isolated leptomeningeal involvement is observed in less than 5% of PCNSL cases [4]. Spinal cord lymphoma is the rarest form of PCNSL and has an inferior prognosis [4,6,7]. In patients with PCNSL, various prognostic scoring systems are used to predict prognosis. The International Extranodal Lymphoma Study Group (IELSG) prognostic scoring is the most widely used one. It is based on age, Eastern Cooperative Oncology Group (ECOG) performance status (PS), lactate dehydrogenase (LDH) level, CSF protein concentration, and involvement of the deep brain structures [8]. Scores 0-1 represents low risk, 2-3 represents intermediate risk, whereas score 4-5 represents high risk [9]. According to the IELSG scoring system, the PCNSL patients with at least two negative factors (IELSG score ≥2) showed poor survival [9]. The survival is similar to those who did not achieve complete response (CR) after two induction chemotherapy [9,10]. Therefore, a more effective treatment strategy is required, especially for PCNSL patients with an IELSG score of ≥2, or whom can not be achieved CR1 after two induction chemotherapy [8-10]. The standard treatment approach of PCNSL consists of induction and consolidation. The PCNSL is sensitive to both chemotherapy and radiotherapy. High-dose methotrexate (HDMTX)-based chemotherapy can cross the blood-brain barrier, an essential part of the treatment. Nevertheless, whole-brain radiotherapy (WBRT) as the sole therapy has been associated with poor survival and an increased risk of treatment-related neurotoxicity [10,11]. For most patients with PCNSL, the induction involves HDMTX-based chemotherapies mostly. However, there is still no standard approach for consolidation therapy. In recent years, regardless of the patients' initial prognostic scores, consolidation with autologous stem cell transplantation (ASCT) after high-dose chemotherapy has been widely used in the treatment of PCNSL [12-14]. The efficacy of upfront ASCT in PCNSL is not clearly defined due to the limited number of studies and a limited number of patients enrolled in these studies [15]. For this reason, we aim to evaluate the

results of PNSCL patients who underwent ASCT in our center.

Methods

The data of PCNSL patients diagnosed in Hematology Unit of Dr. Abdurrahman Yurtaslan Ankara Oncology Hospital between 2010 and 2021 were analyzed retrospectively. Primary central nervous system lymphoma was defined as histologically confirmed NHL restricted to the CNS, including the brain parenchyma, spinal cord, eyes, cranial nerves, or menings [16]. Diagnosis of PCNSL was histologically confirmed by stereotactic brain biopsy, surgical resection, or CSF cytology in all patients. All patients underwent pre-evaluation with contrast-enhanced brain magnetic resonance imaging (MRI), positron emission tomography to exclude systemic NHL, unilateral bone marrow aspiration and biopsy, and lumbar puncture for CSF analysis unless contraindicated. High CSF protein concentration was defined as more than 0.45 g/l in patients younger than 60 years old and more than 0.6 g /l in patients at the age of 60 and older[8]. Involvement of deep brain structures was defined as involvement of periventricular regions, basal ganglia, brain stem, and cerebellum. At the time of diagnosis, IELSG scoring (age> 60 years, ECOG PS ≥2, high LDH, high CSF protein concentration, and deep brain structures involvement) was performed in all patients [8,10]. Response to induction chemotherapy was evaluated by comparing brain MRI performed before and after the second induction course and after the induction regimen. Response to treatment was assessed according to the criteria of the International PCNSL Collaborative Group [17]. Patients with a chemosensitive response to induction therapy underwent upfront ASCT consolidation. Patients who had chemo-refractory response received a salvage regimen. Among ASCT, the engraftment definition for neutrophil was defined as the first day when the absolute neutrophil count (ANC) was >500/mm^3 or 1000/mm^3 for three consecutive days, and thrombocyte engraftment was defined as the first day when thrombocyte count was >20000/mm^3 for three consecutive days without transfusion. All patients received weight adapted G-CSF before the neutrophil engraftment. Overall survival was calculated from the date of histological diagnosis to death or the last date of follow-up. Progression-free survival was calculated from the date of histological diagnosis to disease progress, death, or date of the latest follow-up for progression-free patients, whichever occurred first. Transplant-related mortality (TRM) was defined as death within the first 100 days after ASCT [18].

The local human research ethics committee approved this study. All procedures performed in studies involving human participants were under the national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was carried out with the permission of the Ethics Committee of Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital (Permission granted /Decision number: 2020-12/910).

All statistical analyses were conducted using SPSS V21.0 (SPSS Inc., Chicago, IL) software. Descriptive statistics were applied to summarize the data. The categorical data were reported as rates, and numeric data were reported as medians and average ± standard deviations. The Kruskal Wallis Test was used to analyze engraftment times between the groups. Kaplan Meier test was used to analyze PFS and OS

Results

Eleven patients were diagnosed with PCNSL diagnosis at our center. The patients' median age included in the study was 53.5 years (range 38-68 years). The male to female ratio was 4.5. All patients' disease stage was

Table 1: Patients' characteristics

Patients	N (number)
Age (median)	53.5 (38-68)
Gender (Female /Male)	2/9
DLBCL Histological Subtype	
Germinal center B cell	4
Activated B cell	5
Other	1
Unknown	1
Induction regimen	
R-HyperCVAD	4
MATRIX	4
MTX-Ara-C	2
R-CHOP	1
ASCT	
Received	8
Not Received	3
ASCT conditioning	
TECA	5
Thiotepa- carmustine	3
Radiotherapy	
Received	6
Not received	5
The quantity of infused $CD34^+$ Stem Cells (median)	10.06 $x10^6$ /kg (4.1-16)

DLBCL: Diffuse Large B cell Lymphoma; R-HyperCVAD course A: cyclophosphamide, vincristine, doxorubicin, dexamethasone, and rituximab; Course B, methotrexate, cytarabine, and rituximab; MATRIX: High dose methotrexate, high dose cytarabine, thiotepa, and rituximab; MTX-Ara-C: methotrexate, cytarabine; R-CHOP rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; ASCT: autologous stem cell transplantation; TECA: thiotepa, etoposide, and carboplatine

localized to the central nervous system; two had bulky disease. All of the patients had Diffuse Large B cell Lymphoma (DLBCL) histological subtype of NHL. Four patients had germinal center B cell-like, five patients had activated B cell-like, one patient had T-cell and histiocyte-rich type DLBCL, and one patient's immunohistochemical subtype was not determined. The patients' clinical features are shown in Table 1.

The IELSG score was low risk in three patient; the score was ≥ 2 in eight patients that means intermediate and high risk. One patient with an IELSG score of 1 died due to sepsis in the first month of the transplant. As the induction regimen, R-HyperCVAD (the drugs used in course A: cyclophosphamide, vincristine, doxorubicin, dexamethasone, and rituximab; Course B consists of methotrexate, cytarabine, and rituximab) was given to four patients, methotrexate cytarabine to two patients, MATRIX (HDMTX, high dose cytarabine, thiotepa, and rituximab) to four patient, and R-CHOP (rituximab, cyclo-phosphamide, doxorubicin, vincristine, and prednisone) to one patient.

Seven patients achieved CR1 after induction therapy. One patient received methotrexate cytarabine, and one patient who received R-CHOP had stable disease after first-line induction, so they had hyperCVAD for salvage and achieved CR2. Of the two patients who had MATRIX for induction had refractory disease, they had RT and temozolomide for salvage.

Six patients received radiotherapy among follow-up, of the three patients received radiotherapy after upfront ASCT. Six patients received a median of four intrathecal chemo-therapy during the induction regimen.

Eight patients underwent upfront ASCT. The TECA (thiotepa, etoposide, and carboplatine) regimen was used as the conditioning regimen in five patients, and the thiotepa-carmustine regimen was used in three patients. Median neutrophil engraftment duration was 12 (range 8-16) days, and median platelet engraftment was 12 (range 9-14) days.

Seven patients achieved CR three months after ASCT; one patient was not evaluated due to exitus in the first month of the transplant. Three patients could not achieve ASCT due to transplantation ineligibility (n:2) patients and mobilization failure (n:1). Who did not received ASCT, one of them had temozolomide for consolidation, two of them had temozolomide for salvage.

The median follow-up period in the study was 26 months (range 8 month-82 months). The median follow-up time after ASCT was 20 months (range 1-63 months).

The median overall survival was not reached. Transplant-related mortality was 12.5%, and the mortality rate was 27% in the whole cohort. Three patients died in the first year of the ASCT due to infection. In patients who received ASCT, the median survival time from diagnosis was 29 months (range 8-82 months), and 62.5% of patients had an almost two-year survival advantage. For the whole cohort, 73% of the patients had change for more prolonged survival among follow-up.

Discussion

Primary central nervous system lymphomas are rare and aggressive malignancies. Besides low incidence, the data are limited about standard induction and consolidation treatment. We aim to evaluate the outcome of the PCNL patients' who received ASCT. In our cohort, IELSG score was ≥2 of 72% of patients and had mostly high-risk disease. However, 73% of the patients could receive ASCT, and at the same rate of the patients had long-time survival.

The two-year OS is 80% in patients with an IELSG score of 0-1 points, 48% in those with 2-3 points, and 15% in those with 4-5 points [8]. As the expected 2-year OS is less than 50% in patients with an IELSG score of 2 at the time of diagnosis, intensive treatments are required, especially for this high-risk group of patients. Furthermore, failure to achieve CR after HDMTX-based induction chemotherapy is associated with poor survival, requiring a more effective treatment strategy for these patients [10,19]. Consolidation with WBRT or ASCT should be considered, especially in high-risk patients, due to the low response rates of standard HDMTX-based induction chemotherapy alone [8,20]. In a recent randomized phase III study, no survival advantage was detected when WBRT was used as consolidation treatment [19]. Additionally, WBRT has been associated with neurotoxicity and a high relapse rate [21]. WBRT should be considered in refractory patients or patients who cannot tolerate high-dose chemotherapy. In our cohort, nearly half of the patients had WBRT for palliation or salvage regimen. We preferred ASCT initially for consolidation instead of WBRT to avoid neurotoxicity.

Since ASCT was first used in relapsed PCNSL in 1996, most centers have been used as consolidation treatment in patients with PCNSL [22]. In a multicenter phase II study, 23 PCNSL patients younger than 65 years received HDMTX based induction followed by ASCT with carmustine-thiotepa conditioning regimen. Twenty-one patients received WBRT (45 Gy, two doses of 1 Gy/d) for consolidation after ASCT. With a median follow-up of 63 months, 5-year estimated OS was 87%, and the 5-year probability of relapse-related death was 8.7% [23]. In another study, 13 patients with PCNSL received HDMTX based induction followed by ASCT with carmustine-thiotepa conditioning regimen. Radiotherapy was restricted to patients who did not achieve CR. With a median follow-up of 25 months, 3-year disease-free survival (DFS), and OS was 77% [24]. In a review, 2-year PFS was 69% (range 54% -81%) and 2-year OS was 84% (range 83%-91%) and TRM was 3% for PCNSL patients undergoing upfront ASCT following regimens containing thiotepa and / or WBRT. In the same review, 2-year PFS was 44% (range 25% -62%), 2-year OS was 65% (range 60% -70%), and TRM was 4% in patients who underwent upfront ASCT following thiotepa-free regimens. Thiotepa containing regimens were found to be associated with better PFS and OS when compared to thiotepa-free regimens. Addition of WBRT has not been shown to affect OS [15]. In the study conducted by Cho et al., 2-year OS was 93.3%

in patients who underwent upfront ASCT and 72.9% in patients who underwent ASCT after salvage therapy, but this difference did not achieve statistical significance. However, PFS was significantly higher in patients consolidated with upfront ASCT than those who underwent ASCT after salvage therapy (91.7% and 25.0%, respectively; P = 0.001) [25]. In our center, if the patient were eligible for ASCT, patients were performed upfront ASCT instead of waiting for relapse. We observed that 62.5% of the PCNSL patients who underwent upfront ASCT could survive among follow-up. The median durations for neutrophil and platelet engraftment were ten days (9–13 days) and 14 days (11–24 days) in the same study during ASCT [22]. We administered thiotepa based conditioning mostly, and the median neutrophil and platelet engraftment duration was 12 days in our study.

We administered HDMTX based induction regimens, and we preferred ASCT consolidation for transplantation eligible patients mostly. However, IELSG score mostly intermediate and high risk in our cohort, 73% of the patients had change for long time survival among follow-up. One of our limitations was the number of the cohort, and it was lower due to the low incidence of PCNSL. The other limitation was the retrospective design of our study.

In conclusion, PCNSL is a rare subtype of NHL and DLBCL is the most seen NHL subtype. There is no standard induction treatment nor consolidation, or conditioning regimen. Therefore, bone marrow transplantation centers need to report PCNSL patients' outcomes to contribute to the literature to determine the optimum induction treatment, consolidation, or conditioning regimen.

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Original Article

Evaluation of Bone Mineral Densitometry Measurements in Patients with BCR/ABL-Negative Chronic Myeloproliferative Neoplasm

BCR / ABL-Negatif Kronik Miyeloproliferatif Neoplazili Hastalarda Kemik Mineral Dansitometrisi Ölçümlerinin Değerlendirilmesi

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ABSTRACT

Introduction: This study aims to determine the changes in and the factors related to the bone mineral density in patients with bcr/abl-negative chronic myeloproliferative neoplasm (cmpd).

Methods: The data of 38 males (52.1%) and 35 females (47.9%) patients diagnosed with cmpd were analyzed retrospectively. The age, gender, diagnosis (polycythemia vera (pv), essential thrombocythemia (et)), jak2v617f mutation positivity, the presence of the cmpd complications and the bone mineral densitometry (bmd) measurements of femur neck and lumbar spine carried out during the diagnosis were recorded for each patient. The patients were divided into four groups: under 65 and over 65 years old for men, premenopausal and postmenopausal status for women. They were also grouped according to the t score of the femur neck and lumbar spine with normal and decreased bone density.

Results: Female patients were found to have more bone loss in the lumbar spine (p=0.031). In female patients, the reduction in the lumbar spine bmd was greater in the postmenopausal group (p=0.012). The decrease in bone density in the femur neck was found to be greater in the group above 65 years of age (p=0.01). There was no relationship between bmd and et, pv, jak2v617f mutation positivity, and cmpd complications such as thrombosis and hemorrhage.

Discussion and conclusion: according to the results obtained in our study, the presence of cmpd increases bone loss in lomber area in female patients. Therefore, the bmd measurement and calcium-d supportive treatment planning in the premenopausal group are thought to be beneficial in female patients.

Keywords: bone mineral densitometry, BCR/ABL-negative chronic myeloproliferative neoplasm, polycythemia vera, essential thrombocythemia

ÖZET

Giriş ve amaç: Bu çalışmada, BCR / ABL-negatif kronik miyeloproliferatif neoplazmı (KMPH) olan hastaların, kemik mineral dansitometri ölçümlerindeki değişiklikleri ve prognostik faktörlerle ilişkisini incelemeyi amaçladık.

Yöntem ve gereçler: KMPH tanısı alan 38 erkek (%52.1) ve 35 kadın (%47.9) olgunun verileri retrospektif olarak incelendi. Tanı sırasında gerçekleştirilen femur boynu ve lomber omurganın yaş, cinsiyet, tanı (polisitemia vera (PV), esansiyel trombositoz (ET)), JAK2V617F mutasyon pozitifliği, KMPH komplikasyonlarının varlığı ve kemik mineral dansitometrisi (KMD) ölçümleri her hasta için kaydedildi. Hastalar; erkekler için 65 yaş altı ve 65 yaş üstü, kadınlar için menopoz öncesi ve menopoz sonrası durum olarak dört gruba ayrıldı. Ayrıca T skoru; femur boynu ve lomber omurgaya göre normal ve azalmış kemik yoğunluğu olarak gruplandırıldı.

Bulgular: Kadın hastalarda lomber omurgada daha fazla kemik kaybı olduğu bulundu (p = 0.031). Kadın hastalarda, lomber omurga KMD'sindeki azalma postmenopozal grupta daha fazlaydı (p = 0.012). Femur boynundaki kemik yoğunluğunda azalma 65 yaş üstü grupta daha fazla bulundu (p = 0.01). KMD ile ET ya da PV tanısı, JAK2V617F mutasyon pozitifliği, tromboz ve kanama gibi KMPH komplikasyonları arasında ilişki yoktu.

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Tartışma ve sonuç: Çalışmamızda elde edilen sonuçlara göre, KMPH varlığı kadın hastalarda lomber bölgede kemik kaybını arttırmaktadır. Bu nedenle, menopoz öncesi grupta KMD ölçümü ve kalsiyum destekleyici tedavi planlamasının kadın KMPH hastalarda faydalı olduğu düşünülmektedir.

Anahtar Kelimeler: kemik mineral dansitometri, BCR / ABL-negatif kronik miyeloproliferatif neoplazm, polisitemia vera, esansiyel trombositoz

Introduction:

BCR/ABL-Negative Chronic Myeloproliferative Neoplasm (CMPD) is a clonal hematopoietic stem cell disease. Polycythemia vera, essential thrombocythemia, primary myelofibrosis take part in this group. There are studies showing that inflammatory processes play an important role in the pathogenesis of the CMPD. Inflammatory cytokines such as lipocalin, TNF-alpha, and IL-6 have been shown to increase in the PV and ET cases [1,2,3].

Osteoporosis is characterized by an increase in the risk of fracture as a result of the decreased bone mass and microarchitectural bone deterioration [4]. It has been reported in the literature that one out of every three females and one out of six males are at risk for osteoporotic fractures throughout their lifetime [5].The relationship between BMD and the incidence of fracture has been demonstrated by several large-scale studies. The BMD measurement using the DXA method is the gold standard for the diagnosis of osteoporosis[6-11]. Studies have shown that major inflammatory cytokines such as c-reactive protein (CRP), interleukin -6 (IL-6), and monocytechemotactic protein-1 (MCP-1) are increased in patients with osteoporosis and osteopenia, but no consensus has been reached on this issue [12,13,14].There is a small number of studies suggesting that osteoporosis may develop as a complication in CMPD cases [15]. In a study, CMPD cases were shown to have an increased risk of fracture relative to the general population, but the relevant pathophysiology was not fully explained.

Our study aims to investigate the BMD changes in CMPD cases comprising of ET and PV and the factors that may be cause in these changes.

Methods

Patients and samples: The study includes patients diagnosed with PV and ET among those who applied to our hematology clinic. There were a few myelofibrosis cases, so they were excluded from the study. The data were analyzed retrospectively. Male patients were divided into those under 65 and over 65 years old, female patients were divided into those under premenopausal period and post-menopausal period. Patients with additional systemic diseases (celiac disease, chronic obstructive pulmonary disease, asthma bronchiale, hyperparathyroidism, hyperthyroidism, inflammatory bowel disease, kidney stone disease, rheumatoid arthritis, solid neoplasia, steroid use history, alcohol use) were not included in the study. Presence of JAK2V617F mutation, the presence of the story of the hemorrhage and thrombosis from the CMPD complications, the status of receiving the ASA-hydroxyurea treatment, 25-OH-D levels, and calcium levels were recorded. The vitamin D level <20 ng/ml was accepted as a deficiency. Patients with a calcium value of <8.5 mg/dl were considered to have hypocalcemia. The BMD measurements were performed on the femur neck and lumbar spine using the DXA method. Those with BMD T score above -1 were considered normal, those with BMD T score -1 and below were considered to have decreased bone mineral density. The relationship between the lumbar spine and femur neck bone density and gender, age, JAK2V617F positivity, diagnosis (ET, PV), hypocalcemia and D vitamin levels, ASA and hydroxyurea use, and the thrombosis and hemorrhage complications were investigated.

Statistical analysis: The statistical analysis was performed by the chi-square test using the SPSS 16.0 program (SPSS inc. Chicago, IL, USA). Values below $P < 0.05$ were accepted to be statistically significant.

Table 1: Demographic features of patients

	JAK2V617F positive (n=47)	JAK2V617F negative (n=26)	Total (n=73)	p
Age[1]	62.49±14.72	54.58±11.21	59.67±14.02	0.013
Gender[2] (male)	25 (53.2)	13 (50.0)	38 (52.1)	0.987
Subtype of diagnosis (ET)	15 (31.9)	16 (61.5)	31 (42.5)	0.027
Treatment with HU+ASA	37 (78.7)	14 (53.8)	51 (69.9)	0.051
Thrombosis	9 (19.1)	1 (3.8)	10 (13.7)	0.086
Hemorrhage	3 (6.4)	3 (11.5)	6 (8.2)	0.659
Hemoglobine[3](g/dL)	16.4 (11.8 - 21.3)	15.2 (11.5 – 20.3)	16.0 (11.5 - 21.3)	0.330
White Blood Cell(x10[3])	12.870 (0.18– 30.500)	8.515 (2.930– 18.760)	11.120 (0.18–30.500)	<0.001
Platelets (x10[3])	612 (179 - 2021)	682.5 (155 - 2130)	629 (155 - 2130)	0.876

[1] mean±ss, [2] n(%), [3] median (min-max)

Table 2: The bone mineral densitometry results according to JAK2V617F mutation positivity

	JAK2V617F positive N:47 (65.3%)	JAK2V617F negative N:25 (34.7%)	p
Femur Neck T score			
Normal	23 (48.9%)	11 (44%)	0.805
Abnormal	24 (51.1%)	14 (56%)	
Lomber Spine T score			
Normal	30 (63.8%)	17 (68%)	0.799
Abnormal	17 (36.2%)	8 (32%)	

Results:

The study included a total of 73 CMPD patients consisting of 38 males (52.1%) and 35 females (47.9%). The median age of the patients was 60 years (min=29, max=83). Of the patients, 42(57.5%) were diagnosed with PV and 31(42.5%) with ET. The demographic data of the patients were shown in Table 1. The JAK2V617F positivities of the patients were 76.2% in the PV group and 51.6% in the ET group. The results of bone mineral densitometry according to JAK2V617F mutation positivity are shown in Table 2. Of the patients, 51 (69.9%) were using hydroxyurea and ASA, and 22 (31.1%) were using only ASA. From the CMPD complications, thrombosis was seen in 10 patients (13.7%) and hemorrhage was seen in 6 patients (8.2%). Of the female patients, 9 premenopausal (26.4%) and 26 were postmenopausal (73.6%). The number of patients with vitamin D level below 20 was 51(69.9%) and the number of patients with hypocalcemia was 8 (10.9%). In the lumbar spine, 47.1% of females and 23.1% of males had a decrease in bone density. BMD results according to menopausal status in women and under or over 65 years age of men, are shown in table 3. The decrease in the lumbar spinal bone density of the females was statistically significant (p=0.04). Of the female patients, 9(26.4%) premenopausal and 26(73.6%) were postmenopausal. In postmenopausal female patients, the reduction in lumbar spine BMD was significantly greater than in male and premenopausal female patients (p=0.012). There was no statistically significant correlation between the decrease in lumbar spine bone density and age, JAK2V617F positivity, diagnosis (ET, PV), hypocalcemia and 25-OH-D level, ASA and hydroxyurea use, thrombosis and hemorrhage comp-lications. The decrease in the femur neck bone density of the patients over 65 years was

Table 3: Distribution of bone mineral densities according to localization of the body

Women Premenopausal			Postmenopausal	Men ≤ 65 years old	≥ 65 years old
The bone mineral density of the femur neck	Normal	5 (14,3%)	9 (25,7%)	15 (42,9%)	6(17,1%)
	Abnormal	4(10,5%)	17 (44,7%)	9 (23,7%)	8 (21,1%)
The bone mineral density of the lumbar spine	Normal	6 (12,5%)	11 (22,9%)	19 (39,6%)	12 (25%)
	Abnormal	3 (12%)	15*(60%)	5 (20%)	2 (8%)

*(p=0,012)

70%, while this frequency was below 39.5% in patients under the age of 65. The decrease in BMD in the femur neck of older CMPD patients was significantly higher (p=0.01). The relationship between the decrease in the femur neck BMD and gender, JAK2V617F positivity, diagnosis (ET, PV), hypocalcemia and vitamin D levels, ASA and hydroxyurea use, and the thrombosis and hemorrhage complications was not statistically significant.

Discussion:

In our study, the BMD measurements showed and increased bone loss in the femur neck and lumbar spine in ET and PV patients with increasing age and this bone loss was even more in the lumbar spine in female patients. In the normal population, while the decrease in femur neck BMD was more frequent in female patients in the postmenopausal period, it was remarkable that BMD decrease was observed more frequently in the lumbar spine in patients included in this study. It is known that osteoporosis is a common health problem in both genders in advanced age. Many studies have shown that premenopausal and postmenopausal bone loss in women occurs in the femur neck, and bone loss in the lumbar vertebra occurs in the postmenopausal first decade [16-23]. There are studies showing that bone loss in males increases with age and there is a lifelong loss of bone, especially in the femur neck regionally [24-30]. In a study including PV and ET patients, it has been shown that there is a tendency for trabecular bone loss in a bone biopsy performed to investigate the bone loss detected by the DXA method [31,32]. In another study, PV and ET patients were shown to have increased femoral fracture risk within the next 5 years of diagnosis [33].

The fact that there is no difference between 25 OH D and calcium levels of the patients with normal or decreased BMD suggests that reduced BMD may be due to the disease-related factors. Our study has some limitations. Firstly, a relatively small group was evaluated, and, consequently, the different results that the studies with the larger groups could reveal cannot be ignored. In addition, patients who have already undergone osteoporosis treatment were excluded from the study, which may have led to a bias in this study.

In the literature, it has been shown that the T-score measurements of postmenopausal patients with PV fell from -1.7 to -4.3 in 8-year follow-up. After excluding other causes that could lead to bone loss increase, it has been suggested that this situation may be related to the presence of CMPD [34]. A normal bone remodeling is based on the maintenance of the balance between osteoclasts and osteoblasts [35]. Any imbalance of the bone turnover leads to changes in the skeletal structure and clinical findings that can result in fractures. A study comparing 339597 healthy individuals without the osteoporosis and fracture history with 7595 CMPD patients showed increased fracture risk in ET, PV, and CML patients [33]. A recent study comparing the risk of osteoporotic fracture of a healthy control group with that of 45 patients with ET and PV suggests that fatigue and inactivity caused by disease-related constitutional symptoms may

be effective in increasing fracture risk in patients with ET and PV [15].

In general, it is known that systemic inflammatory diseases are associated with osteoporosis and that the fracture risk increases as a consequence of the decrease in bone density. CMPD patients have also been shown to be under the influence of chronic inflammation [36]. Symptoms including reduced muscle mass, weight loss, decreased activity and fatigue can also lead to BMD deterioration in CMPD patients [37, 38]. The effect of EPO dependent and EPO independent erythropoiesis on bone remodeling was investigated in a rat model. While there were reductions in the osteoblast count and bone formation in both JAK mutation model and erythropoietin-enhanced model, the osteoclast count and activity were found to be normal, and, consequently, the bone loss was associated with the myeloproliferative process [39]. However, there was no significant relationship between the JAK2 mutation positivity and the BMD measurements in this study.

Osteoporosis is associated with low body mass index and increased fracture risk, and progresses without being noticed until a fracture occurs [40]. Many cross-sectional studies have shown that osteoporosis awareness is inadequate both in the groups at risk and in the general population [41, 42, 43, 44]. The osteopenic group with a T score of between -1 and - 2.5 in BMD measurements has also been reported to have a high fracture rate [45]. It has been suggested that the potential risk of fall and impaired bone quality may be influential in these factors [46, 47]. In the patient population in this study, it is considered that factors associated with the myeloproliferative process are also risk factors for osteoporosis and fracture in addition to advanced age.

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Conclusion

Our current result showed that unlike the expected femur neck osteoporosis in the general population, the fact that there is a decrease in the BMD in the lumbar spine in the study group consisting of PV and ET cases. As well as a decrease in the BMD also in patients under the age of 65 shows that this group of patients should be evaluated for osteopenia and osteoporosis, so that the vitamin D support therapy should be planned earlier

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Original Article

Prognostic Value of Lymphovascular and Perineural Invasion in Colon Cancer

Kolon Kanserinde Lenfovasküler ve Perinöral İnvazyonun Prognostik Önemi

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ABSTRACT

Aim: Colon cancer is one of the most common types of malignant tumours in the world. Colon cancer ranks fourth among cancer-related deaths. Lymphovascular invasion (LVI) is the tumour involvement of small lymphatic or blood (typically venous) vessels while perineural invasion (PNI) is the growth of the tumour in, around, and along the nerves and nerve sheaths. In our study, we investigated the effects of LVI and PNI on prognosis, tumour stages, and lymph node metastasis in colon cancer patients.
Methods; The data of patients, who underwent colon cancer surgery in our clinic in the period between February 2013 and January 2020, were analyzed retrospectively. The surgery and pathology reports, demographic characteristics, and overall survival of the patients were examined.
Results: A total of 248 patients were operated due to colon cancer. LVI and PNI were present in 120 (48.3%) and 105 (41.9%) patients, respectively. The relationship of the grade, of the presence of positive lymph nodes, LVI, and PNI, and of the T- and N-stages with the survival were statistically significant with the following p-values of 0.028, <0.001, <0.001, <0.001, 0.005, and <0.001, respectively. When the relationship of LVI and PNI with other factors was examined, it was shown that there was a statistically significant relationship in both groups in terms of T and N stage, metastatic lymph node and survival time. (p <0.001)
Conclusion: Because the presence of LVI and PNI is a determinant of prognosis and lymph node metastasis especially in early-stage patients; we think that close follow-up of patients with LVI and PNI and the evaluation of such patients by a multidisciplinary council for the administration of adjuvant therapy, may affect prognosis favourably.

Key Words: Colon Cancer; Lymphovascular invasion; Perineural invasion

ÖZET

Amaç: Kolon kanseri tüm dünyada en sık görülen maligniteler arasındadır. Kansere bağlı ölüm nedenleri arasında 4. sıradadır. Lenfovasküler invazyon (LVI), küçük lenfatik veya kan (tipik olarak venöz) damarların tümör tarafından tutulması iken perinöral invazyon (PNI) ise sinirlerin ve sinir kılıflarının içinde, çevresinde ve boyunca tümörün büyümesidir. Çalışmamızda LVI ve PNI'nın kolon kanserli hastalarda prognoz, tümör evresi ve lenf nodu metastazına etkisi araştırdık.
Yöntem: Şubat 2013-Ocak 2020 tarihleri arasında kliniğimizde kolon kanseri nedeniyle opere edilen hastaların verileri retrospektif olarak inceledi. Hastaların ameliyat ve patoloji raporları, demografik özellikleri, genel sağkalımları incelendi.
Bulgular: 248 hasta kolon kanseri nedeniyle opere edildi. 120 hastada LVI, 105 (41.9%) PNI hastada vardı. Grade, lenf nodu pozitifliği, LVI, PNI, T ve N evrelerinin sağkalımla ilişkisi istatistiksel olarak anlamlı bulunmuş olup sırasıyla p değerleri: 0.028, <0.001, <0.001, <0.001, 0.005 ve <0.001'dir. LVI ve PNI'nin diğer faktörlerle ilişkisi incelendiğinde, T ve N evresi, metastatik lenf nodu ve sağkalım süresi açısından her iki grupta da istatistiksel olarak anlamlı bir ilişki olduğu gösterilmiştir. (p <0,001)
Sonuç: LVI ve PNI varlığı özellikle erken evre hastalarda prognozun, lenf nodu metastazının bir belirleyici olmasından dolayı; LVI ve PNI pozitif olan hastaların yakın izlemin, adjuvan tedavi için multidisipliner konseyde değerlendirilmenin prognoza olumlu etkilerinin olabileceğini düşünmekteyiz.

Anahtar Kelimeler: Kolon Kanseri, Lenfovaskuler invazyon, Perinöral invazyon

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Introduction

Colon cancer is one of the most common types of malignant tumours in the world. Colon cancer ranks fourth among cancer-related deaths [1]. Despite the advances in treatment and technology, recurrences and metastasis continue to reduce patient survival [2]. The lymphatic system is the main route of metastasis in colon cancer as in many types of cancer. The status of lymph nodes is used to determine the stage of the disease, predict prognosis and select treatment modalities [3]. While surgical treatment is often curative for stage 1-2 colon cancers [4], adjuvant chemotherapy is recommended for stage 3-4 colon cancer [5-7]. Lymphovascular invasion (LVI) is the tumour involvement of small lymphatic or blood (typically venous) vessels while perineural invasion (PNI) is the growth of the tumour in, around, and along the nerves and nerve sheaths [8,9]. Recent advances in histopathological analysis techniques have suggested that histopathological factors such as LVI and PNI are unfavourable prognostic characteristics and may be harbingers of lymph node metastasis in early-stage colon cancers [6] thus, such findings can help identify patients at risk, who will benefit from adjuvant chemotherapy [10]. National Comprehensive Cancer Network (NCCN) guidelines described several additional factors such as LVI and PNI to identify stage II colorectal cancer patients with a high risk for metastasis [7]. In our study, we investigated the effects of LVI and PNI on prognosis, tumour stages, and lymph node metastasis in colon cancer patients.

Material-Method

The data of patients, who underwent colon cancer surgery in the period between February 2013 and January 2020, were analyzed retrospectively. All patients underwent standard surgical therapy. Patients; who were younger than 18 years old, who had distant metastases, who underwent additional visceral organ resection (resection of the liver, pancreas, colon, or small intestine), who had a cancer diagnosis other than adenocarcinoma, who had recurrences, who were operated due to emergency conditions, or who previously underwent surgery due to other types of cancer, were excluded from the study. The study included 248 patients. The surgery and pathology reports, demographic characteristics, and overall survival of the patients were examined. Electronic radiological examination records of chest radiograms, computed tomography, ultrasound, endoultrasound, magnetic resonance imaging, and PET-CT were examined retrospectively. Tumours were categorised based on location as the right colon, transverse colon, and left colon tumours. The 8th edition of the Union for International Cancer Control TNM classification system was used for staging [11]. PNI was defined as the presence of tumour cells in the perineural sheath involving at least 1/3 of the nerve's circumference [9]. LVI was defined as the involvement of the lymphatic or vascular channels by the tumour [12]. The patients were followed at 3-month intervals for 2 years, at 6-month intervals for the next 3 years, and annually thereafter.

Statistical Analysis

SPSS 25.0 software was used in the analysis of the data. For descriptive analysis, quantitative variables mean ± standard deviation and median (minimum-maximum), and qualitative variables were presented as number of patients (percentage). The mean distributions of the quantitative data were tested with the Shapiro-Wilk test and histogram curves. In terms of the quantitative variable, the difference between the categories of the qualitative variable with two categories was examined using the Mann-Whitney U test for those who provided normal distribution assumptions and those who did not provide the Student-t-test. The Chi-squared test was used to evaluate the relationship between two qualitative variables. The statistical significance level was accepted as 0.05.

Results

A total of 248 patients were operated due to colon cancer. Open surgery and laparoscopic surgery were performed on 24 (9.6%) and 224

Table 1. Descriptive characteristics

Variables		
Age	Mean±SD	58.23±13.22
Survival time	Mean±SD	33.6±19.4
Gender, n(%)	Male	143 (57.7)
	Female	105 (42.3)
Location, n(%)	Right	81 (32.7)
	Transvers	5 (2.0)
	Left	162 (65.3)
Operation, n(%)	Laparoscopic	224 (90.3)
	Open	24 (9.6)
Grade, n(%)	1	20 (8.1)
	2	176 (71.0)
	3	41 (16.5)
	4	11 (4.4)
Mortality, n(%)	Yes	186 (75.0)
	No	62 (25.0)
T stage, n(%)	1	31 (12.5)
	2	36 (14.5)
	3	130 (52.4)
	4	51 (20.6)
N stage, n(%)	0	136 (54.8)
	1	76 (30.6)
	2	34 (13.7)
	3	2 (0.8)

SD: standard deviation

(90.3%) patients, respectively. Of the study patients, 105 (42.3%) were women and 143 (57.7%) were men. The mean ± standard deviation and median (minimum-maximum) values of the patients' age were 61.50 ± 12.98 and 62.00 (22.00-93.00) years, respectively. The tumour was located in the right colon in 81 (32.7%) patients; in the transverse colon in 5 (2.0%) patients, and in the left colon in 162 (65.3%) patients. The T-stage of the tumour was T_1 in 31 (12.5%) patients, T_2 in 36 (14.5%) patients, T_3 in 130 (52.4%) patients, and T_4 in 51 (20.6%) patients. The distribution of the patients by the N-stages revealed 136 (54.8%) patients in N_0, 76 (30.6%) patients in N_1, 34 (13.7%) patients in N_2, and 2 (0.8%) patients in N_3. The mean number of resected lymph nodes was 17.78±9.72 and the mean number of the metastatic lymph nodes was 2.02±3.87. Patient characteristics are shown in Table 1.

LVI and PNI were present in 120 (48.3%) and 105 (41.9%) patients, respectively. As for the overall survival, 186 (75.0%) patients survived and 62 (25.0%) patients died out of 248 patients. The mean survival time was 33.6 (2-82) months. The relationship of the grade, of the presence of positive lymph nodes, LVI, and PNI, and of the T and N stages with the survival were statistically significant with the following p-values of 0.028, <0.001, <0.001, <0.001, 0.005, and <0.001, respectively. The results of the Kaplan-Meier analysis were shown in Table 2 (Figure 1-2).

The ratio of the number of metastatic lymph nodes to the total number of the lymph nodes in patients was evaluated as the lymph node ratio. The mean value of the lymph node ratio was 0.11±0.20. The values of the lymph node ratios were classified under 4 categories as Rosenberg et al. described [13]. The lymph node ratio categories were as follows: Category 1 included ratios in the range of 0.01-0.17, category 2 included ratios in the range of 0.18-0.41, category 3 included ratios in the range of 0.42-0.69, and category 4 included ratios of ≥0.70. There were 186 patients in category 1, 38 in category 2, and 12 patients in each of the categories 3 and 4. There were no statistically significant correlations between lymph node ratios and survival (p=0.477). When the relationship of LVI and PNI with other factors was examined, no statistically significant relationships were found with age or gender with p-values of 0.232 and 0.321 for LVI and 0.742 and 0.681 for PNI, respectively. Both LVI and PNI were statistically significantly related to the categorical variables of the tumour grade and the T and N stages. Of the qualitative variables, the relationships of the survival time and metastatic lymph nodes with LVI and PNI were statistically significant. The relationships of LVI and PNI with the investigated factors are shown in Table 3.

Discussion

LVI and PNI are the two major histopathological features that were shown to predict tumour biology and progression in colorectal cancers [14]. In the 1970s, LVI was

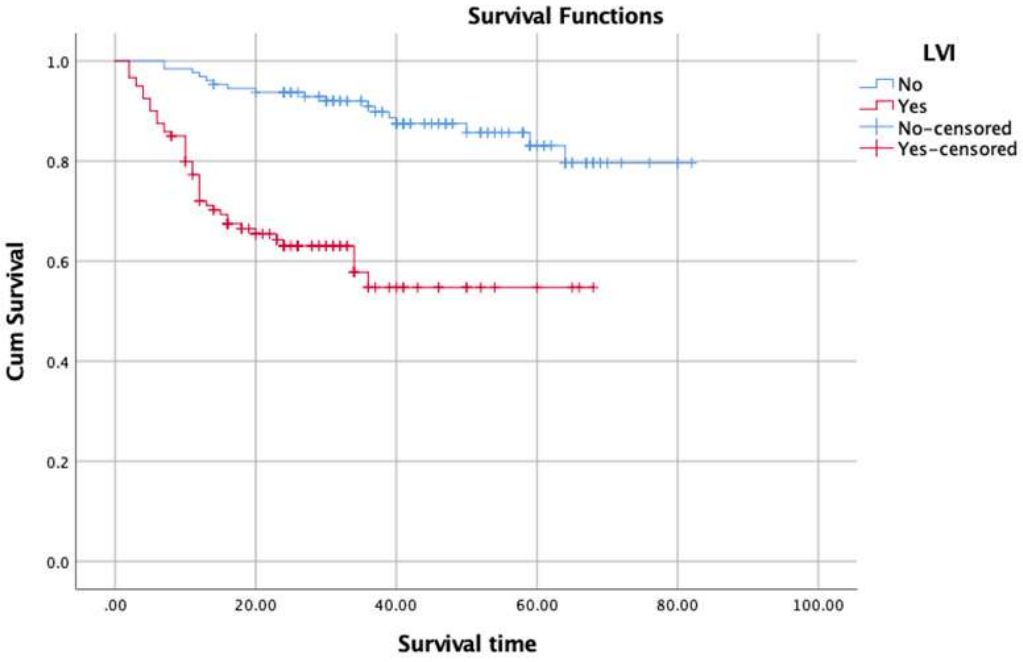

Figure 1. LVI survival curve

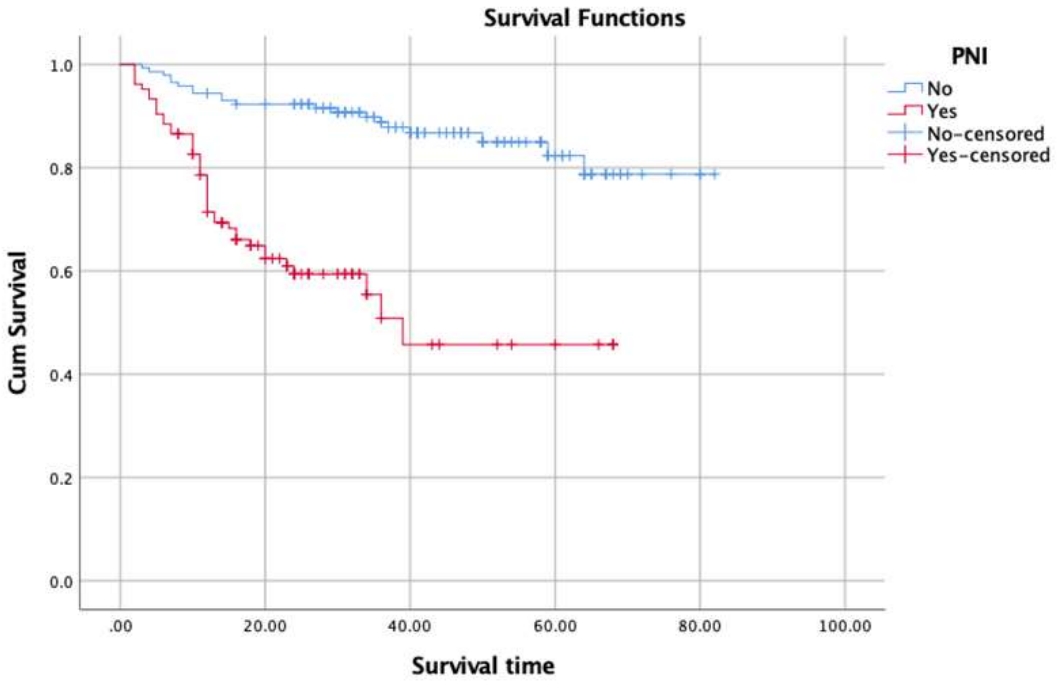

Figure 2. PNI survival curve

Table 2. Kaplan Meier analysis results

Variables		Survival 1 year (%)	3 years (%)	5 years (%)	Life Time Mean±SD	p-value
General		95.2	87.9	81.6	33.6±19.4	-
LVI	No	93.7	92.0	87.5	44.26±17.41	<0.001
	Yes	90.0	77.3	65.4	22.37±14.64	
Grade	1	89.5	89.5	67.1	45.21±19.74	0.028
	2	93.2	85.0	82.5	34.69±19.00	
	3	82.9	57.5	57.5	26.60±18.75	
	4	72.7	54.5	54.5	22.54±17.73	
Lymph node positivity	No	94.0	87.9	84.6	42.01±18.12	<0.001
	Yes	90.4	74.1	68.2	23.86±16.22	
PNI	No	92.3	90.7	87.8	42.59±17.64	<0.001
	Yes	88.5	71.4	62.4	21.44±14.80	
T stage	1	100.0	96.2	88.8	48.03±15.17	0.005
	2	88.9	75.6	75.6	36.19±18.70	
	3	89.2	75.6	68.1	34.67±19.76	
	4	76.4	63.6	63.6	20.60±13.21	
N stage	0	91.2	80.4	75.5	40.08±19.34	<0.001
	1	86.8	72.4	64.3	29.25±17.11	
	2	72.3	68.3	-	19.23±13.55	
	3	0	-	-	11.00±1.41	
Lymph node ratio	0.01-0.17	88.7	77.5	72.5	35.09±20.20	0.477
	0.18-.41	84.2	73.1	53.2	30.05±15.52	
	0.42-0.69	83.3	72.9	54.7	26.58±21.29	
	≥0.70	83.3	83.3	83.3	30.16±15.58	

SD: standard deviation

for the first time associated with poor prognosis in colorectal cancers with blood vessel involvement. In 1989, Minsky et al. described LVI as an independent prognostic factor [15]. In the same years, PNI was defined as the invasion of the nerves around the tumour and was identified as a poor prognostic factor in many cancer types such as the cancers of the colon, pancreas, and prostate [16]. In the 2000s, the American Joint Committee on Cancer (AJCC) identified LVI and PNI as adverse prognostic factors and recommended that they should be addressed every time in pathology reports [17]. We evaluate the status of LVI and PNI of all our patients in our clinic and in every tumour board.

Despite some previous studies arguing about LVI and PNI contrarily, unfavourable prognostic effects of LVI and PNI have been shown in many studies [18-20]. The study by Al-Sukhni et al. reported LVI and PNI rates of 26.3% and 11.1% at all stages, respectively. [21] However, the ratios reported for LVI (33%) and PNI (15-22%) vary in the literature [22,23]. In the same study; the positivity of LVI and PNI was associated with advanced T stages, high tumour grade, a high number of lymph node metastases, and distant metastases. Similarly, Huh et al. found that; in T1 and T2 tumours, the positivity of LVI and PNI was statistically significantly associated with the depth of tumour invasion [24]. In our study; the rates of LVI and PNI were 48.3%

Table 3. Association of LVI and PNI with clinicopathological features

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Variables		LVI		p value	PNI		P
		Yes (n=120)	No (n=128)		Yes (n=120)	No (n=128)	
Grade(%)	1	1 (0.8)	19 (14.8)	<0.001[c]	2 (1.9)	18 (12.6)	<0.001[c]
	2	77 (64.1)	99 (77.30)		68 (65.4)	107 (74.8)	
	3	32 (26.6)	9 (7.1)		26 (25.0)	15 (10.5)	
	4	10 (8.5)	1 (8.8)		8 (7.7)	3 (2.1)	
Survival time	Mean±SD	22,37±14,64	44,26±17,41	<0.001[a]	21,44±14,80	42,59±17,64	<0.001[a]
Lymph node ratio	Mean±SD	0.13±0.20	0.10±0.19	0.421[b]	0.13±0.21	0.10±0.19	0.276[b]
T stage, n(%)	T1	3 (2.5)	28 (21.9)	<0.001[c]	2 (1.9)	28 (19.6)	<0.001[c]
	T2	18 (15.0)	18 (14.1)		13 (12.5)	23 (16.1)	
	T3	59 (49.2)	71 (55.5)		58 (55.8)	72 (50.3)	
	T4	40 (33.3)	11 (8.6)		31 (29.8)	20 (14.0)	
N stage, n(%)	N0	45 (37.5)	91 (71.1)	<0.001[c]	45 (30.8)	103 (72.0)	<0.001[c]
	N1	46 (38.3)	30 (23.4)		46 (41.3)	33 (23.1)	
	N2	27 (22.5)	7 (5.5)		45 (26.0)	7 (4.9)	
	N3	2 (1.7)	0 (0)		46 (1.9)	0 (0)	
Metastatic lymph node	Mean±SD	3.55±4.98	0.58±1.27	<0.001[a]	2.17±5.05	0.59±1.61	<0.001[a]

a. Mann Withney U b. Student t c. Ki-kare

and 41.9% at all stages, respectively. We demonstrated in our study that the posivity of LVI and PNI was statistically significantly associated with both the tumour invasion depth (p<0.001, p<0.001) and high histological grades (p<0.001, p<0.001).

One of the most important prognostic factors in colorectal cancers is the presence of lymph node metastases [3,25]. Many studies have been conducted so far to predict the emergence of lymph node metastases [26,27]. Huh et al. found LVI and PNI as the only factors that could be used to predict lymph node metastases [24]. In the same study, it was shown that the presence of PNI increased the presence of lymph node positivity by 10 times. In our study, the effects of the presence of LVI and PNI on lymph node metastasis were found to be statistically significant (p<0.001, p<0.001). We found that the presence of LVI and PNI increased the likelihood of lymph node metastasis 3 and 5 times, respectively.

There is still no consensus in the literature, especially for early-stage (stage 1-2) patients, about whether such patients should receive adjuvant chemotherapy [28,29]. Studies are available showing that adjuvant chemo-therapy increases survival and decreases mortality in Stage 2 patients with high-risk factors [30,31]. According to the NCCN guidelines; poorly differentiated histology, LVI, bowel obstruction, resection of less than 12 lymph nodes, PNI, tumour or colon perforation, and close, vague, or positive surgical margins can be considered as high-risk factors for colon cancer. We think that the statistically significant relationship of the presence of LVI and PNI with the tumour grade, lymph node metastasis, and tumour invasion depth found in our study may effectively help identify high-risk patients and make treatment decisions, especially in stage-2 patients in whom the administration of adjuvant chemotherapy remains to be controversial. In our clinical practise, we recommend adjuvant chemotherapy especially in stage-2 patients, whose histological features are unfavourable and who have LVI and PNI.

As for the overall survival, Lim et. al showed that 5-year overall survival and 5-year

disease-free survival were statistically significantly reduced in patients with LVI [32]. In another study; the effect of LVI on survival could not be shown but PNI was found to reduce the overall survival significantly [20]. In our study; we found out that the presence of LVI and PNI statistically significantly reduced the one-year, three-year, and five-year survival ($p<0.001$, $p=0,001$).

Many molecular markers other than LVI and PNI have recently been investigated for prognostic value in colon cancers but the results are controversial [33,34]. Micro-satellite instability, allelic loss, tumour expression mechanisms, and molecular markers such as MLH, MSH, and PMS are insufficient to predict the prognosis and disease course. The use of such markers in clinical practise continues to be studied [35-37].

The retrospective study design, the absence of potential prognostic factors such as microsatellite instability and lympho-plasmacytic infiltration in the pathology report of some patients, and the lack of evaluation of clinical factors including comorbidities, operative times, the administration of perioperative transfusion, and postoperative complications are the limitations of this study.

In conclusion, several factors exist affecting prognosis in colorectal cancers. Because the presence of LVI and PNI is a determinant of prognosis and lymph node metastasis especially in early-stage patients; we think that close follow-up of patients with LVI and PNI and the evaluation of such patients by a multidisciplinary council for the administration of adjuvant therapy, may affect prognosis favourably.

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Original Article

Juxtacortical Chondrosarcoma: Analysis of 52 Cases

Jukstakortikal Kondrosarkom: 52 Vakanın Analizi

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ABSTRACT

Introduction: Juxtacortical (surface) chondrosarcoma (JCC) is a very rare subtype of chondrosarcoma, mostly seen in young adults. In this study, we aimed to report demographic data and average survival rates of JCC.

Methods:In this study we used the latest version of the Surveillance, Epidemiology and End Results (SEER) database, patients were examined under the titles of gender, age, race / ethnic origin, lesion location in the body, degree of tumor differentiation, applied surgeries and follow-up periods. Descriptive statistics were given as mean ± standard deviation, frequency and percentage.

Results: A total of 52 patients were included in the study, of which 16 were female and 36 were male (69%), with a mean age of 41.3 ± 20.0 years (range: 8-84 years). When examined for ethnicity, it was found that it was seen most in white race (84.6%).The most common localization of tumor lesions was the lower extremity long bones (50%).Low grade tumor (45% of all tumors) was the most common tumor differentiation. The mean follow-up was 125,5 months (range 4 to 358 months).

Discussion: JCC is a very rare type of chondrosarcoma, and often seen as lower grade compared to other types of chondrosarcoma. It's a little more common in men. It tends to settle on the long bones, most commonly on the lower extremity, especially the femoral diaphysis.

Keywords: Juxtacortical chondrosarcoma, SEER, demographic, survive

ÖZET

Giriş: Jukstakortikal (yüzey) kondrosarkom (JKK), kondrosarkomların daha çok genç erişkinlerde görülen çok nadir bir alt tipidir. Bu çalışmada JKK'ye ait demografik verilerin ve ortalama sağ kalım oranlarının raporlanması amaçlandı.

Yöntemler: Sürveyans, Epidemiyoloji ve End Results (SEER) veritabanının en son versiyonu kullanılarak yapılan bu çalışmada, hastalar, cinsiyet, yaş, ırk/etnik köken, lezyonun vücutta yerleşim yeri, tümörün diferansiasyon derecesi, uygulanan cerrahiler ve takip süreleri altbaşlıklarında incelendi. Tanımlayıcı istatistikler ortalama ± standart sapma, sıklık ve yüzde olarak verildi.

Bulgular: Çalışmaya toplam 52 hasta dahil edildi, bu hastaların 16'sı kadın 36'i (%69) erkekti ve yaş ortalamaları 41,3±20,0 std (8-84 yaş arası) idi. Etnik köken açısından incelendiğinde en fazla beyaz ırkta (%84,6) görüldüğü saptandı. Tümöral lezyonların en sık görüldüğü lokalizasyon alt ekstremite uzun kemikleri ve ilişkili eklemleri (%50) idi. Tümör diferansiasyonları açısından incelendiğinde en sık düşük dereceli tümörler (tüm tümörlerin %45'i) görüldü. Ortalama takip süresi 125,5 ay (4-358 ay arası) idi.

Sonuç: JKK çok nadir bir kondrosarkom türüdür ve sıklıkla diğer kondrosarkom tiplerine göre daha düşük grade'li olarak görülür. Erkeklerde biraz daha sıktır. En sık alt ekstremiteye, özellikle de femur diafizine olmak üzere, uzun kemiklere yerleşim eğilimindedir.

Anahtar Kelimeler: Jukstakortikal kondrosarkom, SEER, demografi, sağkalım

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Introduction

Chondrosarcomas are the third most common malignant bone tumors and contain about 16% of all bone tumors. Chondrosarcomas have many subtypes such as classical, mesenchymal, myxoid, dedifferentiated, clear cell and secondary chondrosarcoma, and it is difficult to differentiate between osteochondromas and grade I chondro-sarcoma [1]. Juxtacortical chondrosarcoma (JK), also known as periosteal chondrosarcoma, is a rare chondrosarcoma variant originating from the outer surface of the bone [2,3]. This tumor accounts for about 2% of all chondrosarcomas [4].

Periosteal chondrosarcomas tend to be seen most frequently between the second and fourth decades and are more common in men [2,4,5].

These tumors most often settle in long bones, such as the femur and humerus. Clinically, as with most other malignant tumors, the most common admission symptoms are pain and / or swelling. Generally, it is a long-term and slowly progressing tumor [3,4,6].

Periosteal chondrosarcomas are often seen as a soft tissue mass with a juxtacortical location on radiographs. They may include features such as peripheral calcification, mottled appearance, popcorn appearance, which are features of cartilaginous tumors. As with many tumors in MRI, they show hypo-intense in T1 sequences and hyper-intense in T2 sequences. Some patients may have punctate mineralization foci. They show peripheral and septal enhancement after gadolinium injection [2,5,6].

The gold standard treatment method of JKs, as with many other malignant tumors, is the resection of the tumor with wide limits. Since it is a periosteal located tumor, it is vital to determine whether the tumor contains the medullary cavity before surgery, because if there is medullary involvement, it should be treated like central chondrosarcoma [2,3,6].

To our knowledge, there is no study on SEER database data and juxtacortical chondrosarcoma that analyzes the epidemiology and survival rates of the tumor. In this study, we studied demographic data, surgical treatments, and survival rates for this disease.

Materials and Methods

In this study, we used the data of the database Surveillance, Epidemiology and End Results (SEER), which was released in 2018. We evaluated juxtacortical chondrosarcoma cases retrospectively. The cases included in the study in this version include patients diagnosed between 1983 and 2016.

As it is known, the SEER database is the most comprehensive registry of tumor patients in the United States. The SEER database contains data on patients' demographic data, cancer incidence and survival times [7]. This database has been used in many surgical subfields and cancer-related studies today and has been used in many studies in the past. In the United States, all cancer data of the country age has been collected through this database since 1973, and the information for this database currently represents data for about 28% of the entire US population [7,8]. This data base is a valuable resource especially for the examination of very rare diseases. It is a database where statistical inferences can be made about these diseases thanks to its ability to provide extensive data. Juxtacortical chondrosarcoma is also one of the very rare subtypes of chondrosarcoma.

In this study, we included cases with Juxtacortical chondrosarcoma (ICD O3 code 9221/3) among the cases with Chondrosarcoma in the International Classification of Diseases for Oncology, Third Edition (ICD-O 3) morphology [9] coding system. The patients were evaluated in terms of age, gender, race (ethnicity), marital status, the direction of the disease, tumor location, tumor grade, follow-up time and follow-up results. In addition, we classified all patient ages in 5 slices. The races of the patients were examined in 3 groups, white races, black races and other races (Asian Pacific, American Indian etc.). Their marital status was grouped as single or married, divorced patients were included in the single group. Histological grades of tumors were examined at four degrees.

As it is known, the studies written with the use of SEER database data are exempt from the approval of the Ethics Review Board. Therefore, no ethics committee application was made for our study.

Statistical Analysis

All statistical analyzes were done using IBM SPSS 22.0 statistical software (IBM Corp, Armonk, NY, USA). Descriptive statistics were expressed as mean ± standard deviation, frequency and percentage.

Results

A total of 52 patients, 36 (69%) males and 16 females, with an average age of 41.3 ± 20.0 std (8-84years), were included in this study.44(84.6%) of the patients were from the white race, 4 were from the black race and 4 were from the other races group.

When patient ages are examined in groups of 5 years, 1 patient is in the 5-9 age group, 5 patients are in the 10-14 age group, 1 patient is in the 15-19 age group, 6 patients are in the 20-24 age group, 3 patients are in the 25-29 age group, 4 patients in the 30-34 age group, 7 patients in the 35-39 age group, 4 patients in the 40-44 age group, 4 patients in the 45-49 age group, 3 patients in the 50-54 age group, 3 patients in the 55-59 age group, 2 patients In the 60-64 age group, 4 patients were in the 65-69 age group, 2 patients were in the 70-74 age group, 1 patient was in the 75-79 age group, and 2 patients were in the 80-84 age group. As stated, there was no aggregation in any age group.

When tumor localizations were examined, the tumor was found in 8 patients in the region containing the upper extremity long bones, scapula and associated joints, in 1 patient in the region containing the upper extremity short bones and related joints. In 50% of 52 patients, the region containing the lower extremity long bones and associated joints, in the area of the lower extremity short bones and associated joints in 1 patient, in the vertebral column in 1 patient, in the costa, sternum, clavicle and associated joints in 8 patients, and in the pelvic area in 6 patients bones were in the region containing sacrum, coccyx and related joints. In one patient, the tumor was located on the bone, but its localization was not reported.

When the histological grades of the tumors were examined, 23 patients (44.2%) had grade I (good differentiated) level, 19 patients had grade II level (moderately differentiated), 5 patients had grade III level (poorly differentiated) and 2 patients had grade IV level (un- differentiated, anaplastic) tumor. Grade information of 3 patients was not available.

When evaluated in terms of tumor direction, in 24 patients (46.1%) the tumor was located on the left side of the body, 20 tumors were located on the right side. Eight patients did not have direction information.

Surgical resection in 24 (46.1%) patients, local excision in 13 patients, partial resection in 5 patients, hemipelvectomy in one patient was performed. One patient refused surgical treatment, one patient died before surgery, the information about the surgery performed in seven patients was not available in the data.

The mean tumor size of 26 patients was 82.4 mm (between 27 mm and 220 mm).

The mean follow-up period of the patients was 125.5 months (4-358 months). Forty-six patients were still alive and had an average follow-up time of 130 months, while eight patients (15.3%) died in an average of 98 months.

Finally, 24 of the patients were single (19 were never married, five were divorced), 24 were married (46.1%), and there was no information about the marital status of 4 patients.

Discussion

The aim of this study was to identify patients diagnosed with juxtacortical chondrosarcoma, a very rare variant of chondrosarcoma, using the SEER database of the United States, and to determine their demographic characteristics, surgeries, and survival rates. The database we have mentioned provides us with a high number of patients about very rare tumors such as juxtacortical chondrosarcoma

Table 1. Demographic Data of Patients Included

Data	n=52
Gender, n (%)	
Male	36 (69.0)
Woman	16 (31.0)
Age, years	
Mean ± SD Median (min-max)	41.3 ± 20.0 38 (8.0-84.0)
Race, n (%)	
White	44 (84.6)
Black Other	4 (7.7) 4 (7.7)
Marital Status (n = 48), n (%)	
Single	24 (50.0)
The married	24 (50.0)
Direction (n = 44), n (%)	
Left Right	24 (46.1) 20 (53.9)
Tumor Localization, n (%)	
Long extremity bones, scapula Short limbs	8 (15.3)
of the upper limb	1 (1.9)
Lower extremity long bones, joints Lower	26 (50)
extremity short bones, joints Vertebral	1 (1.9)
column	1 (1.9)
Costa, sternum, clavicle	8 (15.3)
Pelvis, sacrum, coccyx, joints	6 (11.5)
Tumor Degree n (%)	
Grade I	23 (44.2)
Grade II	19 (36.5)
Grade III	5 (41.6)
Grade IV	2 (3.8)
Follow-up Time, months	125.5
Average	176 (4-358.0)
Median (min-max)	
Follow-up Results, n (%)	
Live	44 (84.7)
Exitus	8 (15.3)

and enables us to obtain detailed information about these diseases [8].

When the data of our country related to the musculoskeletal system tumors are examined, it is seen that it is quite similar to the world literature. According to studies conducted in our country, chondrosarcomas are the third most common malignant bone tumor following osteosarcoma and Ewing's sarcoma [10].

Not much data is available in the literature about the rare variant of chondrosarcoma JKK. Since it is a very rare tumor, there are usually case reports or case series [4,11,12,13]. In this study, a total of 52 patient data were obtained using SEER data between

1983-2016. To our knowledge, we offer the largest series in the literature.

In the literature, it has been reported that the most common age of periosteal chondrosarcoma is between the 2nd and 4th decades, and periosteal chondrosarcoma tendsto occur more frequently in men [2,4,5]. In our studies, it was found that it is more common in men. In terms of age, the average age of the patients was 41 in our study, and it was found that the age of the patients was distributed over a wide age range, with no frequency observed in any age range.

Classical chondrosarcomas of ten tend to settle in the pelvic bones and proximal appendicular skeleton [10]. When the archive data were examined in this study, the most common locations were the lower limb long bones and associated joints.

On the radiographs of the chondrosarcoma, a smoothly bounded soft tissue mass is located on the bone surface, and specific findings can be detected for cartilage tumors. Periosteal bridge formation is frequently found at the borders of the tumor due to chronic periosteal reaction. In radiographic examination of periosteal chondrosarcoma, MRI is superior to other imaging modalities, as with many musculoskeletal tumors, because it is very useful in studying intramedullary space and soft tissue extension with superior contrast resolution [2,3,6].

In the differential diagnosis of periosteal chondrosarcoma, especially the benign tumor that should be kept in mind is the periosteal chondroma and the malignant tumor is the periosteal osteosarcoma. Although it is known that osteosarcoma is seen at an earlier age (often 1st and 2nd decade) compared to chondrosarcoma, juxtacortical chondrosarcoma can be seen in a wide age range including these ages. The presence of extensive cortex destruction and periosteal reaction on radiographs should raise suspicion in terms of osteosarcoma [5,6,14]. Periosteal chondroma also tends to occur at an earlier age and is a benign tumor, often without symptoms. Apart from these tumors, it is important to make differential diagnosis in terms of other benign and malignant tumors and tumor-like lesions [1,10].

Treatment of periosteal chondrosarcoma is resection of the tumor with wide surgical margins. The response of chondrosarcoma to chemotherapy and radiotherapy is low [11]. Recurrence of the disease is most often associated with the inability to remove the tumor with wide limits. [2,4,6,11-13]. Since the chondrosarcoma is a tumor of the periosteal location, central site location is very rare, but patients should be checked for intramedullary placement in pre- operative evaluation [2,3,6,15]. Cases with intramedullary localization should be treated like centrally located chondrosarcoma.

Histological ratings of chondrosarcoma are associated with the clinical behavior of the disease and prognosis. Grade I tumors have better prognosis, while the prognosis worsens as grade level increases. Grade III tumors tend to metastasize 70% [3]. In general, the prognosis of JK is much better than central chondrosarcoma. Metastases are very rare and occur very late in parallel with the slow course of the disease [2,3,6]. In our study, more than 40% of patients were grade I tumors, and approximately 85% of patients on average 125-month follow-up were still alive and underfollow-up.

This study has some limitations. Firstly, the data were analyzed retrospectively and the number of patients was relatively low as it is a rare disease. Because this study was a study analyzing a database data and detailed data could not be found in the database, some patients could not be included in statistical studies. Some of the demographic data and all symptoms, treatments and similar data were not mentioned. For this reason, there is a need for large multicentre studies with a higher number of patients in the future.

Conclusion

Periosteal chondrosarcoma is a malignant tumor that can be seen in a wide age range and tends to occur more frequently in men. Its prognosis is quite good compared to central chondrosarcoma after appropriate surgical treatments.

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Original Article

The Effects of Laxative Use on Rectum Volume and Doses During Radiotherapy in Patients With Prostate Cancer

Radyoterapi Uygulanan Prostat Kanseri Hastalarında Tedavi Süresince Laksatif Kullanımının Rektum Hacim ve Dozlarına Etkisi

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ABSTRACT

Objective: Changes in rectum volume can affect dose delivery during prostate radiotherapy. The aim of this study was to evaluate how laxative use affected rectum volumes and doses in patients with prostate cancer treated with Volumetric Modulated Arc Therapy (VMAT).

Material and Methods: Treatment planning computed tomography (simCT) and cone-beam computed tomography (CBCT) images collected from 20 patients with prostate cancer were retrospectively evaluated. These patients were divided into two groups as those who either used or did not use laxatives during radiotherapy. Rectum volumes were re-contoured on 160 CBCT images, and VMAT treatment plans were re-calculated to determine rectum doses.

Results: In all patients, CBCT images showed increased mean rectum volumes and doses relative to simCT images. Furthermore, the percent volume (Vx) of the rectum receiving 40, 50, 60, and 70 Gy doses based on CBCT results were larger than those based on simCT. The Dmean values in the treatment plans for the group with laxative use were 39 Gy on simCT images and 43 Gy on CBCT images ($p = 0.009$). Alternatively, in the group without laxatives, the Dmean values in the treatment plans were 40 Gy on simCT images and 44 Gy on CBCT images ($p = 0.047$).

Conclusion: In patients undergoing prostate radiotherapy, rectum volume and doses increase regardless of laxative use. Although laxatives can limit volume expansion of the rectum, they do not have the expected effect on rectum doses and actually significantly increase all dosimetric parameters.

Key words: Cone-beam computerized tomography, laxatives, prostate cancer, radiotherapy, volumetric-modulated arc therapy.

ÖZET

Amaç: Rektumdaki hacim değişiklikleri prostat radyoterapisi sırasında uygulanan dozu etkilemektedir. Hacimsel Ayarlı Ark Terapi (VMAT) uygulanan prostat kanseri hastalarında tedavi süresince laksatif kullanımının rektum hacim ve dozlarına etkisinin değerlendirilmesi amaçlandı.

Gereç ve Yöntem: Kliniğimizde tedavi edilen 20 hastanın tedavi planlama tomografi (simCT) ve koni ışınlı bilgisayarlı tomografi (CBCT) görüntüleri retrospektif olarak değerlendirildi. Laksatif kullanan (L+)(n:10) ve laksatif kullanmayan (L-)(n:10) hastalar olacak şekilde 2 grup belirlendi. Toplam 160 tane CBCT görüntüsünde rektum hacimleri yeniden konturlandı. Her hastanın kendi VMAT planı kendi CBCT görüntüleri üzerinde tekrar hesaplatılarak rektum dozları elde edildi.

Bulgular: Tüm hastaların rektum hacimleri CBCT görüntülerinde simCT görüntülerine göre artış göstermiştir. Belirli bir dozu alan rektum hacminin % değeri (Vx) için simCT ve CBCT planları karşılaştırıldığında 40Gy, 50Gy, 60Gy ve 70Gy dozlarının hepsi için tüm hastalarda tedavi süresince artış bulunmuştur. Laksatif kullanan hasta grubunun tedavi planlarındaki Dmean değerleri simCT görüntülerde 39 Gy ve CBCT görüntülerde 43 Gy bulunmuştur ($p = 0.009$). Alternatif olarak, laksatifleri

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kullanmayan hasta grubunda, tedavi planlarındaki Dmean değerleri simCT görüntülerinde 40 Gy ve CBCT görüntülerinde 44 Gy bulunmuştur (p = 0.047).
Sonuç: Prostat radyoterapisi uygulanan hastalarda tedavi sırasında rektum hacmi ve aldığı radyasyon dozu, laksatif kullanımından bağımsız olarak, başlangıç tedavi planına göre artmaktadır.

Anahtar Kelimeler: Hacimsel Ayarlı Ark Terapi, Laksatif, Prostat kanseri, Prostat radyoterapisi, koni ışınlı bilgisayarlı tomografi

Introduction

Radiotherapy is a well-established treatment modality for managing clinically localized prostate cancer [1]. To be effective, the radiotherapy treatment volume should encompass the prostate gland and any extension of the primary tumour [2]. Importantly, both the localization of the prostate gland and seminal vesicle and the volumes of normal tissues can vary during daily radiotherapy [3]. Changes in prostate gland and seminal vesicle localization are more affected by changes in rectum volume and length than changes in bladder volume [4,5]. The excess of rectal filling on treatment planning tomography (simCT) reduces the probability of biochemical and local control [6]. Several methods such as evacuation techniques, dietary interventions, laxatives, and enemas have been applied to limit changes in rectum volume during treatment planning tomography and daily treatment; however, which of these methods is optimal is unknown [7].

Furthermore, radiotherapy in prostate cancer patients can produce side effects that persist for up to 10 years after diagnosis. In particular, bowel-related side effects lower the patient's quality of life [8]. Relative to conformal radiotherapy, intensity-modulated radiotherapy (IMRT) reduces acute and late rectal toxicity by limiting the radiation dose delivered to surrounding organs, including the rectum, while increasing biochemical control by applying high radiation doses to the prostate gland [9,10,11]. Volumetric modulated arc therapy (VMAT) provides a more homogeneous dose distribution for shorter treatment times with gantry rotation when compared to IMRT [12]. These daily radiotherapy treatments can be evaluated with cone-beam computed tomography (CBCT) to assess internal organ movements and reduce daily setup uncertainties [13,14]. Specifically, during daily treatment, the dose given to the target and surrounding critical organs can be calculated from CBCT images [15,16].

Patient positioning errors and changes in rectum filling create uncertainty in radiotherapy applications [17]. However, in the daily CBCT images used to minimize this uncertainty, rectum filling and the received rectum dose do not appear as planned in the simCT [18]. The percent change in rectal volume during prostate radiotherapy can also affect the calculated rectum dose [19]. Indeed, the actual dose received by the rectum is higher than the planned dose as calculated by simCT images [20].

The aim of this study was to assess the effects of laxative use on rectal volume changes to estimate potential effects on rectal dose changes using weekly CBCT images, which were collected during VMAT in patients with prostate cancer.

Material and methods

Patient selection

In our clinic, retrospective assessment of treatment files between November 2015 and October 2017 showed that laxatives (magnesium hydroxide) were prescribed before treatment in 10 patients with prostate cancer, and were used during treatment. We randomly selected another 10 patients with prostate cancer who were treated with radiotherapy in our clinic, but did not use any laxatives during treatment. SimCT images, treatment plans, and CBCT images were obtained for these 20 patients, who were subdivided into 2 groups based on whether or not they used laxatives.

We used the following inclusion criteria: patients who completed definitive or adjuvant

radiotherapy with VMAT; laxative use began at least four days prior to simCT imaging; and laxatives were used without interruption during treatment. Patients that received hybrid treatment with 3D-CRT or laxative use that was interrupted during treatment, were excluded.

The study was approved by the local ethics committee on 08/02/2018 with decision number of 3/7.

Simulation and treatment

Patients were immobilized with a knee support and footrest while in the supine position. SimCT images (Aquilion-LB, Toshiba, Japan) were obtained with a 2-mm cross-sectional thickness that covered the entire pelvis. All patients were recommended to drink 500 mL of water after emptying their bladder and wait for about 1 hour before simCT imaging. The same procedure was repeated before each radiotherapy treatment fraction. Our clinic did not routinely use a specific protocol to achieve an empty rectum. Low gas and well-balanced diet with plenty of fiber was advised to all patients. For patients with excessive rectum filling, simCT imaging was repeated after defecation. Laxatives were prescribed to patients whose excessive rectum filling had not changed between repeated simCT images. In patients with laxative use, magnesium hydroxide (30-60 mL/day peroral at bedtime) was initiated at least four days prior to simCT imaging and was recommended for use during treatment.

Patients received one of three different treatment volumes defined as follows. The first was radical radiotherapy, in which the prostate gland and seminal vesicle were contoured as the clinical target volume (CTV). A 5-mm posterior margin and 8-mm margins in all other directions were used to create a planning target volume (PTV). The second treatment volume was pelvic treatment, which was delivered to the prostate gland, seminal vesicle, and pelvic lymph nodes, followed by a prostate gland and seminal vesicle boost. The CTV of this treatment included the obturator, external iliac, internal iliac, and S1-2 sacral lymph nodes. For pelvic treatment, 5-mm margins in all directions were used to define the PTV. The final treatment volume was given to patients treated with salvage radiotherapy to control biochemical failure after radical prostatectomy. This CTV included the clips of the prostate gland and seminal vesicle bed. The Radiation Therapy Oncology Group (RTOG) contouring atlas [21] was used as the reference to create the surgical bed, with the same PTV margins that were used for radical radiotherapy. Finally, the bladder, the rectum from the anal canal to the sigmoid curve, bilateral femoral heads, and small intestine were contoured as the organs at risk (OARs).

All patients were treated with fraction doses of 1.8-2 Gy in 38-41 fractions, and CBCT imaging was performed with the Elekta XVI pelvis M20 protocol every day in the absence of fiducial markers. In our clinic, placement of gold seed markers into the prostate was not performed routinely during VMAT preparation. Radiotherapy schedules of each group of patients were summarized at Table 1. VMAT treatment plans were created with double arc on the CMS Monaco 5.1 treatment planning system using 6 MV photon energy. In all treatment plans, 95% of the prescribed dose was positioned to cover 98% of the target volume. OAR doses were kept below the tolerance dose limits of the Quantitative Analysis of Normal Tissue Effects in the Clinic (QUANTEC) [22].

Cone-beam computed tomography

To evaluate the original treatment plans on the CBCT images, we first matched patient CBCT and simCT images using bone tissue matching. The original treatment plans were re-calculated in the same isocentre after CBCT electron density information was specified in the treatment planning system for Elekta XVI Pelvis M20 imaging.

CBCT images were obtained daily during treatment and assessed by bone tissue matching. CBCT imaging was repeated before treatment in the following three cases: 1) after defecation of patients with excess rectum filling, 2) after prolonged waiting in patients with an empty bladder, and 3) after appropriate corrections for the PTV.

Table 1: Treatment characteristics of 20 patients with prostate cancer whose separated into two groups as those who either used (L+) or did not use (L-) laxatives during radiotherapy (RT).

Treatment Volume	Dose (Gy)	L (+) (n:10)	L (-) (n:10)
Radical RT	76- 78	6	4
Pelvic RT	74	0	2
Salvage RT	70.2- 72	4	4

The CBCT image obtained on the first day of treatment was accepted as the first week image, and eight CBCT images for each patient were included in the study. Dosimetric calculations were performed on a total of 160 images. The rectum was contoured in every CBCT image. The VMAT treatment plan of each patient was re-calculated at the same treatment centre on corresponding CBCT images. The rectum volumes and doses were evaluated on CBCT images. The rectum mean dose (Dmean) and percent rectum volume (Vx) receiving 40, 50, 60, 70, and 75 Gy were evaluated in the dose volume histogram (DVH).

Statistical analysis

IBM SPSS Statistics version 21 (www.spss.com) was used for the statistical analysis. The Wilcoxon test was used to analyse the rectum variations of each group of patients and to evaluate the planned and delivered dose parameters. A p-value < 0.05 was considered statistically significant.

Table 2: Comparison of mean rectum doses (±standart deviations) between the treatment plans using treatment planning tomography (simCT) and cone-beam computed tomography (CBCT)

	L (+)			L (-)		
	simCT	CBCT	p-value	simCT	CBCT	p-value
D_{mean} (Gy)	39 (±5.1)	43 (±5.6)	0.009	40 (±4.9)	44 (±5.8)	0.047
V_{40} (%)	50 (±8)	58 (±9)	0.013	54 (±11)	57 (±10)	0.44
V_{50} (%)	35 (±7)	43 (±9)	0.013	35 (±8)	41 (±15)	0.16
V_{60} (%)	23 (±6)	31 (±9)	0.013	21 (±6)	30 (±14)	0.028
V_{70} (%)	9 (±5)	17 (±8)	0.005	9 (±6)	20 (±13)	0.013

Abbreviations: D_{mean}: Mean rectum dose, L (+): Group with laxative use, L (-): Group without laxative use, V_x: % volumes of the rectum receiving 40, 50, 60, and 70 Gy.

Table 3: Comparison of mean rectum doses (±standart deviations) between the patient groups with and without laxative use.

		simCT	p-value	CBCT	p-value
D_{mean} (Gy)	L (+)	39 (±5.1)		43 (±5.6)	
	L (-)	40 (±4.9)	0.50	44 (±5.8)	0.87
V_{40} (%)	L (+)	50 (±8)		58 (±9)	
	L (-)	54 (±11)	0.38	57 (±10)	0.72
V_{50} (%)	L (+)	35 (±7)		43 (±9)	
	L (-)	35 (±8)	0.87	41 (±15)	0.95
V_{60} (%)	L (+)	23 (±6)		31 (±9)	
	L (-)	21 (±6)	0.64	30 (±14)	0.87
V_{70} (%)	L (+)	9 (±5)		17 (±8)	
	L (-)	9 (±6)	0.64	20 (±13)	0.87

Abbreviations: CBCT: Cone-beam computed tomography, D_{mean}: Mean rectum dose, L (+): Group with laxative use, L (-): Group without laxative use, simCT: Treatment planning tomography, V_x: % volumes of the rectum receiving 40, 50, 60, and 70 Gy.

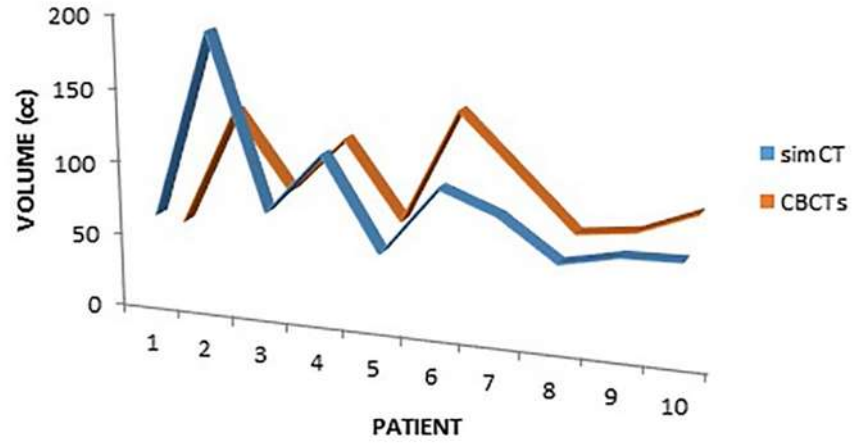

Figure 1: Comparison diagram of rectum volume changes in patients with laxative use, based on treatment planning-tomography (simCT) and mean cone-beam tomography (CBCTs) evaluations of 10 patients.

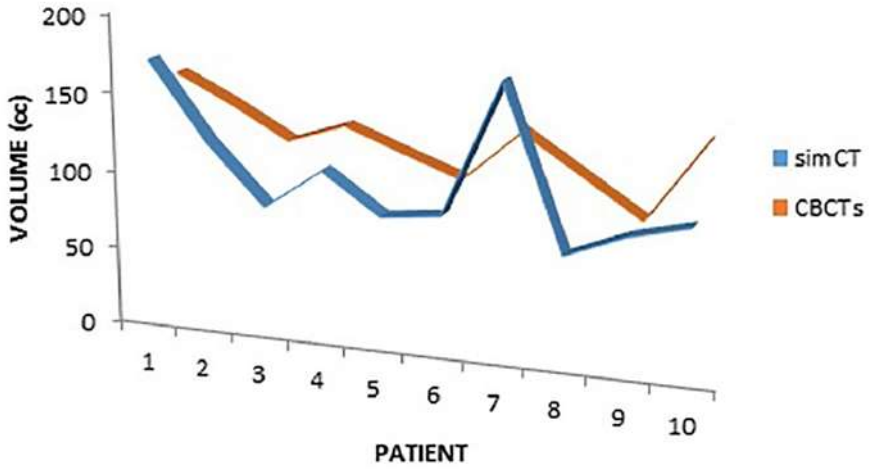

Figure 2: Comparison diagram of rectum volume changes in patients without laxative use, based on treatment planning-tomography (simCT) and mean cone-beam tomography (CBCTs) evaluations of 10 patients.

Results

This study included 820 daily CBCT images obtained from a total of 20 patients. In the group of patients with laxative use, a mean of 39 (38-43) CBCT images were collected during a mean of 39 (39-43) treatment fractions. In the group without laxative use, a mean of 42 (39-46) CBCT images were collected during a mean of 40 (39-43) treatment fractions. The total number of repeated CBCT images due to changes in treatment position or rectum and bladder filling were 34 and 40 in the groups with and without laxative use, respectively. When the CBCT images were examined individually, the number of daily repeated CBCT images after defecation due to excess rectum filling was five in three patients from both groups.

We then evaluated changes in rectum volume during treatment on a total of 160 weekly CBCT images. This revealed that in the group with laxative use, the mean rectum volumes were 85.69 cc (± 41.57) in simCT images and 89.17 cc (± 31.16) in CBCT images (Figure 1). Thus, the mean rectum volume increase during treatment was 4%. Alternatively, in the group without laxative use, the mean rectal volumes were 106.87 cc (± 36.93) in simCT images and 120.96 cc (± 20.58) in CBCT images (Figure 2). In this group, the mean rectum volume increase during treatment was 13%. Overall, CBCT images showed higher mean rectal volumes than simCT images in the groups with and without laxative use ($p = 0.24$ and $p = 0.13$, respectively). Furthermore, analysing these results based on laxative use revealed that the group without laxative use had a higher mean rectal volume compared with the group with laxative use in both simCT and CBCT images ($p = 0.11$ and $p = 0.028$, respectively).

We next evaluated the resulting changes in rectum doses on weekly CBCT images. The Dmean values in the treatment plans for the group with laxative use were 39 Gy on simCT images and 43 Gy on CBCT images ($p = 0.009$). Thus, the 4% increase in mean rectal volume resulted in a 10% increase in Dmean during treatment. In the group without laxative use, the Dmean values in the treatment plans were 40 Gy on simCT images and 44 Gy on CBCT images ($p = 0.047$). In this group, a 13% increase in mean rectal volume resulted in a 10% increase in mean rectum dose during treatment.

In the group with laxative use, CBCT images showed significantly elevated V40, V50, V60, and V70 values relative to the simCT images ($p = 0.013$, $p = 0.013$, $p = 0.013$, and $p = 0.005$, respectively) (Table 2). Thus, a 4% increase in rectal volume during treatment resulted in an 8% increase in V40, V50, V60, and V70 values. In 6 of these patients, the total doses prescribed were 76-78 Gy, CBCT images showed higher V75 values relative to simCT. Specifically, the mean V75 values estimated from the simCT and CBCT images were 4% (0.3%-9%) and 10% (5%-13%), respectively.

In the group without laxative use, CBCT images showed significantly elevated V40, V50, V60, and V70 values relative to the simCT images ($p = 0.44$, $p = 0.16$, $p = 0.028$, and $p = 0.013$, respectively) (Table 2). Thus, a 13% increase in rectal volume during treatment resulted in 3%, 6%, 9%, and 11% increases in V40, V50, V60, and V70, respectively. In 4 of these patients, the total doses prescribed were 76-78 Gy, CBCT images showed higher V75 values relative to simCT. Specifically, the mean V75 values estimated from the simCT and CBCT images were 8% (4%-10%) and 20% (5%-39%), respectively.

Finally, individual evaluation of DVH parameters in both simCT and CBCT images revealed that there were no significant differences between the groups with and without laxative use (Table 3).

Discussion

Treatments such as evacuation techniques, dietary interventions, laxatives, and enemas have been applied to limit changes in rectum volume during daily radiotherapy treatment. Of these methods, one of the most common is the use of laxatives. However, reports on the efficacy of laxative use differ [7]. Indeed, Smitsmons et al. [23] showed that laxative use and adherence to dietary plans significantly lower faeces and gas percentages observed on 576 CBCT images ($p < 0.001$). Alternatively, Oates et al. [24] analysed 435 CBCT images and reported that laxative use does not significantly affect general rectum filling ($p = 0.13$). Here, our results revealed that patients with laxative use had smaller rectal volumes than patients without in both simCT and CBCT images. During treatment, patients with laxative use had smaller changes in rectal volume than in those without. Furthermore, in the group with laxative use, all DVH parameters were significantly affected. Alternatively, in the patients without laxative use, significant changes were found only in the V60 and V70 values despite having larger rectal volumes.

Previously, Collery et al. [25] evaluated a total of 360 CBCT images from 9 prostate cancer

patients who were treated with 7-field-IMRT. This revealed that the treatment significantly increased all DVH parameters relative to the initial treatment plan ($p < 0.05$). Furthermore, rectum V50 and V60 fell below the tolerance limits despite the increase in treatment, while the rectum V70 and V75 increased above the tolerance limits. Here, we observed an increase in all DVH parameters on the CBCT images, which were evaluated in both patient groups. Importantly, in prostate radiotherapy applications with VMAT, the actual mean values did not exceed the QUANTEC tolerance limits for any of the significantly altered DVH parameters. We did not statistically evaluate the V75 values in both groups due to the small number of patients.

Ariyaratne H. et al. [14] reported that in prostate cancer patients treated with 7-field-IMRT, daily rather than weekly CBCT imaging decreases the rectum dose and increases the coverage dose of the CTV. In that study, a total number of 844 CBCT images were collected from 20 patients. The current study included 820 CBCT images taken from 20 prostate cancer patients who received VMAT. Importantly, fewer CBCT images were collected from the group with laxative use relative to the group without because this group received fewer treatment fractions. Although the total number of daily repeated CBCT images was lower in the group with laxative use, the number of daily repeated CBCT images due to rectum fullness was the same in both groups.

This study had several limitations, including the retrospective design, inhomogen distribution of treatment variables between groups and the absence of clinical side effect assessment. Thus, future prospective studies should evaluate daily CBCT images in combination with a clinical side effect assessment to provide more information on the actual doses of the OARs.

In conclusion, in patients undergoing prostate radiotherapy, rectum volumes and radiation doses increase regardless of laxative use. However, laxative use during treatment decreases the likelihood of having an increased rectal volume. Furthermore, contrary to the expected outcomes, laxative use significantly increases all DVH parameters. Thus, dose constraints should be kept as low as possible during treatment planning to anticipate that rectal DVH parameters can increase during treatment.

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Original Article

Expression of CD10, BCL-6 and MUM-1 Markers and Their Effects on Prognosis in Diffuse Large B Cell Lymphoma

Diffüz Büyük B Hücreli Lenfoma'da CD10, BCL-6 ve MUM-1 Markerların Ekspresyonu ve Prognoz Üzerine Etkisi

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ABSTRACT

Background and Aim: Immunohistochemistry may serve as a surrogate to define Diffuse large B-cell lymphoma (DLBCL) cases as germinal center B cell-like (GCB) or non-GCB subtypes and to provide prognostic information. In this study, we aimed to investigate the frequency and prognostic impact of CD10, B-cell lymphoma 2 and 6 (BCL2 and BCL6) and multiple myeloma oncogene 1 (MUM1) expressions in pathology sections of patients with DLBCL to determine the response of these subgroups to the rituximab including chemotherapy regimens.

Materials and Method: Patients were grouped into 2 regarding the chemotherapy regimens they were treated, as cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) or Rituximab-CHOP. The treatment response, follow-up periods and outcomes of patients were recorded. The immunohistochemical panel was stained in pathology sections for CD10, BCL6 and MUM1/IRF 4 biomarkers. The patients were subgrouped as GCB or ABC regarding the immunohistochemical panel.

Results: Totally 81 patients, (39 male, 42 female) were included in the study. At the time of diagnosis, CD10 was positive in 31 patients (38.3%); BCL-6 in 53 patients (65.4%); MUM-1 was positive in 47 patients (58%) and BCL-2 was positive in 53 patients (65.4). With these results, 36 patients (44.4%) were in the GCB group and 45 patients (55.6%) were in the ABC group. No significant difference was found between the individual markers and subgroups in survival analyses.

Conclusion: We did not determine any significant effect of CD10, BCL-6, MUM-1 or BCL-2 positivity or GCB and non-GCB groups in the survival of patients with DLBCL.

Keywords: Diffuse large B-cell lymphoma; Prognostic Impact, ABC and GCB

ÖZET

Giriş ve Amaç: İmmünohistokimya kullanılarak, Diffüz büyük B hücreli lenfoma (DBBHL) vakaları germinal merkez B hücresi benzeri (GCB) ve ABC grubu olmak üzere prognostik açıdan önemli olan alt tiplere ayrılabilir. Biz bu çalışmada DBBHL'li hastaların patoloji preparatlarında CD10, B hücreli lenfoma 2 ve 6 (BCL2 ve BCL6) ve multipl miyelom onkogen 1 (MUM1) markerlarının sıklığını ve rituksimab içeren kemoterapi gruplarındaki prognostik etkisini araştırmayı amaçladık.

Yöntem ve Gereçler: Hastalar, kemoterapi rejimleri açısından, siklofosfamid, doksorubisin, vinkristin ve prednizon (CHOP) veya Rituksimab-CHOP olarak 2'ye ayrıldı. Hastaların tedaviye yanıtı, takip süreleri ve tedavi sonuçları kaydedildi. Tanı anındaki patoloji preparatlarından CD10, BCL6 ve MUM1 / IRF 4 biyomarkerları için boyama yapılarak hastalar immünohistokimyasal panel açısından GCB veya ABC olarak alt gruplara ayrıldı.

Bulgular: Toplam 81 hasta (39 erkek, 42 kadın) çalışmaya dahil edildi. Tanı sırasında 31 hastada (% 38,3) CD10; 53 hastada BCL-6 (% 65,4); MUM-1 47 hastada (% 58) ; BCL-2 53 hastada (65,4) pozitifti.

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Bu sonuçlarla 36 hasta (% 44.4) GCB grubu, 45 hasta (% 55.6) ABC grubu olarak tanımlandı. Sağkalım analizinde gruplar ve biyomarkerlar arasında istatistiksel olarak anlamlı bir farklılık saptanmamıştır.
Tartışma ve Sonuç: DLBCL hastalarının sağkalımında CD10, BCL-6, MUM-1 veya BCL-2 pozitifliği veya GCB ve ABC gruplarının anlamlı bir etkisi saptanmamıştır.

Anahtar Kelimeler: Diffüz Büyük B Hücreli Lenfoma; Prognositk Etki, ABC ve GCB

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma type worldwide accounting for 30-40% of the cases. DLBCLs are derived from the germinal center or post-germinal center B cells. They are exceedingly heterogeneous with a wide variation in genetic features, morphology, and patient outcomes [1]. In more than half of the patients, long-term disease-free survival is expected but on the other hand, approximately 30-40% of patients do not respond properly to the current treatments [2-4]. In that aspect, some biomarkers are required to define the outcomes of DLBCL subgroups.

DLBCLs are classified as germinal center B cell-like (GCB) group, activated B cell-like (ABC) group and unclassified group by gene expression profiling. Not only the morphology but also the therapeutic responses to these subgroups are known to be different from each other [5, 6]. Immunohistochemistry may serve as a surrogate to define DLBCL cases as GCB or non-GCB subtypes and to provide similar prognostic information as complementary DNA microarray does [7] because gene expression profiling studies are often long, costly and require fresh frozen tissue samples present in very few cases [8]. In that aspect, using some antibodies such as CD10, B-cell lymphoma 2 or 6 (BCL2 or BCL6), and multiple myeloma oncogene 1 (MUM1) algorithms have been developed to identify these subtypes [9].

In this study, we aimed to investigate the frequency and prognostic impact of CD10, BCL2, BCL6, and MUM1 expressions in pathology sections of patients with DLBCL to define the GCB and ABC subgroups and to determine the prognostic impact of these subgroups to the rituximab including chemotherapy regimens.

Material and Method

Though 104 patients included in the study, 23 of them excluded due to lack of data. Totally 81 (39 male, 42 female) patients diagnosed with DLBCL and treated in Gazi University Medical Faculty, Medical Oncology Department, between January 1997 and October 2010 were analyzed in this study. The study was approved by the local ethics committee.

The patient records were retrospectively reviewed. The stages of patients were determined regarding Ann Arbor classification and IPI scores were recorded in all patients [10].

Patients were grouped into 2 regarding the chemotherapy regimens they were treated, as cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) or Rituximab-CHOP. The treatment response, follow-up periods and outcomes of patients were recorded.

The pathology sections at the time of the first diagnosis were reached. Hematoxylin-Eosin stained and immunohistochemical sections of all patients were evaluated retrospectively. Moreover, in all patients, the immuno-histochemical panel was stained in pathology sections for CD10, BCL-6, BCL-2 MUM1/IRF 4 biomarkers.

An immunohistochemical examination was performed to determine the CD 10, BCL-6, MUM-1 and BCL-2 expressions on formalin-fixed paraffin sections using the streptavidin-biotin triple indirect immunoperoxidase method. Sections were cut to 4 μm thickness, de-paraffinized in xylene and rehydrated through graded alcohol in distilled water. Antigen retrieval was done in a commercial buffer by autoclaving for 12 hours at 56 ºC. After autoclaving, sections were allowed to cool at room temperature for 20 minutes,

Table 1. Antibodies used for immunohistochemical staining

Antibody	Clon	Isotype	Dilution	Brand Name
CD10	56C6	Mouse monoclonal	Ready to use	NovoCastra (UK)
BCL-6	PG-B6p	Mouse monoclonal	1:50	DakoCytomation (Denmark)
MUM1	MUM1p	Mouse monoclonal	Ready to use	DakoCytomation (Denmark)
BCL-2	124	Mouse monoclonal	Ready to use	DakoCytomation (Denmark)

rinsed thoroughly with distilled water and placed in phosphate buffer saline (PBS). Endogenous peroxidase activity was blocked with hydrogen peroxide and, subsequently, slides were washed with PBS. All primary antibodies were incubated for 2 hours at room temperature. Following primary antibody incubation, sections were washed thoroughly with PBS and incubated with secondary antibody (Ultra streptavidin detection system-HRP; Signet, Massachusetts, USA) for 20 minutes at room temperature. Subsequently, sections were washed with PBS and incubated with Streptavidin-biotin complex (Dako Cytomation, Denmark) for 30 minutes at room temperature. Following a final wash with PBS, immunoreactivity was visualized with AEC (3-Amino 9-Ethylcarbazole; Lab-Vision, Neomarkers, USA). Finally, sections were briefly counterstained with Mayer's hematoxylin and examined by light microscopy. Reactive tonsil tissues that were known to express the markers of interest were used as positive controls.

All specimens were analyzed in a semi-quantitative way after a thorough examination of the whole immunostained slides. The ratio of positively stained tissues to all tumor tissues was determined. As defined before by Hans et al, more than 30% staining in tumoral cells was defined as positive [9]. The patients were subgrouped as GCB or ABC regarding the immunohistochemical panel.

Antibodies used for immunohistochemical staining and their brand names are summarized in Table 1.

Statistical Analysis

The Statistical Package for the Social Sciences program was used for data analysis (for Windows 17.0, SPSS Inc., USA). Variables were analyzed with the One-Sample Kolmogorov-Smirnow test for the determination of normal distribution. Normally distributing data were expressed as mean± standard deviation and non-normally distributing data were expressed as median (minimum-maximum). The Mann-Whitney test was used to compare ABC and GCB subgroups. In the comparison of more than 2 groups, Kruskal-Wallis variance analysis was performed. Pearson correlation analysis was performed to evaluate the correlation between CD10, BCL-6, MUM1, BCL-2, and other parameters.

Complete remission (CR) was defined as the total disappearance of tumors after treatment. Partial remission (PR) was defined as a more than 50% decrease in the tumor while stable disease (SD) was defined as less than 50% decrease or less than a 25% increase in the tumor. Progressive disease (PD) was defined as a more than a 25% increase in tumor or determination of new tumor foci.

The Kaplan-Meier survival curves and Log-rank test were used to represent the overall survival (OS) or progression-free survival (PFS) distributions. Overall survival was defined as the period from the time of diagnosis to death from any cause or the last contact. The significance level was set as $p \leq 0.05\%$.

Results

Totally 81 patients, (39 male, 42 female) were analyzed in the study. The mean age of the patients was 56.1±14.8 (range: 18–82) years. The demographic and clinical features of patients regarding the immunohistochemical subgroups are summarized in Table 2. At the time of diagnosis, CD10 was positive in 31 patients (38.3%), BCL-6 in 53 patients (65.4%), MUM-1 was positive in 47 patients (58%) and BCL-2 was positive in 53 patients (65.4). According to the algorithm defined by Hans et al, 36 patients (44.4%) were in the GCB group and 45 patients (55.6%) were in the ABC group. Patients were divided into two groups as < 60 years (younger), ≥ 60 years (elderly). While 47% of the patients in the GCB subgroup were young, 62% of the patients in the ABC subgroup were young, but the difference between these two groups was not statistically significant (p =0.17).

The median follow-up time was 27 ± 24.08 months in study participants. No significant difference was found between the individual markers and subgroups in terms of OS and PFS in survival analysis. In younger patients than in elderly patients and low-risk patients according to IPI risk scoring, OS and PFS durations were found to be higher. Besides, OS and PFS durations were significantly longer in patients with CR (Table 3).

Twenty-five (69%) of the 36 patients in the GCB group received the R-CHOP regimen, and 11 (31%) of them received the CHOP regimen, while 25 (56%) of the 45 patients in the ABC group received R-CHOP and 20 (44%) of them received the CHOP regimen. The 5-year OS rate in the GCB group was 78% in the R-CHOP group and 49% in the CHOP group (p = 0.90), while the 5-year OS rate in the ABC group was 73% in the R-CHOP group and it was 75% in the CHOP group (p = 0.54) (Figures 1a and 1b).

Of the 28 BCL-6 (-) patients, 21 (75%) received the R-CHOP regimen while 7 (25%) received the CHOP regimen. Of the 53 patients with BCL-6 (+), 29 (55%) received the R-CHOP regimen and 24 (45%) received the CHOP regimen. In the BCL-6 (-) patient group, the 5-year OS rate was 54% in the R-CHOP regimen group and 57% in the CHOP regimen group (p = 0.51). In the BCL-6 (+) patient group, the 5-year OS rate was 84% in the R-CHOP regimen group and 72% in the CHOP regimen group (p = 0.60).

When the CHOP and R-CHOP regimens were compared according to the 5-year OS and 3-year PFS rates, OS and PFS rates were higher in the group receiving R-CHOP regimen but did not reach a statistically significant level (5-year OS rates were 76% and 64%, respectively) (p = 0.39).

When patients were analyzed according to their chemotherapy (CT) regimens, adding rituximab to CT increased the 5-year OS rate in the GCB group, but this did not reach a statistically significant level (Table 4).

Discussion

In this study, we have analyzed the role of immunostaining in predicting the prognosis of patients with DLBCL who were treated with CHOP or R-CHOP. We determined that, among study participants, 44.4% were in the GCB group and 55.6% were in the ABC group. The rates of CD10, BCL-6, MUM-1 and BCL-2 positivity were 38.3%, 65.4%, 58% and 65.4%, respectively. Any significant difference was not found between the individual markers and subgroups in terms of OS and PFS in survival analysis.

Regarding the morphological features, DLBCLs are classified as GCB, ABC and unclassified groups by gene expression profiling. Masir et al [11] reported the GCB subtype in 37% and ABC subtype in 49% of patients. Wawire et al [12] reported the GCB cell type in 40.6% of 165

DLCBL patients in their study. We reported the GCB and ABC prevalence as 44.4% and 55.6%, respectively; and our data were compatible with the literature.

Prognostic factors in DLBCL are highly important in management and determining appropriate treatment modalities. For that reason, immunohistochemical markers have been studied before, but conflicting results are present in previous literature.

Table 2: Demographical and clinical features of patients regarding the immunohistochemical subgroups

Characteristics	ABC n (%)**	GCB n (%)**	Total (n/%)*	p[a]
Gender				
Male	21 (46.7)	18 (50)	39 (48.1)	0.765
Female	24 (53.3)	18 (50)	42 (51.9)	
Age(years)				
<60	28 (62.2)	17 (47.2)	45 (55.6)	0.177
≥60	17 (37.8)	19 (52.8)	36(44)	
Stage (Ann-Arbor)				
1	6 (13.3)	8 (22.2)	14 (17.3)	NA
2	24 (53.3)	12 (33.3)	36 (44.4)	
3	11(24.4)	8 (22.2)	19 (23.5)	
4	4 (8.9)	8 (22.2)	12 (14.8)	
B symptom				
Present	24 (53.3)	18 (50)	42 (51.9)	0.765
Absent	21 (46.7)	18 (50)	39 (48.1)	
Bulky mass				
Present	22 (48.9)	6 (16.7)	28 (34.6)	0.002
Absent	23 (51.1)	30 (83.3)	53 (65.4)	
LDH				
≤N	20 (44.4)	19 (52.8)	39 (48.1)	0.456
>N	25 (55.6)	17 (47.2)	42 (51.9)	
ECOG performance				
0	10 (22.2)	4 (11.1)	14 (17.3)	NA
1	21 (46.7)	21 (58.3)	42 (51.9)	
2	5 (11.1)	5 (13.9)	10 (12.3)	
3	7 (15.6)	5 (13.9)	12 (14.8)	
4	2 (4.4)	1 (2.8)	3 (3.7)	
EN involvement				
+	29 (64.4)	23 (63.9)	52 (64.2)	0.959
-	16 (35.6)	13 (36.1)	29 (35.8)	
IPI risk group				
0-1	25 (55.6)	21 (58.3)	46 (58.6)	NA
2	10 (22.2)	4 (11.1)	14 (17.3)	
3	7 (15.6)	6 (16.7)	13 (16)	
4-5	3 (6.7)	5 (13.9)	8 (9.9)	
IPI				
0	15 (33.3)	10 (27.8)	25 (30.9)	NA
1	10 (22.2)	11 (30.6)	21 (25.9)	
2	10 (22.2)	4 (11.1)	14(17.3)	
3	7 (15.6)	6 (16.7)	13 (16)	
4-5	3 (6.7)	5 (13.9)	8 (9.9)	
Treatment				
R-CHOP	25 (55.69	25 (69.4)	50 (61.7)	0.201
CHOP	20 (44.4)	11 (30.6)	31 (38.3)	
Treatment Response				
Unevaluated	1 (2.2)	1 (2.8)	2 (2.5)	NA
CR	37 (82.2)	30 (83.3)	67 (82.7)	
PR	5 (11.1)	1 (2.8)	6 (7.4)	
SD	1 (2.2)	1 (2.8)	2 (2.5)	
PD	1 (2.2)	3 (8.3)	4 (4.9)	

p[a]: Pearson chi-square analysis *: column % **: row % NA: not applicable; IPI: International prognostic index; EN:Extranodal involvement; CR:Complete remission; PR: Parita remission; SD:Stabil disease; PD:Progressive disease

Table 3: The effects of subgroups and general patient characteristics on OS and PFS

		Number (%)	5 year OS (%)	p	3 year PFS (%)	pA
Age	<60	45(56)	91	6	85	4
	>60	36(44)	47		51	
Stage	1	14(17)	61	0.34	77	0.32
	2	36(44)	79		74	
	3	19(24)	53		50	
	4	12(15)	75		75	
IPI risk	0-1	46(57)	84	5	83	6
group	2	14(17)	45		55	
	3	13(16)	29		42	
	4-5	8(10)	62		62	
LDH	<N	39(48)	45	0.25	70	0.18
	>N	42(52)	68		69	
EN involvemer	Yok	29(36)	69	0.45	63	0.30
	Var	52(64)	65		72	
KT group	RCHOP	50(62)	73	0.60	76	0.39
	CHOP	31(38)	67		64	
Response	D*	2(2)	0	<0.001	0	<0.001
	CR	67(84)	79		79	
	PR	6(7)	33		25	
	SD	2(2)	50		50	
	PD	4(5)	25		25	
CD10	(-)	50(62)	74	0.43	77	0.39
	(+)	31(38)	65		57	
BCL-6	(-)	28(35)	62	0.20	67	0.15
	(+)	53(65)	74		72	
MUM-1	(-)	34(42)	62	0.43	59	0.37
	(+)	47(58)	77		79	
BCL-2	(-)	28(35)	58	0.46	67	0.42
	(+)	53(65)	72		68	
GCB group		36(44)	63	0.55	60	0.51
ABC group		45(56)	77		78	

Table 4: 5-year OS rates in subgroups according to chemotherapy regimens

		5 year OS (%)	p
R-CHOP	GCB	78	0.64
	ABC	73	
CHOP	GCB	62	0.55
	ABC	75	

* Pearson chi-square analysis

Akyurek et al [13] reported that BCL-6 rearrangement predicted significantly shorter overall survival while BCL-2 rearrangement had no prognostic impact on outcome in 239 patients with DLBCL. Bodoor et al [14] investigated the role of BCL-6, CD10, CD138 and MUM-1 expressions in the prognosis of patients with DLBCL and reported that BCL-6 expression was associated with better overall survival without any impacts of CD10 and MUM-1 on survival.

Culpin et al [15] assessed the prognostic validity of immunohistochemical markers and algorithms identified in the CHOP era in immunochemotherapy-treated DLBCL patients and reported that low CD10 (<10%), low LMO2 (<70%) or high BCL-2 (≥80%) predicted shorter OS and high BCL-2 (≥80%), low BCL-6 (<60%), low GCET1 (<20%) or low LMO2 (<70%) predicted shorter PFS. Hassan et al [16] reported that CD-10 expression in DLBCL was associated with

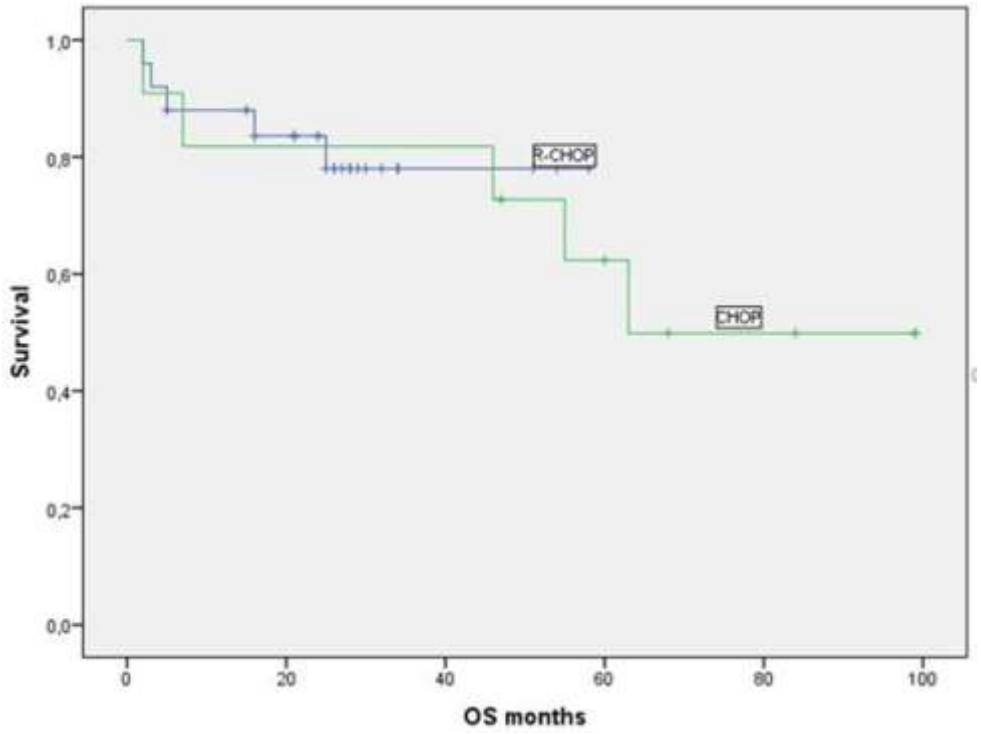

Figure 1a: Chemotherapy-related OS durations in the GCB subgroup (Kaplan-Meier curve)

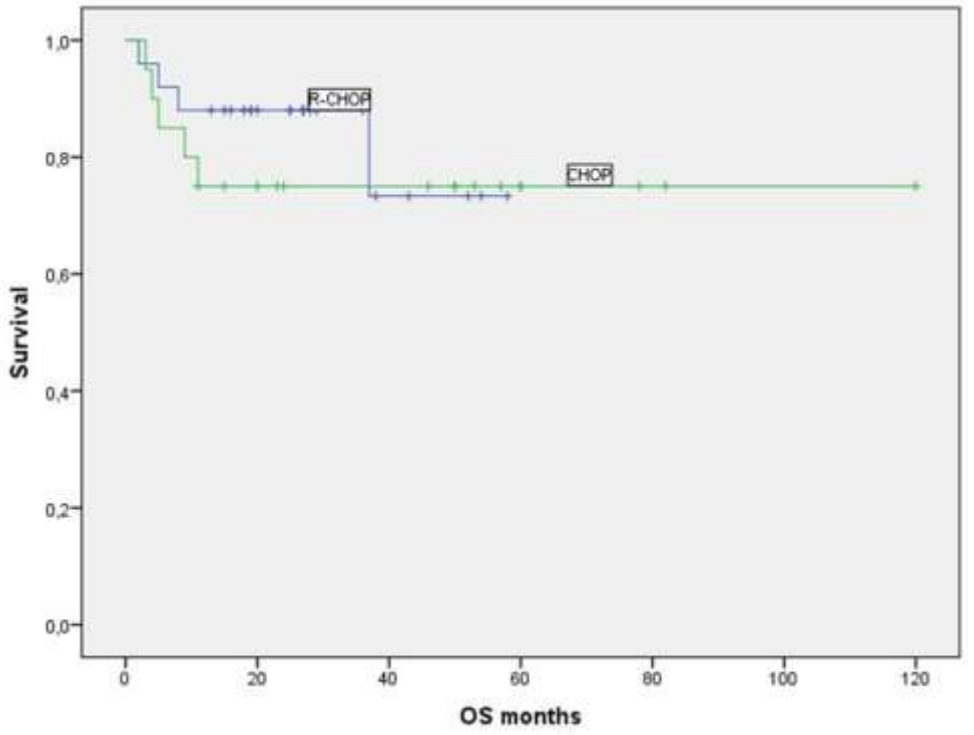

Figure 1b: Chemotherapy-related OS durations in the ABC subgroup (Kaplan-Meier curve)

better immediate clinical response whereas MUM-1 expression was associated with poor immediate clinical response. However, there was no statistically significant association of BCL-6 with an immediate clinical response that was noted after 6 cycles of chemotherapy. Jia et al [17] reported that, among Uygur patients diagnosed with DLBCL, expression of germinal center B (GCB) cell-expressed transcript-1, FOXP1, CD10, BCL-6, and MUM1 was associated with a significantly higher 3-year OS. Dwivedi et al [18] did not determine any statistically significant difference regarding the survival rates between GCB and non-GCB subtypes of DLBCL patients. Recently, in a detailed study of Lu et al [19], using immunohistochemistry and fluorescence in situ hybridization, the prognostic value of Hans algorithm was investigated in 306 cases who were treated with chemoimmunotherapy and GCB subtype was reported to have a better OS and PFS than non-GCB cases. On the other hand, it was also reported that double-positive (CD10(+), MUM1(+)) patients showed similar OS and PFS with the non-GCB group while the (triple-negative (CD10(-), BCL6(-), MUM(-) group) showed similar OS and PFS with the GCB group. Similarly, Chang et al [20] also did not determine any difference in survival of patients with GCB or non-GCB tumors. Although GCB tumors more frequently expressed CD10 and BCL-6 as well as the BCL2 and MYC rearrangements with less frequent expression of MUM1. Abdulla et al reported that concurrent expression of MYC and BCL-2 proteins was strongly associated with inferior survival in DLBCL patients treated with R-CHOP [21].

The main limitations of this study are the retrospective design, the low number of patients and the low follow up time.

In the present study, the 5-year OS rate in patients receiving R-CHOP therapy was 78% in the GCB group, while it was 49% in the group receiving CHOP therapy. The 5-year OS difference in the GCB subgroup is remarkable at 29% among the treatment groups. A similar difference was not detected among the treatment groups in the ABC subgroup (73% in the R-CHOP group, 75% in the CHOP group). Despite this remarkable difference, the difference between the treatment groups in the GCB group was statistically nonsignificant and may be related to the limitations of the study, the low number of patients who reach the primary endpoint, and especially the number of patients.

In conclusion, we did not determine any significant effect of CD10, BCL-6, MUM-1, or BCL-2 positivity or GCB and non-GCB groups in the survival of patients with DLBCL. The addition of rituximab can contribute to treatment success, particularly in the GCB subgroup. Further, prospective, larger studies are warranted to determine the exact role of these markers in the prognosis of DLBCL.

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Original Article

Kinesiophobia in Breast Cancer Survivors and its Relationship with Quality of Life, Comorbidity and Other Clinical Parameters

Meme Kanseri Hastalarında Kinezyofobinin Hayat Kalitesi, Komorbidite ve Diğer Klinik Özellikler ile İlişkisi

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ABSTRACT

Objectives: This cross-sectional study aims to determine the frequency of kinesiophobia in breast cancer survivors and evaluate its relationship with mainly quality of life and comorbidities, also fatigue, lymphedema, and depression.

Material Methods: This study included 54 women with breast cancer who were followed in remission in Aydın Atatürk State Hospital Medical Oncology Clinic between November-December 2020. Clinicopathological characteristics of the patients were recorded. Kinesiophobia was assessed using the Turkish version of Tampa Scale for Kinesiophobia (TSK). Lymphedema was evaluated with bilateral upper extremity measurements. Depressive status and quality of life were determined using the Beck Depression Inventory (BDI) and European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Version 3.0 (EORTC QLQ-C30 v3). Comorbidities were assessed using the Charlson Comorbidity Index (CCI) while fatigue was evaluated by 10 cm visual analogue scale (VAS). The relationship between the TSK and CCI, BDI, VAS-F, EORTC-30 were investigated.

Results: The mean age of patients was 52.11±11.10 years. Of the patients, 36 (66.7%) had kinesiophobia based on having TSK scores above 37. The rate to recieve adjuvant radiotherapy was higher in kinesiophobic group. Kinesiophobic patients had statistically significantly lower global health, physical functioning, and emotional functioning scores, and higher depression and VAS-fatigue scores. Kinesiophobic patients also had higher financial difficulty and higher sypmtom scale scores. Comorbidities and presence of lymphedema did not differ between groups ($p>0.05$). All EORTC QLQ-30 sub-parameter scores except for financial difficulty and symptom severity had negative significant correlations with TSK scores while VAS-fatigue, BDI, EORTC QLQ-30 symptom scale, and financial difficulties showed significant positive correlations. TSK score was not correlated with CCI.

Conclusion: Kinesiophobia is rather frequent in breast cancer survivors and has associations with worse quality of life and higher depression and fatigue scores. These patients should be trained and encouraged for an active life style.

Keywords: breast neoplasms; quality of life; comorbidity; kinesiophobia

ÖZET

Amaç: Bu kesitsel çalışma meme kanseri hastalarında kinezyofobi sıklığının belirlenmesi ve hayat kalitesi ve komorbidite başta olmak üzere yorgunluk, lenfödem, depresyon gibi klinik özellikler ile ilişkisini araştırmayı amaçlamaktadır.

Materyal Metod: Çalışmaya Kasım-Aralık 2020 tarihlerinde Aydın Atatürk Devlet Hastanesi Tıbbi Onkoloji Polikliniği'nde meme kanseri tanısı ile remisyonda izlemde olan 54 kadın dahil edilmiştir. Hastaların tanı anındaki klinikopatolojik özellikleri kaydedildi. Hareketten kaçınma durumu Tampa Kinezyofobi Skalası (TKS) ile değerlendirildi. Lenfödem varlığı her iki koldan çap ölçümüleri ile değerlendirildi. Depresyon varlığının araştırılması için Beck depresyon ölçeği (BDÖ), hayat kalitesinin değerlendirilmesi için Avrupa Kanser Araştırma ve Tedavi Organizasyon Grubu (EORTC) hayat kalitesi formu QLQ-C30 (versiyon 3.0) kullanıldı. Komorbiditelerin belirlenmesi için Charlson Komorbidite

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Indexi (CKI) ve yorgunluk için 10 cm'lik vizüel analog skala (VAS) kullanılmıştır. TKS ile CKI, BDÖ, VAS-yorgunluk, EORTC-30 arasındaki ilişki değerlendirildi.
Sonuçlar: Hastaların ortalama yaşı 52.11±11.10 yıl idi. Hastalardan 36'sı (%66,7) TKS skorlarına göre kinezyofobik olarak sınıflandırıldı (TKS>37). Kinezyofobik grupta adjuvan radyoterapi alma oranı anlamlı olarak daha yüksekti. Ayrıca kinezyofobik hastaların EORTC-30 hayat kalitesi anketinin genel sağlık, fiziksel ve duygusal işlev puanları anlamlı olarak daha düşük; maddi zorluk, semptom skalası, BDÖ ve VAS-yorgunluk puanları ise anlamlı olarak daha yüksekti. Lenfödem varlığı, CKI ve diğer klinik parametrelerde fark tespit edilmedi (p>0.05). Maddi zorluk ve semptom skalası dışındaki tüm EORTC QLQ-30 sub-grup skorları ve TKS arasında negatif yönde anlamlı ilişki saptanırken, VAS-yorgunluk, BDÖ, EORTC QLQ-30 semptom skalası ve maddi zorluk puanları ile anlamlı pozitif ilişki gözlendi. TSK ile CKI arasında ilişki bulunmadı.
Sonuç: Kinezyofobi meme kanseri hastalarında oldukça sık görülmekte olup hayat kalitesi, depresyon ve yorgunlukla ilişkili bulunmuştur. Hastaların bu konuda biligilendirilip daha aktif bir hayat tarzı için motive edilmesi yararlı olabilir.

Anahtar kelimeler: meme kanseri; hayat kalitesi; komorbidite; kinezyofobi

Introduction

Breast cancer is the most common cancer type and leading cause of cancer-related death among women in Turkey [1]. Cancer survivors may experience depression, anxiety, and poor health-related quality of life [2]. Furthermore, fatigue and pain are frequent in cancer patients and survivors and might lead to low level of physical activity and sedentary lifestyle [3-6]. Kinesiophobia is defined as the fear and anxiety of movement and activity due to belief of fragility or susceptibility to injury [7]. The Cognitive Fear Avoidance Model suggests that pain-related fear leads to escape mechanisms resulting in avoidance of movement and activity. Thereafter, in prolonged avoidance, they run a vicious circle occurring due to non-use, disability, and depression [8]. Although there are several papers on kinesiophobia and chronic back pain, musculoskeletal disorders, and cardiovascular diseases, there are few studies about breast cancer and kinesiophobia [9-11]. Previous studies showed that approximately 40% of pain-related disability could be attributed to kinesiophobia [12]. Also, it was propounded that kinesiophobia increased the risk for lymphedema, depression, and poorer upper extremity function in breast cancer survivors [13].

A previous study assessing the relationship between kinesiophobia and global health status on 1236 cancer survivors concluded that fear of movement was significantly related to global health status. The paper further reported that kinesiophobia decreased significantly after rehabilitation with graded activity in high TSK scorers [14].

Approximately 60%-96% of the cancer patients are reported to have high levels of fatigue during or after cancer treatment, which often leads to diminished quality of life [15]. In a study evaluating the quality of life using the Short form-36 (SF-36) in breast cancer survivors, SF-36 physical component score (PCS) and mental component score (MCS) were lower in kinesiophobic patients, where only SF-36 PCS difference was statistically significant. Kinesiophobic patients had also significantly higher mean scores of depression and significant correlations between presence of lymphedema, depression scores, and the TSK score were noted [13]. In this study on 81 breast cancer survivors, a significantly higher rate of lymphedema was also reported in patients with kinesiophobia [13].

We consider that the desire to engage in physical movement may be hampered in breast cancer survivors along with depression and fatigue particularly in case of comorbid diseases. Therefore, we aimed to determine the frequency of kinesiophobia in breast cancer survivors and investigate its relationship with mainly quality of life and comorbidities, secondly lymphedema, fatigue, and depression in the current study.

Material Methods:

This cross-sectional study included 54 patients with breast cancer who were followed in remission in Aydın Atatürk State Hospital Medical Oncology Clinic beetwen November and December 2020. The inclusion criteria were having breast surgery at least a year ago and being followed in remission with diagnosis of breast cancer, at least 6 months since last adjuvant chemotherapy cycle or radiotherapy, being over 18 years, and being able to read and write in Turkish. The exclusion criteria were having metastatic disease, co-existent second malignancy, chronic inflammatory diseases, and cognitive or psychiatric disturbances that may impede with fulfilling the questionnaires.

The demographic characteristics including age, marital and educational status, body mass index, stage of disease, side and type of surgery, hormon receptor status, history of adjuvant chemotherapy, radiotherapy and hormonotherapy were recorded. Lymph-edema of the arm was evaluated by circumferenial measurements of bilateral upper extremities. Measurements were performed at four levels of metacarpal, wrist, 10-cm below and above the lateral epicondyle using a standard retractable fiberglass tape in sitting position with 90° shoulder flexion, elbow extension, and forearm pronation. Assessments were fulfilled by the same physician and noted in cm unit in cm unit. A difference of ≥2 cm at any single location in the affected arm led to the diagnosis of lymphedema.

Kinesophobia

Kinesophobia was evaluted using the Turkish version of Tampa Scale for Kinesophobia (TSK) [16, 17]. This scale is a 17-item scale widely used for chronic musculoskeletal disorders. A 4- point likert questionnaire ranging from “I fully disagree” to “I fully agree” is applied for each item. After reversing the 4th, 8th, 12th, and 16th items, total score of 17-68 is reached. The higher scores indicate higher kinesiophobia level. While the use of total score is advocated in studies, the cut off point of 37 is determined to define severe and mild kinesiophobia [8].

Comorbidity

The presence and intensity of comorbidities was assessed using the Charlson Comorbidity Index (CCI). The index predicts 10-year mortality for patients with various comorbid conditions depending on their strength of association with mortality. Total score is counted summing the scores of 16 available clinical conditions. Higher scores indicate higher mortality risk [18].

Quality of Life

The quality of life was evaluated using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Version 3.0 (EORTC QLQ-C30 v. 3.0). The EORTC QLQ C- 30 is a cancer-specific QOL questionnaire with four-point likert scale with items ranging from 1:not at all to 4: very much. This 30-item self-administered questionnaire evaluates five functional scales physical (PF), role (RF), cognitive (CF), emotional (EF) and social (SF) functioning, and three symptom scales (fatigue, pain, and nausea/vomiting), a global Quality of Life scale, and some single items for evaluation of other complaints of cancer patients (e.g. dyspnea, loss of appetite, sleep disturbances, constipation, and diarrhea), and financial effects of the disease and treatment. All functional scales and individual item scores are indicated on a 0–100 scale. Higher functional scale values indicate better functioning while increased scores of symptom scales and financial difficulties suggest worse symptoms and poorer financial status [19].

Beck Depression Invantory

The depressive status was assessed using the Beck Depression Invantory (BDI). It is a 21-item scale scored 0-3 for each item. Higher scores indicate more severe depressive state, namely 10 refers to minimal depression; 10-18 to mild to moderate depression, 19-29 to severe depression; and above 30 is determined as severe depression [20]. Fatigue severity according to visual analogue scale (VAS 10 cm) was noted where increased scores corresponded to more intense fatigue. The ethics committee aproval was obtined from

Table 1. Demographic and clinical characteristics of breast cancer survivors (n=54)

Clinical variable	% or Median (Interquartile range
Age (years), [Median (Interquartile range)]	53 (16.25)
Education level, n (%)	
Primary and secondary school	35 (64.8)
High school	10 (18.5)
University graduate	9 (16.7)
Body Mass Index (kg/m^2) [Median (Interquartile range)]	28.26 (8.33)
Occupation, n (%)	
Retired	2 (3.7)
Worker	16 (29.6)
Housewife	36 (66.7)
Histological type, n (%)	53 (98.1)
Invasive ductal carcinoma	1 (1.9)
Medullary carcinoma	
Stage at diagnosis, n (%)	
Stage 1	15 (27.8)
Stage 2	26 (48.1)
Stage 3	13 (24.1)
Type of surgery, n (%)	
Breast conservative surgery	34 (63)
Modified radical mastectomy	20 (37)
Side of surgery, n (%)	
Right	25 (46.3)
Left	28 (51.9)
Bilateral	1 (1.8)
Hormone receptor status, n (%)	
Positive	43 (79.6)
Negative	11 (20.4)
Lymph node dissection, n (%)	
ALND	32 (59.3)
SLND	22 (40.7)
Menopausal status, n (%)	
Premenapouse	20 (37)
Menapouse	34 (63)
Adjuvant chemotherapy, n (%)	
Yes	40 (74.1)
No	14 (25.9)
Adjuvant hormonotherapy, n (%)	
Tamoxifen	21 (38.9)
Aromatase inhibitor	22 (40.7)
None	11 (20.4)
Adjuvant radiotherapy, n (%)	
Yes	49 (90.7)
No	5 (9.3)
Lymphedema, n (%)	
Yes	29 (53.7)
No	24 (46.3)
EORTC QLQ-C30 subscales [Median (Interquartile range)]	
Global health status	75 (33.3)
Physical functioning	80 (25)
Role functioning	100 (16.67)
Emotional functioning	83.3 (39.59)
Social functioning	100 (33.33)
Cognitive functioning	83.3 (33.33)
Symptom scales	13.88 (27.78)
Financial difficulties	33.33 (33.33)
Beck Depression Inventory [Median (Interquartile range)]	8 (14)
Charlson Comorbidity Index [Median (Interquartile range)]	3 (2.25)
Tampa Scale of Kinesiophobia (mean±SD)	39.31 ±5.89
VAS-Fatigue [Median (Interquartile range)]	3 (4)

ALND: Axillary lymph node dissection, SLND: Sentinel lymph node dissection, EORTC QLQ-C30: The European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire, SD: Standard deviation, VAS: Visual analogue scale

Table 2. Comparison of clinical characteristics of patients with and without kinesiophobia

Clinical variable	Kinesiophobic (n:36)	No kinesiophobia (n:18)	*p*
Age (years) [Median (Interquartile range)]	53.5 (13)	50.5 (22.5)	0.575
Education level, n (%)			0.059
Primary and secondary school	25 (46.3)	10 (18.5)	
High school	8 (14.8)	2 (3.7)	
University graduate	3 (5.6)	6 (11.11)	
Body Mass Index (kg/m^2) [Median (Interquartile range)]	28.26 (8.47)	28.11 (9.16)	0.811
Occupation, n (%)			0.185
Housewife	27 (50)	9 (16.7)	
Worker	8 (14.8)	8 (14.8)	
Retired	1 (1.9)	1 (1.9)	
Histological type, n (%)			0.667
Invasive ductal carcinoma	35 (64.8)	18 (33.3)	
Medullary carcinoma	1 (1.9)	0 (0)	
Disease duration (months), Median (Interquartile range)]	47.5 (51.25)	43 (43.25)	0.847
Type of surgery, n (%)			
Breast conservative surgery	25 (46.3)	9 (16.7)	0.163
Modified radical mastectomy	11 (20.4)	9 (16.7)	
Side of surgery, n (%)			0.526
Right	15 (27.8)	10 (18.5)	
Left	20 (37)	8 (14.8)	
Bilateral	1 (1.9)	0 (0)	
Hormone receptor status, n (%)			0.232
Positive Negative	16 (50) 9 (16.7)	27 (29.6) 2 (3.7)	
Lymph node dissection, n (%)			0.845
ALND SLND	21 (38.9) 15 (27.8)	11 (20.4) 7 (13)	
Menopausal status, n (%) Premenapouse Menapouse	 13 (24.1) 23 (42.6)	 7 (13) 11 (20.4)	0.842
Adjuvant chemotherapy, n (%)			0.380
Yes No	28 (51.9) 8 (14.8)	12 (22.2) 6 (11.1)	
Adjuvant hormonotherapy, n (%)			0.365
Tamoxifen	12 (22.2)	9 (16.7)	
Aromatase inhibitor	15 (27.8)	7 (13)	
None	9 (16.7)	2 (3.7)	
Adjuvant radiotherapy, n (%)			0.038
Yes No	35 (64.8) 1 (1.9)	14 (25.9) 4 (7.4)	
Lymphedema, n (%)			0.335
Yes No	21 (38.9) 15 (27.8)	8 (14.8) 10 (18.5)	
EORTC QLQ-C30 subscales Median (Interquartile range)]			
Global health status	66.67 (47.92)	83.32 (31.25)	0.014
Physical functioning	73.34 (25.42)	80 (18.3)	0.009
Role functioning	100 (29.16)	100 (0)	0.091
Emotional functioning	75 (47.92)	100 (14.61)	0.015
Social functioning	91.67 (50)	100 (16.69)	0.162
Cognitive functioning	83.33 (33.33)	91.67 (16.70)	0.139
Symptom scales	19.4 (30.55)	11.11 (10.43)	0.013
Financial difficulties	33.33 (33.33)	0 (33.32)	0.034
Beck Depression Inventory Median (Interquartile range)]	12 (17)	5 (9.25)	0.006
Charlson Comorbidity Index Median (Interquartile range)]	4 (1.75)	3 (3)	0.340
Tampa Scale of Kinesiophobia Median (Interquartile range)]	41.5 (6.75)	34 (3.25)	<0.001
VAS-Fatigue Median (Interquartile range)]	4.5 (4.75)	2 (4.5)	0.040

ALND: Axillary lymph node dissection, SLND: Sentinel lymph node dissection, EORTC QLQ-C30: The European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire, VAS: Visual analogue scale.

Adnan Menderes University Clinical Researches Ethics Committee. All patients were provided with information about the study and were given informed consent forms.

Statistical analysis

The SPSS version 11.5 software (SPSS Inc., Chicago, IL, USA) was used for analyses. Histogram and p-plots were examined and Kolmogorov Smirnov test was used to assess data normality before statistical analyses. Continuous variables were presented as mean ± standard deviation and median (interquartile range) and tested by student's t test or Mann Whitney U test. Categorical variables were described as numbers and percentages, and tested by Chi-square or Fisher's Exact tests. Pearson correlation coefficient was used to determine the relationship between continuous variables. Patients were determined to be kinesiophobic if TSK scores were above 37 [8]. Patients with and without kinesiophobia were compared in terms of clinical parameters. $p<0.05$ was considered statistically significant.

Results

The mean age of patients was 52.11±11.10 years. The mean disease duration of 54 patients was 51.61±5.91 months. The clinical characteristics of patients were given in Table 1. Of the patients, 36 (66.7%) had kinesiophobia based on having TSK scores above 37. The mean TSK score was 39.31±5.89 in the whole group. When patients were further divided into as kinesiophobic patients and others, the rate to have recieved adjuvant radiotherapy was higher in kinesiophobic group (p=0.038). Furthermore, kinesiophobic patients had statistically significantly lower global health, physical functioning, and emotional functioning scores and higher depression and VAS-fatigue scores (p=0.014, p=0.009, p=0.015, p=0.006, p=0.040 respectively). Kinesiophobic patients also had higher scores of financial difficulty and sypmtom scales indicating worse financial and somatic symptom burden (p=0.034, p=0.013 respectively). Comorbidities, presence of lymphedema, and other clinical factors did not differ between groups (p>0.05). (Table 2). The Kruskal Wallis test revealed no difference among patients with stage 1, 2, and 3 breast cancer in terms of TSK scores (X^2=2.139, p=0.343).When the correlations between TSK and EORTC scores were analysed, all sub-parameters had significant negative correlations with TSK scores except for scores of the symptom scale and financial difficulties which demonstrated significant positive correlations. Also there were positive significant correlations between TSK scores and VAS-fatigue and BDI scores (p=0.001, r=0.438, p<0.001, r=0.597 respectively). Table 3 shows the correlations between fear of movement, fatigue severity, and comorbidities in patients with breast cancer.

Table 3. Correlations between TSK scores and other clinical characteristics

Clinical variable	p	r
Age (years)	0.973	-0.005
Body Mass Index (kg/m^2)	0.627	0.068
Disease duration (months)	0.931	-0.12
EORTC QLQ-C30 subscales		
Global health status	0.005	-0.381
Physical functioning	<0.001	-0.523
Role functioning	0.002	-0.422
Emotional functioning	<0.001	-0.513
Social functioning	0.027	-0.307
Cognitive functioning	0.027	-0.307
Symptom scales	<0.001	0.522
Financial difficulties	0.022	0.317
Beck Depression Inventory	<0.001	0.597
Charlson Comorbidity Index	0.377	0.123
VAS-Fatigue	0.001	0.438

EORTC QLQ-C30: The European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire, VAS: Visual analogue scale.

Discussion

In this study, the frequency of kinesiophobia and its association with quality of life and comorbidities were investigated in breast cancer survivors. This study revealed that almost two third of breast cancer survivors suffer from kinesiophobia. Also we have observed that patients with kinesiophobia had poorer quality of life, higher depression, and fatigue scores. Comorbidity and lymphedema were not found to have significant relationship with kinesiophobia in breast cancer survivors. In our study group, 66.7% of patients had kinesiophobia which seems higher than

previous studies [13]. The socioeconomic differences of groups assessed might have led to this discrepancy. In the prior studies, it was stated that cancer survivors with increased fear of movement might benefit from rehabilitation programs with graded activity [14]. Therefore, we consider these programs may be offered to all patients with suspected fear of movement.

In a study on 1236 cancer survivors among whom 615 had breast cancer, which evaluated the relationship between kinesiophobia and global health status, kinesiophobia was reported to be inversely associated with global health status determined by the EORTC QLQ-30, similar to our results [14]. Similarly, another study on 62 women with breast cancer evaluating the quality of life using the functional assessment of cancer therapy-breast (FACT-B+4), a significant negative relationship between kinesiophobia and quality of life was reported [21]. In the current study, we also detected significant relationship between kinesophobia and physical and emotional functioning sub-scales of the EORTC QLQ-30.

The breast cancer patients with upper extremity lymphedema may have a belief that their arms might become swollen if they move them and avoid daily activities which can contribute to kinesiophobia [21]. The frequency of lymphedema is 53.7 % in our study group. While previous papers suggest distinct results, a report remarks a cumulative incidence of 41.1% [22]. The study by Gencay et al. noted a significant association between presence of lymphedema and the TSK scores whereas we did not observe such a relationship [13]. Similar to their results, our patients with kinesiophobia had worse quality of life and higher depression scores [13]. The relationship between depression and fear of movement was also revealed in other patient groups [23, 24].

It is known that up to 40% -80% of cancer patients undergoing active treatment suffer from cancer related fatigue which can result in decreased quality of life [25]. In a study evaluating the relationship between kinesiophobia and fatigue, fatigue severity was found to be associated with kinesiophobia in patients with chronic obstructive pulmonary disease [26]. Similarly, we observed a positive correlation between fatigue and kinesiophobia scores in breast cancer survivors and statistically significant difference in terms of fatigue between patients with and without kinesiophobia in our study group. In that study by Vardar et al., kinesiophobia was strongly associated with multisystemic comorbidities in patients with chronic obstructive pulmonary disease [26]. However, we detected no relationship between kinesiophobia and comorbidity in breast cancer survivors in contrast to the case in aforementioned study.

As far as we are concerned, this is the first study to evaluate the relationship between kinesiophobia and comorbidity in breast cancer survivors. Another strong element of the current study is assessing the quality of life with a cancer-specific scale. The limitation of our study is the small sample size. Further studies are required to assess the role of comorbidity on kinesiophobia in these patients.

In this study, we found that kinesiophobia is rather frequent in breast cancer survivors and has relationship with quality of life, depression, and fatigue. These patients should be encouraged for an active life style.

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Original Article

Disease and Clinical Characteristics of Patients with Chronic Myeloproliferative Neoplasms: 11-year Single Center Experience

Kronik Myeloproliferatif Neoplazi Tanılı Hastalarda Klinik Özellikler ve Hastalık Karakteristikleri: 11 Yıllık Tek Merkez Deneyimi

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ABSTRACT

Introductıon: BCR/ABL-negative myeloproliferative neoplasms are characterized by over-production myeloid lineages in the bone marrow. Polycythemia vera, essential thrombocythemia and primary myelofibrosis are the most common myeloproliferative neoplasms. The aim of this study was to analyse patient demographic characteristics, laboratory findings, mutational status together with complications, clinical course and survival.

Methods: This study was conducted on patients diagnosed with myeloproliferative neoplasms between 2008 and 2019. Blood parameters, demographic information, mutation analysis, management, complications and follow-up periods were recorded. Survival rates were calculated and the effect of the parameters on overall survival was analyzed.

Results: Evaluation was made of 247 patients, comprising 105 polycythemia vera, 126 essential thrombocythemia and 16 primary myelofibrosis patients. During follow-up, 11 polycythemia vera, 14 essential thrombocythemia and 2 primary myelofibrosis patients developed thromboembolic complications. Median overall survival could not be reached in polycythemia vera and essential thrombocythemia patient and determined as 70.3 months in primary myelofibrosis patients. Age, LDH, ferritin and platelet/lymphocyte ratio at the time of diagnosis and thromboembolic complications were determined to have a statistically significant effect on survival in all patients. Lower survival rates were seen in the primary myelofibrosis patients although thromboembolic complications were observed at similar rates in all 3 disease subgroups.

Discussion and conclusion: In addition to known risk factors such as age and thromboembolic complications, parameters such as LDH, ferritin and PLR, which may be considered to indicate disease activity and inflammation, can also be used as prognostic markers.

Keywords: myeloproliferative neoplasia, prognosis, thrombosis

ÖZET

Giriş ve amaç: BCR / ABL-negatif miyeloproliferatif neoplaziler, kemik iliğindeki miyeloid öncül hücrelerin aşırı üretimi ile karakterizedir. Polisitemi vera, esansiyel trombositemi ve primer miyelofibrozis en sık görülen miyeloproliferatif neoplazilerdir. Bu çalışmanın amacı hastaların demografik özelliklerini, klinik seyir ve laboratuvar bulgularını komplikasyonlar ve sağkalım ile birlikte analiz etmektir.

Yöntem ve gereçler: Bu çalışmaya 2008-2019 yılları arasında miyeloproliferatif neoplazi tanısı alan hastalar dahil edildi. Kan parametreleri, demografik bilgiler, mutasyon analizi, tedavi, komplikasyonlar ve takip süreleri kaydedildi. Sağkalım oranları hesaplandı ve parametrelerin genel sağkalım üzerindeki etkisi analiz edildi.

Bulgular: 105 Polisitemi vera, 126 esansiyel trombositemi ve 16 primer miyelofibrozis olmak üzere oluşan toplam 247 hasta değerlendirildi. Takip sırasında 11 polisitemi vera, 14 esansiyel trombositemi ve 2 primer miyelofibrozis hastasında tromboembolik komplikasyon gelişti. Polisitemi vera ve esansiyel trombositemi hastalarında ortalama genel sağkalıma ulaşılamadı, ancak primer miyelofibrozis

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hastalarında 70.3 ay olarak belirlendi. Tanı anında yaş, LDH, ferritin ve trombosit / lenfosit oranı ve tromboembolik komplikasyonlarının tüm miyeloproliferatif neoplazi hastalarında sağkalım üzerinde istatistiksel olarak anlamlı bir etkisi olduğu belirlenmiştir. Primer miyelofibrozis hastalarında daha düşük sağkalım oranları görülmesine rağmen, tromboembolik komplikasyon oranı 3 hasta alt grubunun hepsinde benzer oranlarda izlenmiştir.
Tartışma ve sonuç: Yaş ve tromboembolik komplikasyonlar gibi bilinen risk faktörlerine ek olarak, LDH, ferritin ve PLR gibi hastalık aktivitesini ve inflamasyonu gösteren parametreler de bu hastalıkların takibinde prognostik belirteç olarak kullanılabilir.

Anahtar Kelimeler: miyeloproliferatif neoplazi, prognoz, sağkalım, tromboz

Introduction

BCR/ABL-negative chronic myeloproliferative neoplasms (MPNs) are a group of diseases associated with hematopoiesis and over-production of one or more myeloid lineages in the bone marrow (BM). Polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) are the most common MPNs. Although they share common clinical and hematological features, the disease course and therapeutic requirements are different for the different forms [1].

Recent studies have reported that JAK2 (Janus kinase 2; located on chromosome 9p24) mutations are found in virtually all patients with PV and approximately half of ET and PMF patients [2, 3]. Mutations in the MPL (myeloproliferative leukemia virus oncogene; located on chromosome 1p34) and CALR (calreticulin; located on chromosome 19p13.2) have also been described, and are usually somatic [4].

Over-proliferation of all myeloid lineages is seen in PV. In ET, the main problem is megakaryocyte proliferation and thrombocytosis [5]. In the clinical course of both diseases, fibrosis may develop in the BM, and PV and ET may transform to post-PV and post-ET myelofibrosis, which is associated with anemia, splenomegaly and shortened survival [6]. Unlike PV and ET, the main problems of PMF, in addition to clonal proliferation, are BM fibrosis and extramedullary hematopoiesis [7].

When all MPNs are considered, the most common symptoms are fatigue, abdominal pain or discomfort, headache, night sweats and itching [8]. Microcirculatory disorders such as erythromelalgia, visual and neurological symptoms may be seen due to activation of leukocytes, endothelium, platelets or coagulation cascade [9].

Assessments of the survival of these diseases have shown that life expectancy is shorter in PMF. ET affects the quality of life of patients more than survival whereas PV is associated with both impaired quality of life and a reduction in life expectancy [10-13]. The most important complications of MPNs are thromboembolism (TE), bleeding or progression to myelofibrosis or acute myeloid leukemia (AML). Thrombosis is a major cause of mortality and morbidity in PV and ET [14, 15], with a reported incidence of 7-39% and 7–22%, respectively [16-18].

The aim of this study was to analyze the demographic characteristics, clinical features, and the mutational status of MPN patients together with complications, clinical course and survival.

Patients and Methods

This retrospective study was conducted on patients diagnosed with BCR-ABL negative chronic MPN in the Hematology Department of Diskapi Yildirim Beyazit Training and Research Hospital between 2008 and 2019. The diagnosis of MPN was made according to the WHO diagnostic criteria of myeloid neoplasms [5, 19]. The date of diagnosis, demographic information, mutation analysis, treatment management, complications and follow-up periods were recorded for all patients. At the time of diagnosis, hematological parameters were examined, including hemoglobin (Hb) level, hematocrit (Hct) level, platelet count, white blood cell

Table1. Distribution of demographic and disease characteristics of patient subgroups

Parameters (n=247)	PV (n=105)	PMF (n=16)	ET (n=126)
Age,median(ranges),years	64 (20-86)	66.5 (39-85)	65 (22-91)
Cardiovascular risk factor			
Yes	68 (%64.8)	7 (%43.8)	69 (%54.8)
No	37 (%35.2)	9 (%56.2)	57 (%45.2)
Bone marrow fibrosis at diagnosis			
Yes	6 (%5.7)	16 (%100.0)	20 (%15.9)
No	99 (%94.3)	-	106 (%84.1)
Risk score			
Low	44 (%44.8)	3 (%18.8)	48(%38.1)
Int-1	-	5 (%31.2)	-
Int-2	-	4 (%25.0)	-
High	58 (%55.2)	4 (%25.0)	78 (%61.9)
Mutational frequency (%)	96.1	*75*	*71.4*
Driver mutations			
JAK2 V617F (+)	101/105	10/16	81/126
Exon 12 (+)	0/1	0/2	0/11
CALR (+)	0/1	2/4	7/23
MPL (+)	0/1	0/4	0/23
Splenomegaly			
Yes	49 (%47.6)	15 (%93.8)	35 (%27.8)
No	54 (%52.4)	1 (%6.2)	91 (%72.2)
Thromboembolic complication			
Yes	11 (%10.5)	2 (%12.5)	14 (%11.1)
No	94 (%89.5)	14 (%87.5)	112 (%88.9)
Median follow-up, month	28.1[0.0-265.7]	14.0[1.0-93.4]	45.0[0.8-190.2]
Final status			
Nonsurvivor	1 (%1.0)	5 (%31.2)	10 (%7.9)
Survivor	104 (%99.0)	11 (%68.8)	116 (%92.1)

count (WBC), neutrophil count, lymphocyte count, monocyte count, platelet count, platelet distribution width, mean platelet volume (MPV), lactate dehydrogenase (LDH), ferritin and B12 vitamin levels. Survival rates were calculated and the effect of the parameters on overall survival (OS) was analyzed. High-risk and low-risk categories were evaluated in PV and ET patients according to age and previous thrombosis history [20]. The International Prognosis Scoring System (IPSS) was used for PMF patients [21].

Statistical analysis

Data obtained in the study were analysed statistically using SPSS Statistics 20 software (IBM, Armonk, NY, USA). Descriptive data were given as percentages. The Independent Samples t-test (t-table value) was used to compare two independent groups with normal distribution of measurement values, and the Mann-Whitney U test (Z-table value) was applied to data not showing normal distribution. $\chi 2$-cross tables were used to examine the relationship between qualitative variables. Only variables which were statistically significant in the univariate analysis were included in the multivariate Cox regression model. Two-sided p values<0.05 were accepted as statistically significant. Survival was estimated from Kaplan-Meier curves. Comparisons between the patient groups were made using the log-rank test.

Ethical approval and informed consent

All procedures performed in this study were conducted in accordance with the ethical standards of the institutional and / or national research committee and the 1964 Declaration of Helsinki and its subsequent amendments or comparable ethical standards.

Approval for this study was given by the Local Ethics Committee (No: 86/14 Date: 20.04.2020).

Table 2. Distribution of some biochemical blood values according to diagnoses

Variable (N=247)	PV (n=105) Median [Min-Max]	PMF (n=16) Median [Min-Max]	ET (n=126) Median [Min-Max]
Hb, gr/dl	17.5[15.0-23.0]	11.5 [6.3-15.4]	13.5[8.3-17.4]
Hct	53.6[47.3-68.6]	34.1[19.2-48.8]	41.4[25.6-53.0]
WBCcount, $x10^9$/L	11.6[6.5-23.8]	21.9[4.8-43.3]	10.1[3.2-29.5]
Neutrophil count, $x10^9$/L	8.8[3.5-21.7]	17.7[2.5-37.5]	6.9[2.0-25.1]
Lymphocyte count, $x10^9$/L	2.1[0.6-4.6]	2.2[0.6-8.0]	1.9[0.7-7.1]
Monocyte count, $x10^9$/L	0.6[0.1-3.5]	0.8[0.1-1.7]	0.6[0.1-2.4]
PLT count $x10^9$/L	474.0[110.0-1932.0]	261.5[119.0-1074.0]	813.0[412.0-3199.0]
Pct %	0.4[0.1-1.5]	0.3[0.1-0.9]	0.6[0.2-2.7]
MPV	8.2[6.2-11.4]	8.5[6.6-10.0]	7.9[5.1-12.2]
LDH U/L	254.0[140.0-485.0]	663.0[212.0-1157.0]	255.5[152.0-625.0]
Ferritin ng/mL	21.3[4.8-349.0]	96.6[7.6-602.0]	42.5[2.0-454.0]
Vitamin B12 pg/mL	247.0[97.0-957.0]	482.0[228.0-1076.0]	301.0[79.0-1219.0]
PLR	241.8[48.4-685.7]	127.9[18.9-716.0]	457.1[95.9-1999.4]
NLR	4.2[1.1-27.1]	7.5[1.8-23.7]	3.5[1.1-12.9]
MLR	0.3[0.1-5.1]	0.3[0.1-1.1]	0.3[0.1-1.5]
Erythropoietin mlu/ml	2.0[0.5-11.1]	5.0[3.0-7.0]	7.4[1.0-99.0]

Hb: Hemoglobin, Hct: Hematocrite, Plt:Platelet, WBC: White blood cell, PCT: Plateletcrit, MPV: Mean platelet volume LDH: Lactate dehydrogenase, PLR: Platelet to lymphocyte ratio, NLR: Neutrophil to lymphocyte ratio, MLR: Monocyte to lymphocyte ratio

Table 3. Treatment characteristics in disease subgroups

	PV (n=105)	PMF (n=16)	ET (n=126)
First linetreatment			
Hydroxyurea	76 (%72.4)	13 (%81.2)	102 (%81.0)
Anagrelide	-	-	6 (%4.7)
Interferon	-	-	2 (%1.6)
Ruxolitinib	-	3 (%18.8)	-
No treatmentindications	29 (%27.6)	-	16 (%12.7)
1th linetreatmentduration[median, month]	28.5[1.0-222.0]	13.5[1.0-92.0]	36.0[1.0-187.0]
Indication of 2nd linetreatment			
Unresponsivetomedication			
Inadequate	-	-	2 (%6.9)
Side effect, intolerance	1 (%50.0)	2 (%28.6)	7 (%24.1)
Loss of response	1 (%50.0)	3 (%42.8)	9 (%31.1)
Other	-	1 (%14.3)	11 (%37.9)
	-	1 (%14.3)	-
Second line treatment			
Hydroxyurea	-	-	5 (%16.7)
Anagrelide	2 (%100.0)	1 (%16.7)	23 (%76.8)
Ruxolitinib	-	5 (%83.3)	-
Interferon	-	-	2 (%6.7)
2th line treatment duration, [median, month]	23.5[15.0-32.0]	12.0[1.0-28.0]	20.5[1.0-128.0]

Table 4. Examination of factors affecting overall survival in all patients with Cox-Regression model

All patients	B	Standart error	Wald	df	Sig.	OR	95.0% confidenceinterval OR	
							Lower	Upper
Age at diagnosis	0.062	0.029	4.595	1	0.032	1.064	1.005	1.127
Thromboembolism	2.237	0.656	11.618	1	0.001	9.367	2.588	33.905
LDH	0.005	0.001	15.759	1	0.000	1.005	1.003	1.008
Ferritin	0.006	0.002	7.532	1	0.006	1.006	1.002	1.010
PLR	0.003	0.001	11.220	1	0.001	1.003	1.001	0.004

Results:

Evaluation was made of 247 patients, comprising 105 PVR, 126 ET and 16 PMF. The median age was 64 years (20-86) for PV, 66.5 years (39-85) for MF and 65 years (22-91) for ET patients. Of the total 247 patients, 51.6% were female (35.3% of PV patients; 58% of ET patients and 43.8% of PMF patients). The overall frequency of driver mutations was 96.1% for PV, 71.4% for ET and 75% for PMF patients. The demographic and clinical characteristics of the patients are given in Table 1. The hematological and biochemical parameters of the patients are given in Table 2.

Hydroxyurea (HU) was administered as first-line treatment to 76 (72.4%) patients with PV for a median duration of 28.5 months. The most common indications for switching to the second-line treatment were inadequate response (50%) and side-effects (50%). There were no indications for treatment in 29 (27.6%) PV patients, so they were followed up with anti-aggregant agents. HU was administered as the first line treatment to 102 (81.0%) ET patients for a duration of median 36.0 months. The most important indication for switching to the second-line treatment was side-effects (37.9%). In 23 ET patients (76.8%), anagralide was administered as the second-line treatment for a median of 20.5 months, and 16 (12.7%) ET patients had no indication for treatment and were followed up with anti-aggregant agents. Of the patients diagnosed with PMF, the first-line treatment of HU was administered to 13 (81.2%) for a median of 13.5 months, and second-line treatment of ruxolitinib was administered to 5 (83.3%) for median 12 months. Indications for second-line treatment wee mainly side-effects (42.8%). The treatment characteristics of the disease subgroups are given in Table 3.

During follow-up, 11 (10.5%) PV patients, 14 (11.1%) ET patients and 2 (12.5%) PMF patients developed thromboembolic (TE) complications. When the survival rates of the patients were examined, OS of the whole sample could not be calculated because median survival could not be reached in the PV and ET subgroups. The median OS of the PMF patients was determined to be 70.3 (14.0-126.7) months. The survival rates of all the patients and disease subgroups are shown in Figure 1.

As a result of the Cox-Regression model for all patients, it was determined that age, LDH, ferritin and PLR values at the time of diagnosis and the development of TE had a statistically significant effect on survival for all MPN patients ($p < 0.05$). The Cox regression model and Odds Ratio values are shown in detail in Table 4. This analysis could not be performed for disease subgroups due to the low number of patients in the PMF group and the low number of non-survivors in the PV and ET groups.

Discussion

The main purpose of this study was to retrospectively examine our patients diagnosed with MPN that we followed up in our clinic and compare them with the literature.

The median age at diagnosis for PV and PMF has been reported to be 63-64 years, and 55 years for ET [22]. In the current study, the median age at diagnosis for PV, ET and PMF was 64, 65 and 66.5 years, respectively. In contrast to recent reports, the current study ET patients were older. In the ET subgroup, there was a predominance of female patients (58% F vs. 42% M), which was similar to findings of the International Prognostic Score of thrombosis in World Health Organization-Essential Thrombocythemia (IPSET) study [15]. Male predominance was determined in the PV group in accordance with the literature [23]. No gender difference was determined in the PMF patients.

JAK2 mutation has been found to be present in approximately 98% of PV, 60% of ET and 55% of PMF cases according to many recent studies [24-26]. In the current study the overall frequency of driver mutations was 96.1% for PV, 71.4% for ET and 75% for PMF patients. JAK V617F mutation was detected in 96% of PV patients and 64% of ET patients in accordance with current data in

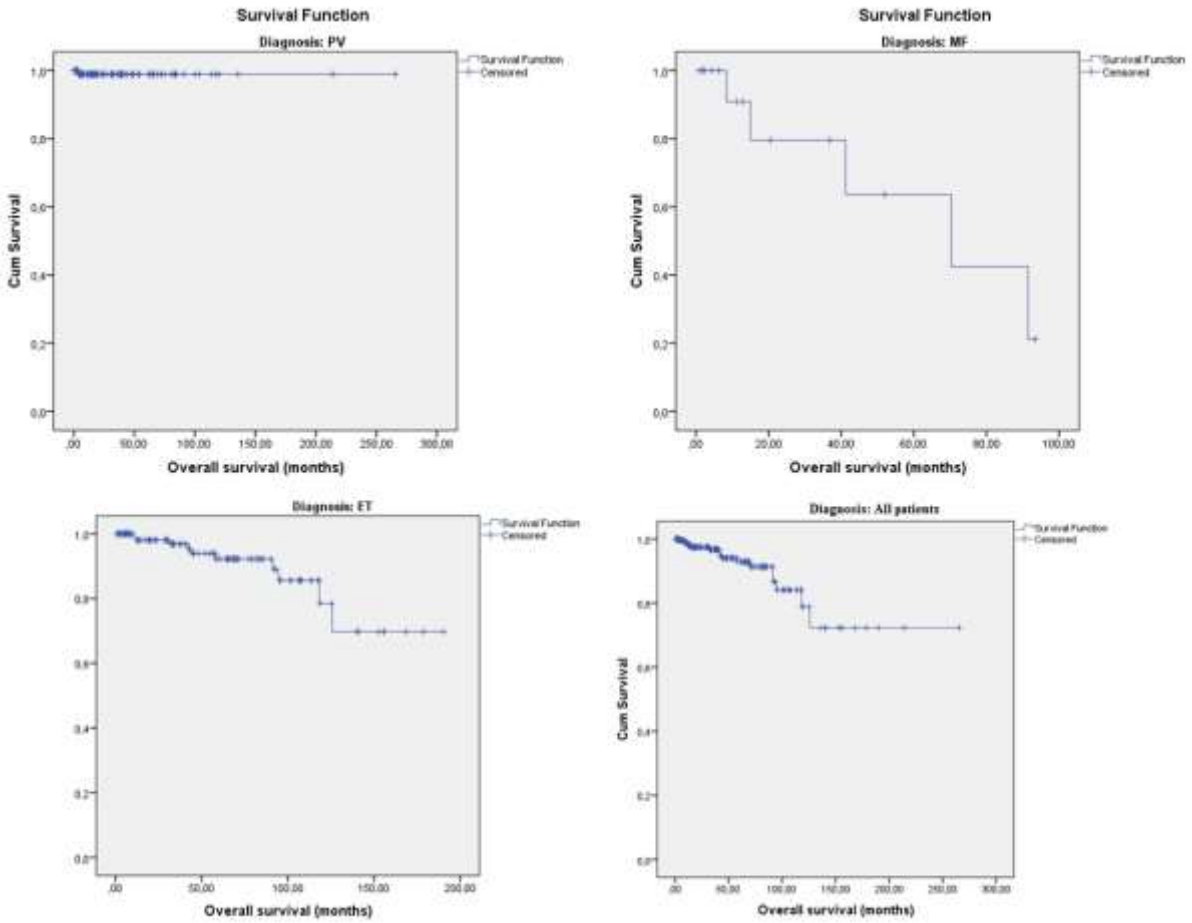

Figure 1: The survival rates of all the patients and disease subgroups

literature. However, in the current study PMF patients, JAK V617F mutation frequency was 62.5%, which was a higher rate than previously reported. The results of the current study supported that adding the analysis of JAK2 Exon 12, CALR and MPL to the JAK2 V617F mutation increased the overall mutational frequency. The CALR analysis in particular increased the frequency from 64.2% to 71.4% for ET patients and from 62.5% to 75% for PMF patients. Therefore, for patients with suspicion of ET or PMF, adding the CALR mutation analysis at the beginning of assessments rather than waiting for JAK2 results can be considered to increase diagnostic capability.

The major complication of Ph-negative MPNs was TE complications. The TE rates of PV patients was reported to be 38.4% in the European Collaboration on Low-dose Aspirin in Polycythemia Vera (ECLAP) study, which was a prospective study of 1638 patients [27]. Tefferi et al., found the incidence of thrombosis in PV patients to be 23.4% in an international study [28]. In the above-mentioned IPSET study, the incidence of thrombosis was 12% in ET patients [15]. In another study from Turkey, the frequency of thrombosis was seen to be 41.1% in ET, 35% in PV and 32% in PMF patients [29]. In the current study, thrombosis rates were 10.5%, 11.1%, and 12% in PV, ET, and PMF, respectively. With the exception of the IPSET study, the incidence of thromboembolism was lower in the current study compared to other studies. This may have been due to some missing data because of the retrospective design of the current study. Patient files may not have been processed and the follow-up time was not sufficient. Although it was

determined that the current study patients with ET developed TE more frequently than patients with PV, this result was not statistically significant.

The expected survival of PV and ET patients is almost the same as that of the general population. In a study from Sweden, the 8-year relative survival rate was 0.84 in PV patients and 0.91 in ET [30]. In a study by the Mayo Clinic Italian collaborative group with 826 patients, the median survival rates were ~20 years for ET, 14 years for PV and 6 years for PMF [22]. In the current study, similar to recent reports, OS was not reached for ET and PV patients, whereas it was determined to be 5.8 years for PMF patients.

In a large study of 1545 PV patients, advanced age (>67 years), leukocytosis ($\geq 15\times10^9$/L), history of thrombosis and abnormal karyotype were determined to be independent risk factors for survival [28]. When evaluated in terms of leukemic transformation and BM fibrosis in PV patients, which is very important for survival, advanced age, leukocytosis and abnormal karyotype have been shown to be related to leukemic transformation [28, 31].

In respect of ET, the IWGMRT study of 867 patients found that advanced age (>60 years), leukocytosis ($\geq 11\times10^9$/L), and a history of thrombosis were significant for survival [15].

In the current study, Cox regression analysis could not be applied to the disease subgroups, but in the analysis of all the MPN patients, age at diagnosis and thrombosis history were found to have a significant effect on survival, which was consistent with recent reports. In addition, a higher platelet-to-lymphocyte ratio (PLR), serum LDH and serum ferritin levels were related to lower survival. Many studies have determined that eleveted PLR is associated with inflammatory, metabolic, prothrombotic and neoplastic diseases [32]. Morever, in many studies, it has been mentioned that PLR is a better inflammatory marker than WBC and is associated with poor prognosis and the risk of developing venous thrombosis [33]. In the current study, OS was lower in patients with higher PLR. In the literature, there is no study showing that PLR affects MPN or separately, ET and PV. Lucijanic et al demonstrated that the PLR was significantly higher in myelofibrosis than in healthy control subjects. They also found that higher PLR was associated with the absence of blast phase disease, constitutional symptoms, and massive splenomegaly, and this was attributed to PLR in MF being less related to inflammation than bone marrow fibrosis [34]. In the current study, a negative relationship was found between the serum ferritin levels and OS in patients with MPN. Although there is no information about this subject in the literature, it has been shown in a previous study that ferritin levels are higher in reactive thrombocytosis than in essential thrombocytosis [35]. High levels of ferritin in MF may be related to severe anemia and the need for blood transfusion. In addition to the known risk factors such as thrombosis and age, inflammatory markers such as ferritin and PLR are also associated with survival in MPN patients, which may indicate that inflammatory processes have a role in the pathogenesis of these diseases. On the other hand, low ferritin may be observed in PV patients as a secondary to phlebotomy. However, since ferritin at the time of diagnosis was evaluated in our study, the effect of low ferritin that may be observed after phlebotomy on overall survival could not be determined.

A previous study found that serum LDH levels were higher (89%) in PMF patients and elevated serum LDH levels were associated with disease burden [36]. In the current study, elevated serum LDH was related with shorter OS in all MPN patients.

Higher LDH levels, which indicate disease activity, may have had a negative effect on survival in this context.

The main goal of treatment in PV and ET is to reduce TE complications and secondarily, to control symptoms. In PMF, the aim is to decrease the patient's constitutional symptoms and improve the quality of life [37]. Acetylsalicylic acid to prevent cardiovascular complications is the main treatment for PV and ET. However, when the platelet count is $>1{,}000\text{x}10^9$/L in ET, aspirin should be

avoided due to acquired vWD. Phlebotomy should be kept in mind to keep the hemotocrit value <45 in PV. Cytoreductive agents such as HU, anagralide or interferon are indicated in patients at high risk of thrombosis. Ruxolitinib may be used when there is insufficient response to HU [38]. For the treatment of PMF, most patients are administered symptom-based therapy as blood transfusion, HU or ruxolitinib. Allogeneic stem cell transplantation should be considered in fit and young PMF patients with high risk features as a curable treatment option [20]. In the current study,72.4% of PV patients and 87.3% of ET patients had treatment indications. Hydroxyurea was the most commonly used first-line treatment agent, and the development of side-effects was the most common indication for switching to second-line treatment in all the disease subgroups. As second-line therapy in PV and ET, the most preferred agent was anagralide, whereas in MF, it was ruxolitinib. Limitations of this study were the Retrospective design and that the parameters associated with TE or prognosis were not evaluated due to insufficient data. Cox regression analysis could not be performed for the disease subgroups due to the low number of PMF patients and the low number of non-survivors in the PV and ET subgroups. The total follow-up period should be longer for diseases such as ET and PV, which have survival rates close to those of the normal population. There is a need for larger prospective studies to analysis TE complications.

In conclusion, the results of this study showed that PMF patients had lower survival rates than patients with other MPNs although TE was observed at similar rates in all 3 disease subgroups. In addition to the known risk factors such as age and TE complications, parameters such as LDH, ferritin and PLR indicating disease activity and inflammation can also be used as prognostic markers. There is a need for larger population-based epidemiological studies to be able to better understand the course of the diseases and to assist in their management.

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Original Article

Predictive Value of Complete Blood Count Inflammatory Parameters for Epithelial Ovarian Cancers in Women During Reproductive Period

Reprodüktif Dönemdeki Kadınlarda Epitelial Over Kanserleri İçin Tam Kan Sayımı İnflamatuar Parametrelerin Prediktif Değeri

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ABSTRACT

Background: Comparison of predictive values of complete blood count inflammatory parameters and serum CA-125 levels in the differential diagnosis of patients with epithelial ovarian tumor (EOTs) diagnosed in the reproductive period.

Materials: In this study, 105 women patients in the reproductive period were retrospectively analyzed. The patients included were examined in terms of clinical, laboratory and pathological features.

Results: The mean age of the patients was 41.5 ± 8.6 years and BMI was 28.6 ± 5.8 kg/m2. Of the whole study population, 54 (51.5%) were benign EOTs, 18 (17.1%) were borderline EOTs and 33 (31.4%) were malignant EOTs. When comparing these three tumor groups, a significant difference was found in terms of serum CA-125 [14.7 U/mL (2.7-238.6) vs 60.2 U/mL (7.8 – 557) vs 416.5 U/mL (2.4-7695), p<0.001, respectively], platelet count [294 x103/ µL (133-744) vs 303, x103/ µL (160-468) vs 383 x103/ µL (128-725), p = 0.018, respectively], neutrophil count [4.2, x103/ µL (1.9 -8.9) vs 4.9 x103/ µL (2.6-10.9) vs 5.1 x103/ µL (2.2 – 10.7), p = 0.012], neutrophil-to-lymphocyte ratio (NLR) [2.1 (0.1-8.2) vs 2.6 (1.2 – 5.9) vs 3.1 (1-6.8), p = 0.021, respectively] and platelet-to-lymphocyte ratio (PLR) [154.4 (82.1-658.4) vs 168.1 (91.1-377.8) vs 201.5 (29.6-499.2), p = 0.008, respectively] values. In the ROC analyses, serum CA-125 levels (AUC= 0.818, p <0.001), platelet count (AUC= 0.673, p = 0.005) and PLR (AUC= 0.690, p = 0.002) values significantly predicted malignant EOTs.

Conclusion: Preoperative platelet count, neutrophil count and PLR predict epithelial ovarian cancers significantly. However, diagnostic predictive values of all three parameters are lower than CA-125. Therefore, CA-125 is still the most valuable serum biochemical marker that can be used in the preoperative differential diagnosis of EOTs.

Keywords: Epithelial ovarian cancer, whole blood count, Inflammatory Parameters

ÖZET

Giriş ve amaç: Reproduktif dönemde tanı alan epitelyal over tümörlerinin (EOTs) ayırıcı tanısında tam kan sayımı inflamatuar parametreleri ile serum CA-125 seviyelerinin prediktif değerlerinin karşılaştırılması.

Yöntem ve gereçler: Bu çalışmada reproduktif dönemde olan 105 kadın sırasıyla retrospektif olarak incelenmiştir. Dahil edilen kadınlar klinik, laboratuar ve patolojik özellikleri açısından incelenmiştir.

Bulgular: Hastaların ortalama yaşı 41.5 ± 8.6 ve BMI 28.6 ± 5.8 idi. Tüm çalışma populasyonunun 54 (51.5%) tanesi benign EOTs, 18 (17.1) tanesi borderline EOTs ve 33 (31.4%) tanesi malign EOTs idi. Bu üç tümör grubunun karşılaştırılmasında serum CA-125 [14.7 U/mL (2.7-238.6) vs 60.2 U/mL (7.8 – 557) vs 416.5 U/mL (2.4-7695), p<0.001, sırasıyla], platelet count [294, x103/ µL (133-744) vs 303, x103/ µL (160-468) vs 383, x103/ µL (128-725), p= 0.018, sırasıyla], neutrophil count [4.2, x103/ µL (1.9 -8.9) vs 4.9, x103/ µL (2.6-10.9) vs 5.1, x103/ µL (2.2 – 10.7), p= 0.012], neutrophil-to-lymphocyte ratio (NLR) [2.1 (0.1-8.2) vs 2.6 (1.2 – 5.9) vs 3.1 (1-6.8), p= 0.021, sırasıyla] ve platelet-to-lymphocyte ratio (PLR) [154.4 (82.1-658.4) vs 168.1 (91.1-377.8) vs 201.5 (29.6-499.2), p= 0.008, sırasıyla]

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değerleri açısından anlamlı fark saptandı. ROC analizinde, serum CA-125 seviyesi (AUC= 0.818, p<0.001), platelets (AUC= 0.673, p= 0.005) ve PLR (AUC= 0.690, p= 0.002) değerleri malign EOTs'lerini anlamlı olarak öngörmekte idi.
Tartışma ve sonuç: Preopratif platelet sayısı, nötrofil sayısı ve PLR epitelyal over kanserlerini anlamlı olarak öngörmektedir. Ancak her üç parametrenin de tanısal öngörü değerleri CA-125'den daha düşüktür. Bu nedenle CA-125 hala EOTs'lerinin preoperative ayırıcı tanısında kullanılabilecek en değerli serum biyokimyasal belirteçtir.

Anahtar Kelimeler: Epitelial over kanseri, Tam kan sayımı, İnflamatuar parametreler

Introduction

Ovarian tumors are the second most common gynecological tumors. Epithelial ovarian tumors (EOTs) account for 95% of these tumors [1]. EOTs are divided into three histopathological subgroups: benign, borderline and malignant. Although these tumors are frequently seen in the postmenopausal period, preoperative differential diagnosis of these three subgroups is vital for women at all ages [2, 3]. In the differential diagnosis, many methods such as clinical, laboratory and imaging have been described to date. For this purpose, one of the most used biochemical markers in clinical practice is to measure the serum CA-125 level [4-6].

CA-125 has a glycoprotein structure. It rises in 80% of advanced malignant EOTs. However, in addition to genital malignancies, it also increases in many other system malignancies and benign diseases. Therefore, sensitivity and specificity values are low for routine clinical use. Since endometriosis, tubo-ovarian abscess, and functional ovarian cysts are frequently seen in women's life, especially in the reproductive period, the diagnostic value of CA-125 in these diseases is even lower. This leads to researchers look for different diagnostic tests [1, 7].

Recently, in many articles have been emphasized the importance of complete blood count inflammatory parameters. Complete blood count is a simple, easily accessible, inexpensive, automated and standardized method [8]. Also, complete blood count is measured routinely in the preoperative period in patients to be operated on. For all these reasons, the idea of being able to be used in the differentiation of benign and malignant ovarian tumors has caused wide repercussions [9]. The basis of this idea is the inflammatory response associated with cancer [10]. The most emphasized hematological parameters are leukocyte count, platelet count, neutrophil count, neutrophil leukocyte ratio (NLR) and platelet lymphocyte ratio (PLR) [6, 11].

The aim of this study is to compare the predictive values of preoperative serum CA-125 levels and complete blood count inflammatory parameters for the diagnosis of malignant EOTs in reproductive women diagnosed with EOTs.

Material Methods

105 reproductive women (15-49 years) who underwent an adnexial mass indication and laparotomy between 2015 and 2020 at the Etlik Zübeyde Hanım Women's Health Training and Research Hospital and who had EOTs as a result of pathology were examined in the study. The study protocol has been approved by a suitably constituted Local Ethics Committee. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Postmenopausal patients, patients having any other systemic disease such as cardiovascular disease, diabetes, acute inflammation, blood disease, kidney disease or other cancers, patients receiving chemotherapy and / or radiotherapy and recent blood transfusion within the previous 3 months and incomplete medical records patients were excluded from the study. In addition, despite the premenopausal period, patients with other ovarian diseases such as endometriosis, tubo-ovarian abscess, hydrosalpinx or other ovarian tumors such as germ cell ovarian tumors, sex cord stromal tumors and patients with tumors belonging to other systems such as the gastrointestinal system was excluded from the study. Preoperative age, gravity, parity, body

mass index (BMI), serum CA-125 level, white blood cell count (WBC), platelet count, neutrophil count, lymphocyte count, monocyte count, neutrophil-lymphocyte ratio, platelet-lymphocyte ratio results and post-operative final pathology results were evaluated.

Statistical analysis

Statistical analysis was performed using the Statistics Package for Social Sciences software (ver. 21.0, SPSS Inc., Chicago, IL, USA). The distribution of data was calculated using the normality test. In the comparison of three groups, the oneway ANOVA test was used for parametric variables and the Kruskal-Wallis test was used for nonparametric data. When statistical differences were found between three groups, each was compared separately. Continuous variables with normal distributions were compared using independent sample t-test and non-normal distributions were compared with the Mann-Whitney U test. To define relationship between numeric parameters, Pearson's correlation analysis was used for variables with normal distributions and Spearman's correlation analysis for variables with non-normal distributions. Receiver operating characteristic (ROC) analysis was used to calculate cutoff values. $p < 0.05$ values were accepted as statistically significant.

Results

The mean age of the patients included was 41.5 ± 8.6 (years), BMI 28.6 ± 5.8 (kg/m2) and the median CA-125 value was 34.5 U / mL (range 2.4-7695). Of the whole study population, 54 (51.5%) were benign EOTs, 18 (17.1%) were borderline EOTs and 33 (31.4%) were malignant EOTs. 35 (64.8%) of benign EOTs were serous cystadenoma, 18 (33.3%) were mucinous cystadenoma and 1 (1.9%) were benign Brenner tumor. 8 (44.4%) of the Borderline EOTs were serous and 10 (55.6%) were of mucinous histological type. Of the malignant EOTs, 22 (66.7%) were serous cystadenocarcinoma, 7 (21.2%) were endometrioid carcinoma, 3 (9.1%) were mucinous carcinoma and 1 (3%) were mixed (serous + mucinous) carcinoma. Clinical, laboratory and pathological features of the whole study group are summarized in Table 1.

Comparing Groups

In the comparison of benign, borderline and malign EOTs; no significant difference was found in terms of age, gravida, parity, BMI, WBC, lymphocyte count and monocyte count ($p>0.05$). In the comparison of the three groups were found to differ significantly in terms of serum CA-125 level ($p<0.001$), platelet count ($p = 0.018$), neutrophil count ($p = 0.012$), NLR ($p = 0.021$) and PLR ($p = 0.008$). In the pairwise comparisons between these groups, which were found to be statistically significant between the three groups, using Bonferonni correction (Adjusted); serum CA-125 value was found to be highest in the malign EOTs groups (Adj $p <0.001$), but similar between borderline EOTs group and malign EOTs groups (Adjusted $p = 0.247$). Platelet count, Neutrophil count, NLR and PLR values were different only between benign EOTs group and malign EOTs group (Adjusted $p = 0.014$, Adjusted $p = 0.041$, Adjusted $p = 0.038$, Adjusted $p = 0.006$, respectively). Comparison of the three groups is summarized in Table 2.

Correlations

In the correlation analysis between variables; serum CA-125 with WBC ($r = 0.290$, $p = 0.003$), platelets ($r = 0.290$, $p = 0.003$), neutrophils ($r = 0.330$, $p = 0.001$), monocytes ($r = 0.273$, $p = 0.005$) NLR ($r = 0.307$, $p = 0.002$) and PLR ($r = 0.292$, $p = 0.003$) values were statistically significant.

ROC analysis

Serum CA-125 level (AUC = 0.818, $p <0.001$), platelet count (AUC = 0.673, $p = 0.005$) and PLR (AUC = 0.690, $p = 0.002$) predicted that malignant EOTs were statistically significant, while neutrophil count (AUC = 0.621, $p = 0.050$) and NLR (AUC: 0.620, $p = 0.053$) could not achieve this. Optimal cutoff value for serum CA-125 level 51.2 U / mL with 69% sensitivity and 70% specificity, for platelet count 325 x103 / µL with 62% sensitivity and 63% and for PLR 172.2 with 62% sensitivity and 63%

Table 1. Clinic, laboratory and pathological characteristics of whole study group

Characteristics	*n*= 105
Age, years, mean ± SD	41.5 ± 8.6
Gravidity, median (range)	3 (0-8)
Parity, median (range)	2 (0-6)
BMI, kg/m^2, mean ± SD	28.6 ± 5.8
CA-125, U/mL, median (range)	34.5 (2.4-7695)
WBC, x10^3/ µL, mean ± SD	7.4 ± 1.9
Platelets, x10^3/ µL, median (range)	308 (128-744)
Neutrophils, x10^3/ µL, median (range)	4.5 (1.9 -10.9)
Lymphocytes, x10^3/ µL, median (range)	1.8 (0.7-4.3)
Monocytes, x10^3/ µL, median (range)	0.37 (0.15-1.21)
NLR, median (range)	2.4 (0.1-8.2)
PLR, median (range)	166.3 (29.6-658.4)
Histological type, n (%)	
Benign EOTs	
Serous cystadenoma	35 (64.8)
Mucinous cystadenoma	18 (33.3)
Benign Brenner Tumor	1 (1.9)
Borderline EOTs	
Serous BOTs	8 (44.4)
Mucinous BOTs	10 (55.6)
Malign EOTs	
Serous cystadenocarcinoma	22 (66.7)
Endometrioid carcinoma	7 (21.2)
Mucinous carcinoma	3 (9.1)
Mixed (serous + mucinous) ovarian carcinoma	1 (3)

Abbreviations: BMI, body mass index; CA125, cancer antigen 125; Hb, Hemoglobin; Hct, hematocrit; WBC, white blood cell count; NLR, neutrophil-to- lymphocyte ratio; PLR, platelet-to- lymphocyte ratio; EOTs, Epithelial Ovarian Tumors; BOTs, Borderline Ovarian Tumor

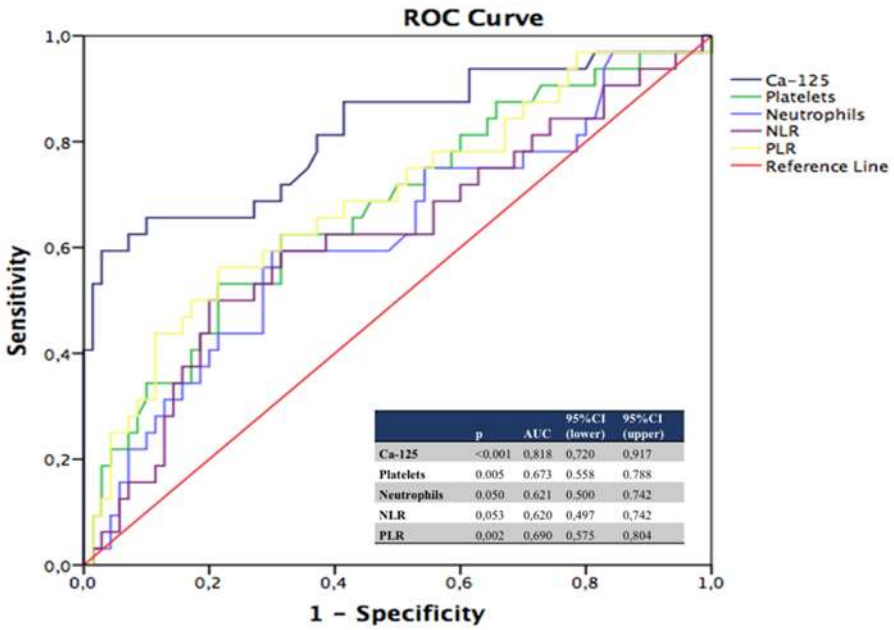

Figure 1. Predictive values of serum CA-125 levels, platelet count, neutrophil count, NLR and PLR for malign EOTs.

Abbreviations: EOTs, Epithelial Ovarian Tumors; AUC: area under the ROC curve; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio

Table 2. Comparisons of the three groups

	Benign EOTs *n*: 54	Borderline EOTs *n*: 18	Malign EOTs *n*: 33	*p*
Age, years, mean ± SD	42.5 ± 9.4	37.1 ± 8.5	42.5 ± 6.7	0.052
Gravidity, median (range)	3 (0-8)	3 (0-4)	2 (0-7)	0.393
Parity, median (range)	2 (0-6)	1(0-4)	2 (0-4)	0.493
BMI, kg/m^2, mean ± SD	29.6 ± 6.3	27.4 ± 6.6	27.8 ± 4.5	0.350
CA-125, U/mL, median (range)	14.7 (2.7-238.6)	60.2 (7.8 – 557) *	416.5 (2.4-7695) *	<0.001
WBC, x10^3/ µL, mean ± SD	6.9 ± 1.7	7.9 ± 2.3	7.7 ± 2.2	0.054
Platelets, x10^3/ µL, median (range)	294 (133-744)	303 (160-468)	383 (128-725) *	0.018
Neutrophils, x10^3/ µL, median (range)	4.2 (1.9 -8.9)	4.9 (2.6-10.9)	5.1 (2.2 – 10.7) *	0.012
Lymphocytes, x10^3/ µL, median (range)	1.8 (1.1-3.7)	1.8 (1.1-3.9)	1.8 (0.7-4.3)	0.917
Monocytes, x10^3/ µL, median (range)	0.35 (0.16-1.21)	0.38 (0.15-0.719)	0.38 (0.20-0.91)	0.433
NLR, median (range)	2.1 (0.1-8.2)	2.6 (1.2 – 5.9)	3.1 (1-6.8) *	0.021
PLR, median (range)	154.4 (82.1-658.4)	168.1 (91.1-377.8)	201.5 (29.6-499.2) *	0.008

Table 3. Correlations between CA-125 and Hematologic Inflammatory Parameters.

	WBC, x10^3/ µL	Platelets, x10^3/ µL	Neutrophils, x10^3/ µL	Lymphocytes, x10^3/ µL	Monocytes, x10^3/ µL	NLR	PLR
Ca -125, U/mL							
r	0.290	0.290	0.330	-0.028	0.273	0.307	0.292
p	0.003	0.003	0.001	0.779	0.005	0.002	0.003

CA125, cancer antigen 125; Hb, Hemoglobin; Hct, hematocrit; WBC, white blood cell count; NLR, neutrophil-to- lymphocyte ratio; PLR, platelet-to- lymphocyte ratio

specificity were calculated. ROC analysis results are shown in figure 1.

Discussion

In this study, we investigated the clinical, laboratory and pathological characteristics of patients with benign, borderline and malignant EOTs managed at our clinic. At the end of the study, we found that serum CA-125 levels, platelet counts, neutrophil counts, NLR and PLR were higher in malignant EOTs group compared to benign EOTs group. In ROC analysis; only CA-125 levels, platelet count and PLR ratios significantly predicted malignant EOTs. We found the highest predictive value in serum CA-125. In the correlation analyses, there was a significant correlation between serum CA-125 value and WBC, platelet count, neutrophil count, monocyte count, NLR and PLR values.

So far, the increased preoperative platelet count was reported in many of gynecological malignancies such as ovarian, endometrial, vulvar and cervical cancer [12-14]. Of these cancers, ovarian cancer is the most emphasized in the literature. Yilmaz et al. showed that platelet count in the detection of malignant ovarian tumors have been evaluated as useful new marker [15]. In another study by Watrowski et al. found that a total of 44.8 % malignant and 8.6 % benign ovarian cases presented with thrombocytosis (PLT ≥350/nl) and the sensitivity and specificity of preoperative thrombocytosis and CA-125 were calculated as %45/%91 and 88%/76%, respectively. So, they concluded that platelet count is an ubiquitously available parameter that could be useful in the diagnostic evaluation of pelvic mass [16]. Similarly, in our study, the number of platelets had a significant predictive value for malign EOTs. However, this predictive value was lower than CA-125.

PLR and NLR are both easily to calculate markers. These markers evaluated in many studies on ovarian neoplasms. In a study by Yıldırım at al. showed that patients with ovarian cancer exhibited significantly higher

NLR and PLR values. Also, they found that higher NLR and PLR values predicted ovarian cancer at the cut-off value of 3.35, sensitivity of 55% and specificity of 81% for NLR and at the cut-off value of 572.9, sensitivity of 100% and specificity of 0.38% for PLR. [17]. In a retrospective study, Bakacak et al. İnvestigated the utility of preoperative NLR, PLR and lymphocyte count as biomarkers to distinguish malignant from benign ovarian masses. They identified NLR (68.8% sensitivity and 54.1% specificity) and PLR (81.8% sensitivity and 50.8% specificity) as markers for distinguish malignant from benign masses [18]. In another study by Yıldırım et al. reported that NLR and PLR were useful methods that could be applied together with CA-125 due to the relatively high sensitivity values for the malign-benign differentiation of ovarian masses [19]. In addition to, Prodromiduo et al. and Eo, Wan Kyu, et al. suggested that both PLR and NLR seemed to be promising screening and prognostic factors of epithelial ovarian cancer [20, 21]. In the present study, NLR and PLR rates were higher in malignant EOTs than benign EOTs. However, only PLR significantly predicted malignant EOTs in the ROC analysis.

Our study has two important limitations. The first is that our number of participants is relatively low. The second is that it is a retrospective study. Despite these limitations, retrospective studies reflect real life data and make important contributions to the body of knowledge. Our study also has two important strengths. First, all patients included in the study have histopathological diagnoses. Secondly, histological subtypes of EOTs, benign, malignant and borderline, were compared completely.

In conclusion, complete blood count inflammatory parameters in the reproductive period are associated with CA-125. Among these inflammatory parameters, platelet counts, neutrophil counts, NLR and PLR values are significantly higher in Malign EOTs. Also, platelet count and PLR can be used to distinguish malign EOTs from benign EOTs. However, the area under curve of these parameters is not higher than CA-125. Therefore, CA-125 is still the most valuable diagnostic marker that can be used preoperatively for the differential diagnosis of EOTs in reproductive aged women. Whereas, platelet count or PLR can be used in combination with CA-125.

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Original Article

The Comparison of the Biosimilar Filgrastim (Tevagrastim) and the Original Filgrastim (Neupogen) in the Autologous Hematopoetic Stem Cell Mobilization: A Single Center Experience

Otolog Hematopoetik Kök Hücre Mobilizasyonunda Biobenzer Filgrastim (Tevagrastim) ile Orjinal Filgrastimin (Neupogen) Karşılaştırılması (Tek Merkez Deneyimi)

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ABSTRACT

Objective : Autologous hematopoietic stem cell transplantation is a significant treatment modality for several hematological malignancies and some solid tumors. A sufficient dose of CD34+ stem cell mobilization is a prerequisite for successful autologous hematopoietic stem cell transplantation. Granulocyte colony stimulating factor (G-CSF) is usually used alone or in combination with chemotherapy for mobilization. Two types of G-CSF are used in peripheral blood stem cell mobilization; filgrastim (original or biosimilar) and lenograstim. It was aimed to compare the original filgrastim (Neupogen) with the biosimilar filgrastim (Tevagrastimin) in terms of effectiveness in peripheral blood stem cell mobilization.

Materials and Methods: Ninety-five patients who underwent stem cell mobilization between January 2015 and November 2018 in the Bone Marrow Transplant Service of Ankara Numune Training and Research Hospital were analyzed retrospectively. We used Cyclophosphamide (low dose: 2g/m^2 or high dose: 3-4 g/m^2), as a mobilization regimen, on the first day and G-CSF (Neupogen or Tevagrastim) at a dose of 5 g/kg was started one day after chemotherapy, as a mobilization regimen in all patients.

Results: Thirty-nine patients were female and 56 were male. The mean age was 51.24±12.38 years. Sixty-four (67.4%) patients had Multiple Myeloma (MM), 20 (21.1%) patients had non-Hodgkin lymphoma (NHL), and 11 (11.6%) patients had Hodgkin lymphoma (HL). Stem cell mobilization was performed with Neupogen in 50 of ninety-five patients and with Tevagrastim in 45 of them. While there was no statistically significant difference between the demographic characteristics of the patients in terms of age, diagnosis, and whether they received radiotherapy or not ($p> 0.05$); There was a significant difference in terms of the dose of cyclophosphamide (higher dose cyclophosphamide was used more in the arm receiving Neupogen) and gender (gender distribution in the Neupogen arm was equal, there were more male patients in the Tevagrastim arm) ($p<0.05$). No statistically significant difference was found between the use of original and biosimilar products in terms of achieving the target of 2x10^6/kg, which is the minimum CD34$^+$ cell dose required for adequate engraftment, and 4x10^6/kg for double transplantation ($p>0.05$). However, if the target level of CD34$^+$ stem cell was above 6x10^6/kg, a statistically significant difference was observed in favor of Neupogen compared to the Tevagrastim arm ($p=0.021$). When the neutrophil engraftment days after autologous stem cell transplantation were compared, it was observed that the neutrophil engraftment was shorter in the Neupogen arm compared to the Tevagrastim arm (approximately 1 day) ($p=0.015$), while the platelet engraftment was similar in the Neupogen and Tevagrastim arms ($p=0.186$).

Conclusion: While the original and biosimilar filgrastim CD34$^+$ cell dose show similar efficacy in reaching the target of 2x10^6/kg for transplantation and 4x10^6/kg for double transplantation. The difference was observed in favor of the Neupogen arm, when stem cell collection was aimed at above 6x10^6/kg. The reason for this difference was thought to be related to the higher dose of cyclophosphamide used in the original molecule (Neupogen) arm. We need further studies that were included more cases which are used high-dose cyclophosphamide for comparing the biosimilar molecule (Tevagrastim) with the original molecule (Neupogen).

Keywords: autologous stem cell transplantation, biosimilar G-CSF, stem cell mobilization

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ÖZET

Giriş ve amaç: Başarılı otolog hematopoetik kök hücre nakli için yeterli dozda $CD34^+$ kök hücre mobilizasyonu ön koşuldur. Yapmış olduğumuz çalışmada orjinal filgrastim (Neupogen) ile biyobenzer filgrastimin (Tevagrastimin) çevre kanı kök hücre mobilizasyonunda etkinlik açısından karşılaştırılması amaçlanmıştır.

Yöntem ve gereçler: Ankara Numune Eğitim ve Araştırma Hastanesi Kemik İliği Nakil servisinde Ocak 2015 ile Kasım 2018 tarihleri arasında kök hücre mobilizasyonu yapılan 95 hasta retrospektif olarak analiz edildi. Tüm hastalara mobilizasyon rejimi olarak birinci gün siklofosfamid (düşük dozda: 2g/m^2 veya yüksek dozda: 3-4 g/m^2) ve kemoterapiden 1 gün sonra 5 g/kg dozunda G-CSF başlanmıştır.

Bulgular: Doksan beş hastanın 50'sine neupogen ile, 45'ine tevagrastim ile kök hücre mobilizasyonu yapıldı. Hastaların demografik özellikleri arasında yaş, tanı, radyoterapi alıp almaması açısından istatistiksel anlamlı farklılık yok iken ($p> 0.05$); alınan siklofosfamid dozu (neupogen alan kolda daha çok yüksek doz siklofosfamid kullanılmıştır.) ve cinsiyet (neupogen kolunda cinsiyet dağılımı eşit iken, tevagrastim kolunda daha çok erkek hastalar vardı.) açısından farlılık tespit edildi ($p<0.05$). Yeterli engraftman için gereken minimum $CD34^+$ hücre dozu olan $2x10^6$/kg ve çift nakil için $4x10^6$/kg lık hedefe ulaşmak açısından orjinal ve biyobenzer ürün kullanımı arasında istatistiksel olarak anlamlı bir fark saptanmadı ($p>0,05$). Ancak $6x10^6$/kg'ın üzerinde kök hücre hedefine bakıldığında neupogen lehine tevagrastim koluna göre istatistiksel olarak anlamlı bir fark izlenmiştir ($p=0,021$). Otolog kök hücre nakli sonrası nötrofil engraftman günleri karşılaştırıldığında, nötrofil engraftman zamanının neupogen grubunda tevagrastim grubuna göre daha kısa olduğu gözlenirken (yaklaşık 1 gün) ($p=0.015$), trombosit engraftman zamanı neupogen ve tevagrastim gruplarında benzerdi ($p=0.186$).

Tartışma ve sonuç: Çalışmamızda $6x10^6$/kg'ın üzerinde kök hücre toplanması hedeflendiğinde neupogen lehine farklılık gözlenmiştir. Bu farklılığın nedeni kullanılmış olan siklofosfamid dozunun orjinal molekül (Neupogen) kolunda daha yüksek olması ile iliskili düşünülmüştür. Daha çok vaka serileri içeren, yüksek doz siklofosfamidin kullanıldığı, biyobenzer molekül (tevagrastim) ile orjinal molekülün (neupogen) kıyaslandığı çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: otolog kök hücre nakli, biyobenzer G-CSF, kök hücre mobilizasyonu

Introduction:

In recent years, peripheral blood stem cells have been preferred to use in stem cell transplantation. Granulocyte colony-stimulating factors (G-CSF) are often used either as a single agent or in combination with the chemotherapy at peripheral stem cell mobilization. Physicians have been using two types of G-CSF: filgrastim (original or biosimilar) and lenograstim Lately, the biosimilar filgrastim products have been using in neutropenia after chemotherapy and in stem cell mobilization for getting benefit for the cost. A Biosimilar product is produced from the biotechnical product of the original molecule and it is also similar to the original molecule. However, we could not say that the biosimilar product is identical to the original. For this reason, it is very important for comparing the biosimilar molecule with the original molecule.

In recent years, various biosimilar filgrastim products have been used in neutropenia and stem cell mobilization after chemotherapy. Biosimilar G-CSFs (Zarzio®, Sandoz International GmbH, Holzkirchen, Germany; Nivestim®, Hospira Worldwide, Lake Forest, Illinois, USA; Tevagrastim®, Teva Pharmaceutical Industries, Petah Tiqwa, Israel; Ratiograstim®, in all indications of original filgrastim Ratiopharm, Ulm, Germany) has been approved by the European Medicines Agency (EMA). This approval is based on prospective randomized studies in which biosimilar filgrastim have similar efficacy and side effect profile compared to the original filgrastim in chemotherapy-induced neutropenia [1,2]. However, there are no prospective studies about its use in peripheral stem cell mobilization.

Biosimilar G-CSFs has been approved by the European Medicines Agency (EMA) in all indications of the original filgrastim. In Turkey, Tevagrastim (filgrastim XM02) was licensed and approved in 2015 and has been used in our hospital since 2016. There are few data in the literature which was comparing the Tevagrastim and Neupogen at peripheral stem cell mobilization, also there is not any report from Turkey in the literature about the issue [3]. This study's aim is to compare the effectiveness and the side effects of biosimilar Filgrastim (Tevagrastim) and original product (Neupogen) in peripheral stem cell mobilization.

Materials And Methods :

We retrospectively evaluated 95 patients who had a stem cell mobilization in Ankara Numune Training and Research Hospital Bone Marrow Transplant Service between January 2015 and November 2018. The patients of the study were divided into two arms as patients using the Original Filgrastim (Neupogen) and Biosimilar Filgrastim (Tevagrastim). None of the patients had a stem cell mobilization before.

All patients received cyclophosphamide (2-4 g/m^2) on day 1 and G-CSF (Neupogen or Tevagrastim) at a dose of 5 µg / kg one day after the chemotherapy. Stem cells were collected using an apheresis device (Fresenius, Com. Tec cell separator) via a peripheral or central venous catheter. When the total number of peripheral blood leukocytes increased up to 1500/mm^3 in the patients and the peripheral CD34 positive cell count was at least $15x10^6$/lt, the leukapheresis was started." The FacsCanto, Becton Dickinson" flow cytometry device was used for counting the CD 34^+ cells. Apheresis products were frozen at - 196°C, using 10% dimethylsulfoxide and 40% patient plasma.

The minimum CD34+ cell dose for adequate engraftment was reported as $2x10^6$/kg for a single transplant and 4 x 10^6 / kg for a double transplant. Optimal $CD34^+$ cell dose was $>4x10^6$/kg for single transplants and 8-$10x10^6$/kg for double transplants (4). Therefore, patients in the Neupogen and Tevagrastim arms were compared based on these values.

The patients were re-hospitalized to our clinic for autologous peripheral stem cell transplantation 3-4 weeks after the mobilization procedure. The transplantation was performed using a melphalan regimen (200 mg/m^2/day) in multiple myeloma and performed using a high-dose BEAM (Carmustine 300mg/m^2, (-7 day); Etoposide 200 mg/m^2, (-6/-3 days); Cytarabin 200 mg/m^2, (-6/-3 days); Melphalan 140 mg/m^2, (-2 day)) regimen in non-Hodgkin and Hodgkin lymphoma patients.

This retrospective study was approved by the Ankara Numune Training and Research Hospital Local Ethics Committee (11/04/2018-R=E-18-1882).

Statistical Analysis

Descriptive statistics were presented as mean ± standard deviation and median (minimum-maximum) for numerical variables, and as number (percentage) for categorical variables. The compliance of numerical variables to normal distribution was determined by the Shapiro-Wilk test. The Mann-Whitney-U Test was used to compare the mean of the two groups in terms of numerical variables, and the Kruskal Wallis Test was used to compare the means of more than two groups. Pearson Chi-Square Test or Fisher's Exact Test was used to comparing two categorical variables. The change between two measurements of a numerical variable and the interaction of this change with a categorical variable was evaluated by analysis of variance (repeated measures ANOVA) in repeated measures. All statistical evaluations were made using the Statistical Package for Social Sciences (SPSS) for Windows 14.01 (IBM SPSS Inc., Chicago, IL) program. Statistical significance was accepted as $p < 0.05$ in all analyzes.

Results

The research group consisted of 95 patients, including 39 women and 56 men. Patient characteristics and demographics were summarized in table 1. The mean age of the patients was 51.24 ± 12.38 years. Neupogen in 50 patients and Tevagrastim in 45 patients were used during the peripheral stem cell mobilization. 64 (67.4%) of the patients had MM, 20 (21.1%) of NHL, and 11 (11.6%) of HL. The age range of the patients having received the Neupogen and Tevagrastim did not differ. The rates of the patients diagnosed with MM, NHL, and HL and those receiving and not receiving radiotherapy were similar in the Neupogen and Tevagrastim arms ($p > 0.05$). The proportion of male patients was higher in the Tevagrastim arm compared to

Table.1: Patient characteristics and demographics

	All population n=95	Neupogen n=50	Tevagrastim n=45	*p*
Age	51.24±12.38	50.26±12.02	52.33±12.83	0.203
	55(18-70)	54(21-70)	57(18-69)	
Gender				
Female	39(41.1)	29(58.0)	10(22.2)	<0.001
Male	56(58.9)	21(42.0)	35(77.8)	
Diagnosis				
MM	64(67.4)	37(74.0)	27(60.0)	
NHL	20(21.1)	7(14.0)	13(28.9)	0.202
HL	11(11.6)	6(12.0)	5(11.1)	
Radiotherapy				
Yes	26(27.4)	15(30.0)	11(24.4)	0.544
No	69(72.6)	35(70.0)	34(75,6)	
Cyclophosphamide dose				
2 g/m^2	33	7	26	<0,001
3 g/m^2	23	11	12	
4 g/m^2	39	32	7	

Table- 2. Comparing the original and biosimilar filgrastim according to the CD34$^+$ cells

CD34$^+$ cells	Allpopulation n=95	Neupogen n=50	Tevagrastim n=45	*p*
<2x10^6/kg	3(3.2%)	0(0.0%)	3(6.7%)	0.103
≥2x10^6/kg	92(96.8%)	50(100.0%)	42(93.3%)	
<4x10^6/kg	14(14.7%)	4(8.0%)	10(22.2%)	0.051
≥4x10^6/kg	81(85.3%)	46(92.0%)	35(77.8%)	
<6x10^6/kg	37(38.9%)	14(28.0%)	23(51.1%)	0.021
≥6x10^6/kg	58(61.1%)	36(72.0%)	22(48.9%)	

Categorical variables are shown as number (%)

Table.3: Generic-original difference in terms of PLT before and after mobilization

Variables	All population n=95	Neupogen n=50	Tevagrastim n=45	*P*		
				PLT	G-CSF	PLT*G-CSF
PLT level before mobilization	232(107-630)	235(109-630)	229(107-443)	<0.001*	0.357**	0.658***
PLT level after mobilization	64(24-223)	67(24-223)	61(24-178)			

Numeric variables are shown as median (minimum-maksimum)
*PLT levels before and after collection showed a statistically significant difference.
**While pre-collection platelet values were normal in both Neupogen and Tevagrastim arms, post-collection platelet values decreased
***There was no statistically significant difference between the Neupogen and Tevagrastim groups in terms of the change in PLT levels before and after collection

Table-4: Biosimilar and original difference in terms of neutrophil and platelet engraftment day

Variables	All population n=95	Neupogen n=50	Tevagrastim n=45	*p*
Neutrophil engraftment day	11(7-19)	10(7-13)	11(9-19)	0.015
PLT engraftment day	11(7-37)	11(7-27)	11(7-37)	0.186

Numeric variables are shown as median (minimum-maksimum)

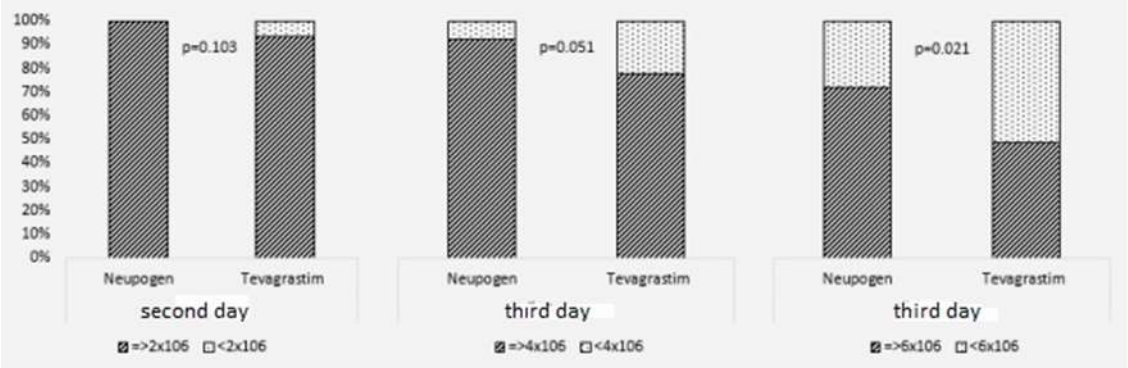

Figure-1. Comparing the orginal and biosimilar filgrastim according to the $CD34^+$ cells

the Neupogen arm (p <0.001). The cyclophosphamide dose was significantly higher in the Neupogen arm compared to the Tevagrastim arm (p<0.001).

No statistically significant difference was found between the use of the original and biosimilar products in terms of achieving the target of $2x10^6$/kg, which is the minimum $CD34^+$ cell dose required for adequate engraftment, and $4x10^6$/kg for double transplantation. With both drugs, the minimum and optimum amount of $CD34^+$ stem cells required for a single transplant could be collected at similar rates. However, if target level of $CD34^+$ stem cell target above $6x10^6$/kg, a statistically significant difference was observed in favor of Neupogen compared to the Tevagrastim arm. (p = 0.021) (Table 2, Figure 1).

PLT levels before and after collection showed a statistically significant difference. While pre-collection platelet values were normal in both Neupogen and Tevagrastim arms, post-collection platelet values decreased (p<0.001). However, there was no statistically significant difference between the Neupogen and Tevagrastim groups in terms of the change in PLT levels before and after collection (p=0.658). (Table 3) (Figure 2). Both products caused a similar decrease in platelet values.

When the neutrophil engraftment days after autologous stem cell transplantation were compared, it was observed that the neutrophil engraftment time was shorter in the Neupogen arm compared to the Tevagrastim arm (approximately 1 day) (p=0.015), while the platelet engraftment time was similar in both arms (p=0.186). (Table 4).

Discussion

Granulocyte colony stimulant factor or G-CSF and chemotherapy have been using in peripheral stem cell mobilization. In cases which stem cell mobilization could not be performed, the mobilization process is completed with plerixafor. G-CSF followed by cyclophosphamide was preferred to use in stem cell mobilization in this study. There are very few data in the literature that comparing the biosimilar and original molecule after using cyclophosphamide. The main goal during autologous stem cell transplantation is to provide patients with adequate number and quality of $CD34^+$ stem cells for neutrophil and platelet engraftment as early as possible. For

this reason, it is important to collect sufficient number and quality $CD34^+$ cells in stem cell mobilization. While all of these are targeted, the low cost are getting importance [5]. In recent years, low cost biosimilar molecules are preferred rather than original molecule. For this reason, the studies which are comparing the cost and efficacy in biosimilar and original molecule, are getting importance. We concluded that: If $CD34^+$ stem cells collected below the $6x10^6$/kg ($2x10^6$/kg, p =0.103 and $4x10^6$/kg, p = 0.051), there was no difference between the two products (biosimilar versus original). The other result we obtained that no significant differences were found on the platelet engraftment day (p=0.186, however, the neutrophil engraftment day was 1 day shorter in favor of the original molecule (10.34±1.02 days in the Neupogen arm, 11.00±1.65 days in the Tevagrastim arm; p=0.015) after autologous stem cell transplantation. This difference in neutrophil engraftment days between original and biosimilar products is important in terms of shortening the hospitalization period of the patients and reducing the infection rates. On the other hand, considering that other factors have an effect on the duration of hospitalization and infection rates. We think that 1-day differences in neutrophil engraftment was an acceptable for original product and the biosimilar product. Ergene et al. examined the factors that might have an effect on the neutrophil engraftment after autologous stem cell transplantation. These factors are age, gender, amount of $CD34^+$ cells, conditioning regimen, type of disease, and time to initiate G-CSF after autologous stem cell transplantation. They concluded that only the amount of $CD34^+$ cells had a significant effect on the neutrophil engraftment [6]. Other studies in the literature also support this finding: The most important factor affecting hematopoietic reconstruction after autologous stem cell transplantation is the quantity and quality of $CD34^+$ cells [7,8,9].

There are some studies in the literature that showing that biosimilars are as effective as the

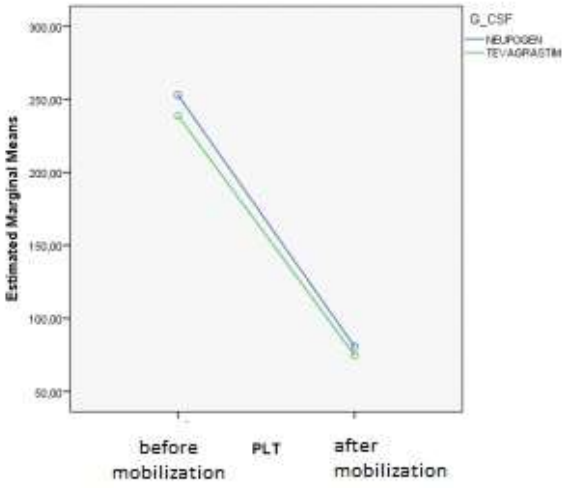

Figure-2. Generic-original difference in terms of PLT before and after mobilization

original molecule in neutropenia caused by chemotherapy as a result of the comparison of the original molecule and biosimilars [10,11,12]. Waller [10] and et al. compared the Amgen filgrastim with the biosimilar Hospira filgrastim in the phase-III study, in patients of breast cancer, and it was observed that the biosimilar Hospira filgrastim was as effective as the original molecule on neutropenia. Engert [11] et al. examined the effects of Tevagrastim (XMO2) and original filgrastim in patients with NHL. They found that both molecules could be similarly effective in neutropenia and febrile neutropenia. Sevic et al. were compared the original filgrastim (Neupogen) and biosimilar filgrastim (Tevagrastim) in oncologic patients [12]. This was a multicenter study that was included 337 patient from 14 centers. This studies' result indicate that original and biosimilar filgrastim have comparable efficacy in treating neutropenia [12]. Bassi et al. also compared two biosimilar products (Tevagrastim (®) and Zarzio (®)). Fifty-six MM and lymphoma patients who underwent autologous stem cell transplantation, were included in this study. They compared Tevagrastim® and Zarzio® according to the cost effect and engraftment days. While there is no difference between the two biosimilar products in terms of neutrophil engraftment

days, however, Tevagrastim has been shown to reduce costs by 56% and Zarzio by 86% [13]. In the literature, comparative studies of the original molecule and biosimilar product regarding peripheral stem cell mobilization are still insufficient [14,15]. Lefrere et al. examined 40 patients who had peripheral stem cell mobilization. They found no differences between the biosimilar molecule (Zarzio) and the original molecule (Neupogen) in terms of the median $CD34^+$ cell ratio obtained by [14]. Other study done by Schmitt et al. The original molecule and its biosimilars XM02 (Ratiograstim, Tevagrastim and Biograstim) were compared in 22 healthy donors, and no significant difference was found in terms of the amount of $CD34^+$ cells collected by peripheral stem cell mobilization [15]. Şıvgın and et al. reported another study from Turkey, which was compairing the orginal filgrastim (Neupogen) with biosimilar filgrastim (Leucostim) and lenograstim (Granulosyte). A total of 96 patients who were planned to undergo peripheral autologous stem cell transplantation, were included in this study. The highest level of $CD34^+$ cell were observed in the biosimilar filgrastim (leucostim) arm as a result of peripheral stem cell mobilization. This study has been emphasized that; the biosimilar filgrastim (leucostim) may be more advantageous than the original molecule and lenograstim [16].

Healthy donors of acute myeloid leukemia and myelodysplastic syndrome patients' were examined by Danylesko et al. There were two arms ıın thiıs study. $CD34^+$ cells were collected with the original molecule in one arm, and biosimilar filgrastim in the other arm. No significant difference were found between the median $CD34^+$ cells and the quality of the graft. The quality of graft was decided according to the similarity of neutrophil and thrombocyte engraftment days in allogeneic hematopoietic stem cell transplant patients [17]. Short time and rapidly engraftment means that high quality and quantity of $CD34^+$ cells according to Danylesko and et al [17]. Consequently; Danylesko et al, were concluded that the biosimilar filgrastim can be used in CD34+ cell mobilization from healthy donors as like as the original molecule[17].

We found no difference between the two products in terms of single transplantation or two transplantation in the quantity of $CD34^+$ cells ($<6x10^6$ $CD34^+$ cells). The thrombocyte engraftment days are the same in two product, while the neutrophil engraftment day is only 1 day shorter in favor of the original molecule. This was showing that the graft quality is similar for the two products. The difference between the two products had been arised, when the target level of $CD34^+$ cell is higher than the $6x10^6$. We thought that, this difference may depend on cyclophosphamide doses. The mean dose of cyclophosphamide in the original molecule arm was statistically significant higher than the dose of cyclophosphamide in the biosimilar arm ($p <0.001$). Hamadini et al. concluded the same result in the literature. They compared the patients who had low/ median/ high level of cyclophosphamide in peripheral stem cell mobilization. They observed that quantity of $CD34^+$ cells ($> 5x10^6$ $CD34^+$) was higher in the patients who had high level of cyclophosphamide [18]. Similarly to the data, the original and biosimilar filgrastim showed similar efficacy in stem cell collection in our study; however; different cyclophosphamide doses in the two arms cause a limitation for this study. Therefore, we need more studies involving case series with using high-dose cyclophosphamide which will compare the biosimilar molecule and the original molecule.

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Original Article

Evaluation of the Relationship Between Cytomegalovirus Replication and Acute Graft-Versus-Host Disease in Patients with Allogeneic Hematopoietic Stem Cell Transplantation

Allojenik Hematopoietik Kök Hücre Transplantasyonlu Hastalarda Sitomegalovirüs Replikasyonu ve Akut Graft-Versus-Host Hastalığı Arasındaki İlişkinin Değerlendirilmesi

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ABSTRACT

Introduction: It is controversial that cytomegalovirus (CMV) replication is a cause of graft-versus-host disease (GVHD). The aim of this study is to evaluate whether CMV replication causes acute GVHD development or not.

Materials and methods: The study is retrospective. Patients diagnosed with acute GVHD with a prior history of allogeneic hematopoietic cell transplantation (allo-HCT) were included in the study. All the included patients were followed-up in the bone marrow transplantation unit between 01/01/2013-10/31/2019. As the control group, patients without a history of acute GVHD after allo-HCT were included. The data of patients with acute GVHD after allo-HCT and patients with out known acute GVHD were compared.

Results: Fifty nine patients with acute GVHD and 178 patients without acute GVHD after allo-HCT were included in the study. Sixteen of the patients with acute GVHD were female and 43 were male. Sixty two of the patients without acute GVHD were female and 116 were male. The average CMV-DNA level was found as 1871.0 [821.0-16720.0] copies/ml in patients who had CMV replication in the acute GVHD group. On the other hand, the average of CMV-DNA level was found as 1607.5 [601.0-119181.0] copies/ml in the non-acute GVHD group. There was no statistically significant difference between the two groups.

Discussion: CMV replication does not seem to contribute to acute GVHD development after allo-HCT. Suppression of CMV replication may not prevent acute GVHD development.

Keywords: CMV replication, acute GVHD, allo-HCT

ÖZET

Giriş: Sitomegalovirus (CMV) replikasyonunun, graft-versus-host hastalığının (GVHH) bir nedeni olduğu tartışmalıdır. Bu çalışmanın amacı CMV replikasyonunun akut GVHH gelişimine neden olup olmadığını değerlendirmektir.

Gereç ve yöntemler: Çalışma, geriye dönüktür. Daha önceden allojenik hematopoietik hücre nakli (allo-HHN) öyküsü olan akut GVHH tanısı alan hastalar çalışmaya dahil edilmiştir. Dahil edilen tüm hastalar 01/01/2013-31/10/2019 tarihleri arasında kemik iliği nakil ünitesinde takip edilmiştir. Kontrol grubu olarak, allo-HHN sonrası akut GVHH öyküsü olmayan hastalar alınmıştır. Allojenik

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hematopoietik hücre naklinden sonra akut GVHH'si olan hastalar ile nakil sonrası bilinen akut GVHH'si olmayan hastaların verileri karşılaştırılmıştır.
Bulgular: Allojenik hematopoietik hücre naklinden sonra akut GVHH'li 59 hasta ve akut GVHH'si olmayan 178 hasta çalışmaya dahil edilmiştir. Akut GVHH'li hastaların 16'sı kadın, 43'ü erkektir. Akut GVHH olmayan hastaların 62'si kadın, 116'sı erkektir. Akut GVHH grubunda CMV replikasyonu olan hastalarda ortalama CMV-DNA düzeyi 1871,0 [821,0-16720,0] kopya/ml bulunmuştur. Akut olmayan GVHH grubunda ise CMV-DNA düzeyi ortalaması 1607,5 [601,0-119181,0] kopya/ml bulunmuştur. İki grup arasında istatistiksel olarak anlamlı bir fark saptanmamıştır.
Tartışma: CMV replikasyonunun, allo-HHN sonrası akut GVHD gelişimine katkıda bulunmadığı görülmektedir. CMV replikasyonunun baskılanması akut GVHH gelişimini engellemeyebilir.

Anahtar kelimeler: CMV replikasyonu, akut GVHH, allo-HHN

Introduction

Hematopoietic cell transplantation is accepted as a part of the treatment for many serious congenital or acquired diseases of the bone marrow [1]. Despite the progress in immunosuppressive and antiviral treatments, acute graft-versus-host disease (GVHD) and cytomegalovirus (CMV) infection remain major complications after allogeneic hematopoietic cell transplantation (allo-HCT) [2].

Multiple studies have shown that GVHD and its treatment put patients at risk for CMV replication [3-5]. In contrast, it is controversial that CMV replication might be a cause of GVHD. Data directly associating CMV replication and GVHD development are missing [6]. In a recent study reported by Wang LR et al, the presence of CMV replication was shown in the development of acute GVHD [7].

Hereby in this study, we aimed to evaluate whether CMV replication causes acute GVHD development or not.

Materials and method

The study is retrospective. Approval was obtained from the Ethics Committee of Tertiary Hospital (Approval date: 01/22/2020 and decision no: 2020-01/510).

Patients diagnosed with acute GVHD with a prior allo-HCT were included in the study. All the patients included in the study were followed-up in the bone marrow transplantation unit of tertiary hospital between 01/01/2013-10/31/2019. As the control group, patients without a history of acute GVHD after allo-HCT were included.

The present study was included data that were obtained from patients with acute GVHD and patients without acute GVHD after allo-HCT in tertiary hospital bone marrow transplantation unit.

Records of the patients were obtained electronically as a retrospective file scan. Demographic data and other information of the patients were recorded in a previously prepared form. The patients' gender, age during allo-HCT, underlying disease [Acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), chronic myeloid leukemia (CML), Hodgkin's lymphoma (HL), non-Hodgkin lymphoma (NHL), multiple myeloma (MM), myelodysplastic syndrome (MDS), other diseases], CMV replication status, measured CMV-DNA level in the patient file, status of acute GVHD presence, acute GVHD type [skin GVHD, liver GVHD, gastrointestinal tract (GIS) GVHD, lung GVHD, other GVHD] were recorded.

After allo-HCT, patients were monitored for CMV-DNA levels once a week.

In patients with CMV replication, initial values of CMV-DNA level above 1000 copies/ml or close to 1000 copies/ml were recorded. The data of patients with acute GVHD after allo-HCT and patients without known acute GVHD after allo-HCT were compared. Fifty nine patients with acute GVHD and 178 patients without acute GVHD (as a control group) were included in the study.

Table 1. Evaluation of the effect of age, gender, donor match status, CMV replication status and disease type on GVHD.

Variable	Acute GVHD (n=59)	No GVHD (n=178)	Total (N=237)	Statistical analysis* Possibility
Gender				
Female	16 (27.1%)	62 (34.8%)	78 (32.9%)	χ^2=1.194 p=0.275
Male	43 (72.9%)	116 (65.2%)	159 (67.1%)	
Underlying disease				
AML	20 (33.9%)	86 (48.3%)	106 (44.8%)	χ^2=14.581 p=0.029
ALL	21 (35.6%)	45 (25.3%)	66 (27.8%)	
CML	1 (1.7%)	8 (4.4%)	9 (3.8%)	
HL	9 (15.2%)	11 (6.2%)	20 (8.4%)	
NHL	1 (1.7%)	14 (7.9%)	15 (6.4%)	
MM	-	2 (1.1%)	2 (0,8%)	
MDS	3 (5.1%)	3 (1.7%)	6 (2.5%)	
Other diseases	4 (6.8%)	9 (5.1%)	13 (5.5%)	
CMV replication				
Yes	27 (47.4%)	95 (54.0%)	122 (52.4%)	χ^2=0.754 p=0.385
No	30 (52.6%)	81 (46.0%)	111 (47.6%)	

*"Pearson-χ2 cross tables" were used to analyze the relations of two qualitative variables.
GVHD: Graft-versus-host disease, AML: Acute myeloid leukemia, ALL: Acute lymphoid leukemia, CML: Chronic myeloid leukemia, HL: Hodgkin's lymphoma, NHL: Non-Hodgkin lymphoma, MM: Multiple myeloma, MDS: Myelodysplastic syndrome.

Patients aged 15 years or older, patients who had allo-HCT with or without acute GVHD were included in the study.

Statistical analysis

SPSS (IBM SPSS Statistics 24) was used to analyze the data. Frequency tables and descriptive statistics were used to interpret the findings. "Pearson-χ2 cross tables" was used to analyze the relationship between the qualitative variables. In the data without normal distribution, "Mann-Whitney U" test (Z-table value) statistics were used to compare the two independent groups with the measured values. P <0.05 was considered statistically significant.

Results

Fifty nine patients with acute GVHD and 178 patients without acute GVHD after allo-HCT were included in the study. Sixteen (27.1%) of the patients with acute GVHD were female and 43 (72.9%) were male. Sixty two (34.8%) of the patients without acute GVHD were female and 116 (65.2%) were male. There was no significant difference between the two groups in terms of gender (Table 1). The age at the time of transplantation was 35.0 [15.0-63.0] years in patients with acute GVHD and 36.5 [15.0-64.0] years in patients without acute GVHD. There was no statistically significant difference between the two groups in terms of the age at the time of transplantation (Z = -0.943, p = 0.346) (Table 2).

A statistically significant difference was found between the groups in terms of underlying disease type ($\chi2$ = 14.581; p = 0.029).

The incidence of acute GVHD was the highest among patients with ALL, on the other hand, the incidence of acute GVHD was the least among patients with AML (Table 1).

There was no statistically significant difference between the groups regarding CMV replication status (p> 0.05). In acute GVHD group, CMV replication occured in 27 (47.4%) patients wheras 95 (54.0%) patients had CMV replication in the non-acute GVHD group (Table 1).

The average CMV-DNA level in patients who had CMV replication in the acute GVHD

Table 2.The effects of transplantation age, CMV-DNA level on GVHD development.

Variable	Acute GVHD (n=59)	No GVHD (n=178)	Statistical analysis* Possibility
Transplantation age (years)	35.0 [15.0-63.0]	36.5 [15.0-64.0]	Z=-0.943 p=0.346
CMV-DNA level (copies/ml)	1871.0 [821.0-16720.0]	1607.5 [601.0-119181.0]	Z=-0.890 p=0.373

*In the data without normal distribution, "Mann-Whitney U" test (Z-table value) statistics were used to compare the two independent groups with the measured values.
GVHD: Graft-versus-host disease.

Table 3. Distribution of acute GVHD types

Distribution of acute GVHD types	Acute GVHD	
	n	%
Skin	23	39.0
Skin and liver	4	6.8
Skin and gastrointestinal tract	7	11.9
Liver	12	20.3
Liver and gastrointestinal tract	1	1.7
Gastrointestinal tract	12	20.3

GVHD: Graft-versus-host disease.

group was found as 1871.0 [821.0-16720.0] copies/ml. Besides, the average CMV-DNA level was found as 1607.5 [601.0-119181.0] copies/ml in non-acute GVHD group. There was no statistically significant difference between the two groups in terms of CMV-DNA level (Z= -0.890, p = 0.337) (Table 2).

Acute skin GVHD which was detected in 23 (39%) of the patients, was the most common form of acute GVHD in patients. Gastrointestinal system acute GVHD and liver acute GVHD were nearly equally detected in patients (Table 3).

Discussion

Acute GVHD, which is one of the most important complications of allo-HCT [8]. Acute graft-versus-host disease remains a major cause of morbidity and mortality following allo-HCT [9]. Despite prophylactic treatment, acute GVHD affects 30%–70% of recipients depending on the type of transplant, patient characteristics and GVHD prophylaxis regimen [10].

The National Institutes of Health consensus criteria use clinical findings to distinguish between acute and chronic [11]. Therefore, patients presenting with typical acute GVHD findings before the 100th day are considered to be "classical acute GVHD", whereas patients presenting with the same findings after the 100th day are typically categorized as "late-onset acute GVHD" upon reduction of immunosuppression [11].

In a study, it has been reported that skin, GIS and liver are the main target organs in patients with acute GVHD [12]. In the study of Martin et al, they found that 81% of patients had skin involvement, 54% had GIS involvement, and 50% had liver involvement at the onset of acute GVHD [13].

In this study, we found that skin, GIS and liver GVHDs were observed more frequently in patients with acute GVHD.

Risk-bearing allo-HCT recipients should be screened at least once a week from the 10th day to the 100th day after transplant, regardless of the treatment method chosen for the presence of CMV viremia or antigenemia [14].

In this study, CMV replication before acute GVHD was detected in 27 (47.4%) of 59 patients with acute GVHD. On the other hand, CMV replication was detected in 95 (54.0%) of 178 patients in the non-acute GVHD group.

There was no statistically significant difference found between the two groups in terms of the frequency of patients with CMV replication status.

Endothelial cells infected with CMV might produce inflammatory cytokines, such as interleukin 6, which play an important role in the initial phase of GVHD [15].

In patients with CMV replication after allo-HCT, the inflammatory response can thus contribute to the initiation of acute GVHD [16]. However, data directly linking CMV replication with acute GVHD development are not yet available [17,18]. In the study of Meyers et al, it was reported that the frequency of acute GVHD increased in relation to the CMV replication [19].

In the study of Cantoni et al, they reported that acute GVHD incidence and transplant-related mortality increased in patients with CMV replication after allo-HCT [20]. On the other hand, we did not find any statistically significant difference between the two groups in terms of the frequency of patients with CMV replication.

CMV-DNA levels should be monitored from days of engraftment started to at least 100 days in the recipients after allo-HCT [21].

In conclusion, this study shows that CMV replication may not contribute to acute GVHD development after allo-HCT. When CMV replication is detected in patients, preemptive treatment is generally started. However, it should be kept in mind that suppression of CMV replication would not prevent acute GVHD development.

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Original Article

Parosteal Osteosarcoma: Radiologic and Prognostic Features

Parosteal Osteosarkom: Radyolojik ve Prognostik Özellikler

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ABSTRACT

Aim: The aim of this study is to evaluate significance of Magnetic Resonance Imaging (MRI) characteristics and their effects on surgical margins, prognosis, oncological and functional outcomes of parosteal osteosarcoma in our clinic.

Patients and method: Fifteen patients (4 male/11 female) who operated with the diagnosis of parosteal osteosarcoma in our clinic were retrospectively reviewed. The epidemiological data, biopsy method and delay in diagnosis are noted. The lesions maximum circumferential extension, intramedullary involvement and neurovascular extension in MRI sections were evaluated. The resection type (segmental articular/hemicortical) and surgical margins were noted. Functional and oncological results at last follow-up were assessed.

Results: The mean age was 31.7 (17-71) years; mean follow-up was 50.1 (24-176) months. The most common site was distal femur. The closed biopsies were related with increased number of re-biopsies and misdiagnosis/improper interventions. ($p<0.001$,$p=0.023$) Intramedullary involvement percentage was related with maximum circumferential extension percentage ($p=0.006$) The intramedullary involvement ratio of ≤25% suggested no recurrence or metastasis. The mean MSTS score was 87.8% (range, 60-100%). The neurovascular involvement was related with metastatic disease, deep infections and complication related surgeries. ($p=0.017$, $p=0.002$,$p=0.005$) The most common resection type was segmental articular resection (9 patients). Hemicortical resections with biological reconstructions had the best MSTS scores. ($p=0.002$) The maximum circumferential extension percentage, intramedullary involvement percentage and neurovascular involvement showed lower MSTS scores. The 5-year overall survival was 92.3%, local recurrence-free survival was 86.2% and metastasis-free survival was 86.2%.

Conclusion: The reliability of needle biopsy sampling is controversial in parosteal osteosarcomas. The lesions extent of intramedullary involvement, neurovascular bundle proximity and maximum periosteal circumferential extension on MRI should be considered when planning the surgery. The evaluation of maximum circumferential extension on MRI is crucial for the resection margins.

Keywords: Parosteal Osteosarcoma, Radiological evaluation, Prognostic criteria, Biopsy method, Tumor endoprosthesis

ÖZET

Giriş ve Amaç: Parosteal osteosarkomlar nadir gözlenen bir osteosarkom tipidir. Klasik olarak kemoterapi ve radyoterapiye duyarsız, düşük dereceli bir lezyondur. Ancak kolaylıkla çevre dokulara invazyon gösterebilmektedirler.Amacımız parosteal osteosarkomlarda manyetik rezonans görüntüleme (MRG) ile tespit edilen özelliklerin; cerrahi sınır, prognoz, onkolojik ve fonsiyonel sonuçlara etkilerini değerlendirmektir.

Yöntem ve Gereçler: Kliniğimizde 2006-2018 yılları arasında parosteal osteosarkom tanısı ile cerrahi tedavi uygulanmış asgari 2 yıl takip edilmiş 15 hastanın (4 Erkek/11 Kadın) klinik ve radyolojik verileri retrospektif olarak değerlendirildi. Epidemiyolojik verilerin yanında tanı için uygulanan biyopsi yöntemi (açık/kapalı) ve tanıda gecikme süresi not edildi. Hastaların tanısal MRG kesitlerinde lezyonun azami çevresel uzanım oranı, intrameduller tutulum oranı ve nörovasküler paket tutulumu

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değerlendirildi. Rezeksiyon tipleri (segmental ekleme uzanan/hemikortikal) ve rezeksiyon sonrası cerrahi sınırlar değerlendirildi. Hastaların son kontrollerindeki fonksiyonel ve onkolojik sonuçları değerlendirildi.
Bulgular: Çalışma grubumuzda ortalama yaş 31.7(17-71), ortalama takip süresi 50.1(24-176) ay idi. En sık tespit edilen tutulum distal femurdu. Kapalı biyopsi yöntemlerinin tekrarlayan biyopsiler/yanlış tanı/uygunsuz girişimlere yol açtığı belirlendi($p<0.001$, $p=0.023$). Lezyonun intrameduller tutulum oranının, azami çevresel uzanım miktarı ile ilişkili olduğu belirlendi($p=0.006$). %25'in altında intrameduller tutulum oranının yapılan uygun rezeksiyonlarla nüks veya metastazlara yol açmadığı belirlendi. Ortalama MSTS skoru %87.8 idi. Nörovasküler tutulum varlığının metastazlar, derin enfeksiyonlar ve tekrarlayan cerrahiler ile ilişkili olduğu belirlendi ($p=0.017$, $p=0.002$, $p=0.005$). En sık uygulanan rezeksiyon tipi segmental, ekleme uzanan rezeksiyon (9 hasta) idi. Hemikortikal rezeksiyon ve biyolojik rekonstrüksiyonların en iyi MSTS skorlarına sahip olduğu belirlendi ($p=0.002$). Yüksek azami çevresel uzanım miktarı, intrameduller tutulum oranı ve nörovasküler tutulum varlığının düşük MSTS skorları ile ilişkili olduğu belirlendi. 5-yıllık genel sağkalımın %92.3, lokal-progresyonsuz sağkalımın %86.2 ve metastassız sağkalımın ise %86.2 olarak tespit edildi.
Tartışma ve Sonuç: Parosteal osteosarkomlarda iğne biyopsisinin güvenilirliği sorgulamaya açıktır. Tanısal MRG'de ekleme uzanım kadar lezyonun azami çevresel uzanım miktarı, intrameduller tutulum oranı ve nörovasküler tutulum varlığı cerrahi öncesi planlamada ve rekonstrüksiyon yöntemi belirlemede önem taşımaktadır. MRG'de lezyonun azami çevresel uzanım miktarı cerrahi sınırları belirleyebilmek açısından önem taşımaktadır.

Anahtar Kelimeler: Parosteal osteosarkom, Radyolojik değerlendirme, Prognostik kriterler, Biyopsi yöntemi

Introduction

Parosteal osteosarcoma is a rare surface osteosarcoma variant which commonly involves the metaphyseal ends of the bones [1-4]. The well-differentiated, insidious character of the tumor may lead to delayed diagnoses and symptoms [1,3-6]. Wide resection is the recommended treatment regarding its low-grade malignant potential and low potential to metastasize [4,6,7]. Although parosteal osteosarcoma are mostly encountered as low grade, intermediate and dedifferentiated high grade types are not rare [4,6-9]. Low and intermediate grade variants mostly show better survival however dedifferentiated high grade variants have worse outcomes by means of both local progression and metastasis [4,7]. The incidence of local recurrences in parosteal osteosarcoma mostly depends on the quality of surgical margins and can even be detected within twenty years postoperatively [3,4,6-10].

The lesions in close proximity to the neurovascular structures is a major problem particularly as a result of recurrent biopsies or operations [3]. The other most common pitfall encountered in parosteal osteosarcoma patients is being misdiagnosed and improperly intervened as benign lesions [3-6]. There is also a debate in determining the treatment method and prediction of local recurrence in detection of intramedullary extension on magnetic resonance imaging (MRI) [2,3,11,12]. A meticulous pre-operative planning by MRI is recommended to define resection margins and histologic grade [11,13].

In our study we sought to evaluate significance of MRI findings and surgical margins on prognosis with oncological and functional outcomes by the retrospective single center experience of parosteal osteosarcoma.

Patients and Method:

The clinical and radiological data of 23 patients who received surgical treatment with

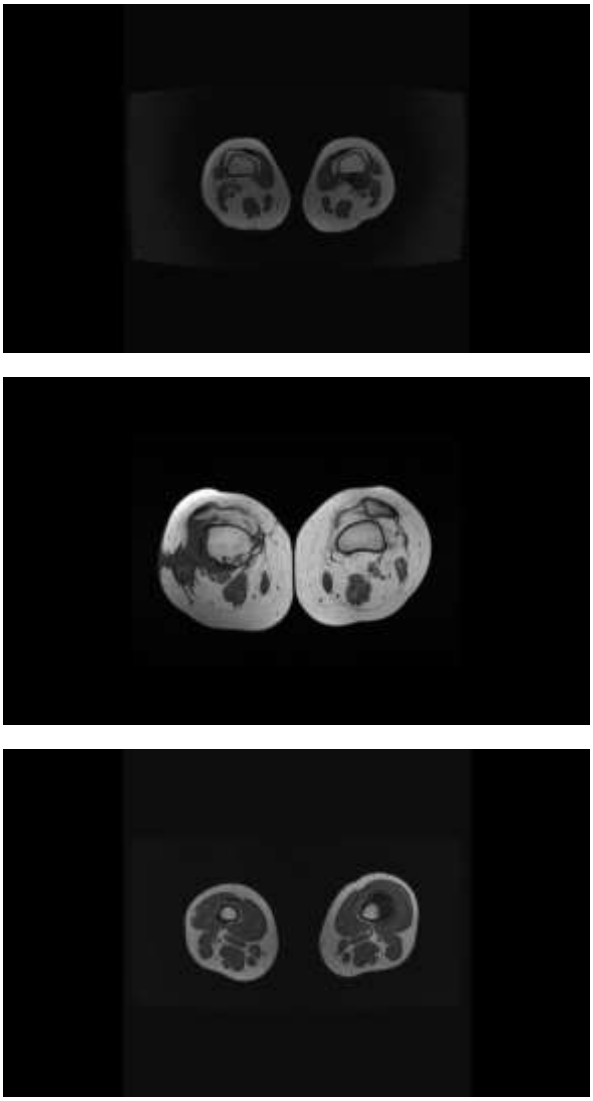

Figure 1. The examples of circumferential extension of the cases in our study. T1 weighted contrast enhanced series of the patients are evaluated and approximately (A) 30% circumferential extension, (B) 50% circumferential extension and (C) 75% circumferential extension of the parosteal osteosarcoma examples are demonstrated.

the diagnosis of parosteal osteosarcoma in our institution between May 2006 and February 2018 were retrospectively reviewed. Five patients had limited radiological data, two patients had their parosteal osteosarcoma diagnosis and management in elsewhere center and a patient was lost to follow-up. Thus, eight patients were excluded and 15 patients (4 male/11 female) were included in the study group.

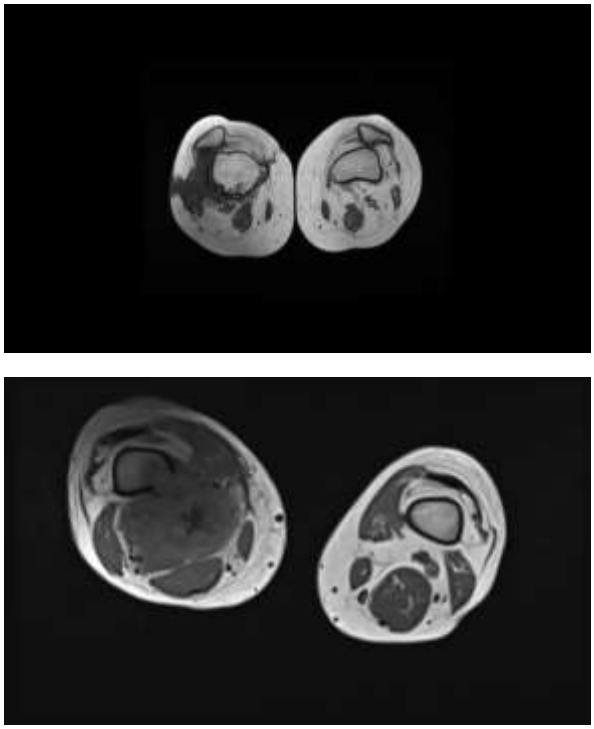

Figure 2: The examples of intramedullary involvement percent of the cases in our study. T1 weighted contrast enhanced series of the patients are evaluated and approximately (A) <10% intramedullary involvement, (B) 75% intramedullary involvement examples from series are shown.

This study (no. 280) was assessed by Institutional Review Board of Baltalimanı Bone Diseases Training and Research Hospital in the 68th board meeting held in 02.03.2021. The board approved the research on compliance with the rules of ethics and scientific guidelines.

The epidemiological data was evaluated regarding site of the lesion and biopsy method. The histopathological diagnosis of longer than 3 months is accepted as delay in diagnosis. The delay in diagnosis and Enneking stage of the lesion were also noted.

Preoperative MRI assessment was performed regarding the lesion's maximum circumferential extension percentage in transverse sections (Figure 1). Intramedullary involvement was evaluated in T2 sections and

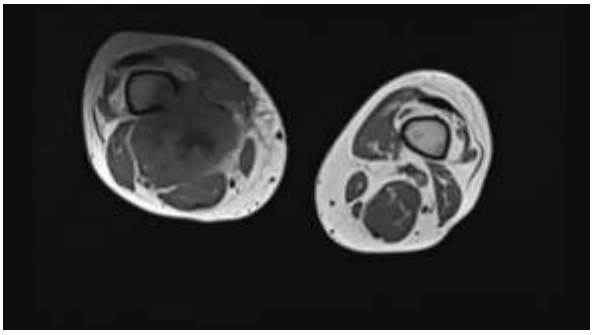

Figure 3: The examples of neurovascular involvement types in our series. T1 weighted contrast enhanced series demonstrate involvement of the neurovascular structures by the tumor.

contrast enhanced MRI series and the percentage of the involvement was noted (Figure 2). The neurovascular bundle involvements were also evaluated. (Figure 3) The patients are further evaluated regarding resection type (segmental intraarticular/ hemicortical), histopathological margins. The occurrence of complications (infections, local recurrences, implant failures) during follow-up were noted. The MSTS scores of the patients at latest follow-up were also evaluated. The oncologic status at the latest follow-up was noted (continuous disease-free (CDF), no evidence of disease (NED), alive with disease (AWD) and died of disease (DOD)). The overall survival was defined as the interval in months, from the time of definitive surgical treatment to the last follow-up visit or death.

Recurrence-free survival and metastasis-free survival were defined as the interval in months with no evidence of local disease and metastatic disease after definitive surgical treatment, respectively. The statistical analysis of the observed values was performed using Fischer's exact test or Kruskal-Wallis test. The survival of the patients was evaluated by the Kaplan–Meier method with a log rank test. The independent factors effecting survivals were identified by cox regression analysis. Statistical analyses were performed by using SPSS version 20.0 (SPSS, Chicago, IL). A p-value of < 0.05 was considered statistically significant

Results:

A total of 15 patients were included in the study group. The mean age was 31.7 years (median, 30; range, 17-71), mean follow up was 50.1 months, median follow-up was 30 (range, 24-176) months. The most common site was distal femur in 13 patients. Ten patients had previous biopsies in elsewhere centers. Our series suggested that previous core needle biopsies in elsewhere center was significantly related with the increased number of re-biopsies and misdiagnoses and/or improper interventions. ($p<0.001$, $p=0.021$) There was delay in diagnosis in 7 patients. Closed biopsy methods were significantly related with delayed diagnosis. ($p=0.044$) Nine patients were staged as Enneking IB, five patients were IIB and a patient with lung metastasis was staged as III. (Table 1) The maximum circumferential extension percentage was a crucial indication for selection of resection method in our series. The mean circumferential extension percentage was 39.0% in hemicortical resections whereas 47.8% in intraarticular resections. (Table 2) Intramedullary involvement was evident in 8 patients. Intramedullary involvement ratio was significantly correlated with the maximum circumferential extension percentage ($p=0.006$) The intramedullary involvement ratio of $\leq 25\%$ suggested no recurrence or metastasis. Metastasis was related with intramedullary involvement ratio. ($p=0.004$) The neurovascular bundle proximity (<5 mm) or involvement was detected in 4 patients. Neurovascular involvement was significantly related with maximum circumferential extension percentage and intramedullary involvement percentage ($p=0.032$,$p=0.041$). Besides the presence of neurovascular

Table 1: Epidemiological data of the parosteal osteosarcoma patients in our series.

Variable		Value
Mean age		31.7 (Range: 17-71, Median: 30)
Gender		4 Male, 11 Female
Localization	Distal Femur	13
	Proximal Tibia	1
	Distal Tibia	1
Biopsy method	Open Biopsy	9
	Needle Biopsy	6
Previous Biopsies	None	6
	Needle Biopsy	9
Misdiagnosis/Improper intervention		7
Delay in diagnosis	>6 months	3
	3-6 months	4
Lung Metastasis	Diagnosis	1
	Follow-up	1
Enneking stage	IB	9
	IIB	5
	III	1

involvement was found to significantly related with metastatic disease, complication related surgeries and lower MSTS (p=0.017,p=0.005,p=0.026).

The most common resection type was segmental articular resection in 9 patients. (Table 3) Histopathologically evaluated surgical margins were all tumor free. Prosthetic reconstructions were applied in nine patients, biologic reconstruction (liquid nitrogen recycled bone and hemicortical fixation) was performed in six. (Figure 4)

The parosteal osteosarcoma should meet two radiological criteria in preoperative MRI evaluation for consideration of hemicortical resections. Hemicortical resections with biological reconstructions demonstrated the best MSTS scores. (p=0.002) (Figure 4) The maximum circumferential extension ratio, intramedullary involvement ratio and neurovascular involvement were inversely correlated with MSTS scores. (p=0.003, p=0.044, p=0.025) In two patients vascular resections required reconstructions.

Table 2: The localization and preoperative radiological evaluation criteria of the patients in our series.

Patient No.	Localization	Maximum Circumferential Extension (%)	Intramedullary Involvement (%)	Neurovascular Bundle Involvement
1	Distal Femur	20	65	N/A
2	Distal Femur	40	75	Involvement
3	Distal Femur	80	<25	N/A
4	Distal Femur	40	<25	N/A
5	Distal Femur	25	<25	N/A
6	Distal Femur	40	<25	N/A
7	Proximal Tibia	75	<25	N/A
8	Distal Femur	50	80	Close Proximity (<5 mm)
9	Distal Femur	50	<25	N/A
10	Distal Tibia	50	100	Close Proximity (<5 mm)
11	Distal Femur	30	<25	N/A
12	Distal Femur	10	50	Involvement
13	Distal Femur	60	<25	N/A
14	Distal Femur	60	<25	N/A
15	Distal Femur	40	<25	N/A

The postoperative deep infections were encountered in two pateints, local recurrences in two and distant metastases in two patients. Regarding the oncological status 15 patients were CDF and NED. The mean MSTS score was 87.8% (range, 60-100%). (Table 3)

The 5-year overall survival was 92.3%. (Figure 5) 5-year local recurrence-free survival was 86.2%. (Figure 6) 5-year metastasis-free survival was 86.2 %. (Figure 7)

Discussion

The low grade and well-differentiated character of parosteal osteosarcoma may lead to delayed symptoms and/or misdiagnoses [1,3-6]. Also repetitive biopsies or improper interventions with misdiagnosis may result in adhesions and local seeding of this malignancy [3-6]. As popliteal region is commonly involved, the lesion is mostly in close proximity to major popliteal neurovascular structures, which may also complicated by repetitive procedures [3]. Regarding its low-grade character, limited local extension and low potential to metastasize, wide resection is recommended as the mainstay of treatment [4,6,7]. Determination of the treatment method and prediction of local recurrence in presence of intramedullary extension on magnetic resonance imaging (MRI) is debated [2,3,11,12]. We sought to evaluate the MRI findings, common diagnostic problems and their effects on prognosis with oncological and functional outcome differences in parosteal osteosarcoma.

We have found that seven of the fifteen patients in our study had more than one biopsy to achieve the definitive diagnosis and five of them was needle biopsy. The frequency of previous biopsies/delayed diagnosis ratio was higher in needle biopsy group. ($p<0.001$). The reliability of needle biopsies reported to be limited in determining not only the diagnosis but also the degree of anaplasia in parosteal

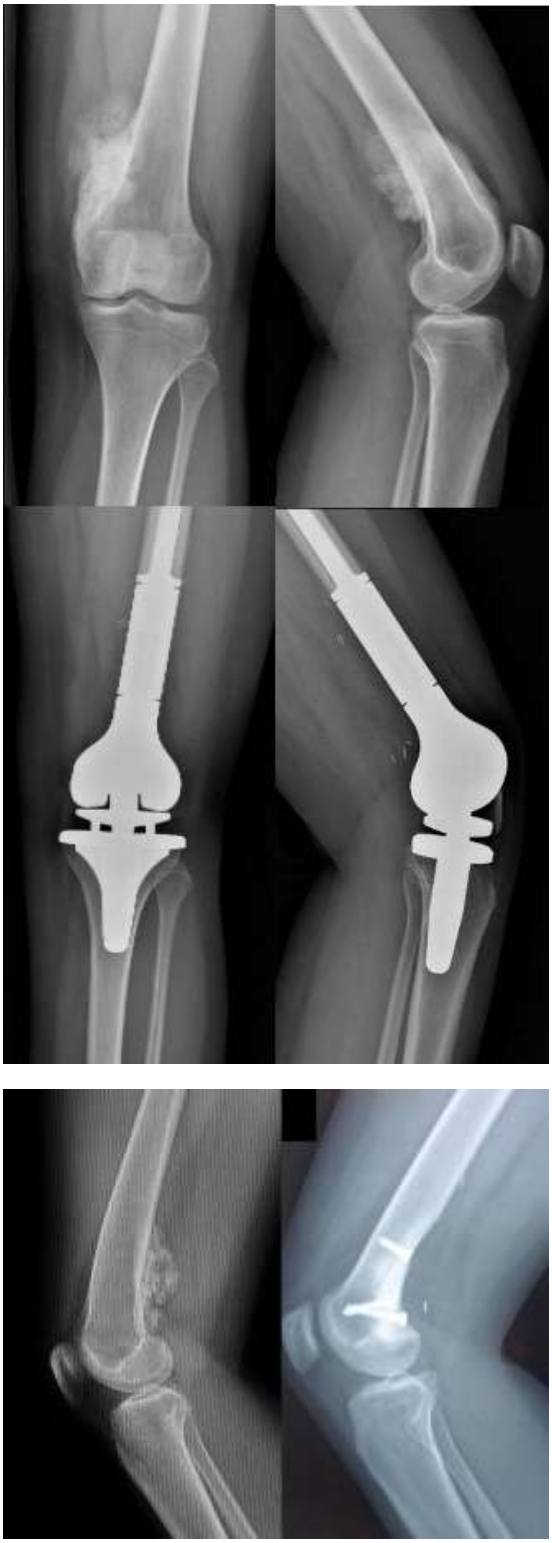

Figure 4: (A) Pre- and post-operative 1st year anteroposterior and lateral x-ray views of a 40-year-old patient who was applied segmental extraarticular resection and tumor endoprosthetic reconstruction. (B) Pre- and post-operative 2nd year lateral x-ray views of a 37-year-old patient who was applied hemicortical resection followed by fixation with screws is demonstrated.

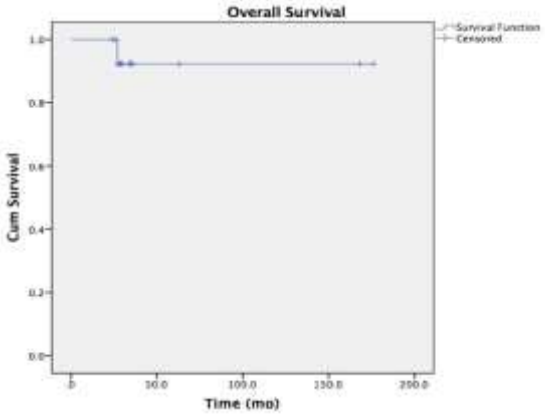

Figure 5: The Kaplan Meier survival curve demonstrating the overall survival of parosteal osteosarcoma in our series.

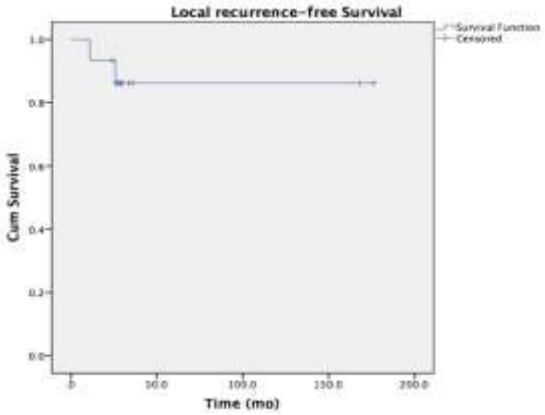

Figure 6: The Kaplan Meier survival curve demonstrating the local recurrence-free survival of parosteal osteosarcoma in our series.

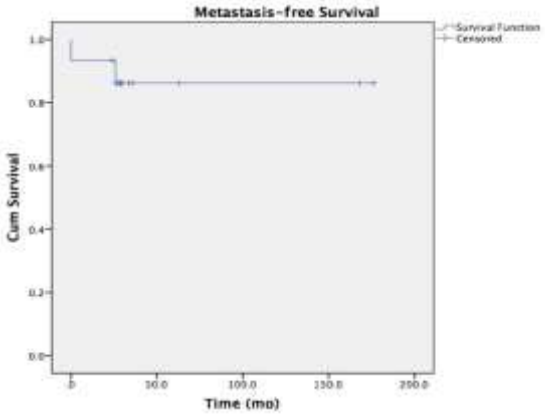

Figure 7: The Kaplan Meier survival curve demonstrating the metastasis-free survival of parosteal osteosarcoma in our series.

Table 3: The resection, reconstruction and surgical margin features with the outcome data of the patients in our series.

Patient No.	Resection Type	Surgical Margin	Reconstruction Type	Follow-up Time (mo)	Oncological Status	Local Recurrence (mo)	Metastasis (mo)	Deep Infection (mo)	MSTS Score (%)
1	Segmental	Negative	Tumor Endoprosthesis	36	NED	N/A	N/A	N/A	90
2	Segmental	Negative	Tumor Endoprosthesis	29	NED	N/A	N/A	4	87
3	Hemicortical	Negative	Liquid Nitrogen and Screw fixation	34	NED	N/A	N/A	N/A	100
4	Segmental	Negative	Tumor Endoprosthesis	29	NED	N/A	N/A	17	90
5	Hemicortical	Negative	Liquid Nitrogen and Screw fixation	168	CDF	N/A	N/A	N/A	100
6	Hemicortical	Negative	Liquid Nitrogen and Screw fixation	34	NED	N/A	N/A	N/A	100
7	Segmental	Negative	Tumor Endoprosthesis	30	NED	N/A	N/A	N/A	93
8	Hemicortical	Negative	Liquid Nitrogen and Screw fixation	176	CDF	N/A	N/A	N/A	100
9	Hemicortical	Negative	Liquid Nitrogen and Screw fixation	27	NED	N/A	N/A	N/A	100
10	Segmental	Negative	Tumor Endoprosthesis	35	NED	26	26	N/A	70
11	Hemicortical	Negative	Liquid Nitrogen and Screw fixation	28	NED	N/A	N/A	N/A	80
12	Segmental	Negative	Tumor Endoprosthesis	27	DOD	N/A	0	N/A	60
13	Segmental	Negative	Tumor Endoprosthesis	26	NED	N/A	N/A	N/A	76
14	Segmental	Negative	Tumor Endoprosthesis	63	NED	11	N/A	N/A	83
15	Segmental	Negative	Tumor Endoprosthesis	24	NED	N/A	N/A	N/A	87

osteosarcoma [14]. Some authors reported a high rate of histological difference after total excision, which 60% of the needle biopsy revealed accuracy in final degree of the lesion, whereas this rate was 88% in open biopsy in the same study [4]. Previous biopsies and improper interventions constitute a major problem in achieving diagnosis and planning the treatment of parosteal osteosarcoma [4,14]. Each intervention on parosteal osteosarcoma, particularly in the popliteal region, requires extensive dissection of the surrounding soft tissues and neurovascular structures, which may cause adhesions [12]. These adhesions and scar tissue may complicate the diagnostic evaluation thus a detailed information on previous interventions is essential. One patient in our series had 4 closed biopsies in 5 years in elsewhere center however final diagnosis was achieved at 5th biopsy (open) in our clinic. Deep infections and complication related surgeries were thought to be related with repetitive interventions in our series.

The intramedullary changes were one of the major factors that helped the decision of resection type in our study. We assume that some intramedullary changes might be originated from the increased pressure inside the medulla, also we have detected limited intramedullary involvements around the vascular entry or tendinous/ligamentous attachment sites to the bone. The hemicortical resections including limited intramedullary involvement may be a method of choice. Bertoni et al. compared the radiologic and histopathologic findings of 18 parosteal osteosarcoma with intralesional radiolucencies and concluded that most of the high-grade components were located in deep radiolucency areas [12]. Han et al. have reported that in radiological evaluation (MRI, CT) 81% of their cases demonstrated intramedullary changes, however only 48% confirmed this radiological change [15]. They have concluded that the disparity may be related to the exaggerated views on MRI [15]. Jelinek et al. had direct anatomic mapping and correlation in four high-grade parosteal osteosarcoma and concluded that three lesions demonstrated intramedullary extension by a low-grade component and only one high-grade component was evident in juxtacortical portion of the tumor [11]. Okada et al. reported that intramedullary involvement is a sign of higher-grade lesion and when present, usually does not involve more than one quarter of the intramedullary canal diameter [3]. The intramedullary involvement is reported to correlate with metastasis, local recurrence and worse prognosis by several authors, however this information was contradicted by some studies [2-4,7,12,14]. We recommend hemicortical resections in cases with a maximum circumferential extension of <50% and intramedullary involvement of <25%.

The neurovascular involvement was found more frequently in patients with higher maximum circumferential extension percentage ($p=0.036$) and intramedullary involvement ($p=0.041$). The assessment of soft tissue extension in primary parosteal osteosarcoma, which is reported as a significant determinant of the grade, should include meticulous evaluation of MRI [3,11,15]. Achieving wide margins in the popliteal region is reported to be difficult without sacrificing the neurovascular bundle [15]. Our study showed that the presence of neurovascular involvements was related with the incidence of metastatic disease, deep infections and complication related surgeries.

Complete excision is reported to be the mainstay of the treatment in parosteal osteosarcomas [2-4,6,7,10,12,15]. It is noted that tumor positive margins were associated with metastasis and local recurrences even after two decades [2,9]. Lewis et al. stated that

tumor free margins remains the critical factor determining overall prognosis [12]. Our study demonstrated that the maximum periosteal circumferential extension percentage of the parosteal osteosarcoma on preoperative MRI could be a good indicator for determining involvement and planning wide resections. Our study also suggested that these lesions have tendency to spread over the bone segment in all dimensions as they become larger thus we recommend meticulous evaluation of the horizontal sections of the MRI for planning resections. Our study suggested that the maximum periosteal circumferential extension percentage of the lesion that exceeds 50% of the bone at horizontal plane could be an indicator of intramedullary involvement. The literature suggests that the accurate definition of the margins on preoperative multiplanar MRI is important for successful wide resection in parosteal osteosarcoma [11,16]. Amputations are recommended in patients with large circumferential tumor with involvement of the neurovascular structures or contaminated surrounding soft tissue as a result of previous interventions [3].

Overall, our results were consistent with earlier studies by means of oncological outcomes. The 10-year overall survival was reported as 80-91% in various series, which, some authors included dedifferentiated cases [3,4,7]. Including dedifferentiated cases, our study suggested a 5-year overall survival as high as 92.3%. The local recurrence rate of parosteal osteosarcoma was reported within a range of 0-57% in published series [1,3,4,6,7,12,15]. Present study not only included primary cases but also improper interventions and misdiagnosed cases, have demonstrated a 5-year local recurrence-free survival ratio of 86.2%. The metastasis rates were reported ranging 8-14.8% in previous series [3,4,7]. Okada et al. suggested 11% cumulative probability rate within 10-year [3]. The distant metastasis patients in our series was 2 with a 5-year metastasis-free survival of 86.2%. One patient who had lung metastasis at the time of diagnosis had history of previous biopsies in elsewhere center. The presence of delayed diagnosis and improper interventions have decreased metastasis free survival ratios in our series.

This study had several limitations. This study is primarily based on MRI findings and their prognostic importance. Some misdiagnosed patients have applied previous operations without the diagnosis of parosteal osteosarcoma. To overcome a potential bias, the evaluations were completed after the diagnosis of parosteal osteosarcoma in our clinic.

The reliability of needle biopsy sampling which, may cause misdiagnoses or improper interventions, is controversial in parosteal osteosarcomas. Previous interventions should be questioned in details especially in lesions, which are in close relation with neurovascular structures. When planning the surgery, the orthopaedic surgeon must consider the lesions extent of intramedullary involvement, neurovascular bundle proximity and maximum periosteal circumferential extension on MRI. Particularly the evaluation of maximum circumferential extension on preoperative MRI is crucial in determining the resection margins.

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Original Article

Immunohistochemical Evaluation of M30 and M65 in Barrett's Esophagus and Esophageal Cancer

Barrett's Özofagus ve Özofagus Kanserinde M30 ve M65'in İmmünohistokimyasal Olarak Değerlendirilmesi

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ABSTRACT

Introduction: Although many markers have been studied in esophageal adenocarcinomas, there is no marker currently available for clinical use. This study aimed to investigate the role of apoptosis in Barrett's esophagus and adenocarcinoma carcinogenesis, determine whether there is a predictive value of apoptotic-necrotic markers M30 and M65, and examine Barrett's mucosa and cancerous tissue by the immunohistochemical method.

Methods: Esophageal tissue biopsy with an upper gastrointestinal endoscopy was performed on participants, who were older than 18 years and newly diagnosed. There were 20 with Barrett's esophagus, 20 esophageal cancer patients and 20 gastroesophageal reflux disease patients as a control group. Among the tissue samples taken, M30 and M65 were stained with immunohistochemical methods. The samples were examined to see whether there was a significant immunohistochemical difference among the groups in terms of M30 and M65 staining.

Results: There was no statistically significant difference among the groups in terms of M30 expression ($p = 0.329$). When compared to the control and the Barrett's esophagus groups, M65 positivity was significantly higher in the adenocarcinoma group ($p = 0.0001$).

Discussion and conclusion: There was no statistically significant difference in M30 expression among the groups in our study. M65 was found to be significantly high in esophageal adenocarcinoma. This suggests that necrosis is more dominant in the pathogenesis of esophageal adenocarcinoma. M65 can be used as a predictive marker in esophageal adenocarcinoma.

Keywords: Barrett's Esophagus, Esophagus Cancer, M30, M65

ÖZET

Giriş ve amaç: Özofagus adenokarsinomlarında birçok belirteç çalışılmış olmasına rağmen, şu anda klinik kullanım için bir belirteç bulunmamaktadır. Bu çalışmada apoptozun Barrett's özofagusu ve adenokarsinom karsinogenezindeki rolünü araştırmayı, apoptotik-nekrotik belirteçler M30 ve M65'in prediktif değeri olup olmadığını belirlemeyi ve Barrett's mukozasını ve kanserli dokuyu immüno-histokimyasal yöntemle incelemeyi amaçladık.

Yöntem ve gereçler: 18 yaşından büyük ve yeni tanı almış katılımcılara üst gastrointestinal endoskopi ile özofagus doku biyopsisi yapıldı. Kontrol grubu olarak Barrett's özofagusu olan 20, özofagus kanseri hastası ve 20 gastroözofageal reflü hastalığı olan hasta vardı. Alınan doku örneklerinden M30 ve M65 immunohistokimyasal yöntemlerle boyandı. Örnekler, M30 ve M65 boyaması açısından gruplar arasında anlamlı bir immünohistokimyasal farklılık olup olmadığını görmek için incelendi.

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Bulgular: Gruplar arasında M30 ekspresyonu açısından istatistiksel olarak anlamlı fark yoktu (p = 0,329). Kontrol ve Barrett's özofagus grupları ile karşılaştırıldığında, adenokarsinom grubunda M65 pozitifliği anlamlı olarak daha yüksekti (p = 0.0001).
Tartışma ve sonuç: Çalışmamızda gruplar arasında M30 ekspresyonunda istatistiksel olarak anlamlı fark yoktu. Özofagus adenokarsinomunda M65 anlamlı olarak yüksek bulundu. Bu, özofagus adenokarsinomunun patogenezinde nekrozun daha baskın olduğunu göstermektedir. M65, özofagus adenokarsinomunda prediktif bir belirteç olarak kullanılabilir.

Anahtar Kelimeler: Barrett Özofagus, Özofagus Kanseri, M30, M65

Introduction

Esophageal cancer is the eighth most common cancer and the sixth most common cause of death worldwide [1]. The major risk factors for esophageal adenocarcinoma are gastro-esophageal reflux disease (GERD) and Barrett's esophagus (BE). Barrett's esophagus develops through the process of metaplasia, in which columnar cells replace squamous cells due to chronic reflux [2]. Reflux of acid and bile salts can cause oxidative DNA damage and double-strand DNA breaks in Barrett epithelial cells, thereby initiating carcino-genesis [3]. The activation–inhibition steps in the apoptosis process play an important role in the onset and progress of carcinogenesis in Barrett's esophagus [4].

Activation of the caspase enzyme family is one of the events that induce apoptosis. Cytokeratins (CK) are from the intermediate filament protein family. They help support the shape and integrity of cells in epithelial tissues. The secretion of CKs from apoptotic or proliferating cells increases. Cytokeratin 18 (CK18) is secreted extensively from epithelial cells of rapidly growing tumours. Its release increases rapidly due to complicated neo-plastic processes [5]. Janti et al. showed that with the serial gene analysis they performed in BE and esophageal cancers, CK18 is strongly stained in all BE and CK20 is expressed more in adenocancer [6,7]. During epithelial cell death, CK18 is cleaved by caspases at aspartate 238 and aspartate 396, resulting in exposure of the CK18Asp396 neoepitope (M30 antigen). In particular, the resulting monoclonal M30 marker identifies and helps measure the cleaved fragment of CK18 at aspartate 396. However, the monoclonal M65 marker measures both intact CK18 and cleaved CK18. While total CK18 (M65) is secreted in all cell deaths, broken CK18 (M30) occurs during apoptosis and is secreted when cells undergo secondary necrosis. An increase in the M30–M65 ratio favours apoptosis, while a decrease favours necrosis [8]. Thus, monoclonal M30 and M65 antibodies can be used as markers of apoptotic–necrotic epithelial cell death [9].

Serum levels of M30 and M65 have been examined in a number of previous studies that evaluated the apoptosis process in various malignancies [10,11,12]. Although many protein–genetic markers have been studied in esophageal adenocarcinomas such as the caspas family or interleukins, there is no prognostic or diagnostic marker currently available for clinical use [13,14]. This study aimed to investigate the role of apoptosis in Barrett's esophagus and adenocarcinoma carcinogenesis, determine whether there is a predictive value of apoptotic–necrotic markers M30 and M65 and examine Barrett's mucosa and cancerous tissue by the immuno-histochemical method.

Materials and methods

Selection of Patients

This study included 20 patients with GERD who underwent upper gastrointestinal endoscopy, the histopathology of which showed esophageal tissue without Barrett or

cancer, 20 patients with esophageal cancer and 20 patients with Barrett's esophagus, older than age 18, who were admitted to the gastrointestinal clinic with the appropriate medical indications between 2008–2015. Tissue samples taken from the pathology clinic archive were re-evaluated and stained with M30 and M65 by immunohistochemical methods. Patients' demographic data, medical history and endoscopy reports were recorded by file scanning. Those with other known malignancies, active inflammatory diseases or infectious diseases were excluded from the study. Our retrospective study was approved by the local ethics committee (B10.4 İSM.406.68.49 11.3.2015 Keçiören Education and Research Hospital Ankara).

Immunohistochemical Method

For immunohistochemical staining, 4 mm thick sections of epithelial cells from each formalin-fixed, paraffin-embedded uterine horn were used. Tissue sections were deparaffinized in xylene, rehydrated in a graded series of alcohol and immersed in distilled water. Endogenous peroxidase activity was blocked by incubating the sections in 1% hydrogen peroxide (v/v) in methanol for 10 min at room temperature (RT). The sections were subsequently washed in distilled water for 5 min, and antigen retrieval was performed for 3 min using 0.01 M citrate buffer (pH 6.0) in a domestic pressure cooker. The sections were transferred into 0.05 M Tris–HCl (pH 7.6) containing 0.15 M sodium chloride (TBS). After being washed in water, the sections were incubated at RT for 10 min with super block (SHP125) (ScyTek Laboratories, USA) to block nonspecific background staining. The sections were then covered with the primary antibodies diluted 1:100 for M30 and 1:50 for anti-M65 in TBS at 48 C overnight (Santa Cruz Biotech Inc., Europe). After being washed in TBS for 15 min, sections were incubated at RT with biotinylated link antibody (SHP125) (ScyTek Laboratories, USA). This was followed with Streptavidin/HRP complex (SHP125) (ScyTek Laboratories, USA). Diamino-benzidine was used to visualise peroxidase activity in the tissues. Nuclei were lightly counterstained with haematoxylin, and then the sections were dehydrated and mounted. Both positive and negative controls were included in each run. Positive controls comprised sections of reactive lymph nodes for M30 and M65. TBS was used in place of the primary antibody for negative controls. The slides were evaluated with the Olympus Bx 51 light microscope. (Olympus Corp., Japan)

Statistical Method

Statistical analyses were performed using the SPSS 16.0 program. Normally distributed numerical data were presented as mean ± standard deviation (SD); non-normally distributed data were presented as median (minimum–maximum). P-values less than 0.05 were considered statistically significant. The correlation between the categorical variables was analysed by Chi-square analysis and between-group comparisons were made by the Kruskall Wallis H test.

Results

Patient Demographics

Our study was performed with tissue preparations taken from 20 patients with BE without dysplasia and 20 with esophageal adenocarcinoma. The control group of GERD patients comprised 20 samples. Of the 60 individuals included in the study, 26 were female and 34 were male. Demographic data on age and gender of the groups are presented in Table 1. There was no statistically significant difference among the groups in terms of gender distribution (p=0.622). There was a statistically significant difference in age among the groups (p=0.03). The adeno-carcinoma group showed significantly higher age values than the other two groups (Table 1).

Table 1. Demographic comparison of groups

	Barrett	Adenoca	Control	*P*
Patients, *n*	20	20	20	
Age (years) mean (range)	55.4 (32-72)	67 (40-80)	53.8 (35-79)	0.003
Male/Female *n* (%)	11/9 (55/45)	13/7 (65/35)	10/10 (50/50)	0.622

Table 2. Comparison of M30 and M65 staining between groups

		Barrett n(%)	Adeno ca n(%)	Control n(%)	P
Patients, n		20	20	20	
M30					
	+	0 (0%)	2 (10%)	0 (0%)	0,329
	-	20 (100%)	18 (90%)	20 (100%)	
M65					
	+	1 (5%)	13 (65%)	2 (10%)	0,0001
	-	19 (95%)	7 (35%)	18 (90%)	

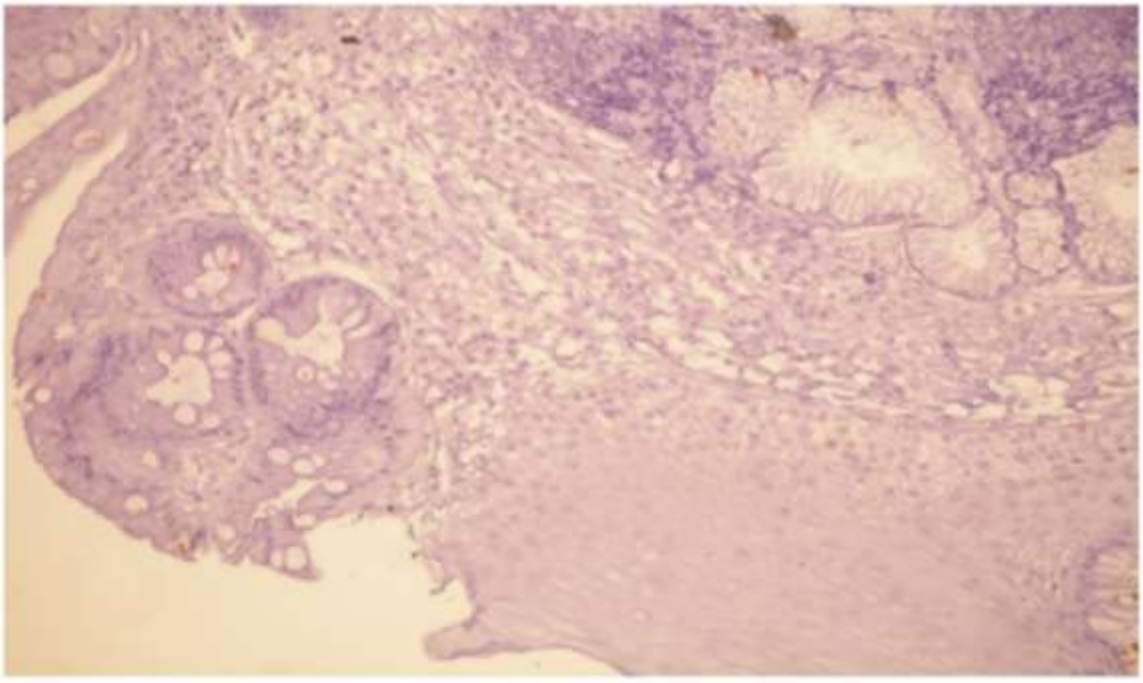

Figure 1. M30 Negative Barrett Esophaguse Area

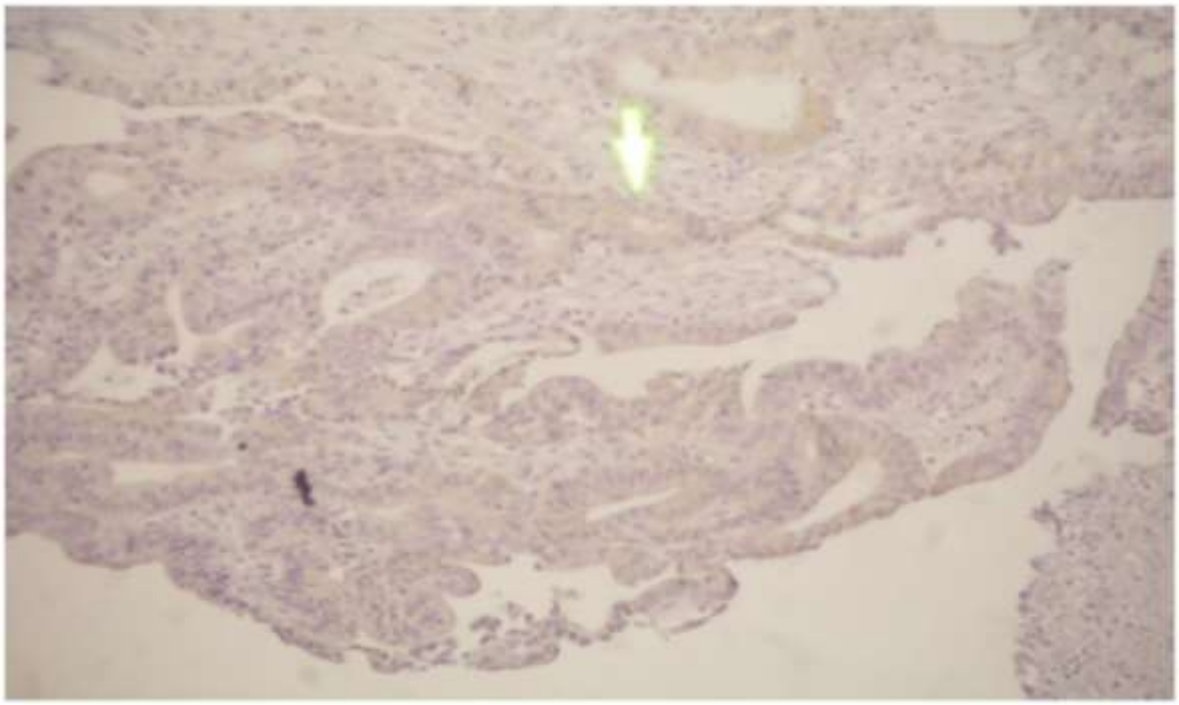

Figure 2. M30 Positive Adenocancer Area

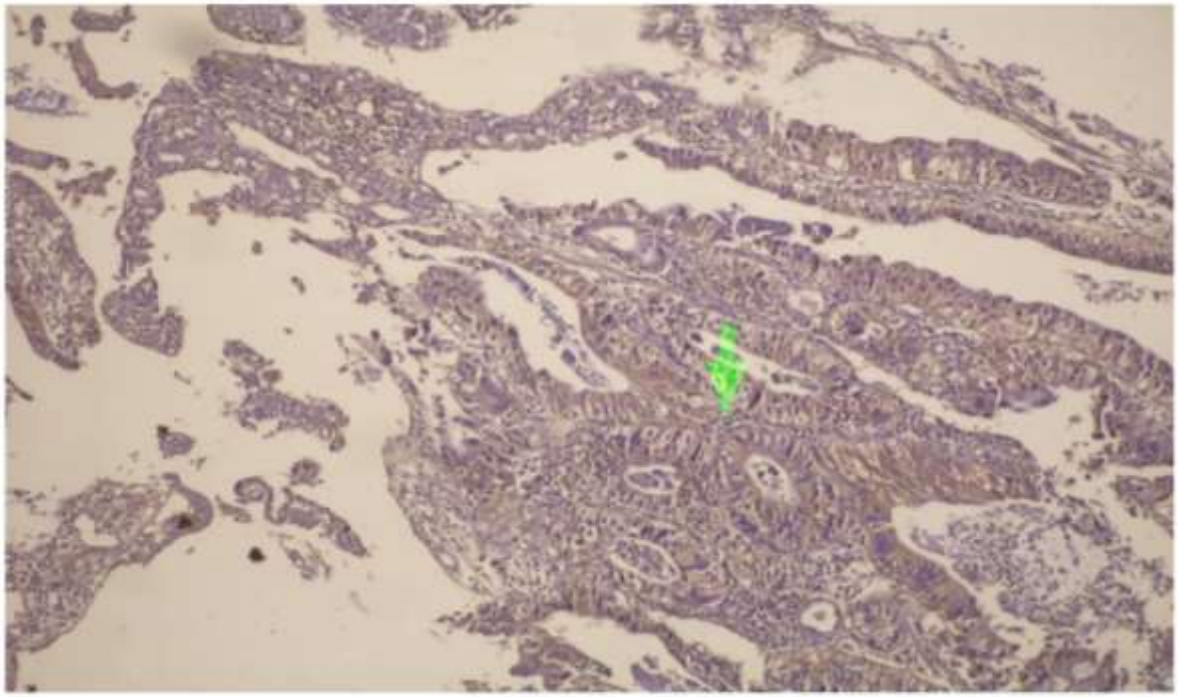

Figure 3. M65 Positive Adenocancer Area

M30 Expression

A total of 3.3% of the cases showed staining with M30. None of the samples in the Barrett's esophagus or the control group were found to be stained, whereas two adenocarcinoma cases (10%) showed staining with M30; there was no statistically significant difference between groups in terms of M30 positivity (p = 0.329) (Figure-1, 2) (Table 2).

M65 Expression

M65 stainings that were evaluated by group showed staining in a total of 26.7% cases. Staining was detected in two control samples (10%) and one Barrett's esophagus sample (5%), and M65 staining was observed in 13 adenocarcinoma cases (65%). M65 positivity was significantly higher in the adenocarcinoma group compared with the control and Barrett's esophagus group (p = 0.0001) (Fıgure-3) (Table 2).

Discussion

M30 expression by the groups showed no statistically significant difference in our study in terms of M30 positivity. M65 expression by the groups was statistically significant in adenocarcinoma cases.

Negative M30 in the control group with GERD patients and Barrett's esophagus suggested that low expression of M30 was present in the esophageal squamous epithelium. M65 was only expressed in one case of GERD and CK18 was usually reported as negative or very low in normal squamous epithelium; this was probably related to the limited use of the M30 pathway in the evaluation of apoptosis in these groups. Also, expression of M30 and M65 might have been limited since the members of the Barrett group did not have dysplasia. In a study in which Dvorakova et al. immunohistochemically investigated apoptosis resistance in BE, biopsy specimens from 10 healthy esophageal squamous epithelium, 13 BE and 4 healthy colon columnar epithelia were examined by seeding the media. Electron microscopy (EM) revealed no apoptotic changes in either the squamous epithelium or the BE epithelium. However, after deoxycholate (DOC), which is both bile acid and an inducer of apoptosis, was added to the media, apoptotic changes were observed in the squamous epithelium in the EM; no changes were observed in BE. When the same tissues were stained with M30, M30 was not expressed in the healthy esophageal squamous epithelium and highly expressed in the DOC-induced healthy colon columnar epithelium; low expression was observed in the DOC-induced BE epithelium. It was thought that M30 is not secreted from the squamous epithelium, which is specific to early apoptotic cells of the glandular tissues; it was also stated that the BE epithelium was resistant to apoptosis [15]. These results are consistent with the absence of M30 positivity in the control group and in the BE cases.

Previous studies showed that the M65 antibody theoretically measures both caspase cleaved and intact CK18; CK18 is usually expressed in glandular epithelium and is found in all BE cases [6]. However, in our study, M65 positivity was detected in only one case of BE. The fact that M65, which reflects the necrotic process, was statistically significant in the adenocarcinoma group compared to the control and the Barrett group can be explained by the fact that the cellular cycles of tumour cells and dysplastic cells are very rapid and high in their production and destruction activities and none of our Barrett patients was dysplastic.

In our study, M30 was only expressed in two patients in the adenocarcinoma group and was not statistically significant; this can be explained by the fact that programmed cell death in cancer cells is inhibited by various mechanisms such as mutations in p53, a tumour suppressor gene, changes in the Fas and FasL molecules that play an important role in the apoptosis mechanism, and

activation of Bcl-2 proto-oncogenin. In a study by Fareed et al. [16], lower M30 staining in the non-chemotherapy group (24.6%) compared with the chemotherapy group (56.7%) and chemotherapy-induced apoptosis that was inhibited in tumour cells also supported this idea. It is also known that cells undergo necrosis instead of apoptosis when cellular Adenosine triphosphate (ATP) production is inadequate [17]. The predominant manner of death might have been necrosis due to the hypoxia of the tumour cells.

In the study by Fareed et al., in which M30 and M65 were examined by the immunohistochemical method to determine the degree of tumour regression by neoadjuvant chemotherapy in patients with gastroesophageal adenocarcinoma, neoadjuvant chemotherapy was not administered to 122 patients with gastric/gastroesophageal carcinoma whereas 97 patients with gastric/ gastroesophageal/ lower esophageal cancer received preoperative platinum-based chemotherapy. M65 was expressed (positive) in most of the patients (92.6%); M30 positivity was detected in 56.7% of patients who received neoadjuvant chemotherapy and 24.6% of those who did not. This was interpreted as the induction of apoptotic cell death in tumour cells exposed to chemotherapy and the expression of M30, an apoptosis marker. Patients who were administered neoadjuvant chemotherapy were found to be correlated with M30 positivity and tumour regression response [16].

M65 may affect carcinogenesis through several signalling pathways, including the phosphoinositide 3-kinase (PI3K)/Akt, Wnt and mitogen-activated protein kinase (MAPK) signalling pathways [18]. Consistent with our study, in previous studies on colonic and gastric cancers, serum M65 was found to be higher than the control group; in gastric and gastroesophageal cancers, M65 was found to be expressed in 92.5% by the immunohistochemical method [8]. In a study by Ausch et al. that examined 62 patients with colorectal cancer and 27 healthy controls, serum M65 levels were higher in patients with cancer, and a significant postoperative decrease was detected in the subgroup of surgery patients (19/31) [19]. In another study by Greystoke et al., M30 and M65 were found to be higher in cancer patients, and M65 tended to increase as the stage progressed [20].

In the literature, there are studies on gastrointestinal cancers that show high serum levels or positively stained M30 by the immunohistochemical method, in contrast to our results. In a meta-analysis that included 11 original studies by Huang et al., the role and prognostic values of M30 and M65 in gastrointestinal cancer were evaluated. Low levels of M30 and M65 were shown to be protective factors for all cancer patients, and low M30 remained a protective factor for metastasized cancer patients [21]. This meta-analysis showed that each cancer has its own specificity. Tumour development, microenvironment, treatment strategies and prognosis are all different in different types of cancer. Also, different measurement techniques for M30 (ELISA, immunohistochemistry) might have caused inconsistent results in different studies. In a study by Brandt et al. on 35 patients with gastrointestinal system cancer, serum M30 levels were found to be higher in cancer patients than in the control group. In the same study, M30 expression was higher in the immunohistochemical examination of tissue samples of patients with colorectal cancer than in the control group. The reason for the positive M30 in serum and tissue in cancer patients is that the apoptosis in the tumour cells continued, though decreased, but apoptosis-resistant cells survived as a result of increased cell turnover [22]. Although not a predominant death pattern in the esophageal adenocarcinoma group, apoptosis was present in varying proportions; however, M30

expression might have been limited to only two patients in our study since the number of patients was not high enough to show this difference and each tumour was at a different stage at the time of histological sampling.

A number of studies have examined the role of serum M30 and M65 in assessing prognosis, survival and response to treatment in gastric cancer. In a study conducted by Oyama et al. that included 54 patients with gastric cancer and 12 healthy controls, M65 was suggested as an independent prognostic indicator [23]. In contrast, some CK18(M65) expressing adenocarcinomas have shown decreased expression with increasing tumour progression, such as breast and colorectal cancer [24,25]. These conflicting results may stem from differences among the types of cancers or among the experimental protocols [20].

There are several potential limitations to this study. The sample size was not adequate and none of our Barrett patients was dysplastic. Additionally this study was a retrospective study with some missing data about patient's tumor stages and treatments protochols.

In conclusion, our study showed that M65 was significantly higher in oesophageal adeno-carcinoma patients. This suggests that necrosis is more dominant in the pathogenesis of oesophageal adenocarcinoma. Despite the fact that many protein–genetic markers have been studied in oesophageal adeno-carcinomas, there is currently no marker for clinical use. M65 can be used as a predictive marker in oesophageal adenocarcinoma. However, there is a need for extensive studies on this topic.

The authors declare that they have no conflict of interest.

Informed consent from patients or families were obtained before the study started.

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Invited Review

The Role of Gene Signatures on Treatment Decisions in Early-Stage Breast Cancer

Erken Evre Meme Kanseri Tedavisinde Gen İmzalarının Rolü

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ABSTRACT

Breast cancer is a heterogenous disease with various morphological, clinical and molecular features. Traditionally, pathologic parameters and clinical stage of the disease are used to determine indication of adjuvant treatment, however these features are not sufficient to identify the patients that need treatment. Prognostic and predictive biomarkers are needed to develop personalized treatments. Predictive factors are indicators of response to a particular treatment. MammaPrint® (Agendia, Amsterdam, the Netherlands), Oncotype DX® (Genomic Health, Redwood City, CA), Prosigna® (Nanostring technologies, Seattle, WA) and Endopredict® (Myriad Genetics) are gene expression profiles that are used to predict the benefit of adjuvant chemotherapy and provide additional prognostic and/or predictive information. In this review, we discuss the role of gene expression signatures in treatment decision of patients with breast cancer.

Keywords: breast cancer, gene, recurrence score

ÖZET

Meme kanseri çeşitli morfolojik, klinik ve moleküler özelliklere sahip heterojen bir hastalıktır. Adjuvan tedavi endikasyonunu belirlemek için geleneksel olarak patolojik parametreler ve hastalığın klinik evresi kullanılır, ancak bu özellikler tedaviye ihtiyacı olan hastaları belirlemek için yeterli değildir. Kişiselleştirilmiş tedaviler geliştirmek için prognostik ve prediktif biyobelirteçlere ihtiyaç vardır. Prediktif faktörler, belirli bir tedaviye yanıtın göstergeleridir. MammaPrint® (Agendia, Amsterdam, Hollanda), Oncotype DX® (Genomic Health, Redwood City, CA), Prosigna® (Nanostring teknolojileri, Seattle, WA) ve Endopredict® (Myriad Genetics) adjuvan kemoterapinin yararı ve ek prognostik ve/veya prediktif bilgileri tahmin etmek için kullanılan gen ekspresyon profilleridir. Bu derlemede, meme kanserli hastaların tedavi kararında gen ekspresyon imzalarının rolünü güncel literatür ışığında tartışmayı hedefledik.

Anahtar Sözcükler: meme kanseri, gen, rekürrens skoru

Introduction

The widespread use of adjuvant systemic therapy in early stage breast cancer resulted in a reduction of up to 30% in 10-year mortality of breast cancer [1]. This benefit was shown to be independent of the patient's age, nodal status, grade and diameter of the tumor [1]. However, the different clinical course observed in the follow-up of patients with the same stage revealed the fact that breast cancer is a heterogeneous disease. Although the adjuvant treatment decisions are based on characteristics of tumor (hormone receptor, HER2 status, Ki67 proliferation marker, tumor size, grade and lymph node involvement) and patient (age and menopausal status), standard clinical and pathological features are insufficient to distinguish between patients who will benefit from the adjuvant therapy and those who will not [2]. Tools that were created to assist in decision making (Adjuvant! Online and PRECIT plus) do not consider biological

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characteristics of the tumor [3–6]. The patients treated with hormonal therapy alone, showed disease recurrence rates of less than 20% by 10 years after diagnosis, thus most patients with hormone receptor positive (HR(+)) lymph node negative disease would not benefit from the addition of chemotherapy [7]. Retrospective clinical studies show that 30-50% of patients with early-stage breast cancer receive unnecessary systemic therapy [1].

In the light of gene-expression profiling, several biological-based prognostic profiles are created using the expression of hundreds of genes. These tests, gene signatures, are developed to provide more precise cost-effective care, better prediction of clinical outcome and to determine the benefit of adjuvant chemotherapy, especially in hormone receptor positive lymph node negative patients since they are known to have low risk of recurrence [8]. With the introduction of gene signatures into practice, personalized treatment came up, that's aim is to protect the patient from overtreatment and toxic effects of chemotherapy. These advances in molecular technology change 30-40% of patients' systemic treatment decision [9]. Guidelines support the use of Oncotype Dx, Mamma Print, EndoPredict, Prosigna (PAM50) and Breast cancer index (BCI) as gene expression assays [10].

Herein, we aimed to evaluate the gene signatures and their aid to treatment decisions in early-stage breast cancer in the light of current literature.

Gene signatures

Recurrence Score

The recurrence score (RS) test (Oncotype DX) is a 21-gene expression profile that became available in 2004 [11]. It is a reverse-transcription polymerase chain reaction-based assay of 5 reference genes and 16 cancer-related genes which uses a continues scale from 1 to 100 to anticipate the 10-year disease recurrence risk and magnitude of adjuvant chemotherapy benefit in patients with early-stage HR(+), mostly lymph node negative but also 1-3 lymph node positive breast cancer [12]. Some of the genes are involved in cell proliferation and hormonal response which are associated with chemotherapy response [7].The RS is categorized into low (<18), intermediate (18–30), and high risk (≥31). The NSABP B-20 trial, one of the first studies seeking the relationship between RS and chemotherapy benefit, exhibited the cutoff points of RS when analyzed retrospectively [13]. The trial revealed that the addition of CMF (cyclophosphamide, methotrexate, 5-Fluorouracil) to tamoxifen reduced the risk of developing metastases in patients with RS greater than 31, but no benefit was seen in patients with lower RS (absolute decrease of recurrence at 10 years were 27.6% and 1.1%, respectively). Patients with RS 0-10 had outstanding outcomes with endocrine therapy alone. The chemotherapy regimen used in NSBAP B-20 trial was CMF or MF; however, in a small study conducted by Gianni et al.[14], it was shown that the relationship between RS and chemotherapy benefit is not regimen specific. Eighty-nine patients with locally advanced hormone receptor positive breast cancer were evaluated in the study and patients with RS<18 rarely had a pathological complete response (pCR) with neoadjuvant treatment consisting of anthracycline/taxane regimen. RS was shown to be positively correlated with the probability of pCR. Recently published studies investigating the impact of RS on neoadjuvant treatment decision showed Oncotype DX as a significant predictor variable of pCR where patients with a RS>25 are more likely to obtain a histological response type 0-1 [15,16]. In addition, in multivariate analysis, it was the most significant predictor in comparison to Ki67, estrogen receptor and initial tumor size. Multiple following studies demonstrated the benefit of 21-gene Oncotype

DX Breast RS in treatment decisions, which prevented overtreatment [17,18]. In a meta-analysis of four studies with more than 500 lymph node negative, HR(+) breast cancer patients, the chemotherapy recommendation rate was found to be decreased from 55% to 35% [19].

The uncertainty about the patients with a midrange score, RS 18-30, was evaluated in TAILORx study [17]. Endocrine therapy was found noninferior to the chemotherapy plus hormone replacement therapy in terms of invasive disease-free survival, local or distant recurrence and overall survival at 9 years (83.3% vs 84.3%, 94.5% vs 95.0% and 93.9% and 93.8%, respectively). Subgroup analysis showed minimal benefit of chemotherapy for patients 50 years of age and younger with RS of 16-25. The results were related to antiestrogenic effect gained by menopause induced by chemotherapy. NSABP B-20 and TAILORx trial together proved that Oncotype Dx can aid physician in chemotherapy treatment decisions, as node negative patients with RS 0-25 can safely receive hormone replacement therapy alone where patients with RS 26-100 derive benefit from chemotherapy followed by endocrine therapy. Accordingly, the National Comprehensive Cancer Network (NCCN) Guidelines prefer the use of Oncotype Dx as the only gene expression assay that predict chemotherapy benefit [20]. (Figure 1)

Although the journey of Oncotype DX started in lymph node negative patients, the usefulness of RS was evaluated in node positive breast cancer patients in the following time period. The SWOG-8814 trial was the first study aimed to evaluate the chemotherapy benefit in postmenopausal HR(+) Her2(-) patients with 1-3 lymph nodes [21]. The study concluded that N1 patients with RS 0-17 could be treated with endocrine therapy alone whereas patients with RS 31-100 achieved strong clinical benefit with anthracycline based chemotherapy. TransATAC study in addition to SWOG-8814 validated the use of Oncotype Dx in the node positive women and showed that the benefit gained with tamoxifen is also valid for anastrazole, with approximately 16% adjustment for the lower risk of disease recurrence with the aromatase inhibitor [22]. The first prospective data, WSG Plan B study, that report clinical outcome on node positive and negative HR(+) early breast cancer patients with RS<11 showed excellent 3-year-disease free survival rates (98%) with endocrine therapy alone [23]. The chemotherapy was omitted in the population, whose actually high risk by traditional parameters. Breast cancer specific mortality had been reported in the secondary analysis of the SEER registry where RS was strongly predictive of breast cancer specific mortality [24]. The subgroup analysis of SEER registry has additional importance in terms of evaluating the patients with tumors $\leqslant$ 5 mm in size, since gene expression profiles are not routinely recommended for T1a tumors. The risk was found elevated in this group with RS >31, even though the estimate lacks precision. The first analysis investigating both distant recurrence and breast cancer death rates were done by Stemmer et al. [18] in a large prospectively designed registry in patients with N1mi or 1-3 positive nodes. The distant recurrence rates and breast cancer death estimates for RS<18, 18-30 and >31 was 3.2%, 6.3%, and 16.9% and 0.5%, 3.4%, and 5.7%, respectively.

Although WSG Plan B study and SEER registry were accepted to be cornerstone of RS validation in node positive patients, recently published results of RxPONDER trial evaluating endocrine therapy alone vs. chemoendocrine therapy in patients with 1–3 positive nodes changed the practical impact of oncologists [25]. The RxPONDER cutoff level was 25 and those with RS of 18-25 received less chemotherapy compared to those with 26-30. The difference in chemotherapy recommendations was independent

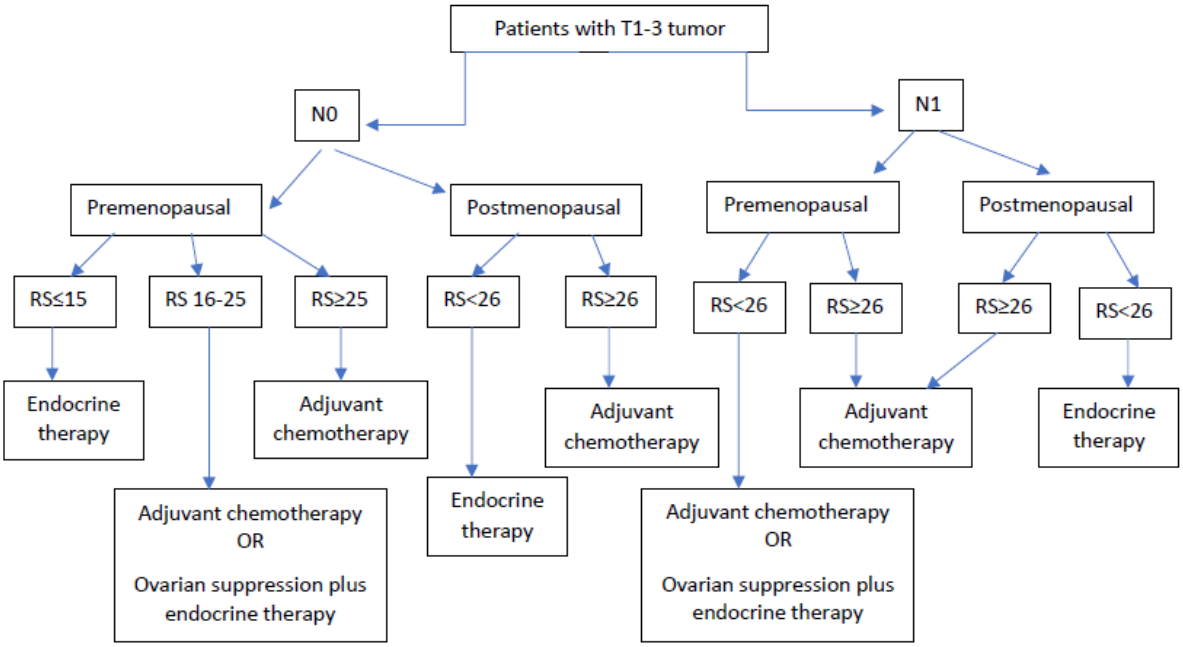

Figure 1: 21-gene (OncotypeDx) expression algorithm for Early-stage HR (+) Her2(-) Breast Cancer

from factors such as age, tumor size, and number of nodes involved. The results of the study changed the guidelines' in node positive patients [20].

MammaPrint

MammaPrint is a platform where 70 gene expressions are examined by microarray method. These genes are associated with cell cycle, invasion, metastasis, and angiogenesis. HR(+) positive patients are grouped as low or high risk. The practice changing done using MammaPrint assay is MINDACT trial, where prospective evidence for the addition of 70-gene signature to standard clinical-pathological criteria in selecting patients for adjuvant chemotherapy was provided [26]. The importance of the study was identifying a subset of patients who have a low risk of distant recurrence despite high clinical risk based on tumor size, grade and nodal status. Patients with low clinical and genomic risk received only endocrine therapy whereas ones with high clinical and genomic risk received chemotherapy. Patients with discordant results were randomized to chemotherapy and no-chemotherapy group. The 5-year survival rate without distant metastases were found 94.7% in high clinical/low genomic risk patients who didn't receive adjuvant chemotherapy. Among patients at low clinical and high genomic risk, 5-year survival with no distant metastases with and without chemotherapy were 95.8% vs 95.0%, respectively. As a result, using 70-gene signature for patients at high clinical risk is recommended to be able to omit chemotherapy. MammaPrint is also valuable in node positive patients as the rates of survival without distant metastases at 5 years were 96.3% vs 95.6% in patients who received and didn't receive chemotherapy, respectively. These data reveal that the benefit of adjuvant chemotherapy is small in patients with high clinical and low genomic risk [26]. In subgroup analyses, the benefit of chemotherapy was mostly seen in patients under <50y.

EndoPredict

EndoPredict (EP) is a 12-gene assay calculating a prognostic score using nine

cancer-related genes and 3 reference genes [27]. It is a platform used to predict distant metastasis in early-stage ER-positive, HER2-negative, lymph node-positive or negative patients. The EP clinical score (EPclin) is calculated using nodal involvement and tumor diameter [28]. Patients are classified as low (<3.3) and high (>3.3) risk. EP can predict both early and late recurrences. Based on the results of ABSCG-6 and 8 trials, patients with a low-risk score had risk of distant recurrence risk of 4% at 10 years [28]. On the other hand, node positive (1-3) patients with low score has a 5.6% risk of distant recurrence at 10 years [29]. In a study done by Müller et al. investigating the performance of EP in clinical practice, comparison of pre- and post-assay treatment decisions showed a change of therapy in 37.7% of patients [30]. 12.3% of patients had a change to an additional chemotherapy while 25.4% of patients changed to an endocrine therapy alone.

Prosigna (PAM50)

Prosigna (PAM50) studies the expression of 50 genes and 8 reference genes [31]. The risk of recurrence (ROR) score is calculated using 46 gene expressions, proliferation score, and tumor size. A score between 0-100 is given and patients are grouped as low (0-40), intermediate (41-60) and high risk (61-100). Risk of early (0-5 years) and late (5-10 years) distant recurrences can be predicted with this method in HR(+) Her2(-) lymph node positive or negative patients [29,32,33]. The prognostic value of PAM50 is well defined in a study done by Danish Breast Cancer Cooperative Group where patients with node negative disease has a distant recurrence risk of 5% after endocrine therapy if they have low ROR and 17.8% if they have high ROR [33]. The same study revealed the distant recurrence risk as <3.5% at 10 years with endocrine therapy alone in the patients with node positive disease. In TransATAC study, the similar group of patients didn't show ant distant recurrence at 10 years [29].

Brest Cancer Index

Breast Cancer Index (BCI) is created by combining two profiles: HOXB13/IL17BR (H/I) expression ratio and the molecular grade index (MGI). It can significantly determine the prognosis regardless of clinical factors (eg, age, tumor size, tumor grade and lymph node status) [34]. In ATAC trial, it was found prognostic in early-stage node negative patients for both early (0-5y) and late (5-10y) distant recurrence [22].BCI can identify patients who will benefit from endocrine therapy and who needs extended endocrine therapy.

Node-negative patients with T1 and T2 tumor and with BCI score between 0-5 (low) is placed into the same prognostic category as T1a-bN0M0, regardless of T score [35]. These tumors demonstrated a lower distant recurrence risk and extending endocrine therapy didn't show any significant improvement in terms of disease-free or overall survival. However, for patients with T1 tumor, BCI score of 5.1-10 (high) showed significant roles of distant recurrence in 5-10y period. Multiple trials investigating T1-3N0/+ HR(+) pre and postmenopausal patients with high BCI revealed significant improvement in disease-free survival with extended adjuvant endocrine therapy (5y of letrozole for postmenopausal and 10y of tamoxifen for premenopausal patients) [36–38].

Conclusion

Multiple studies with >96000 hormone receptor positive HER2(-) early breast cancer patients demonstrated the clinical benefit of Oncotype DX in personalized treatment. Since RS is also useful in determining the benefit from chemotherapy, it is superior to methods that detect similar gene expression profiles. Mamma Print, EndoPredict, Pam50 and BCI are other useful analyses that can be used to help estimate the recurrence risk. Head-to-head prospective comparison of the

assays is needed. Until then clinicians should order one of these assays in early-stage HR(+) HER2(-) breast cancer patients to omit chemotherapy and prevent its toxic effects, especially in patients who are candidates for endocrine therapy alone.

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