Original Article

Primary and Secondary Hematolymphoid Neoplasms of the Gastrointestinal Tract: A Single Institute Experience

Gastrointestinal Sistemin Primer ve Sekonder Hematolenfoid Neoplazileri: Tek Merkez Deneyimi

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ABSTRACT

Introduction: The gastrointestinal tract is the most common extranodal region for hematolymphoid neoplasms. However, when compared to inflammatory lesions or epithelial tumors, this group of neoplasms is quite rare and some of them cause diagnostic difficulties. In this study, the clinicopathological features of common hematolymphoid neoplasms of the gastrointestinal tract were aimed to be represented and observed rare entities were highlighted.

Materials and methods: Forty-six patients who were diagnosed of hematolymphoid neoplasia with gastrointestinal system infiltration between the years 2014 and 2021 were selected retrospectively from the archives of pathology department. Pathology reports, demographic and clinical data, endoscopic and imaging findings were collected from the hospital information system.

Results: Thirty-six of the patients were diagnosed of primary neoplasms of the gastrointestinal tract. Nine patients were accepted as secondary spread of systemic disease to the gastrointestinal tract. For one patient, differential diagnosis regarding primary or secondary disease could not be made with available data. The mean age was 56. Approximately three-quarters (73.9%) of the cases were diagnosed by endoscopic biopsy. Patients frequently (73.9%) presented with nonspecific gastrointestinal symptoms. However, ileus was described in cases with bowel localization. The most common diagnosis was diffuse large B-cell lymphoma (n:26) followed by MALT lymphoma (n:6). We had four patients diagnosed as duodenal-type follicular lymphoma. Rare cases were IRF4-associated large B-cell lymphoma (n:1), EBV positive large B cell lymphoma (n:1), extracavitary/solid variant primary effusion lymphoma (n:1), myeloid sarcoma (n:1).

Discussion: An unexpected difference was that the number of patients with diffuse large B-cell lymphoma was more than four times of MALT lymphoma. Possibility of confusion with poorly differentiated carcinomas is an important handicap especially in rare high grade lymphomas and myeloid neoplasms. Duodenal lymphoid follicles should be carefully evaluated and immunohistochemical evaluation should be performed.

Keywords: Gastrointestinal Neoplasms, Lymphoma, Hematologic Neoplasms

ÖZET

Giriş: Gastrointestinal sistem, hematolenfoid neoplazilerin en sık görüldüğü ekstranodal bölgedir. Bununla birlikte inflamatuar lezyonlar ve epiteliyal neoplazilere kıyasla oldukça nadir gözlenen bu grup neoplazilerde tanı zorlukları yaşanabilmektedir. Bu çalışmada gastrointestinal sistemin sık görülen hematolenfoid neoplazilerinin klinikopatolojik özelliklerini sunmayı, nadir görülen antitelere ise dikkat çekmeyi amaçladık.

Gereç ve yöntemler: Patoloji arşivimizden, 2014-2021 yılları arasında, gastrointestinal sistem infiltrasyonu ile hematolenfoid neoplazi tanısı alan 46 hasta retrospektif olarak tespit edilmiştir. Patoloji raporları yanı sıra demografik ve klinik verilere, endoskopi ve görüntüleme bulgularına hastane bilgi sisteminden ulaşılmıştır.

Bulgular: Otuzaltı hastada primer odak gastrointestinal sistemdi. Dokuz hastada, gastrointestinal infiltrasyon sistemik hastalığın sekonder yayılımı olarak kabul edildi. Hastalardan birinde, mevcut verilerle primer veya sekonder hastalık ayrımı yapılamadı. Ortalama yaş 56 idi. Olguların yaklaşık dörtte

First Received: 06.05.2022, Accepted: 25.07.2022 doi: 10.5505/aot.2023.68916

üçü (%73,9) endoskopik biyopsi ile tanı aldı. Hastaların sıklıkla başvuru sebebi nonspesifik gastrointestinal semptomlardı (%73,9). Barsak yerleşimli orgularda ise ileus tablosu ile karşılaşıldı. En sık tanı diffüz büyük B hücreli lenfoma (n:26), ardından MALT lenfomaydı (n:6). Duodenal tip foliküler lenfoma tanısı alan dört hastamız mevcuttu. Nadir vakalarımız; IRF4 ile ilişkili büyük B hücreli lenfoma (n:1), EBV pozitif büyük B hücreli lenfoma (n:1), ekstrakaviter/solid varyant primer efüzyon lenfoma (n:1), myeloid sarkomdu (n:1).,

Tartışma: Difüz büyük B hücreli lenfoma hasta sayısının MALT lenfomanın dört katından fazla olması beklenmeyen bir farktır. Özellikle nadir görülen yüksek dereceli lenfomalar ve myeloid neoplazilerin, az diferansiye karsinomlarla karıştırılabilme olasılığı önemli bir handikaptır. Duodenal lenfoid foliküller dikkatlice değerlendirilmeli ve immünhistokimyasal değerlendirme yapılmalıdır.

Anahtar kelimeler: Gastrointestinal Neoplaziler, Lenfoma, Hematolojik Neoplaziler.

Introduction

Primary or secondary hematolymphoid neoplasms of the gastrointestinal system (GIS) have a wide spectrum of diagnosis and prognosis. Especially primary lymphoproliferative neoplasms have been better identified, subcategorized and some of them renamed in recent years [1, 2]. On the other hand, rarely, any systemic lymphoma or hematolymphoid neoplasia can also infiltrate GIS secondary.

The gastrointestinal system is the most common site of extranodal lymphomas. With geographic variations, primary gastrointestinal (GI) lymphomas account for approximately 4-20% of all non-Hodgkin lymphomas and 20-45% of extranodal NHLs [3-5]. Depending on the tumor location, they show non-specific GI symptoms such as nausea, vomiting, abdominal pain, or clinical presentations such as bleeding, ileus, perforation. Some subtypes are asymptomatic and can be diagnosed incidentally [2, 6].

It is reported that 44-81% of gastrointestinal lymphoproliferative neoplasms are observed in the stomach. The stomach is followed by the small intestine (often the ileum) and the ileocecal region. Similar to systemic lymphomas, the majority of GIS lymphomas have a B-cell immunophenotype. MALT lymphoma/ Extranodal marginal zone lymphoma and diffuse large B-cell lymphoma (DLBCL) are the most common diagnoses (50% and 30-40% of all gastric lymphomas, respectively). Helicobacter pylori (HP), known to be important in MALT lymphomagenesis, is thought to have a role in the pathogenesis of DLBCL as well. Enteropathy-associated T-cell lymphoma, defined as a complication of celiac disease, also supports the importance of inflammatory processes in the pathogenesis of GIS lymphoma [1-9].

There have been revisions in the current WHO Classification of Tumors of Hematopoietic and Lymphoid Tissue, and primary GIS lymphoid neoplasms. Duodenal-type follicular lymphoma, which is defined as a variant of systemic follicular lymphoma with differences in clinical and pathological features, is a new entity that has taken its place among B-cell lymphomas. Large B cell lymphoma with IRF4 rearrangement is reported as a provisional diagnosis. Indolent lymphoproliferative disorders (indolent T cell lymphoproliferative disorders of the gastrointestinal tract, NK-cell enteropaty) have been added to the new classification of T and NK cell lymphomas, which are known for their aggressive course. Entities such as extramedullary plasmacytoma, plasmablastic lymphoma, classical Hodgkin lymphoma, posttransplant lymphoproliferative disorders, which are mostly reported as case reports, are also rare lymphoid neoplasms of the GIS. Histiocytic, myeloid and dendritic cell neoplasms can be listed as rare hematopoietic tumors of the gastrointestinal tract [1-3].

This study has been aimed to present the single center experience about demographic, clinical and pathological characteristics of gastrointestinal hematolymphoid neoplasias with a wide diagnostic spectrum and to emphasize the points that should be considered in the histopathological evaluation of hematolymphoid neoplasia of the GIS.

Materials and Methods

A total of 46 patients with hematolymphoid neoplasia infiltrating the gastrointestinal tract between January 2014 and June 2021 were obtained retrospectively from archives of pathology department. In all cases, the histopathological diagnoses were made by a single pathologist via endoscopic biopsy, core biopsy, or surgical resection materials. Consultation cases were not included in the study group. Hematoxylin-eosin stained sections for all specimens were prepared in our department from routinely processed formalin-fixed paraffin blocks in a standard procedure, and additional immunohisto-chemical methods (and in situ hybridization-EBER for some cases) were also applied. All diagnoses were reviewed according to the current WHO classification. The number of patients in the diagnostic groups was determined. Cases presenting with GIS symptoms or predominant tumor mass in GIS were grouped primary [10]. Tumors that were first diagnosed from non-GIS locations but later recurred with GI involvement were considered secondary. None of the patients had a history of transplantation, HIV-seropositivity or immunosuppression.

Demographic and clinical data, biopsy indications, biopsy methods, endoscopic findings of all patients were obtained from medical records. In addition, tumors were recorded according to the macroscopic features observed in the resection materials or endoscopy / imaging findings (erythema, ulceration, polyp, increased wall thickness, ulcerovegetative mass, polypoid mass, bulky mass).

Histochemically Giemsa stain was applied to gastric biopsies as a standard procedure, and the presence of HP was evaluated histologically in all cases. The pathology reports of the pre-treatment bone marrow biopsies evaluated in our department were also accessed. Slides of diffuse large B-cell lymphoma cases were re-evaluated to investigate the presence of accompanying MALT lymphoma component. Similarly, MALT lymphoma cases were also reviewed in terms of high grade transformation.

Statistical analyses were performed using the SPSS for Windows (28.0 version, Armonk, NY: IBM Corp). The patient and tumor characteristics were defined as numbers and percentages. Categorical variables were tested with chi-square. The p-value <0.05 was considered as statistically significant difference.

Compliance with Ethical Standards:

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the non-interventional research ethics committee of Bezmialem Foundation University with the document number 23352 dated 09/07/2021.

Results

The distribution of histopathological subtypes was shown in the graph (Figure 1). The average age of the patients was 56 (10-88). The female/male ratio was 1/1.3. However, the number of female and male patients was equal for each one of the most common diagnoses including the DLBCL, MALT lymphoma and Duodenal type follicular lymphoma groups. About three-quarters of the

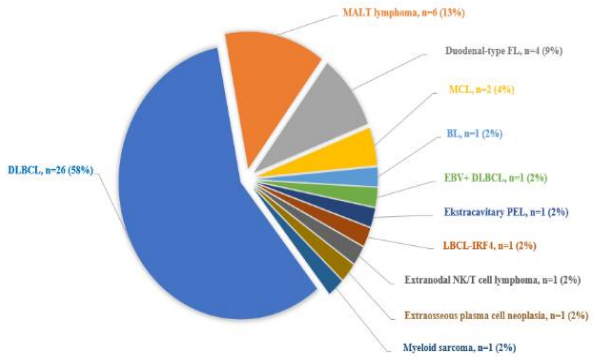

Figure 1: Histopathological subtypes.
DLBCL: Diffuse large B-cell lymphoma, FL: Follicular lymphoma, MCL: Mantle cell lymphoma, BL: Burkitt lymphoma, EBV+ DLBCL: EBV positive large B cell lymphoma, PEL: Primary effusion lymphoma, LBCL-IRF4: IRF4-associated large B-cell lymphoma.

Table1: Patient characteristics

	n	%
Age		
-<60	24	52.2
- ≥60	22	47.8
Gender		
-Female	20	43.5
-Male	26	56.5
Symptom of presentation		
-Gastrointestinal symptoms	34	73.9
-Urgent surgery indication	9	19.6
-Nonspecific	3	6.5
Biopsy method		
-Endoscopic biopsy	34	73.9
-Resection	11	23.9
-Percutaneous core needle biopsy	1	2.2
Macroscopic features		
-Ulcerovegetative mass	19	41.3
-Polypoid mass	11	23.9
-Ulceration	8	17.4
-Other*	8	17.4
Origin		
-Primary	36	78.2
-Secondary	9	19.6
-Undetermined	1	2.2

* Polyp, increased wall thickness, erythema, bulky mass etc.

biopsy specimens were endoscopic biopsy. The main complaints of the patients were GIS symptoms such as nausea, vomiting, dyspepsia, abdominal pain and etc. The patient and tumor characteristics were summarized in Table 1. Approximately half of the lymphoid neoplasms were detected in the stomach (52.2%). Tumors were grouped in terms of anatomical regions in Table 2.

With the assistance of imaging data, thirty-six (78.3%) of the cases were classified as primary GIS lymphoma. Nine cases (19.6%) were considered secondary involvement of the GIS. The most common location of secondary infiltration was the stomach (7/9) (Table 2). Five of the cases had a known history of systemic lymphoma (three DLBCL, one Extranodal NK/T-cell lymphoma, one nodal marginal zone lymphoma). In two DLBCL and two mantle cell lymphoma cases with diffuse lymph node involvement as well as gastric infiltration on imaging, there was not any known history of lymphoma. In the patient diagnosed with myeloid sarcoma presenting with a polypoid mass located in the ileum, primary or secondary differentiation could not be made with the available data.

In diffuse large B-cell lymphoma (26/46), which was the most common diagnostic group, the mean age was 60.69 (39-78). Most of them presented with an ulcerovegetative mass (17/26). Of the cases, 16 (61.5%) were located in the stomach, 5 (19.2%) in the small intestine, four (15.4%) in the colon, and one (3.8%) in the pancreatic head. One case of primary terminal ileum origin had a clinical history of Crohn disease. However, detailed information about the follow-up period and treatment status of Crohn's disease could not be obtained. Sixteen had GCB and nine had ABC immunophenotypes. One DLBCL case could not be classified according to the classical Hans algorithm [11]. A total of five DLBCL cases, three of which had activated B cell immunophenotype, were accepted as secondary infiltration of the gastrointestinal tract. Of the primary GIS DLBCLs, 66.7% were in the GCB phenotype and 28.6% were in the ABC phenotype (p=0.75). In two DLBCL cases, one primary and one secondary,

Table 2: Histopathological tumor subtypes according to anatomical regions

	Anatomic Sites												Total (%)
Histopathology	**Gastric – n:24**					**Small intestine – n:14**			**Large intestine – n:6**			**Pancreas n:1**	
Primary	*Cardia*	*Fundus*	*Corpus*	*Antrum*	*UD*	*Duodenum*	*Jejunum*	*İleum*	*Cecum*	*Colon*	*Rectum*		
DLBCL	3	-	7	3	-	2	1	2	1	2	-	-	21 (45.65)
MALT lymphoma	-	-	1	2	1	1	-	-	-	-	1	-	6 (13.04)
Duodenal-type FL	-	-	-	-	-	4	-	-	-	-	-	-	4 (8.69)
BL	-	-	-	-	-	-	-	1	-	-	-	-	1 (2.17)
EBV+ DLBCL	-	-	-	-	-	-	-	1	-	-	-	-	1 (2.17)
Extracavitary PEL	-	-	-	-	-	1	-	-	-	-	-	-	1 (2.17)
LBCL-IRF4	-	-	-	-	-	-	-	-	1	-	-	-	1 (2.17)
EPCN	-	-	-	-	-	-	-	1	-	-	-	-	1 (2.17)
Seconder													
DLBCL	-	1	2	-	-	-	-	-	-	-	1	1	5 (10.87)
MCL	-	-	1	1	-	-	-	-	-	-	-	-	2 (4.35)
Nodal MZL	-	-	-	1	-	-	-	-	-	-	-	-	1 (2.17)
Ekstranodal NK/T	-	-	1	-	-	-	-	-	-	-	-	-	1 (2.17)
Undetermined													
Myeloid sarcoma	-	-	-	-	-	-	-	1	-	-	-	-	1 (2.17)
Total	3	1	12	7	1	8	1	6	2	2	2	1	46 (100)

DLBCL: Diffuse large B-cell lymphoma, FL: Follicular lymphoma, BL: Burkitt lymphoma, EBV+ DLBCL: EBV positive large B cell lymphoma, PEL: Primary effusion lymphoma, LBCL-IRF4: IRF4-associated large B-cell lymphoma, EPCN: Extraosseous plasma cell neoplasia, MCL: Mantle cell lymphoma, MZL: Marginal zone lymphoma, UD: Undetermined.

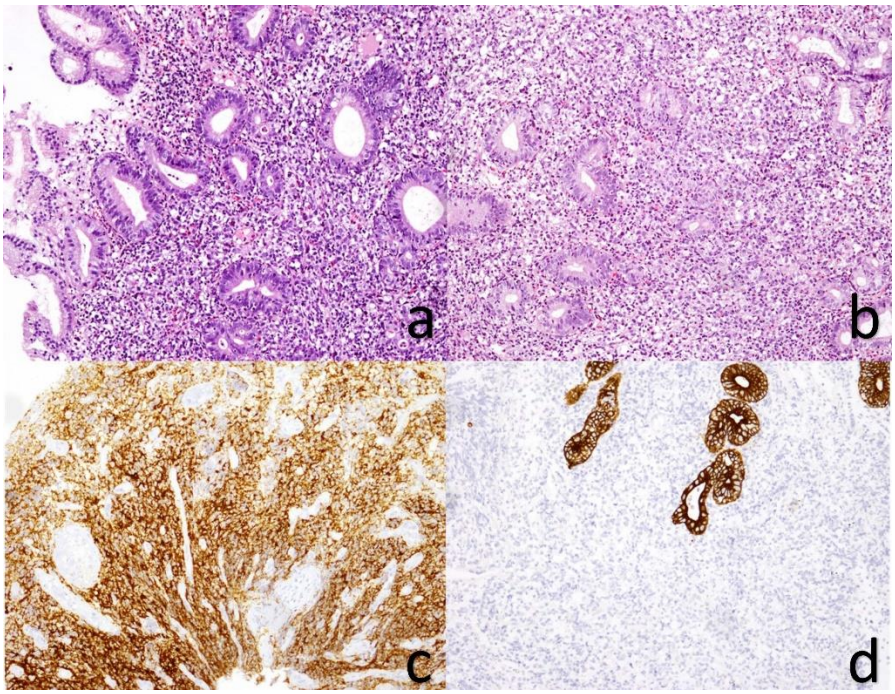

Figure 2: a-b) A case of gastric localized diffuse large B-cell lymphoma. Atypical lymphocytes with moderate amounts of clear cytoplasm resembling signet ring cells with infiltrating between glands, HEx100. c) Diffuse positive reaction with CD20, x100. d) Pancytokeratin positivity in residual glands, x100.

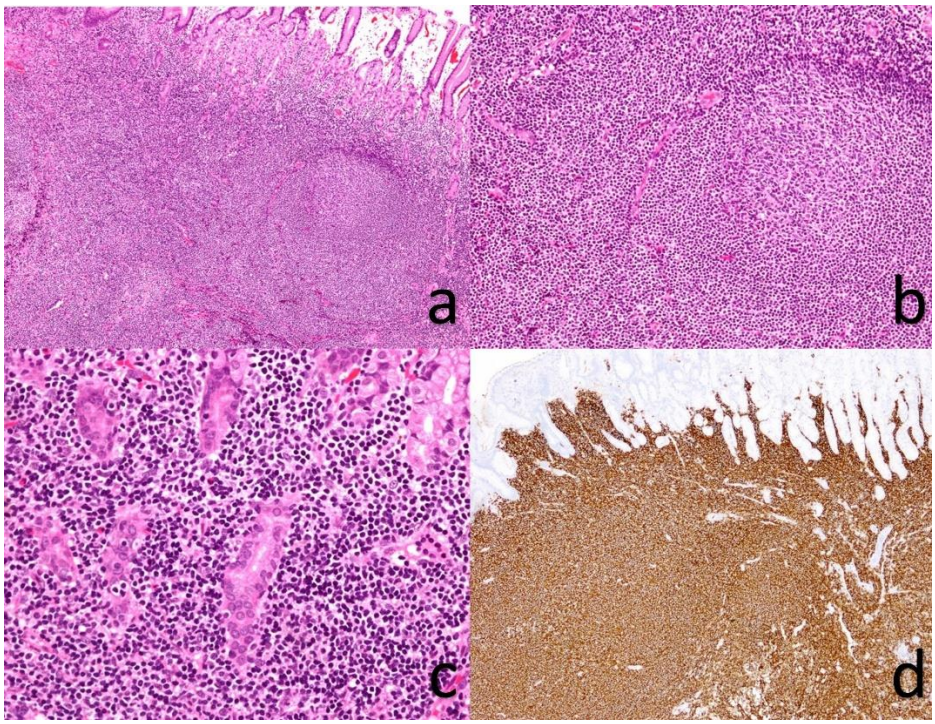

Figure 3: a) A case of MALT lymphoma with polypoid swelling of the mucosa, HEx40. b) Atypical lymphoid proliferation with small size, coarse chromatin, clear cytoplasm infiltrating residual germinal centers, HEx100. c) Lymphoepithelial lesion formation, HEx200. d) Immunohistochemical diffuse CD20 positivity, x20.

immunohistochemically strong and diffuse (>40%) c-myc expression and BCL2-BCL6 co-expression were detected. Concomitant low grade lymphoma (MALT lymphoma) was not found in any of the cases. The mean Ki67 proliferative index was 80.24% (45-98%) in our DLBCL cases known for their aggressive course. Helicobacter pylori was found in only one of thirteen patients with primary gastric DLBCL (Figure 2).

The mean age was younger (48.16, 28-73) in MALT lymphoma cases (6/46), the second most common diagnosis of the study group. All of the cases were diagnosed by endoscopic biopsy. GIS symptoms were described in five cases. The complaint of the patient who presented with a polypoid mass located in the rectum was bloody diarrhea. The other four cases were located in the stomach (two antrum, one corpus, one unknown), and one case was located in the duodenum. Accompanying helicobacter pylori enfection was detected in three gastric and one duodenal neoplasia. Vaguely nodular or diffuse patterned atypical lymphoid proliferation was predominantly consisted of cells with small round nuclei and clear cytoplasm. In all of the biopsies, the presence of lymphoepithelial lesion could be demonstrated, at least by immunohistochemical pancytokeratin. In some of the cases, the dendritic cell network was irregularly expanded. Centroblast or immunoblast-like large cell aggregation, which could be evaluated in favor of transformation into high-grade lymphoma, was not observed. All neoplasms had kappa/lambda monotypic plasmacytoid differentiation supporting the diagnosis. The mean Ki67 proliferation index was 16.60% (5-30%) (Figure 3).

The mean age of the patients with duodenal-type follicular lymphoma was 57.5 (40-69). In the endoscopy of the cases presenting with non-specific GI symptoms, polyp was described in three and erythema in one. Bacilli were observed in favor of Helicobacter pylori in one of the biopsies. The extent of the atypical lymphoid infiltration with nodular features in the mucosa and submucosa was more clearly demonstrated by immunohisto-chemical CD20 staining. Similar to systemic follicular lymphoma, strong positive reaction with CD10, BCL6 and BCL2 was detected in all cases. The CD21 and CD23 positive follicular dendritic network, which appeared to be trapped in the periphery of the atypical follicles, was a key finding observed in all cases. Ki67 proliferation index was 21.25% (15-30%) on average.

Patients who had rare diagnoses presented with acute abdomen symptoms. A ten-year-old male patient who presented with ileus due to intussusception was diagnosed with Burkitt lymphoma. Another case in the pediatric age group underwent ileocecal resection for a mass in the cecum and was diagnosed with IRF4-associated large B-cell lymphoma. The ulcerovegetant mass observed in the ileal resection material of the case who applied to the hospital with perforation was reported as EBV-positive large B cell lymphoma. The surprise diagnosis in the duodenal endoscopic biopsy of the patient who was examined clinically for icterus was extracavitary/solid variant primary effusion lymphoma. One of our rare diagnoses was lambda monotypic positive plasma cell neoplasia presenting with an ulcerovegetative mass in the ileum. Although it was easy to recognize the plasma cells histomorphologically in some of the biopsy sections, the morphology was quite anaplastic in some sections. Concomitant lymphoid cell component was not detected. As a result, infiltration was interpreted in favor of extraosseous plasmacytoma, accompanied by clinical data. Plasma cell myeloma infiltration was not detected in the patient's bone marrow biopsy. Another focus of involvement was not detected on imaging. One patient who underwent resection with ileus had myeloid sarcoma. Gross examination revealed three separate masses located in the ileum. The patient did not have any known history of myeloid neoplasia prior to this diagnosis. There was not any registered bone marrow biopsy in our department for this patient.

Bone marrow biopsies of thirty-three cases which were performed for staging were reviewed. Bone marrow infiltration was detected in only one of the cases grouped as primary gastrointestinal lymphoma. This case with ileus symptoms and an ulcerovegetating mass in the terminal ileum was diagnosed with diffuse large B-cell lymphoma. Gastro-intestinal infiltration was secondary in the other four cases with bone marrow involvement.

Discussion

In recent years, hematolymphoid neoplasms of the gastrointestinal tract have been one of the topics on which revisions have been reported and new entities have been defined. Although the boundaries of definitions about neoplasia have been detailed, lymphoid proliferations with aggressive morphology but indolent course, which are considered as disorders in the new classification, make the pathological diagnosis more stressful. For this tumor group, which may require multiple immunohistochemical examinations, sometimes adequate tissue cannot be obtained with commonly used endoscopic/ colon-oscopic biopsy methods. Ulceration and crush artifact are other problems which are frequently encountered in these biopsies and complicate morphological evaluation. On the other hand, differential diagnosis especially with poorly differentiated malignancies, is another histopathological challenge in GIS where the expectation of epithelial neoplasia

Table 3: Similar retrospective studies in our country [11-15].

Author / Year	Patients	Age *Min-Max (Median)*	Gender *F/M*	Anatomic sites of origin (%) *Stomach/Small intestine/Large intestine/Other*	Diagnostic procedure *Laparotomy/ Endoscopy/ Other*	Diagnoses (%) *DLBCL/ MALT/FL/ MCL/TCL*
Dincol et al. 1992	33	26-75 (ND)	18/15	39.3 / 48.4 / 12.1	21 / 12 / -	ND
Atalay et al. 2003	56	14-81 54.5	26/30	78.5 / 18 / 3.5	12 / 44 / -	ND
Alacacıoglu et al. 2007	24	34-80 64	12/12	83.3 / 8.3 / 8.4	ND	75/25/-/-/-
Erkurt et al. 2009	41	18-90 58	22/18	61 / 26.8 / 7.3	ND	58.5/26.9/2.4/2.4/9.8
Yildirim et al. 2019	22	25-77 47	8/14	31.8 / 50 / 18.2	ND	86.4/-/4.5/4.5/-
Current study	46	10-88 56	20/26	52.2/32.6/13.1	11/34/1	56.5/13/8.7/4.3/2.2

F: Female, M: Male, DLBCL: Diffuse large B-cell lymphoma, FL: Follicular lymphoma, MCL: Mantle cell lymphoma, TCL: T-cell lymphomas, ND: Not descripted.

is higher. Based on these considerations, we tried to collect the histopathological and clinical features of gastrointestinal lymphoma cases in our center. There are very few similar retrospective studies published in our country. Some of our data were compared with these studies in Table 3 [11-15]. To summarize the basic results of this study; i) Almost half of the patients were diagnosed with DLBCL. ii) MALT lymphoma, the first lymphoid neoplasia that comes to mind in the gastrointestinal tract, was only 13% of the cases. iii) Although classical FL immuno-profile was observed in duodenal follicular lymphoma cases with low grade histo-morphology, the dendritic meshwork organization which was pushed to the follicle periphery was distinctive from systemic forms, iiii) In pancytokeratin-negative undifferentiated neoplasms, if positivity can not be detected with common B or T cell immunomarkers, the differential diagnosis spectrum should be expanded and additional markers (plasmacytic, myeloid/ histiocytic/ dendritic, viral, etc.) should be added to the immune panel.

The stomach is prominent primary site of GI lymphomas. Although different rates are given for the incidence of intestinal lymphoma, it is reported to be high in Middle Eastern countries (49-81%) [5, 9]. Tumor location is important in terms of histo-pathological subgroup and therefore prognostic differences. The stomach is the region where mostly indolent lymphoma group is observed, especially MALT lymphoma. In the intestine, mainly high grade lymphomas are detected [5, 9]. However, the GI tract region where DLBCL is observed with the most frequency is the stomach and followed by the ileocecum, in current reviews [2]. Approximately half of our cases showed infiltration in the stomach, and the small intestine was the second most common location of tumors. DLBCL constituted more than half of all our cases and of tumors located in the stomach. Do to the fact that we have not made a clinical-prognostic comparison in our study, indolent or aggressive histological subtype differences depending on tumor location have not been observed.

Gastric DLBCL can transform from MALT lymphoma. It has been reported that a low-grade component accompanying high-grade

lymphoid neoplasia is detected in approximately one-third of the biopsies [9]. We did not have a case in which low-grade and high-grade neoplastic proliferation were detected together, which could be an example of this information.

In various published case series, B-cell non-Hodgkin lymphomas constitute the majority of GIS lymphoproliferative neoplasms, similar to systemic forms [9]. In the study of Nakamura et al. (5), T-cell lymphomas were reported at higher rates in both the gastric and intestinal groups compared to the other series, and this was attributed to the geographical incidence difference. Any subgroup of T-cell lymphoma/disorder of primary GI origin was not detected in our archive. Our patient with extranodal NK/T-cell lymphoma infiltrating secondary to the gastric corpus was the only case with T-cell immunophenotype. The patient, who had primary uveitis symptoms before endoscopy, lost vision three months after the biopsy.

Because of its prognostic importance, GI DLBCL should be classified in terms of GCB or ABC phenotypes, similar to systemic forms [2]. In the study of Lin et al (11), they found no significant difference with regard to numbers or survival between GCB and ABC subtypes in ninety cases grouped according to three algorithms. Similarly, Hwang et al. (12) did not observe prognostic difference between the two groups. Although the GCB immunophenotype was dominant in our DLBCL cases, no statistically significant difference was found. Assessment of prognosis was not performed within the scope of our study. For the differential diagnosis of high-grade B-cell lymphoma c-myc, BCL2 and BCL6 should be added to the immunopanel in gastrointestinal DLBCL cases. Double/triple expressor cases should be referred to FISH for evaluation of MYC, BCL2 and/or BCL6 gene rearrangements. These cases are characterized by a more aggressive course [2]. Contrary to this information, in the study of Choi et al. (11), it was reported that c-myc gene rearrangement is more common in GI DLBCL cases compared to nodal forms, but did not have a negative effect on overall survival. Two of our cases showing coexpression of c-myc, BCL2 and BCL6 relapsed with diffuse disease. However, gene rearrangement with FISH has not been confirmed. One patient with triple expressor DLBCL with primary terminal ileum localization, who also has Crohn's disease, may serve as an example for questioning the role of chronic inflammation in lymphomagenesis. In large population-based cohort studies investigating the risk of lymphoma in inflammatory bowel diseases, no statistically significant increase was found in ulcerative colitis patients. On the other hand, an increase in the risk of lymphoma development has been reported in Crohn patients by being associated with various variables such as disease duration, hospitalization, treatment protocols and etc. [16-18]. Refractory celiac disease can be considered the prototype entity for the relationship between non-infectious inflammatory processes and lymphoma [6]. In fact, the first infectious inflammatory factor which comes to mind as a risk factor, H. Pylori, was detected in only one primary DLBCL case. In MALT lymphomas, H. pylori was seen in two-thirds of the cases. However, the number of cases was insufficient for statistical comparison.

Our cases of duodenal-type follicular lymphoma with histologically low grade morphology and nodular pattern have been diagnosed in the last two years. Similar to those reported in the review of Auerbach et al. (2), atypical follicles without tingible body macrophages, whose mantle region could not be distinguished, consisted of cells with centrocyte morphology. Diagnosis is not difficult with the described histopathological features and immunohistochemical profile (CD10+, BCL6+, BCL2+). The dendritic meshwork of atypical follicles pushed and

trapped at the periphery is another sensitive feature of duodenal-type FL that should be emphasized. However, we think that awareness of this entity is important in differential diagnosis, since reactive lymphoid follicle development can be observed frequently in GIS.

GI lymphomas, especially DLBCL, are neoplasms that are usually diagnosed at an early stage without bone marrow infiltration [6, 19]. Similar to the literature, bone marrow invasion was detected in only one of primary GIS tumors. This was the triple expressor patient with the history of Crohn disease.

Another striking point was the variety of diagnosis in our case group, which also included secondary hematolymphoid neoplasms. Endoscopic biopsy specimens constitute the majority of the materials in most pathology laboratories currently. In these small biopsies, which sometimes show artificial features, aggressive lymphomas and poorly differentiated carcinomas such as signet ring cell carcinomas that do not form a definitive pattern can be confused (Figure 2). Similar to this situation, diagnostic difficulties can be experienced in hematolymphoid neoplasms with partial epithelial morphology. In particular, the diagnosis is even more challenging for hematolymphoid tumors that do not express common B or T cell antigens. It was not easy to finally reach the diagnosis due to the anaplastic component of our extracavitary/solid variant primary effusion lymphoma and extraosseous plasmacytoma cases.

Myeloid sarcoma, of which histopathological diagnosis is always challenging, presents as tumoral masses formed by immature myeloid cells in extramedullary regions. Skin, soft tissue, lymph nodes are the most common locations of these rare tumors. It has been reported that 6.5-10% of cases originate from the GIS, which is even rarer [20, 21]. Tumor can develop de novo, can be a presentation form of acute myeloid leukemia recurrence or can accompany other myeloid neoplasms. In the histopathological diagnosis process, it is important to first consider the entity. Inadequate immunophenotyping, especially in small biopsies, can lead to misdiagnosis (up to 25-47%) [22]. Non-Hodgkin lymphomas, poorly differentiated carcinomas or melanoma can be considered in the differential diagnosis [23]. We would like to remind another rare diagnosis that can be encountered in the evaluation of GIS biopsies through our case of myeloid sarcoma located in the ileum.

In this study, which consists of retrospectively collected cases, the diagnostic variety of gastrointestinal hematolymphoid neoplasms can be observed. However, it was an important limitation that the number of cases was not sufficient for statistical analysis. The lack of treatment/follow-up results and prognostic evaluation can be considered as another limitation.

Conclusion

In this study, we tried to review GI hematolymphoid neoplasma with demographic, clinical, macroscopic and histopathological characteristics in a single center. The most common location of tumors was the stomach and the most common macroscopic feature was an ulcerovegetative mass. The patients frequently described non-specific GI symptoms. Although DLBCL and MALT lymphoma are the two most common subtypes, a wide variety of subtypes should be kept in mind in the differential diagnosis, especially when the rarer secondary lymphoproliferative neoplasms are also considered. While it is difficult to distinguish low-grade lymphoproliferative neoplasms from inflammatory entities and/or benign conditions, high-grade neoplasms can be confused with epithelial tumors. For this reason, in addition to detailed histopathological examination, immunohistochemical evaluation is critical in the

diagnostic approach of hematolymphoid neoplasms. Although the limited number of cases in our single-center study did not allow statistical assessment, we thought that our heterogeneous diagnostic spectrum would contribute to the literature and be useful in the evaluation of this group of neoplasms.

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Doi: 10.5505/aot.2023.68916

Original Article

Evaluation of Awareness, Behavior, and Knowledge Levels of Female Healthcare Professionals About Breast and Cervical Cancer in Southern Turkey

Türkiye'nin Güneyindeki Kadın Sağlık Çalışanlarının Meme ve Rahim Ağzı Kanseri Konusundaki Farkındalık, Davranış ve Bilgi Düzeylerinin Değerlendirilmesi

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ABSTRACT

Purpose: This study aimed to measure the level of knowledge of female healthcare professionals about breast cancer and cervical cancer.

Methods: This cross-sectional study including 310 female healthcare staff working in Hatay Training and Research Hospital was conducted between January 2020 and June 2020. The Turkish version of breast cancer and cervical cancer awareness measure questionnaires were used for the study.

Results: The age of the 310 participants varied from 17 to 62 years, 16.5% of the participants were doctors, 48.1% of them were nurses and midwives, 13.9% of them were laboratory workers and technicians, 16.5% of them were secretaries, and 5.2% of them were students. Regarding breast cancer screening, 63.5% underwent breast self-examination (BSE) routinely. Of the participants, 60.3% never underwent mammography (MMG) and ultrasonography (USG), 27.7% never went for a gynecological examination, and 58.4% had never received a Pap smear test.

Conclusions: The level of knowledge about breast cancer was sufficient among especially doctors and nurses but their attitudes and behaviors were not of the expected level.

Keywords: Breast cancer; cervical cancer; HPV; pap smear; questionnaire

ÖZET

Amaç: Bu çalışma ile amacımız, kadın sağlık çalışanlarının meme ve serviks kanseri hakkındaki bilgi düzeylerini ölçmektir.

Gereç ve Yöntem: Bu kesitsel çalışmada Ocak 2020 ile Haziran 2020 arasında Hatay Eğitim ve Araştırma Hastanesinde çalışan 310 kadın sağlık çalışanı çalışmaya dahil edildi. Çalışma için meme kanseri ve serviks kanseri farkındalık anketlerinin Türkçe versiyonu kullanıldı.

Bulgular: 17-62 yaş aralığındaki 310 katılımcının (ort: 34.3 ± 8.8) %16.5'i doktor, %48.1'i hemşire ve ebe, %13.9'u laboratuvar çalışanı ve teknisyen, %16.5'i sekreter ve %5.2'si öğrencidir. Meme kanseri taramasına yönenlik %63.5'i rutin kendi kendine meme muayenesi (KKMM) yaptı. Katılımcıların %60.3'ü hiç mamografi (MMG) ve ultrasonografi (USG) yaptırmadı, %27.7'si hiç jinekolojik muayene olmadı ve %58.4'ü hiç pap smear test aldırmadı.

Sonuç: Özellikle doktor ve hemşirelerin meme kanseri hakkındaki bilgileri yeterli olmakla birlikte tutum ve davranışları yeterli düzeyde değildi.

Anahtar Kelimeler: Meme kanseri, serviks kanseri, HPV, pap smear, anket

First Received: 12.05.2022, Accepted: 07.07.2022 doi: 10.5505/aot.2023.60376

Introduction

According to the 2018 data of the Global Cancer Observatory, 18.1 million people were diagnosed with cancer worldwide and 10 million people died of it. In 2018, breast cancer ranked first among all cancers in women worldwide with 2 million (24.2%) cases, while cervical cancer ranked fourth with 569,000 (6.6%) cases. Similarly, breast cancer ranked first in terms of death rate with 626,000 (15%) cases and cervical cancer fourth with 311,000 (7.5%) cases [1]. Mammography (MMG) is the gold standard in breast cancer screening. The American Cancer Society (ACS) and the American Institute for Cancer recommend a regular breast self-examination (BSE) at the age of 20–40 years and an annual medical examination and MMG after the age of 40 years [2,3]. In Turkey, the Ministry of Health also recommends undergoing an MMG every 2 years for women aged more than 40 years [4]. Smith et al. [5] reviewed eight randomized controlled trials and reported that MMG decreased mortality to 20% in the age group 40–74 years, to 15% in the age group 40–49 years, to 22% in the age group 50–74 years, and to 30% in all age groups.

More than 85% of deaths due to cervical cancer occur in low- and middle-income countries [6]. The most important risk factor in the development of cervical cancer is the human papillomavirus (HPV) infection [7]. HPV has more than 150 subtypes, and 12 of them lead to cervical cancer. The most common types are HPV-16 and HPV-18; it is known that 300 million people worldwide carry this virus [8,9]. In 2012, the ACS recommended cervical cancer screening for women after 3 years of sexual experience or from the age of 21 years, whether sexually active or not, using the Pap smear test [10,11]. This study aimed to measure the level of knowledge of doctors, nurses, laboratory workers, secretaries, and students, who work in hospitals and have various socioeconomic, cultural, and educational levels, about breast cancer and cervical cancer, which are detectable and preventable diseases, to evaluate their attitudes and habits.

Materials and Methods

This was a cross-sectional study sample consisting of 310 female healthcare staff working aged 17–65 years, including doctors, nurses, midwives, laboratory workers, technicians, students, and secretaries conducted in the Hatay Training and Research Hospital between January 2020 and June 2020.

The Turkish version of breast cancer [12] and cervical cancer [13] awareness measure questionnaires were used for the study. The purpose of the study was explained before distributing the questionnaire forms, and the participants were told not to write their names and identities on the form. The filled-in questionnaires were collected 10–15 min later. The questionnaire comprised 42 items and 3 subparts. The first part consisted of 12 questions regarding sociodemographic characteristics, which included age, body mass index (BMI), educational status, occupation, place of residence, marital status, chronic disease history, smoking and alcohol use, sports activity, menopausal status, and family cancer history. The second part, which consisted of six questions, included the sources of information about cancers, whether the participants underwent BSE, ultra-sonography (USG), and MMG and their results, and the frequency of going for a gynecological examination and doing a Pap smear. The third part consisted of 24 questions, including the knowledge level about breast cancer, cervical cancer, Pap smear, and HPV vaccine.

Inclusion criteria were being a healthcare staff worker for at least 1 year and being between ages 17-65 years. Exclusion criteria were females that declined participation.

Ethical approval

Ethics committee approval was obtained at the meeting of Mustafa Kemal University Tayfur Ata Sökmen Medical Faculty Non-Invasive Clinical Research Ethics Committee (meeting number: 15, decision number: 17) on December 26, 2019. The principles of the Helsinki Statement have been followed. A voluntary informed consent form was signed, stating that the data obtained from the participants of the study would be kept confidential and not be shared with anyone.

Statistical analysis

Mean, standard deviation, median, lowest, highest, frequency, and ratio values were used in the descriptive statistical analysis of data. The distribution of variables was measured using the Kolmogorov–Smirnov test. The Mann–Whitney U test was used for analyzing quantitative independent data, while the chi-square test was used for analyzing qualitative independent data. The Fisher test was used when the chi-square test conditions were not met. Statistical significance was accepted as $P < 0.05$. IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA) was used for statistical analyses

Results

The age of the 310 participants, who filled in the questionnaire, varied from 17 to 62 years, and the mean age was 34.3±8.8 years. All sociodemographic characteristics are shown in Table 1. Behaviors and attitudes about breast and cervical cancer screening of all participants are shown in Table 2.

Regarding questions about breast cancer, the highest rate of a correct answer was 97.7% to the question "There are screening centers for breast cancer" and the lowest rate of a correct answer was 49.7% for the question "The most common symptom of breast cancer is a pain in the breast." Regarding the questions about cervical cancer, the highest rate of a correct answer was 96.5% to the question "Cervical cancer is a disease that can be diagnosed and screened early" and the lowest rate of a correct answer was 36.1% to the question "Pap smear scan begins 3 years after the first sexual experience." All questions and correct answer rates for measuring breast and cervical cancer knowledge levels are shown in Table 3.

The answers with statistically significant differences between the doctors/nurses and other staff groups to the questions about BSE, USG, MMG, gynecological examination, Pap smear, and breast and cervical cancer knowledge level are shown in Table 4. Table 5 shows the comparison between the groups aged ≤39 years and ≥40 years, and Table 6 shows the same between those with and without a family history of cancer.

Discussion

Cervical and breast cancers have a high chance of being cured when diagnosed early. The importance of scanning methods increases every day. The literature states that BSE, USG, and MMG for breast cancer, gynecological examination and with pap smear test for cervical cancer facilitate early diagnosis, thus increasing survival rates [7,14]. The present study found that the rate of having knowledge about early diagnosis and screening was 95.8% for breast cancer and 96.5% for cervical cancer. This knowledge was mostly (61.9%) obtained from healthcare professionals, followed by conferences/ seminars (20.0%). Acikgoz et al. [15] reported, in their study on female workers in hospitals, that information was obtained at the highest rate from healthcare workers (35.4%) and then from friends/neighbors (19.3%). Discigil et al. [16] found, in their study on females living in the Aegean region, that the most common information source was the television (64.7%). In the present study, the sample consisted of hospital employees, and hence the most common information source was healthcare personnel.

Table 1. Distribution of hospital staff by sociodemographic characteristics (*n* = 310)

1. Age, year (mean ± sd)		34.3 ± 8.8	
2. BMI (mean ± sd)		23.8 ± 4.1	
		n	***%***
3. Education status			
Secondary school		3	1.0
High school		54	17.4
University		253	81.6
4. Job			
Doctor		51	16.5
Nurse/midwife		149	48.1
Labor/technician		43	13.9
Student		16	5.2
Secretary		51	16.5
5. Living place	Village	7	2.3
	Town	42	13.5
	City	261	84.2
6. Marital status	Married	181	58.4
	Single	116	37.4
	Divorced	10	3.2
	Widow	3	1.0
7. Chronic diseases	Yes	60	19.4
	HT	10	3.2
	DM	8	2.6
	Others	42	13.5
	No	250	80.6
8. Smoking	Yes	75	24.2
	No	210	67.7
	Ex-smokers	25	8.1
9. Alcohol intake	Yes	103	33.2
	No	207	66.8
	Every day	2	0.6
	Occasionally	5	1.6
	Sometimes	96	31.0
10. Doing sports	Yes	59	19.0
	No	127	41.0
	Irregular	124	40.0
11. Menopause status	Postmenopausal	18	5.8
	Premenopausal	292	94.2
12. Family history of cancer	Yes	60	19.4
	First degree	23	7.4
	Second degree	25	8.0
	Third degree	12	3.8
	No	250	80.6

BMI, Body mass index; DM, diabetes mellitus; HT, hypertension.

Table 2. Behaviors and attitudes about breast and cervical cancer screening (*n* = 310)

	n	%
13. Where did you get information about early diagnosis and screening?		
TV/radio	31	10.0
Magazine/newspaper/brochure	21	6.8
Doctors/nurses/midwifes	192	61.9
Friends/neighbors/relatives	4	1.3
Conference/seminar	62	20.0
14. Do you conduct BSE for breast cancer screening?		
Yes	197	63.5
No	37	11.9
Sometimes	76	24.5
15. How often do you undergo MMG and breast USG?		
Never	187	60.3
Once a year	37	11.9
Twice a year	22	7.1
Irregular	64	20.6
16. What is the result of your last USG and MMG?		
No information	17	13.8
Normal results (control after 1 year)	40	32.5
Normal results (control after 2 years)	40	32.5
Benign breast diseases (cyst, fibroadenoma)	23	18.7
Suspicious mass (biopsy result is normal)	3	2.4
17. How often do you go for the gynecology examination?		
Regularly	44	14.2
In case of complaint	180	58.1
Never	86	27.7
18. How often do you undergo a Pap smear test?		
Never	181	58.4
Once a year	34	11.0
Twice a year	17	5.5
At intervals of 3–5 years	32	10.3
Irregularly	46	14.8

BSE, Breast self-examination; MMG, mammography; Pap smear, Papanicolaou smear

Table 3. Knowledge levels about breast and cervical cancer (*n* = 310)

No	Questions	Correct information	
		n	%
19	Breast cancer is a disease that can be diagnosed and screened early.	297	95.8
20	The most common cause of breast cancer is pain in the breast.	254	49.7
21	Breast cancer occurs only in advanced age.	291	93.9
22	Breast cancer occurs only in women.	246	79.4
23	Turkey has screening centers for breast cancer.	303	97.7
24	Biopsy is absolutely necessary because every mass in the breast makes you think of cancer.	183	59.0
25	Surgery is performed only in the last stage of the breast cancer.	263	94.8
26	Cervical cancer is a disease that can be diagnosed and screened early.	299	96.5
27	Turkey has screening centers for cervical cancer.	292	94.2
28	Early starting of sexual activity is a risk factor for cervical cancer.	215	69.4
29	Multiple partners for cervical cancer are a risk factor.	260	83.9
30	Increased number of pregnancies increases the risk of cervical cancer.	119	38.4
31	Compliance with genital hygiene rules reduces the risk of cervical cancer.	250	80.6
32	HPV is an important factor in the development of cervical cancer.	260	83.9
33	Cervical cancer can be prevented with HPV vaccine.	232	74.8
34	HPV vaccine is available in Turkey.	270	87.1
35	HPV vaccine is available for free.	168	54.2
36	HPV vaccine is administered only for women.	145	46.8
37	For HPV vaccination, sexual experience is required.	222	71.6
38	Men do not get sick with HPV; they are only carriers.	117	37.7
39	HPV has only one type, and it only settles in the genital area.	211	68.1
40	The Pap smear test starts at the age of 21 years.	118	38.1
41	The Pap smear test is started after 3 years of the first sexual activity.	112	36.1
42	Pap smear test is not required after the HPV vaccine.	263	84.8

HPV, Human papilloma virus; Pap smear, Papanicolaou smear.

Table 4. Comparison of the attitudes and statistically significant knowledge levels of the doctors/nurses group and other staff group about breast and cervical cancer

No.	Questions	Doctors/nurses (*n* = 200)		Other staff (*n* = 110)		*P*
		n	%	*n*	%	
14	**Do you conduct BSE for breast cancer screening?**					**0.027**
	Yes	136	68.0	61	55.5	
	No	17	8.0	20	18.2	
	Sometimes	47	23.5	29	26.4	
15	**How often do you undergo MMG and breast USG?**					0.967
	Never	120	60.0	67	60.9	
	Once a year	23	11.5	14	12.7	
	Twice a year	15	7.5	7	6.4	
	Irregular	42	21.0	22	20.0	
17	**How often do you go for the gynecology examination?**					0.619
	Regular	30	15.0	14	12.7	
	In case of complaint	118	59.0	62	56.3	
	Never	52	26.0	34	30.9	
18	**How often do you undergo a Pap smear test?**					0.819
	Never	114	57.0	67	60.9	
	Once a year	21	10.5	13	11.8	
	Twice a year	13	6.5	4	3.6	
	At intervals of 3–5 years	21	10.5	11	10.0	
	Irregular	31	15.5	15	13.6	
19	**Breast cancer is a disease that can be diagnosed and screened early.**					**0.045**
	Correct information	195	97.5	102	92.7	
	Incorrect information	5	2.5	8	7.3	
20	**The most common cause of breast cancer is pain in the breast.**					**0.006**
	Correct information	111	55.5	43	39.1	
	Incorrect information	89	44.5	67	60.9	
22	**Breast cancer occurs only in women.**					**0.001**
	Correct information	170	85.0	76	69.1	
	Incorrect information	30	15.0	34	30.9	
24	**Biopsy is absolutely necessary because every mass in the breast makes you think of cancer.**					**0.000**
	Correct information	135	67.5	48	43.6	
	Incorrect information	65	32.5	62	56.4	
25	**Surgery is performed only in the last stage of the breast cancer.**					**0.015**
	Correct information	177	88.5	86	78.2	
	Incorrect information	23	11.5	24	21.8	
34	**HPV vaccine is available in Turkey.**					**0.040**
	Correct information	180	90.0	90	81.8	
	Incorrect information	20	10.0	20	18.2	
35	**HPV vaccine is available for free.**					**0.006**
	Correct information	120	60.0	48	43.6	
	Incorrect information	80	40.0	62	56.4	
	For HPV vaccination, sexual experience is required.					**0.005**
37	Correct information	154	77.0	68	61.8	
	Incorrect information	46	23.0	42	38.2	
38	**Men do not get sick with HPV; they are only carriers.**					**0.037**
	Correct information	84	42.0	33	30.0	
	Incorrect information	116	58.0	77	70.0	
39	**HPV has only one type and it only settles in the genital area.**					**0.001**
	Correct information	149	74.5	62	56.4	
	Incorrect information	51	25.5	48	43.6	
41	**The pap smear test is started after 3 years of the first sexual activity.**					**0.041**
	Correct information	64	32.0	48	43.6	
	Incorrect information	136	68.0	62	56.4	
42	**Pap smear test is not required after the HPV vaccine.**					**0.001**
	Correct information	180	90.0	83	75.5	
	Incorrect information	20	10.0	27	24.5	

χ^2, Chi square test.
BSE, Breast self-examination; HPV, human papilloma virus; MMG, mammography; Pap smear, Papanicolaou smear; USG, ultrasonography.

Table 5. Comparison of the attitudes and statistically significant knowledge levels of ≤39 years age group and ≥40 years age group about breast and cervical cancer

No	Questions	≤39 years age group (*n* = 209)		≥40 years age group (*n* = 101)		*P*
		n	%	*n*	%	
14	**Do you conduct BSE for breast cancer screening?**					**0.008**
	Yes	124	59.3	73	72.3	
	No	33	15.8	4	4.0	
	Sometimes	51	24.4	24	23.8	
15	**How often do you undergo MMG and breast USG?**					**0.000**
	Never	151	72.2	36	35.6	
	Once a year	14	6.7	23	22.8	
	Twice a year	6	2.9	16	15.8	
	Irregular	38	18.2	26	25.7	
17	**How often do you go for the gynecology examination?**					**0.002**
	Regular	25	12.0	19	18.8	
	In case of complaint	113	54.1	67	66.3	
	Never	71	34.0	15	14.9	
18	**How often do you undergo a Pap smear test?**					**0.000**
	Never	154	73.7	27	26.7	
	Once a year	16	7.7	18	17.8	
	Twice a year	6	2.9	11	10.9	
	At intervals of 3–5 years	10	4.8	22	21.8	
	Irregular	23	11.0	23	22.8	
20	**The most common cause of breast cancer is pain in the breast.**					**0.017**
	Correct information	94	45.0	60	59.4	
	Incorrect information	115	55.0	41	40.6	
35	**HPV vaccine is available for free.**					**0.024**
	Correct information	104	49.8	64	63.4	
	Incorrect information	105	50.2	37	36.6	

χ^2, Chi square test.

BSE, Breast self-examination; HPV, human papilloma virus; MMG, mammography; Pap smear, Papanicolaou smear; USG, ultrasonography.

Table 6. Comparison of the attitudes and statistically significant knowledge levels of the groups with and without a family history of breast and cervical cancer

No	Questions	Family history of cancer + (*n* = 60)		Family history of cancer – (*n* = 250)		*P*
		n	%	*n*	%	
14	**Do you conduct BSE for breast cancer screening?**					0.603
	Yes	39	65.0	158	63.2	
	No	5	8.3	32	12.8	
	Sometimes	16	26.7	59	23.6	
15	**How often do you undergo MMG and breast USG?**					**0.037**
	Never	28	46.7	159	63.6	
	Once a year	11	18.3	26	10.4	
	Twice a year	3	5.0	19	7.6	
	Irregular	18	30.0	46	18.4	
17	**How often do you go for the gynecology examination?**					0.943
	Regular	8	13.3	36	14.4	
	In case of complaint	36	60.0	144	57.6	
	Never	16	26.7	70	28.0	
18	**How often do you undergo a Pap smear test?**					0.071
	Never	28	46.7	153	61.2	
	Once a year	5	8.3	29	11.6	
	Twice a year	5	8.3	12	4.8	
	At intervals of 3–5 years	7	11.7	25	10.0	
	Irregular	15	25.0	31	12.4	
22	**Breast cancer occurs only in women.**					**0.023**
	Correct information	54	90.0	192	76.8	
	Incorrect information	6	10.0	58	23.2	
29	**Multiple partners for cervical cancer are a risk factor.**					**0.026**
	Correct information	56	93.3	204	81.6	
	Incorrect information	4	6.7	46	18.4	
30	**Increased number of pregnancies increases the risk of cervical cancer.**					**0.019**
	Correct information	31	51.7	88	35.2	
	Incorrect information	29	48.3	162	64.8	

χ^2, Chi square test.
BSE, Breast self-examination; HPV, human papilloma virus; MMG, mammography; Pap smear, Papanicolaou smear; USG, ultrasonography.

Breast Cancer

The rate of performing BSEs regularly was 63.5%, while that of performing BSEs irregularly was 24.5% in this study. In the literature, the rate of performing BSEs regularly was 4.3%–38.8%, which were higher compared with the results of the present study [15]. The rate of performing BSE irregularly ranged from 29.5% to 34.5%,, which were lower compared with the results of the present study [17].

The rate of performing BSE was significantly higher in doctors/nurses compared with the other personnel and significantly higher in the ≥40 years age group compared with the ≤39 years age group in this study. No significant difference was found between the participants with and without family history of cancer in this study. In a similar study by Nahcivan et al. [18] performing BSE was not affected by educational status or family history of cancer; married females and those aged more than 40 years had a higher rate of performing BSEs.

Dundar et al. [17] did not find a significant difference between the age groups. In another study, the BSE ratio was found to be significantly higher in nurses than in doctors and other personnel, but no difference was found between age groups. [19] Although the literature supports the lack of effect of family history of cancer on BSE rates, no consensus existed on the effect of age and occupational groups. In this study the rate of undergoing breast USG and MMG was found to be 39.7%. The rate of undergoing them every year was 11.9%. The rate of undergoing regular breast USG and MMG was 3.2% in a study examining women aged 20 years and older, who applied to a family health center [20]; 20% in a study examining hospital employees [15]; and 44% in a survey for other healthcare professionals [19]. The low rate of performing screening tests may be attributed to the low mean age of the participants. In the present study, the USG and MMG performance rate was 64.4% in females aged 40 years and older, 21.8% in females aged 39 years and younger, 53.3% in those with a family history of cancer, and 36.4% in those without a family history of cancer; these differences were found to be statistically significant. Also, 40% of the doctors/nurses and 39.1% of the other staff group underwent USG and MMG, but no significant difference was found between these two groups. In the study by Özcam et al. [19], the screening rates increased as the mean age increased, but no significant difference was observed in relation to the family cancer history and education levels. Another study evaluated females aged between 40 and 69 years. As age increased, the rate of undergoing USGs and MMGs also increased..In addition, those in the 40–49 years age group with a family history of cancer had more USGs and MMGs compared with those without a family history of cancer, and those with higher education undergo USGs and MMGs more often compared with those with lower education; these differences were found to be significant. In the 50–69 years' age group, no significant difference was observed in relation to the education level and family history of cancer [21]. It found that as the mean age increased and the participants had a family history of cancer, they were prone to have breast cancer screenings more regularly, and these findings were consistent with the literature. However, the lack of difference between professions in the present study was attributed to the fact that the mean ages were close to each other.

In the present study, among the seven questions asked for measuring breast cancer knowledge levels, the most correct answer was given to the question "There are screening centers for breast cancer" (97.7%). The question with the lowest rate of correct answers was the question about the method of application of breast cancer, with a rate of 49.7%. Doctors and nurses answered all seven questions more correctly compared with the other staff. A statistically significant difference was observed in the questions regarding early diagnosis, method of application, sex-related difference, biopsy indications, and treatment options. However, no significant difference was noted in questions about screening centers and age of occurrence. Participants aged ≥40 years answered all the questions about breast cancer more correctly compared with those aged ≤39 years. Despite a statistically significant difference in the question regarding the most frequent method of application, no significant difference was observed in other questions with respect to age. Participants who did not have a family history of cancer answered the question regarding the method of application more correctly compared with those who did have a family history of cancer. Those with a family history of cancer answered the other six questions more correctly. Although the rate of giving correct answers to the question about the possibility of breast cancer in both sexes was significantly higher than in those with a family history of cancer, no difference

was observed in other questions. In a study, the participants were asked to list the admission symptoms of breast cancer, and the most common answer was "palpable swelling in the breast," with a rate of 72.4% [22]. Discigil et al. [16] reported the rate of those who had information about the age of occurrence of breast cancer was 51.7%, and the rate of those who knew that breast cancer could develop in males was 49.8%. In another study, females aged 30–70 years were examined in four groups, and their knowledge about breast cancer was questioned. Their knowledge was found to decrease with age, but they underwent more screening [23]. In the present study, correct answers were obtained at rates close to those in the literature regarding the questions about admission symptoms and cancer screening. Contrary to the literature, the rate of correct answers increased with age, but these increased rates did not differ statistically. It was believed that doctors and nurses with a high level of education had more information about breast cancer, education level had less effect on basic issues, and the presence of a family history of cancer did not affect the level of knowledge.

Cervical Cancer

In the present study, no significant difference was found in the rates of undergoing gynecological examination and Pap smear test according to occupational groups and family history of cancer but it was found significantly higher in the age group of more than 40 years. In a study conducted on healthcare professionals, the rate of undergoing a Pap smear test at least once was reported as 43.5%, and the rate of having it done regularly at 1-year intervals was 31.5% [19]. The same study found that doctors and nurses had undergone significantly fewer smear tests compared with other personnel, and that positive family history of cancer had no effect on smear tests [19]. Pinar et al. [24] reported, in their study on nurses aged 20–43 years, that the rate of undergoing regular gynecological examinations was 26.4%, and the rate of undergoing regular Pap smear tests was 30.4%. In another study, the rate of undergoing regular gynecological examination was 30.3%, and the rate of undergoing a Pap smear test was 45.2% [25]. A study conducted in Africa in 2003 found that only 18.7% of healthcare professionals underwent a Pap smear test [26]. In a study conducted with Korean–American females aged more than 60 years, the rate of undergoing a Pap smear test at least once in their life was 58.5%. Participants aged more than 70 years had significantly more Pap smear tests compared with those aged 60–69 years, those who were married underwent more Pap smear tests compared with those who were not, and those who had higher education underwent more tests compared with those who did not [27]. The rate of undergoing a Pap smear test had different distribution ranges in many scientific studies, and the increase in this rate in advanced age groups in this study was in accordance with the literature. Besides the close ratios in employees with different education levels was attributed to in-hospital interaction.

An effective way to prevent cervical cancer is the prophylactic administration of the HPV vaccine. In America, vaccination started for girls aged 9–26 years in 2006. The Food and Drug Administration recommended three doses of vaccination for boys of the same age group in 2009 to protect boys from genital warts and anal cancers [28].

The participants were asked 17 questions to measure their knowledge about cervical cancer. Doctors/nurses answered 14 of 17 questions most correctly, while the other 3 questions regarding the number of pregnancies being a risk factor and screening start age were answered most correctly by the other personnel. A statistically significant difference was observed in 6 of the 14 questions to which doctors/nurses gave more correct answers, and in 1 of the 3 questions to

which the other personnel gave more correct answers. No significant difference was observed in the other questions according to the occupational group. The age group 40 years and older gave more correct answers to 11 questions, and the age group 39 years and younger to 6 questions. A statistically significant difference was found only in the answers to the question "HPV vaccine is available free of charge" among the questions that the age group 40 years and older gave more correct answers to. No significant difference was found in the other 16 questions according to age. Those with a family history of cancer answered 9 questions more correctly, and those without a family history of cancer answered 8 questions more correctly. Despite a statistically significant difference in the two questions that those with a family history of cancer answered more correctly, no significant difference was observed in the other questions according to the family history of cancer. In a survey conducted on female healthcare professionals, it was reported that those who underwent a Pap smear test had given more correct answers, compared with those who did not undergo a Pap smear test, regarding the vaccine protects from cancer, HPV is a risk factor for cervical cancer, and that the age of first sexual intercourse and multiple sexual partners increase the risk [29]. In another study conducted to measure the knowledge level of hospital staff, more than 90% of the doctors gave correct answers to the questions about HPV infection and the mode of transmission. No significant difference was observed between nurses and doctors [30]. In a similar study conducted on nurses in Thailand, 81.8% of the participants knew that having sexual intercourse at a young age was a risk factor, 85.6% knew that having multiple sexual partners was a risk factor, and 72.2% knew that HPV infection was a risk factor [31]. The low mean age and the small number of participants were among the limitations of this study. Large studies questioning more parameters are needed.

Conclusion

The frequency of performing BSE, MMG, and USG increased with age in all occupational groups, but this increase was found to be insufficient. Although doctors and nurses were found to have a high level of knowledge on basic issues about cervical cancer and risk factors, their attitudes and behaviors were not at the expected level. Moreover, the attitudes and knowledge level of other personnel were low. The level of knowledge of all hospital staff, regardless of their age and family history of cancer, was found to be low on specific issues such as the supply of HPV vaccine, when it should be administered, and to whom it could be administered. The HPV vaccine should be included in the vaccination program; audio, visual media, and social media should be used effectively. The informed staff could ensure that correct information was conveyed to their immediate surroundings and thus to the whole society.

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Doi: 10.5505/aot.2023.60376

Original Article

Characteristics and Outcome of Patients with Chronic Myelomonocytic Leukemia: Experience of a Single Center

Kronik Miyelomonositik Lösemi Hastalarının Özellikleri ve Tedavi Yanıtları: Tek Merkez Deneyimi

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ABSTRACT

Introduction: Chronic myelomonocytic leukemia (CMML) is a condition that overlaps with myelodysplastic syndrome and myeloproliferative neoplasms. The prognosis is generally poor, with a median survival of 20 to 40 months and approximately 15-30% of patients progressing to acute myeloid leukemia (AML). We aimed to evaluate the characteristics and outcomes of CMML patients who were treated at our institution.

Material and methods: A retrospective cohort study examined data from 14 CMML patients between January 2013 and January 2022.

Results: The median age of fourteen patients at diagnosis was 66 years (min 43-max 84 years). Only one patient (7.1%) had the JAK 2 V617F mutation. Most of the patients had CMML stage-0 disease (64.3%) and 13 patients had the proliferative type of disease. Nine patients were treated with hydroxyurea, which resulted in two responders. Eight patients were treated with azacitidine, which resulted in three responders. During follow-up, AML transformation was observed in five patients (35.7%) and the median duration between diagnosis and AML transformation was 12 months (10-33 months). In the AML-transformed group, at the time of diagnosis, the percentage of neutrophils was lower (52.5% vs 72.3%), and the percentage of monocytes was higher (27% vs 15.6%). In AML-transformed group the total disease duration was longer (21 (11-44) vs 5 (2-48) months) than non-transformed group.

Discussion and Conclusion: In patients receiving hypomethylating agents and hydroxyurea treatments for CMML, adequate response cannot be obtained. The rate of AML transformation increases with disease duration.

Keywords: Hydroxyurea, Azacitidine, Myelodysplastic Syndromes, Splenomegaly, Monocytes

ÖZET

Giriş ve Amaç: Kronik miyelomonositik lösemi (KMML), miyelodisplastik sendrom ve miyeloproliferatif neoplazmların ortak özelliklerine gösteren, kötü prognozlu, medyan sağkalımı 20-40 ay arasında değişen ve takip sürecinde %15-30 oranında akut miyeloid lösemi (AML) gelişen bir hastalıktır. Bu çalışmanın amacı, merkezimizde takip edilen KMML hastalarının özelliklerini, tedaviye yanıtlarını ve AML dönüşüm oranlarını değerlendirmektir.

Yöntem ve Gereçler: Ocak 2013- Ocak 2022 tarihleri arasında KMML tanısı ile takipli 14 hastanın verilerileri retrospektif olarak değerlendirilmiştir.

Bulgular: On dört hastanın ortalama yaşı 66 (en küçük 43-en büyük 84) olup sadece bir hastada (%7.1) JAK2 V617F mutasyonu saptandı. Hastaların çoğunda Evre-0 KMML (%64.3) olup ve 13 hastada proliferatif tip hastalık olduğu gözlendi. Dokuz hasta (%64.3) hidroksiüre ile tedavi edildi ve iki hastada

First Received: 25.05.2022, Accepted: 21.09.2022 doi: 10.5505/aot.2023.20082

yanıt alındı. Sekiz hasta azasitidin ile tedavi edildi ve üç hastada yanıt elde edildi. Takip sırasında beş hastada (%35.7) AML dönüşümü saptandı. KMML tanısı sonrası AML dönüşümünün medyan 12 ayda (10-33 ay) olduğu gözlendi. AML dönüşümü gözlenen grupta, tanı anında nötrofil yüzdesinin daha düşük (%52,5 ve %72,3) ve monosit yüzdesinin daha yüksek (%27 ve %15.6) olduğu gözlendi. AML dönüşümü gözlenen grupta toplam hastalık süresinin daha uzun (21 ve 5 ay) olduğu saptandı.
Tartışma ve Sonuç: KMML tedavisinde kullanılan hipometile edici ajanlar ve hidroksiüre tedavileri ile hastalarda yeterli yanıt elde edilememektedir. AML dönüşüm oranı hastalık süresi arttıkça artmaktadır.

Anahtar Kelimeler: Hidroksiüre, Azasitidin, Miyelodisplastik Sendromlar, Splenomegali, Monositoz

Introduction

Chronic myelomonocytic leukemia (CMML) is a clonal hematological cancer with proliferative and dysplastic features. It is classified as one of the overlapping conditions of Myelodysplastic syndrome (MDS) and Myeloproliferative neoplasms (MPN) in the 2016 World Health Organization (WHO) classification [1]. Chronic myelomonocytic leukemia is a rare hematological cancer that affects only 4 people out of every million. It primarily affects older people and is more prevalent in men. The disease is classified as proliferative or dysplastic based on peripheral white blood cell (WBC) counts. Blast percentages in bone marrow and peripheral blood determine the stage of disease. The risk group primarily drives treatment selection. Patients with low risk and no symptoms are followed without treatment or with cytoreductive therapy (hydroxyurea), whereas patients with medium or high risk are treated with hypomethylating agents (HMA) or hydroxyurea. The prognosis is generally poor, with a median survival of 20 to 40 months and approximately 15% to 30% of patients progressing to acute myeloid leukemia (AML) [2, 3].

Since the disease is uncommon and the number of randomized controlled studies is limited, the therapy is based primarily on physician discretion, rendering real-world data essential in this context. Here we are presenting the characteristics and outcomes of CMML patients treated at our center.

Methods

This retrospective cohort study examined data from patients with CMML who were treated at our center between January 2013 and January 2022. Patients were diagnosed with CMML according to the WHO classification [1].

Manual file records and electronic medical record systems were used to obtain clinical information from patients. The demographics, comorbidities, CMML diagnosis date, CMML type, disease stage at diagnosis, therapy and response, last control date, and survival status of all patients were documented. The 2016 WHO classification was used for staging [1].

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: 2022-03/1709).

The SPSS software (Version 25.0; Armonk, NY: IBM Corp) was used for statistical analysis. The numerical variables were presented as median (min-max), while the categorical variables were presented as ratios. Disease duration was calculated as the time defined as the period between the date of CMML diagnosis and the date of last follow-up or death from any cause.

Results

The characteristics of the patients

Between January 2013 and January 2022, 14 CMML patients were diagnosed and included

in the study. The median age at diagnosis was 66 years (a range of 43-84 years). At the time of presentation, half of these patients had splenomegaly and hepatomegaly. All patients had been evaluated for the presence of JAK2 V617F and cytogenetic abnormalities. Only one patient (7.1%) had the JAK2 V617F mutation, and no cytogenetic abnormality was found in any of the patients. Most of the patients had CMML stage-0 (64.3%) and 13 patients had proliferative type of disease according to WHO staging. According to Mayo risk stratification, seven patients (50%) were in the high-risk group, six patients (42,9%) were in the intermediate-2 risk group, one patient (7,1%) was in the intermediate-1 risk group, and none of them were in the low-risk group. In the bone marrow examination, six patients had grade 1-2 fibrosis, none of them had grade 3 fibrosis. All patients' characteristics are shown in Table 1.

Table 1. Characteristics of the patients

Parameters	N =14, (%)
Age (years) (median, min-max)	66 (43-84)
Gender (M/F)	10 (71.4) / 4 (28.6)
CMML Stage	
CMML-0	9 (64.3)
CMML-1	4 (28.6)
CMML-2	1 (7.1)
CMML type (Dysplastic/ Proliferative)	1 (7.1) / 13 (92.9)
CMML risk group, n (%)	
Low risk	0
Intermediate-1	1 (7.1)
Intermediate-2	6 (42.9)
High risk	7 (50)
Peripheral blood Parameters, median (min-max)	
WBC count (x10^9/L)	49.2 (9.8-94)
Monocyte percentage	20.1 (2.1-78.3)
Absolute Monocyte counts (×10^9 /L)	5.496 (413-44.574)
Hemoglobin concentration (g/L)	9.47 (4.2-13.4)
Platelet count (×10^9 /L)	83 (15-338)
Lymphocyte percentage	11.6 (3.3-20.1)
Neutrophil percentage	70.2 (5.8-85.4)
Bone marrow blast percentage, median (min-max)	2 (0-15)
JAK 2 V617F mutation	1 (7.1)
Splenomegaly	7 (50)
Hepatomegaly	6 (42.8)
Disease duration, median (min-max), months	8.4 (2-48)

CMML; chronic myelomonocytic leukemia, M; male, F; female, WBC; white blood count, AML; acute myeloid leukemia

Treatment and the response of patients

Nine patients (64.3%) were treated with hydroxyurea and only two patients responded to hydroxyurea according to their peripheral blood count. Four patients (28.6%) were treated with azacitidine and two of them responded to azacitidine. Three of the patients refractory to hydroxyurea were treated with azacitidine and one patient was treated with azacitidine plus ruxolitinib, only one patient responded to azacitidine. One of the hydroxyurea-refractory patients was treated with ruxolitinib, but no response was observed. One patient was treated with the best support due to poor performance status and comorbidities. During follow-up, AML transformation was observed in five patients (35.7%) and the median duration between diagnosis and AML transformation was 12 months (10-33 months). Allogeneic hemato-poietic cell transplantation was performed on a male patient who had AML transformation (allo-SCT). After allo-SCT, he achieved a complete response, and he is still alive and has been in remission for 40 months. During the follow-up period, three patients died (one patient without AML transformation due to

Table 2. AML-transformed and non-transformed group characteristics

Parameters	AML-transformed group, (N=5)	Non-transformed group, (N=9)
Age,years, median (range, min-max)	66 (63-82)	66 (43-84)
WBC count, median (range, min-max) ($X10^9$/L)	56.800 (9.840-60.000)	55.000 (19.710-94.060)
Monocyte percentage, median (min-max), %	27.9 (24.9-78.3)	15.6 (2.1-24.5)
Absolute Monocyte count, median (min-max), ($\times10^9$ /L)	10.2 (2519-44.474)	4.7(413-18.906)
Hemoglobin concentration, median (range, min-max) (g/L)	9.5 (8.0-13.4)	9.2 (4.2-12.9)
Platelet count, median (range, min-max) ($\times10^9$ /L)	84 (41-338.000)	83 (15-121))
Lymphocyte percentage, median (min-max), %	13 (11.6-15.8)	10.9 (3.3-20.1)
Neutrophil percentage, median (min-max), %	52.6 (5.8-54.3)	72.3 (53.4-85.4)
Disease duration, median (min-max), months	21 (11-44)	5 (2-48)
Bone marrow fibrosis (Grade 1-2/3), %	2 (40)	4 (44)

WBC; white blood count, AML; acute myeloid leukemia,

septicemia and two patients due to AML transformation).

There was no difference in patients' gender, age, CMML stage, risk status, first treatment option and responses, WBC, PLT, and lymphocyte percentage at the time of diagnosis between the AML-transformed and non-transformed groups. In the AML-transformed group, at the time of diagnosis, the percentage of neutrophils was lower (52.5% vs 72.3%), and the percentage of monocytes was higher (27% vs 15.6%). The total disease duration was longer (21 (11-44) vs 5 (2-48) months). AML-transformed and non-transformed group characteristics are shown in Table 2.

Bone marrow fibrosis

At the time of diagnosis, six patients were diagnosed with grade 1-2 bone marrow fibrosis; none had grade 3 fibrosis. During follow-up, two patients with bone marrow fibrosis developed AML.

Discussion

In our study, 14 patients were examined. Patients were 66 years old on average, with half having hepatosplenomegaly and the majority having CMML stage 0, proliferative phase, and high-risk disease. Nine patients received hydroxyurea treatment, and eight patients received azacitidine treatment. During the follow-up period, AML-

transformation was observed in five patients; two of these patients died due to disease progression, and one was treated with allo-SCT. The AML-transformed group had a higher percentage of monocytes, a lower percentage of neutrophils, and the disease lasted longer than the non-transformer group.

CMML is a rare hematological disease with heterogeneous disease characteristics, so it is critical for centers to share their experiences. It is extremely beneficial to learn about the factors that influence the outcome and risk of AML transformation. Furthermore, optimal treatment choice according to the type and risk of the disease is another matter of debate.

Hypomethylating agents are the first-line therapy for CMML, particularly in high-risk patients. In CMML patients, HMAs have a response rate of 25–75% [5–9]. Coston et al. reported, HMAs have a response rate of 39% and a median time to response of five months (range 2–5) [9]. Responses of HMAs treatments were similar in dysplastic and proliferative CMML patients in the azacitidine-treated arm, but better responses were seen in dysplastic CMML patients in the decitabine-treated arm [9]. HMA responses were linked to lower serum LDH levels as well as the absence of KRAS, NRAS, and c-KIT [9]. Only one patient in our study had dysplastic CMML. Eight patients (four as first-line treatment and four as second-line treatment) received azacitidine (75 mg/m^2 for 7 days in 28-day cycles), but only three (37.5%) responded. The criteria affecting treatment responses were not evaluated in our study due to the small number of patients.

Hydroxyurea is the most commonly used cytoreductive therapy for the treatment of leukocytosis and organomegaly associated with CMML. In our study, nine patients were treated with hydroxyurea, but only two of them (22.2%) responded.

The median overall survival is 20 to 40 months and approximately 15–30% of patients progress to AML [2, 3, 9]. In our study, during the follow-up period, five patients (35.5%) developed AML. In the AML-transformed group, at the time of diagnosis, the median percentage of peripheral blood monocytes was higher (27.9% vs. 15.6%), while the median percentage of neutrophils was lower (52.5% vs. 72.3%). The disease duration was longer in AML-transformed groups than in non-transformed groups (21 vs 5 months). CMML disease duration was longer in AML-transformed patients (21.9 vs 17.1 months, p =0.0002) than in non-transformed patients [10].

Gur et al. reported that 20% of CMML patients had grade 2-3 fibrosis. In the fibrosis group, WBC and monocyte counts were higher, and splenomegaly was more common. Furthermore, the survival time in this patient group was shorter [4]. In our study, there was no patient with grade 3 fibrosis. Six of our patients had bone marrow fibrosis of grades 1-2. There was no difference in many parameters examined between patients with fibrosis and those without fibrosis.

While the only curative treatment is allo-SCT, the majority of patients are not candidates due to their advanced age and comorbidities [11]. Allo-SCT was performed on one patient in our study, and he has been in remission for 40 months.

Conclusion

In conclusion, CMML is a rare disease with a poor prognosis that shares features with MDS and MPN. The only curative treatment is allo-SCT but many patients are unable to receive it due to their advanced age and comorbidities. The current CMML treatment options and outcomes are far from satisfactory. Therefore, whenever possible, patients should be encouraged to participate in clinical trials.

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Doi: 10.5505/aot.2023.20082

Original Article

Simultaneous Evaluation of Serum Immunofixation Electrophoresis and Serum Free Light Chain Measurements Used in the Diagnosis of Monoclonal Gammopathy and Smoldering Multiple Myeloma of Uncertain Significance in the Presence of Risk Factors

Risk Faktörleri Varlığında Önemi Belirsiz Monoklonal Gammopati ve Smoldering Multiple Myeloma Tanısında Kullanılan Serum İmmünfiksasyon Elektroforezi ve Serum Serbest Hafif Zincir Ölçümlerinin Eş Zamanlı Değerlendirilmesi

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ABSTRACT

Introduction: This study aimed to investigate the effectiveness of using serum Free Light Chain (sFLC) together with traditional examination methods in diagnosing Monoclonal Gammopathy of Undetermined Significance (MGUS) and Smoldering Multiple Myeloma (SMM), especially in patients with Monoclonal Gammopathy (MG) without hypercalcemia, renal dysfunction, anemia, and bone lesions (CRAB) symptoms, by eliminating the need for bone marrow biopsy (BMb).

Methods: A total of 160 patients over 50 years of age with an ESR of 50 mm/h and above were included in the study. Serum Immunofixation Electrophoresis (sIFE) and sFLC levels of these patients were studied simultaneously. Sensitivity and specificity of the sIFE and sFLC for diagnosis of MGUS were estimated by ROC analysis.

Results: MG was detected in 36 (22.5%) patients with sIFE and in 30 (18.7%) patients with sFLC of 160 patients included in the study.There were a total of 44 patients with MG detected by sIFE alone, sFLC alone, and both sIFE and sFLC. BMb were performed on 42 patients with MG who approved the BMb procedure. These patients were diagnosed MGUS, SMM and MM by using test results, BMb results, and clinical and laboratory data. The sensitivity of sIFE in the detection of MG was 82.5% and the specificity was 99.2% ($p<0.001$). sFLC had a sensitivity of 72.5% and a specificity of 99.2% ($p<0.001$). When sIFE and sFLC were performed simultaneously, the sensitivity was 100% and the specificity was 98.3% ($p<0.001$).

Discussion and Conclusion: Simultaneous monitoring of sIFE and sFLC, which is both non-invasive and with the opportunity to achieve results in less time without BMb, may be sufficient and reliable in the diagnosis and follow-up of MGUS in the detection of MGs and especially in asymptomatic patients.

Keywords: MGUS, ESR height, sFLC

ÖZET

Giriş ve Amaç: Bu çalışmada, özellikle hiperkalsemi, böbrek fonksiyon bozukluğu, anemi ve kemik lezyonları (CRAB) semptomları olmayan Monoklonal Gammopatili (MG) hastalarda, Önemi Belirsiz Monoklonal Gammopati (MGUS) ve Smoldering Multipl Miyelom (SMM) tanısında, serum Serbest

First Received: 27.05.2022, Accepted: 29.06.2022 doi: 10.5505/aot.2023.32650

Hafif Zincirinin (sFLC) geleneksel tetkik yöntemleri ile birlikte kullanımının kemik iliği biyopsisi (Kİb) ihtiyacını ortadan kaldırarak etkinliğinin araştırılması amaçladık.
Yöntem ve Gereçler: Çalışmaya ESR 50 mm/h ve üzerinde olan, 50 yaş üstü 160 hasta alındı. Bu hastaların serum İmmünfiksasyon Elektroforezi (sIFE) ve sFLC düzeyleri eş zamanlı çalışıldı. MGUS tanısı için sIFE ve sFLC'nin duyarlılığı ve özgüllüğü ROC analizi ile tahmin edildi.
Bulgular: Çalışmaya alınan160 hastanın yapılan sIFE ile 36(%22,5) hastada ve sFLC ile de 30 (%18,7) hastada MG tespit edilmiştir. Yalnızca sIFE, yalnızca sFLC ve hem sIFE hem de sFLC tarafından saptanan MG'li toplam 44 hasta vardı. MG tespit edilen ve kemik iliği biyopsi (Kİb) işlemine onay veren 42 hastaya Kİb yapılmıştır. Bu hastalara; test sonuçları, Kİb sonuçları, klinik ve laboratuar verileri de kullanılarak MGUS, SMM ve MM tanısı konulmuştur. sİFE'nin MG tespitinde sensitivitesi %82.5, spesifitesi %99.2 ($p<0.001$) olarak saptanmıştır. sFLC'nin sesensitivitesi %72.5, spesifitesi %99.2 ($p<0.001$) olarak saptanmıştır. sİFE ve sFLC eş zamanlı yapıldığında ise sensitivitesi %100, spesifitesi %98.3 ($p<0.001$) olarak saptanmıştır.
Tartışma ve Sonuç: MG'lerin tespitinde ve özellikle asemptomatik hastalarda Kİb yapmadan hem noninvaziv hem de daha kısa sürede sonuç alma fırsatı ile sIFE ve sFLC'nin eşzamanlı bakılması MGUS tanısı koymada ve takip etmede yeterli ve güvenilir olabilir.

Anahtar kelimeler: MGUS, ESR yüksekliği, sFLC

Introduction

Monoclonal Gammopathies (MG) are a group of diseases characterized by uncontrolled proliferation and accumulation of clonal plasma cells capable of synthesizing light and heavy chains of immunoglobulin (Ig), called monoclonal protein (M protein), which can be detected in serum or urine [1, 2]. MGs range from benign Monoclonal Gammopathy of Undetermined Significance (MGUS) to Smoldering Multiple Myeloma (SMM), which usually does not require treatment and has a silent course, to the highly malignant Multiple Myeloma (MM), which requires treatment [3, 4].

MGUS is a MG that is more common in the elderly and is characterized by mature plasma cell proliferation in the bone marrow (BM) [5]. It is seen at a rate of 3.2% above the age of 50, and at a rate of 5.3% above the age of 70 [6]. Its prevalence increases with age and this rate reaches 8.7% over the age of 80 [7-9].

MGUS may also include a heterogeneous group such as SMM, which is an intermediate stage of the disease as it progresses to MM. This group may exhibit biological behaviors similar to MGUS, but may also include clinical manifestations such as hypercalcemia, renal dysfunction, anemia, and bone lesions (CRAB symptoms) requiring treatment for myeloma [10]. MM is a group of malignant diseases characterized by neoplastic transformation of plasma cells and clonal growth of B cells in BM [1, 2].

Comprehensive clinical and laboratory evaluation is required to differentiate MGUS, SMM, and MM patients. It is important to make this distinction. Because while active treatment is required in MM, follow-up is more important in MGUS and SMM due to the risk of progression to MM rather than treatment.

Excessive and disproportionate production of kappa or lambda free light chains occurs in MGUS or MM [11]. Therefore, measurement of free light chain levels in serum has an important place in the diagnosis of diseases that cause abnormal monoclonal or polyclonal light chain concentrations.

Traditional methods such as serum Protein Electrophoresis (sPE), serum immunofixation electrophoresis (sIFE), and nephelometric measurement of serum Ig heavy chains are used in the diagnosis and follow-up of MGs [12)]. However, the lack of standardization,

limited sensitivity and specificity of these methods are the main limitations [13, 14].

With the introduction of serum free light chain (sFLC) measurement method [15], it has gained an important place in hematological diagnoses by changing the diagnostic procedures [16-18]. Detection of sFLC is very useful in the diagnosis, follow-up and prognosis of MGs [19]. There are also studies showing that sFLC is a reliable marker that can be used together with conventional tests in the detection and follow-up of MGs [20].

MGUS was updated again in 2014 to include the criteria used in the differential diagnosis of SMM and MM in the International Myeloma Working Group (IMWG) guide [18] in the sFLC. The diagnostic criteria of these diseases include the demonstration of clonal plasma cell proliferation by BMb [18, 21-23].

In this study, we aimed to investigate the effectiveness of using sFLC together with traditional examination methods in diagnosing MGUS and SMM, especially in patients with MG without CRAB symptoms, by eliminating the need for BMb, which is an invasive procedure, in the presence of certain risk factors.

Materials and Methods:

The study was planned as a prospective study. The study protocol was prepared in accordance with the Declaration of Helsinki and was accepted by the Erciyes University Faculty of Medicine Ethics Committee (Ethics Committee Decision No: 2022/340).

Patients:

Between January 2012 and January 2013, 160 patients who were followed up in with an ESR 50 mm/s and above, over 50 years of age, with bonepain and informed consent form were included followed in the Haematology clinic of Erciyes University Faculty of Medicine. Those below the age of 50 and with ESR below 50 mm/s, plasma cell disorders, other lymphoproliferative diseases, rheumatoid arthritis, Sjogren's, psoriatic arthritis vs soft tissue diseases, infections such as hepatitis C, HIV, scleroderma, urticaria vs dermatological diseases and autoimmune diseases were excluded from the study.

Laboratory:

The serum samples of the patients included in the study were collected in the immunology laboratory for sPE, sIFE and sFLCtests. sIFE, sFLC kappa and lambda from the serums were stored at -20°C to be measured. Completeblood count, biochemical tests (serum calcium, phosphorus, total protein,albumin, creatinine) levels were studied. BMb samples were taken from the patients with a peak in the gamma band in sPE and MG detected in sIFE and sFLC tests, and both microscopic evaluation and histopathological diagnosis were made. Patients who did not accept BMb were evaluated clinically and with other laboratory parameters.

The sPE agarose gel method was studied on a semi-automated SAS-1Plus/SAS-2/Platinum device (Helena Biosciences Europe, Tyne and Wear, England). BMb was planned for patients with sPE and M protein levels of 1.5 g/dL and above. sIFE was performed using the interlabG26 Agarose Gel Electrophoresis Analyzer and the Slide method Paragon IFE kit (Beckman Coulter, Brea, CA). The sensitivity for IFE varied between 50-150 mg/dL depending on the type of monoclonal protein. Interpretation in sIFE was made visually and qualitatively. Measurement of sFLC was performed on an automated nephelometry instrument (BN ProSpec, Dade, Germany) with reagent (Freelite™, The Binding Site Ltd, Birmingham, UK). For each test, a standard dilution of 1/100 was run as specified by the manufacturer. When "antigen excess" was detected, the measurement was repeated with a higher dilution (1/400 and 1/2000).

Statistical Analysis:

Statistical analyzes were performed with IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. Clinicopathological variables of patients associated with MG were evaluated for normal distribution using the Kolmogorov Smirnov test and visual methods. In descriptive statistics, parametric continuous variables were presented as mean (standard deviation), nonparametric variables were presented as median (range), and categorical variables were presented as frequency (percentage). According to the normal distribution status, numerical data were compared between groups with Student's T test or Mann Whitney U test, and categorical data were compared between the groups with Chi-square test or Fisher's exact test according to their suitability. AUC, cut-off values, sensitivity and specificity of the tests used to diagnose MG were evaluated by ROC analysis. Comparison of the areas under the curve of these tests was done by pairwise comparisons. Statistically $p<0.05$ was considered significant.

Results

Both sIFE and sFLC levels of 160 patients included in the study were evaluated. MG was detected in 36 patients (22.5%) with sIFE. MG was detected in 30 patients (18.7%) with sFLC. A total of 44 patients with MG detected by only sIFE, only sFLC, both sIFE and sFLC were recommended to undergo BMb, and BMb could not be performed in two patients with MG in sIFE, since they did not give voluntary consent. MGUS, SMM, MM were diagnosed by looking at the plasma cell percentages and clinics of 40 of the 42 patients who underwent BMb, and the other two biopsy results were determined as B-cell lymphoma and MDS, respectively. MG was detected by the sIFE method in 34 of these patients, and the diagnostic distribution was found to be MGUS 24 patients, SMM three patients, MM six patients, and one other (MDS). Again, in 30 of these patients, MG was detected by the sFLC method, and the diagnostic distribution was found to be MGUS 21 patients, SMM three patients, MM five patients, and one other (B-cell lymphoma). (Figure.1)

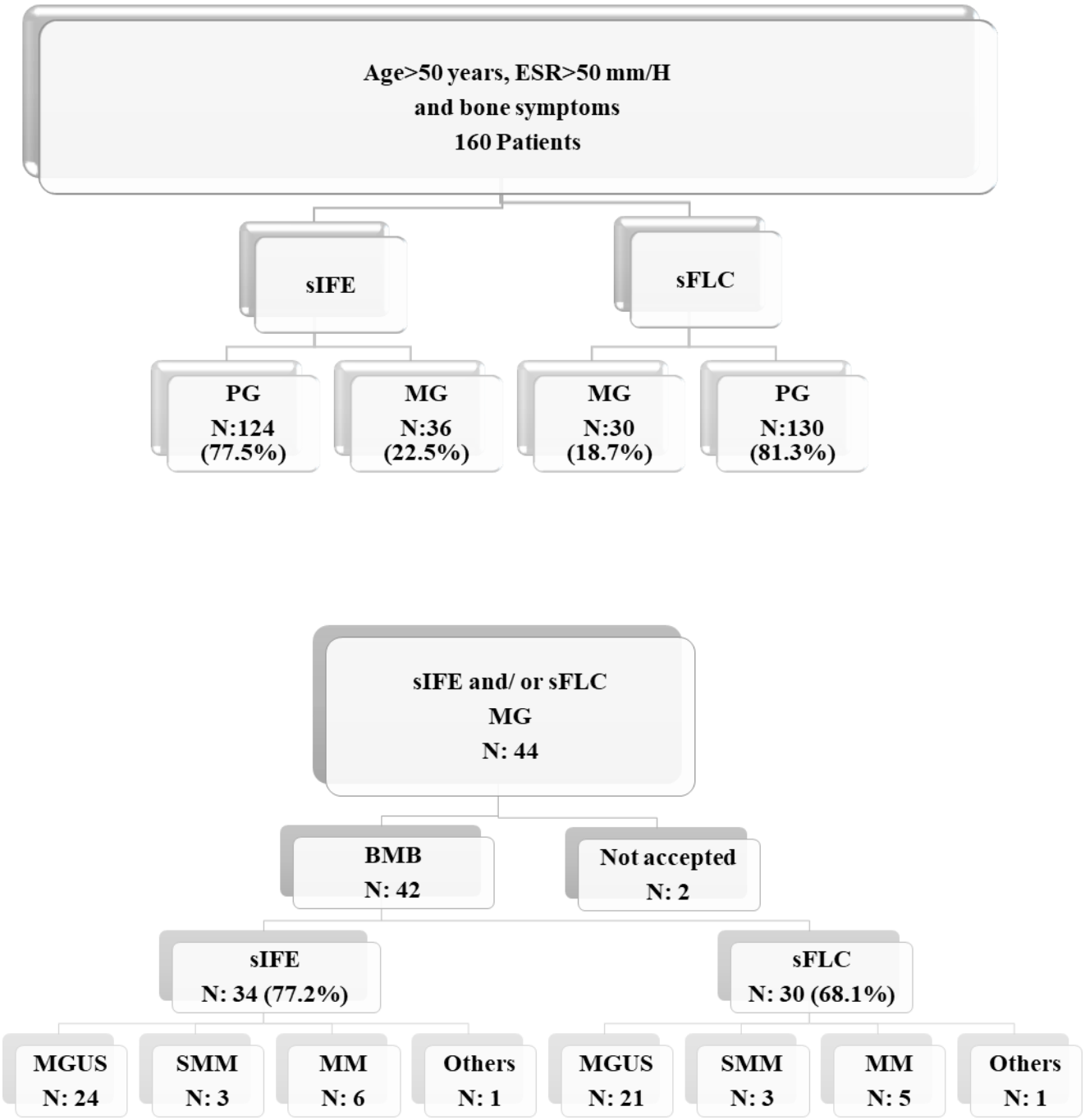
A comparison of 44 patients with MG with sIFE or sFLC was compared with the remaining 116 patients with polyclonal gammopathy (PG) (Table 1). There was a statistically significant difference between the two groups in terms of gender, and the rate of male patients was found to be higher in the MG group (P=0.001).

Patients were evaluated in terms of CRAB parameters included in the MM diagnostic criteria. Hypercalcemia was observed in 15.9% of the patients with MG, while it was observed in 0.9% of the PG patient group (p=0.001). In terms of osteoporosis, when the two groups were compared, osteoporosis was found in 46.3% of the MG patients, while osteoporosis was found in 16.7% of the PG group (p=0.030). When the two groups were compared in terms of laboratory parameters; A statistically significant difference was found in terms of IgA, IgM, sIFE lambda, BUN, creatinine and T score levels. While the median age of the patients with MG was 67.5 (range:60.5-73.5 years), the median age of the PG group was 64.0 (range:56.0-73.0 years, p=0.143) (Table.2).

Descriptive statistics of ROC curve via sIFE, sFLC, sIFE or sFLC according to MG detected in bone marrow biopsy was shown in Table3.

When the area under the curve in the ROC curves was compared for these three methods, no statistically significant difference was found between measuring only sIFE and only sFLC (p=0.350, Table 4). When sFLC was evaluated together with sIFE, it was found that detecting patients with MG was statistically significantly superior to measuring sIFE alone or sFLC alone (p=0.007; p<0.001, respectively) (Figure.2).

Figure 1: Diagnostic Approach Results According to Immunological Tests and Bone Marrow Biopsy



ESR: Erythrocyte Sedimentation Rate, sIFE: Serum Immunofixation Electrophoresis, sFLC: Serum Free Light Chain, PG: Polyclonal Gammopathy, MG: Monoclonal Gammopathy, BMB: Bone Marrow Biopsy, MGUS: Monoclonal Gammopathy of Undetermined Significance, SMM: Smoldering Multiple Myeloma, MM: Multiple Myeloma

Table 1: Comparison of Categorical Data Between Two Groups

		Total N=160	PG N=116	MG N=44	p value
Gender	**Female**	96 (60%)	79 (68.1%)	17 (38.6%)	0.001
	Male	64 (40%)	37 (31.9%)	27 (61.4%)	
Age	**50-69 years**	10 (63.8%)	75 (64.7%)	27 (61.4%)	0.699
	70 year and over	58 (36.3%)	41 (35.3%)	17 (38.6%)	
Diagnosis According to sIFE	**Polyclonal Gammopath**	124 (77.5%)	116 (100%)	8 (18.2%)	
	Ig G Kappa (mg/mL)	19 (11.9%)	0 (0%)	19 (43.2%)	
	Ig G Lambda (mg/mL)	13 (8.1%)	0 (0%)	13 (29.5%)	
	Ig A Kappa (mg/mL)	4 (2.5%)	0 (0%)	4 (9.1%)	
MG Status (According to sIFE)	**No**	124 (77.5%)	116 (100%)	8 (18.2%)	<0.001
	Yes	36 (22.5%)	0 (0%)	36 (81.8%)	
Diagnosis According to sFLC	**Normal serum**	26 (16.3%)	21 (18.1%)	5 (11.4%)	
	MG	19 (11.9%)	0 (0%)	19 (43.2%)	
	MG with RF	11 (6.9%)	0 (0%)	11 (25%)	
	Polyclonal Ig Increase or RF	104 (65%)	95 (81.9%)	9 (20.5%)	
MG Status (According to sFLC)	**No**	130 (81.3%)	116 (100%)	14 (31.8%)	<0.001
	Yes	30 (18.8%)	0 (0%)	30 (68.2%)	
eGFR	**< 60 (mL/dk/1.73m²)**	34 (21.3%)	21 (18.1%)	13 (29.5%)	0.114
	≥ 60 (mL/dk/1.73m²)	126(78.8%)	95 (81.9%)	31 (70.5%)	
Distribution of Diagnoses According to BMB	**MGUS**			31 (64.6%)	
	SMM			3 (6.3%)	
	MM			6 (12.5%)	
	Other			2 (4.2%)	
	None accept			2 (4.2%)	
Corrected Calcium	**>11 (mg/dL)**	8 (5%)	1 (0.9%)	7 (15.9%)	0.001
	≤ 11 (mg/dL)	152 (95%)	115(99.1%)	37 (84.1%)	
R F	**eGFR <40 (mL/dk/1.73m²)**	13 (8.1%)	9 (7.8%)	4 (9.1%)	0.754
	eGFR≥ 40 (mL/dk/1.73m²)	147(91.9%)	107(92.2%)	40 (90.9%)	
Anemia (Female <12; Male <13)	**Yes**	119(74.4%)	87 (75%)	32 (72.7%)	0.769
	No	41 (25.6%)	29 (25%)	12 (27.3%)	
Osteoporosis	**T score < -2.5 (osteoporosis)**	22 (37.3%)	3 (16.7%)	19 (46.3%)	0.030
	T score ≥ -2.5 (normal)	37 (62.7%)	15 (83.3%)	22 (53.7%)	

PG: Polyclonal Gammopathy, MG: Monoclonal Gammopathy, sIFE: Serum Immunofixation Electrophoresis, sFLC: Serum Free Light Chain, RF: Renal Failure, Ig: Immunoglobulin eGFR: estimated Glomerular Filtration Rate, BMB: Bone Marrow Biopsy, MGUS: Monoclonal Gammopathy of Undetermined Significance, SMM: Smoldering Multiple Myeloma, MM: Multiple Myeloma

Table 2: Comparison of Groups According to Laboratory Parameters

	Total (N=160)	PG (N=116)	MG (N=44)	P value
Age, years	66 (57-73)	64 (56-73)	67,5 (60,5-73,5)	0.143
ESR	66,5 (54-86)	68,5 (55-86,5)	59 (52,5-80)	0.095
sIg G (mg/mL)	1660 (1390-2125)	1600 (1390-1990)	1820 (1370-2605)	0.077
sIg A (mg/mL)	323 (209-441)	356 (278-462)	100,6 (50,1-290,5)	<0.001
sIg M (mg/mL)	92,8 (49,3-140,5)	110,5 (68,9-154)	41,5 (23,1-85,2)	<0.001
sIg E (mg/mL)	26,2 (17,3-100,8)	28 (17,3-135)	17,3 (17,3-71,4)	0.219
sIFE Kappa (mg/L)	405,5 (331-499,5)	400 (341,5-488,5)	441,5 (243-655)	0.828
sIFE Lambda (mg/L)	220,5 (163,5-297,5)	235,5 (182,5-291)	193,5 (63,4-345,5)	0.043
sIFE Kappa/Lambda	1,8 (1,5-2,3)	1,8 (1,5-2)	2,8 (0,9-9,4)	0.093
sBeta 2 Microglobulin (µg/mL)	3,1 (2,5-4,3)	3,1 (2,5-4,1)	3,4 (2,6-5,3)	0.094
sFLC Kappa (mg/L)	34 (20,8-54,1)	33,8 (21,8-51,1)	36,7 (14,1-97,6)	0.577
sFLC Lambda (mg/L)	42,3 (22,4-82,9)	42,7 (28,6-79,2)	39,8 (14,1-93,2)	0.396
sFLC Kappa/Lambda	0,8 (0,6-1,1)	0,8 (0,6-0,9)	1,1 (0,2-2,7)	0.143
Calcium Level (mg/dL)	9 (8,6-9,4)	9 (8,6-9,4)	9 (8,6-9,5)	0.757
Corrected Calcium (mg/dL)	9,6 (9,2-10)	9,6 (9,2-10)	9,6 (9,2-9,9)	0.942
Hemoglobin (g/dL)	11,2(10,2-12,2)	11,2(10,3-12,2)	11,1(9,7-12,2)	0.448
Total Protein (g/L)	7,3(6,8-7,7)	7,2(6,9-7,7)	7,4(6,7-8)	0.431
Albumin (g/L)	3,3(2,8-3,7)	3,3(2,8-3,7)	3,4(2,9-3,8)	0.997
BUN (mg/dL)	18(13-26,5)	17(13-24,5)	21(17-29,5)	0.028
Creatinine (mg/dL)	0,7(0,6-1,1)	0,7(0,6-0,9)	0,9(0,7-1,3)	0.002
T score (BMD)	-2,3(-2,7--1,8)	-2(-2,4--0,9)	-2,4(-2,8--2)	0.019
eGFR (mL/dk/1.73m²)	89.5(8.5-179.1)	91.8(9-179)	87(8.5-146)	0.108

PG: Polyclonal Gammopathy, MG: Monoclonal Gammopathy, ESR: Erythrocyte Sedimentation Rate, sIg: serum Immunoglobulin, sIFE: Serum Immunofixation Electrophoresis, sFLC: Serum Free Light Chain, BMD: Bone Mineral Densitometry, eGFR: estimated Glomerular Filtration Rate

Table 3. Descriptive Statistics of ROC Curve According to MG Detected in Bone Marrow Biopsy

	Sensitivity(%)	Spesificity(%)	*p* value	AUC	95% CI	LR(+)	LR(-)
sIFE	82.5	99.2	<0.001	0.908	0.852-0.948	97.4	0.2
sFLC	72.5	99.2	<0.001	0.858	0.794-0.909	85.6	0.3
sIFE or sFLC	100	98.3	<0.001	0.992	0.962-1,000	59	0.0

ROC:Receiver Operator Characteristic, MG: Monoclonal Gammopathy, sIFE:Serum Immunofixation Electrophoresis, sFLC: Serum Free Light Chain, AUC: Area Under The Curve, CI: Confidence Interval, LR(+): Likelihood Ratio For a Positive Result, LR(-) :Likelihood Ratio For a Negative Result

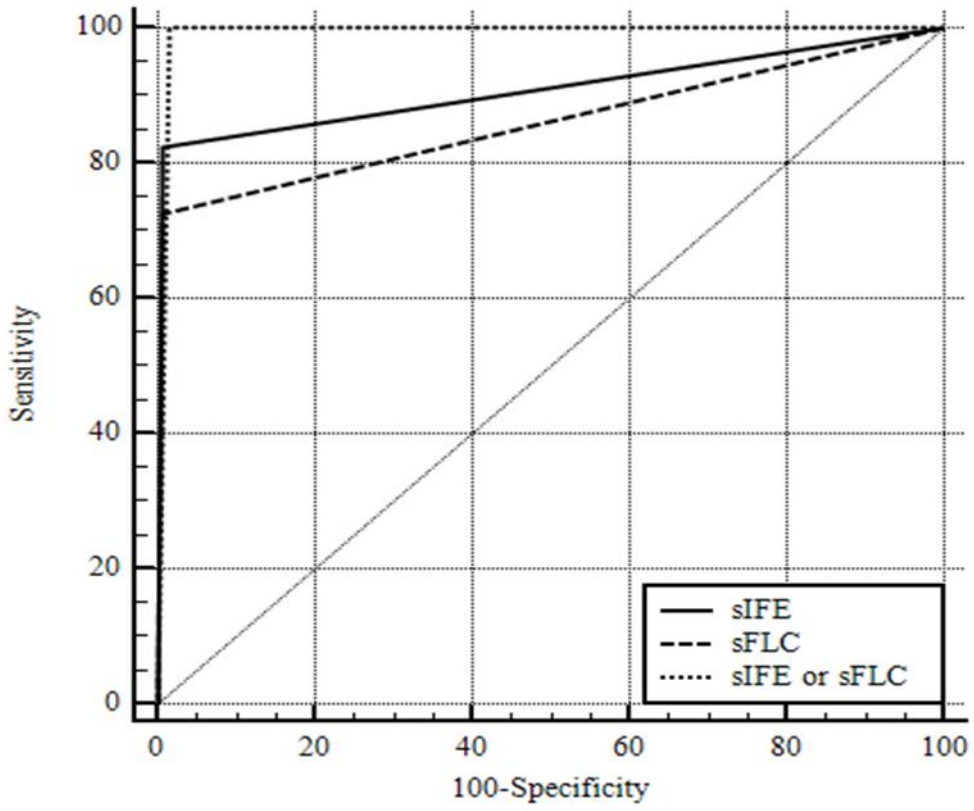


Figure 2: Comparison of ROC Curves

sIFE: Serum Immunofixation Electrophoresis, sFLC: Serum Free Light Chain

Table 4. Pairwise Comparison of ROC Curves

	Difference Between Areas	SE	95% CI	z statistics	*P*-value
sIFE-sFLC	0.050	0.053	-0.055-0.155	0.935	0.350
sIFE-sIFE or sFLC	0.083	0.031	0.023-0.143	2.711	0.007
sFLC-sIFE or sFLC	0.133	0.036	0.063-0.204	3.702	<0.001

ROC: Receiver Operator Characteristic,sIFE: Serum Immunofixation Electrophoresis, sFLC: Serum Free Light Chain, SE: Standard Error, CI: Confidence Interval

Discussion

The present study aimed to detect MGs in the presence of risk factors such as high ESR, bone pain and advanced age, and to diagnose MGUS, SMM, and MM among them. At the same time, our aim is to compare the consistency of the results between sIFE and sFLC measurement tests used in the detection of MGs, to determine which method is more sensitive in diagnosing MGUS, and to show whether serum free light chain (κ and λ) measurements can be an alternative to the sIFE method, which is routinely applied in the Immunology laboratory, and which one is more sensitive in detecting MG. While determining the sensitivity and specificity of the sPE, sIFE and sFLC tests, we aimed to diagnose MGUS and SMM in patients with MG without the need for BMb.

Apart from traditional methods such as sPE and sIFE, there are many studies showing that sFLC has an important role in diagnosis, follow-up and progression of MGUS [16, 18-20, 24]. There are many studies that found that κ/λ measurement with the sFLC method is an important predictor of the progression from MGUS to MM in patients with MG [24, 25]. However, sFLC measurements have been shown to be better than sPE and sIFE in early detection of disease progression [16, 26].

Apart from these methods, evaluation of BMb plays a key role in the differentiation of MGUS patients into SMM and MM [18, 21-23]. In the IMWG guideline, BMb is also included among the diagnostic criteria for MGUS, SMM, and MM from MGs [27].

On the one hand, sFLC shows the variation in the concentration ratio of the two chains without direct evidence for the presence of a monoclonal light chain, while sPE/sIFE reveals the true presence of a monoclonal immunoglobulin or monoclonal light chain, thus suggesting that it is a better test than sFLC [28].

In this study, we diagnosed MG in 22% of patients with sIFE and 18.7% with sFLC. We performed BMb, which we accept as the gold standard method, in 42 of 44 patients with MG detected by both methods. We detected plasma cell percentages in 40 of these patients. Thus, we found the sensitivity of sIFE as 82.5% and of sFLC as 72.5% in detecting MG. Therefore, while sIFE found 19% of patients with MG detected by BMb to be normal, we found 28.5% of these patients to be normal with sFLC.

Beetham et al. reported the specificity of the abnormal FLC κ/λ ratio as 96% and the sensitivity as 76% in patients with MG in a

study conducted by [29]. Jaskowski et al. compared serum IFE and FLC values in 483 cases and found the specificity of the κ-FLC test as 91.4%, the sensitivity as 99.5% and the agreement as 94.6%, the specificity of the λ-FLC as 99.7%, the sensitivity as 98.5% and the agreement as 72.9% [30]. Thus, they concluded that sFLC measurements are less sensitive than sIFE, but more specific for detecting monoclonal proteins in serum. We also reached a similar conclusion in our study. In one study, abnormal serum FLC rates were found in 82% of all patients, including MGUS and SMM patients. In the remaining 18% patient group, MG could not be detected by sFLC [31].

In a similar study, sFLC values were found to be normal in only 50% of patients with MG detected by sIFE. Singhal et al. also found sFLC rates to be normal in 34% of patients with positive sIFE results [32]. While Katzmann et al. found 8% sensitivity of sPE and sFLC measurements alone without sIFE on MGUS scanning, they found an abnormal sFLC rate in only 42.4% of MGUS patients [13]. Again, in a few similar studies, inconsistencies were found between the 2 methods [28, 33]. All of these studies show us that sIFE or sFLC alone is not sufficient to detect MG.

In our study, we found that the sensitivity in detecting MG was 100% when we applied both sIFE and sFLC with sPE to all patients. In other words, when we used sIFE and sFLC simultaneously, we were able to detect all patients with BMb Clonal plasma cell ratios.

It is generally accepted that sPE/sIFE and urinary protein electrophoresis (uPE)/ urinary immunofixation electrophoresis (uIFE) should be evaluated together in adequate screening of MGs. One of the limitations of our study was the lack of 24-hour urine sampling due to logistical problems, loss of time, difficulties in patient cooperation and increased workload.

However, as a result of our study, we were able to detect all MGs with the simultaneous study of sPE, sIFE and sFLC, and showed that the need for a separate urine sampling could be eliminated.

Dispenzieri et al. [16] also found a result in parallel with our study, showing that the combination of sFLC, sPE and IFE is highly sensitive in screening myeloma and related diseases, and 24-hour urine studies are not needed. Sabatino [34] conducted a similar study to our study and showed that the combination of sPE ,sIFE and sFLC are sensitive and simple diagnostic tests for detecting MG . In our study, we tried to question the necessity of performing not only urine tests but also BMb. And indeed, we found that we could detect MG with sPE, sIFE, and sFLC in all patients diagnosed with BMb. In a similar study, Sidiqi et al. [35] showed that it is possible to diagnose with these methods, especially when diagnosing MGUS, without performing BM biopsies.

In other studies, in MG patients, there are studies showing inconsistency between IFE and FLC results [28, 33]. There could be many reasons for this inconsistency. One of these is usually a polyclonal increase in globulins secondary to inflammatory responses, particularly chronic inflammation and chronic liver disease, with an increase in κ light chain usually predominant. This increase can cause an abnormality in the sFLC value, which can lead to false negatives or false positives for MGs [26, 36-39]. In the study in which sPE and sIFE were used together, these two tests detected more than 90% of monoclonal Ig [36]. These methods were able to identify only 50% of intact monoclonal Ig and monoclonal light chain production, and intact Igs were detected in approximately 80% of MG patients. The remaining 20% of cases or light chain monoclonal proteins and nonsecretory lesions were missed by sPE and sIFE methods.

Another reason for false negativity can be explained by the inability to detect the group with free heavy chain disease by the sFLC method. In addition, impaired renal function also causes an increase in serum levels of free Ig light chains. Glomerular filtration depends on the size of proteins, and variability in polymerization of light chains also affects retention of free light chains differently. Normally, sFLC κ/λ is between 0.26-1.65, while this ratio is considered to be between 0.37-3.17 in patients with renal dysfunction due to these reasons [40-42].

For these reasons, sFLC cannot detect all of the intact Ig-induced gammopathies and light chain gammopathies, and its normal finding does not mean that there is no gammopathy [32, 43].

Therefore, abnormal sFLC value alone will not be sufficient to detect MGs.

Despite all these studies, it has also been shown that abnormal sFLC rates can be a good predictor of progression from SMM to MM [44].

Consequently, a test panel is required for screening MGs, as no single test has optimal sensitivity. In serum analysis, IMWG recommends a panel of sPE, siFE, and sFLC κ and λ tests for screening for pathological monoclonal proliferative disorders.

In our study, we found that 61.4% of 44 patients diagnosed with MG were male and 38.6% were female. In a study [5] conducted with 1384 patients, 54% of the patients were male and 46% were female. The study of Bin Xu et al. [45] supported this study and again found more male patients than females.

Clinical manifestations such as CRAB symptoms are among the diagnostic criteria of MM and indicate end-organ damage. MGUS is asymptomatic and has no CRAB findings. In our study, we evaluated patients for CRAB symptoms, and there was a significant difference between those with MG and those with PG in terms of hypercalcemia and osteoporosis. This situation can be explained by the presence of not only MGUS but also SMM and MM patients among the patients diagnosed with MG. In addition, the presence of these symptoms may predict the progression of MGUS to MM and may indicate the need for closer follow-up and even treatment of these patients compared to others.

In our study, we showed that in order to diagnose MGUS in patients with risk factors for MG, instead of looking at sIFE or sFLC alone, sFLC with sIFE can be as sensitive as BMb. Thus, we concluded that simultaneous monitoring of sIFE and sFLC when diagnosing MGUS may eliminate the need for BMb. However, prospective studies with a much larger number of patients are needed for this to be put into practical clinical practice

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Doi: 10.5505/aot.2023.32650

Original Article

Uric Acid and Multiple Myeloma, Unexplored Association

Ürik Asit ve Multiple Miyeloma, Keşfedilmemiş İlişki

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ABSTRACT

Introduction: Multiple Myeloma (MM) is a common hematological malignancy and various factors affect survival. Uric acid (UA) is an easily and quickly accessible laboratory test. UA has been found to affect prognosis and survival in many hematological diseases and its impact on myeloma is not widely investigated.

Methods: Our retrospective study includes 106 MM patients between 2014 and 2021. The influence of UA level at diagnosis on treatment outcomes and survival of patients who received autologous stem cell transplantation (ASCT) was investigated.

Results: The mean UA at diagnosis was 6.05 mg/dL, and 38.7% of our cohort relapsed after a median of 30 months of follow-up, with 22.7% dead. In survival analysis, the level of UA did not significantly differ in both progression-free survival (PFS) and overall survival (OS) (HR, 1.067; 95% CI, 0.947-1.202; p=0.290, HR, 0.941; 95% CI, 0.791-1.121; p=0.497, respectively).

Discussion and Conclusion: In our study, regardless of the cut-off value for the UA level at the time of diagnosis, the UA level had no impact on PFS or OS in MM patients who received ASCT.

Keywords: Hyperuricemia, Plasma Cell Disorders, Hematopoietic Stem Cell Transplantation, Prognosis, International Scoring System

ÖZET

Giriş ve Amaç: Multipl Miyelom (MM) sık görülen bir hematolojik malignitedir ve çeşitli faktörler sağkalımı etkiler. Ürik asit (ÜA), kolay ve hızlı erişilebilir bir laboratuvar testidir. ÜA'nın birçok hematolojik hastalıkta prognozu ve sağkalımı etkilediği bulunmuştur ve miyelom üzerindeki etkisi geniş çapta araştırılmamıştır.

Yöntem ve Gereçler: Retrospektif çalışmamız 2014-2021 yılları arasında 106 MM hastasını içermektedir. Otolog kök hücre nakli (OKHN) uygulanan hastaların tanı anındaki ÜA düzeyinin tedavi sonuçları ve sağ kalım üzerine etkisi araştırılmıştır.

Bulgular: Tanı anında ortalama ÜA 6.05 mg/dL idi ve kohortumuzun %38.7'si medyan 30 aylık takipten sonra nüks etti ve %22,7'si hayatını kaybetmişti. Sağ kalım analizinde, ÜA seviyesi hem hastalıksız sağ kalım (PFS) hem de genel sağ kalım (OS) açısından önemli ölçüde farklı değildi (HR, 1.067; %95 GA, 0.947-1.202; p=0.290, HR, 0.941; %95 CI, 0.791-1.121; p=0.497, sırasıyla).

Tartışma ve Sonuç: Çalışmamızda, OKHN uygulanan MM hastalarında, tanı anındaki ÜA düzeyi için cut-off değeri ne olursa olsun, ÜA düzeyinin PFS veya OS üzerinde etkisi yoktu.

Anahtar Kelimeler: Hiperürisemi, Plazma Hücre Bozuklukları, Hematopoetik Kök Hücre Nakli, Prognoz, Uluslararası Skorlama Sistemi

First Received: 02.06.2022, Accepted: 01.07.2022 doi: 10.5505/aot.2023.25901

Introduction

Multiple Myeloma (MM) constitutes 1% of all cancers and approximately 10% of hematological malignancies [1]. It is an incurable disease and approximately 140,000 new cases are encountered worldwide each year. Despite advances in treatments, the mean overall survival was found to be 5.2 years [2,3]. Disease stage, cytogenetic abnormalities, and response to treatment are among the most important factors affecting survival [4]. The International Scoring System (ISS-including albumin and beta-2 micro-globulin measurements), and the Revised International Scoring System (R-ISS-which is formed by adding cytogenetic tests and lactate dehydrogenase [LDH] to the ISS, have become more popular in recent years), and are used in disease staging. According to the International Myeloma Study Group (IMWG), after a mean follow-up of 46 months, 5-year overall survival rates of R-ISS 1, R-ISS 2, and R-ISS 3 are 82%, 62%, and up to 40%, respectively [5,6]. Among the other factors influencing survival, response to therapy is related to the depth of response and response time, and it has been demonstrated to have a significant impact on disease prognosis [7,8].

Uric acid (UA), which is generated when purine nucleotides are degraded, is a proinflammatory marker in epidemiological research, and some studies suggest it may be a hematological marker [9,10]. Also, adverse effects of UA on prognosis and complications in various hematological diseases and hematopoietic cell transplantation (HCT) have been detected [11-14]. Despite the importance of UA in hematological diseases, very limited information was obtained in our literature review regarding the UA level and the course of the disease in MM patients. One of these studies, which investigated the etiology of MM and oxidative stress, found that the UA level in myeloma patients was not significantly higher than in the healthy control group [15]. In contrast to this study, another investigation found that patients with high UA levels had a worse disease stage, laboratory parameters, and survival [16].

In terms of disease follow-up, simple and rapid tests that can predict treatment response and prognosis in hematological disorders are crucial. Our goal is to see how the UA level at the time of diagnosis affects treatment outcomes and survival in MM patients who had autologous stem cell transplantation.

Methods

This study was designed as a retrospective observational cross-sectional study. Electronic media and archive files of MM patients who were admitted to Ankara Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital Hematology Clinic between 2014 and 2021 were scanned. The study included patients with MM who were between the ages of 18 and 75 and were receiving an autologous transplant. Patients who did not have UA measurement at the time of diagnosis, who underwent second autologous and allogeneic cell transp-lantation, and who used melphalan 140 mg/m^2 as a conditioning regimen in auto-logous stem cell transplantation were excluded from the study. This study was conducted under ethical principles in accordance with the Declaration of Helsinki. Local ethics committee approval was obtained (Date:23/12/2021 Number: 2021-12/24).

The diagnosis of MM was made according to the IMWG-2014 criteria [1]. Patients' demo-graphic information, Eastern Cooperative Oncology Group (ECOG) performance score, UA level, myeloma-related parameters (hemoglobin, calcium, creatinine, LDH, ISS, R-ISS), and hematopoietic cell trans-plantation-specific comorbidity index (HCT-CI) data were all collected. Definitions were made for ISS and R-ISS in accordance with IMWG's criteria in 2005 and 2015, respectively [5,6]. HCT-CI was calculated according to the

Table 1. Demographic features of the patients

Parameters	N=106 (100%)
Age (median, min-max)	57,5 (28-75)
Gender (M/F)	61 (57.5%) / 45 (42.5%)
ECOG (0/ ≥1)	57 (53.8%) / 49 (46.2%)
Comorbidity (present)	30 (28.3%)
MM subtype	
Heavy chain	78 (73.6%)
Light chain	25 (23.6%)
Non-secretory	3 (2.8%)
International Scoring System Stage	
ISS I	39 (36.8%)
ISS II	38 (35.8%)
ISS III	29 (27.4%)
Revised International Scoring System Stage	
R-ISS I	36 (34%)
R-ISS II	45 (42.5%)
R-ISS III	21 (19.8%)
N/A	4 (3.8%)
Laboratory	
Hb (g/dL)	11.55 (6.53-16.3)
Uric acid (mg/dL)	6.05 (1.8-15.5)
Creatinine (mg/dL)	0.88 (0.34-6.9)
Calcium (mg/dL)	9.6 (8.1-16.6)
LDH (U/L)	190 (80-829)

M, Male; F, Female; ECOG, Eastern Cooperative Oncology Group; MM, Multiple Myeloma; ISS, International Staging System; R-ISS, Revised International Staging System; Hb, hemoglobin; LDH, Lactate dehydrogenase

Table 2. Treatment details

Parameters	N=106 (100%)
Induction Chemotherapy	
VCD	99 (93.4%)
VTD	4 (3.8)
VCD + VRD	3 (2.8)
Radiotherapy (applied)	10 (9.4%)
Pre-ASCT Response	
CR	51 (48.1%)
VGPR	25 (23.6%)
PR	28 (26.4%)
SD	2 (1.9%)
HCT-CI score (0 -1 / ≥ 2)	90(84.9%) / 16 (15.1%)
Post-ASCT Response	
CR	65 (61.3%)
VGPR	16(15.1%)
PR	18 (17%)
N/A	7 (6.6 %)
Median follow up (months) (median,min max)	30 (8-75)
Relapse	41(38.7%)
Mortality	24 (22.7%)

VCD, bortezomib-cyclophosphamide-dexamethasone; VTD, bortezomib-thalidomide –dexamethasone; VRD, bortezomib-lenalidomide-dexamethasone;
CR, complete response; VGPR, very good partial response; PR, partial response;
SD, stable disease; N/A, not applicable

definitions of Sorror et al. in 2005 [17]. Data on treatment responses before and three months after autologous stem cell transplantation, progression-free survival (PFS), and overall survival (OS) were collected. PFS was defined as the latest date after the beginning of therapy when there was no evidence of recurrence, death from any cause, or no recurrence. OS was defined as the date from the start of treatment until death from any cause or the last date the patient was known to be alive.

Statistical Analysis

Analyzes were performed with SPSS Software (Version 26.0 Armonk, NY). Descriptive statistics were used to summarize the data. Categorical data were expressed as ratios, and numerical data as median and mean ± standard deviation. Kolmogorov Smirnov test was used as the normal distribution test. Survival analysis Kaplan Meier test was performed. Factors affecting survival were evaluated with log-rank. Factors predicting survival with Cox regression analysis were first analyzed univariate and factors with $p<0.3$ were included in the multiple analysis. $P<0.05$ was considered statistically significant.

Results

A total of 215 patients were screened in the study, 109 of them were excluded and a total of 106 patients were included.

The patients in the study had a median age of 57.5 (ranges:28-75), and 57.5 % of them were male. In terms of performance score, 53.8%

Table 3. Factors influencing overall survival

	Univariate Analysis		Multivariate Analysis	
Factors	Hazard Ratio (95% CI)	*P* value	Hazard Ratio (95% CI)	*P* value
Age	1.016 (0.953-1.083)	0.626		
Gender (ref female)	1.382 (0.617-3.092)	0.432		
ECOG	1.680(0.791-3.569)	0.177	1.645 (0.750-3.611)	0.214
Uric Acid	0.941 (0.791-1.121)	0.497		
LDH	1.003 (1.000-1.006)	0.021	1.002 (1.00-1.005)	0.065
Hb	1.005 (0.838-1.205)	0.958		
ISS	1.267 (0.774-2.075)	0.346		
R-ISS	1.309 (0.772-2.220)	0.318		
HCT-CI score	1.409 (0.983-2.020)	0.062	1.348 (0.931-1.952)	0.114

**p<0.05 was regarded as statistically significant. Factors with p<0.3 in univariate analysis were regarded as eligible for multivariate analysis.*
ECOG, Eastern Cooperative Oncology Group; LDH, Lactate dehydrogenase; Hb, hemoglobin; ISS, International Staging System; R-ISS, Revised International Staging System; HCT-CI score, Hematopoietic cell transplantation-specific comorbidity index

Table 4. Factors influencing progression-free survival

	Univariate Analysis		Multivariate Analysis	
Factors	Hazard Ratio (95% CI)	*P* value	Hazard Ratio (95% CI)	*P* value
Age	1.012 (0.970-1.055)	0.583		
Gender (ref female)	1.160 (0.841-1.599)	0.367		
ECOG	1.492 (0.839-2.653)	0.173	1.430 (0.784-2.608)	0.243
Uric Acid	1.067 (0.947-1.202)	0.290	1.032 (0.909-1.172)	0.623
LDH	1 (0.997-1.003)	0.775		
Hb	0.933 (0.810-1.074)	0.334		
ISS	1.298 (0.880-1.917)	0.190	1.245 (0.805-1.924)	0.325
R-ISS	1.124 (0.748-1.689)	0.573		
HCT-CI score	1.168 (0.894-1.525)	0.255	1.188 (0.902-1.564)	0.220

**p<0.05 was regarded as statistically significant. Factors with p<0.3 in univariate analysis were regarded as eligible for multivariate analysis.*
ECOG, Eastern Cooperative Oncology Group; LDH, Lactate dehydrogenase; Hb, hemoglobin; ISS, International Staging System; R-ISS, Revised International Staging System; HCT-CI score, Hematopoietic cell transplantation-specific comorbidity index

were ECOG 0 and 28.3% had comorbidities. The mean UA at the time of diagnosis was 6.05 mg/dL, and Table 1 summarizes the patients' demographic data, MM subtype information, ISS, R-ISS, and laboratory data.

VCD (bortezomib, cyclophosphamide, dexamethasone) chemotherapy was used as induction chemotherapy in 93% of the patients, and complete response (CR) was achieved in 51% of the patients before autologous transplantation. CR was seen at a rate of 65% in the third month of post-transplant response evaluation. The median follow-up time was 30 months, with a recurrence rate of 38.7% and a mortality rate of 22.7%. Treatment details are summarized in Table 2.

In the analysis of the OS effect performed, it was determined that the UA level had no significant impact on OS (HR, 0.941; 95% CI, 0.791-1.121; p=0.497). Only the level of LDH on OS showed a significant difference in univariate analysis (HR, 1.003; 95% CI, 1.000-1.006; p=0.021); however, this

difference was lost in multivariate analysis (HR, 1.002; 95% CI, 1.000-1.005; p=0.065). There was no significant difference in PFS (HR, 1.067; 95% CI, 0.947-1.202; p=0.290) in the analysis of the effect of UA level, and no factor that showed a significant difference in PFS was found in the statistical analysis (HR, 1.067; 95% CI, 0.947-1.202; p=0.290). Survival analyzes are summarized in Tables 3-4.

Discussion and Conclusion

In our study, we found that the UA value at the time of diagnosis had no effect on progression-free survival or overall survival in our group of MM patients who had VCD induction treatment and consolidation treatment with ASCT in the follow-up.

Although there is previous literature on the negative prognostic feature of UA level at diagnosis for different hematological malignancies, there are very limited and contradictory results on the relationship between UA level and prognosis in MM patients [13,18]. In one of the few studies, Kohsari et al. found that in stage-1 MM patients, the UA level did not increase when compared to the control group [15]. On the other hand, a separate study evaluating the prognosis relationship of UA with 94 MM patients with a sample size similar to ours found that the group with a high UA level (cut-off value; 455.4 mol/L [equivalent to 7.6 mg/dL]) had negative prognosis factors such as advanced disease, high cytogenetic risk, and overall survival decreased, suggesting that it could be a potential biomarker in predicting MM prognosis [16]. However, in our study, we observed that UA level had no effect on PFS or OS in MM patients (HR, 1.067; 95% CI, 0.947-1.202; p=0.290), and (HR, 0.941; 95% CI, 0.791-1.121; p=0.497), respectively. Except for LDH, which had a significant impact on OS in univariate analysis (HR, 1.003; 95% CI, 1.000-1.006; p=0.021), no factor (including ISS and R-ISS) was shown to affect survival in our analysis. This might be due to the small sample size resulting from the exclusion of patients whose uric acid level was not reached at the time of diagnosis, as well as the use of ASCT in patients who achieved a certain level of response.

In our evaluation of the literature, we discovered no studies examining the diagnosis or pre-transplant UA level in patients who underwent ASCT, even though there are many studies examining the consequences of allogeneic stem cell transplantation (allo-SCT). Among these studies, the European Society for Blood and Marrow Transplantation (EBMT) Transplant Complication Working Party published a study in 2020 that divided pre-transplant UA levels in allo-SCT patients into low and high (cut-off value: 4.3mg/dL) cohorts and investigated the impact on the transplant. In the cohort with high UA levels, there was a decrease in PFS and OS, and a significant increase in non-relapse mortality (NRM) and relapse after alloSCT [19]. This may be because of the unfavorable effect of high UA levels on humoral and cellular immunity on the graft in allogeneic transplants [20]. Even though all of our patients had ASCT, we were unable to identify an effect of UA level on PFS and OS at any cut-off value in our study. While UA level does not affect prognosis in patients who have undergone ASCT, UA has a prognostic effect in patients who have undergone allo-SCT, which may be due to the immune system's active role in allo-transplantation patients.

The retrospective design of our study is one of its main limitations. Other than that, to create a homogenous study population in our study, we used very stringent inclusion criteria, and patients who did not have a UA level at the time of diagnosis were also excluded. As a consequence, only 106 patients were included in the study out of 215 screened patients, making our sample group smaller. Both the

negative statistical results of the decrease in our sample group and the selection of all patients in our group from the patients who underwent ASCT had a negative impact on our study's ability to demonstrate the predictive effect of UA. Therefore, while our study provides information on the prognostic value of UA, particularly in MM patients who received ASCT, generalizing it to all MM patients may not be appropriate.

In conclusion, we found that the UA level at the time of diagnosis had no influence on PFS and OS in MM patients who had ASCT in our study. Even though UA is a commonly used test in hematological malignancies for information such as inflammation and tumor burden in current practice, we were unable to demonstrate its influence on the prognosis of MM patients in our study.

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Doi: 10.5505/aot.2023.25901

Original Article

The Evaluation of the Relationship Between ADC Values and Gleason Score in the Prostate Cancer

Prostat Kanserinde ADC Değerleri ile Gleason Skoru Arasındaki İlişkinin Değerlendirilmesi

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ABSTRACT

Introduction: The purpose of this study was to evaluate the apparent diffusion coefficient (ADC) values of different grades of prostate cancer (PC) and determine whether the use of ADC values could predict the tumor aggressiveness in PC.

Materials and methods: Fourtyseven patients (Gleason score (GS) ≥ 6) who underwent prostate multi-parametric MRI (1.5 Tesla) between January 2017 and December 2020 for the evaluation of suspicious findings on clinical or laboratory evaluation were enrolled in this study. The specimens which were obtained from systematic 12-core trans-rectal ultrasound (TRUS)-guided biopsy were used for histopathologic diagnoses. The average ADC values within the tumors were calculated. Independent sample t-test, one-way analysis of variance (ANOVA, Tukey's post-hoc or Tamhane) and receiver operating characteristics (ROC) curve analysis were used.

Results: The mean ADC value of high-risk patients which was 585.4±138.7×10^{-6} mm^2/s, was significantly lower than other subgroups (p=0.036). The mean ADC value in low-risk group (798.1±236.5 ×10^{-6} mm^2/s) was significantly higher (p = 0.012) than others. No significant difference in ADC values was found between low-risk vs intermediate-risk groups (p = 0.149) and intermediaterisk vs high-risk groups (p = 0.419). No statistically significant difference in ADC values between GS 3+4 and GS 4+3 (p, 0.552) was found. ROC analysis revealed an optimal ADC cut-off of 595×10^{-6} mm^2/s for differentiating high-risk group from the other subgroups (sensitivity, 71%; specificity 67.6%, p, 0.038). For the determination of low-risk group, an ADC cut-off of 665×10^{-6} mm^2/s (sensitivity, 80%; specificity, 65.6%, p, 0.017) was found.

Discussion: While ADC values may differentiate the high-risk and low-risk tumors, the strength of ADC in the prediction of intermediate-risk tumors was low. The ADC cut-off value of 665×10^{-6} mm^2/s showed the high sensitivity and moderate specificity for the detection of low-risk tumors.

Keywords: Diffusion-weighted imaging, ADC values, Gleason score, Prostate cancer

ÖZET

Giriş: Bu çalışmanın amacı, farklı derecelerdeki prostat kanserinin (PK) görünür difüzyon katsayısı (ADC) değerlerini değerlendirmek ve ADC değeri kullanımının PK'deki tümör agresifliğini tahmin edip edemeyeceğini değerlendirmekti.

Gereç ve yöntemler: Ocak 2017 ile Aralık 2020 arasında klinik veya laboratuvar bulgularına göre şüpheli olguların değerlendirilmesi için multi-paramterik prostat MRG (1.5 Tesla) çekimi yapılan 47 hasta (Gleason skoru (GS)≥6) çalışmaya dahil edildi. Histopatolojik tanı için trans-rektal ultrason (TRUS) kılavuzluğunda 12 kor sistematik biyopsiden elde edilen örnekler kullanıldı. Tümör içindeki ortalama ADC değerleri hesaplandı. Bağımsız örneklem t testi, tek yönlü varyans analizi (ANOVA, Tukey's post-hoc veya Tamhane) ve "receiver operating characteristics" (ROC) eğrisi analizi kullanıldı.

Bulgular: Yüksek riskli hastaların ortalama ADC'si 585.4±138.7×10^{-6} mm^2/s) ölçülmüş olup diğer risk alt gruplarına göre anlamli olarak daha düşüktü (p = 0.036). Düşük risk grubundaki hastalarda ortalama ADC değeri (798,1±236.5×10^{-6} mm^2/s) diğer gruplara göre anlamlı olarak daha yüksekti (p = 0,012).

First Received: 02.06.2022, Accepted: 17.08.2022 doi: 10.5505/aot.2023.81488

Düşük riskli ile orta riskli gruplar (p = 0.149) ve orta riskli ile yüksek riskli gruplar (p = 0.419) arasında ADC değerlerinde anlamlı bir fark bulunmadı. GS 3+4 ve GS 4+3 arasında ADC değerlerinde istatistiksel olarak anlamlı bir fark bulunmadı (p, 0,552). ROC analiziyle, yüksek risk grubunu diğer alt gruplardan ayırt etmek için optimal ADC eşik degeri 595×10^{-6} mm^2/s olarak hesaplandı (duyarlılık, %71; özgüllük %67,6, p, 0,038). Düşük risk grubunun belirlenmesi için ADC eşik değeri 665×10^{-6} mm^2/s (duyarlılık, %80; özgüllük, %65,6, p, 0,017) olarak bulundu.

Tartışma: ADC değerleri yüksek riskli ve düşük riskli tümorleri ayırt edebiliyorken, ADC'nin orta riskli tümörleri tahmin etme gücü düşüktü. ADC eşik değeri olan 665×10^{-6} mm^2/s, düşük riskli tümorlerin tespiti için yüksek duyarlılık ve orta düzeyde özgüllük göstermektedir.

Anahtar kelimeler: Difüzyon ağırlıklı görüntüleme, ADC değerleri, Gleason skoru, Prostat kanseri

Introduction

Prostate cancer (PC) is the most frequently diagnosed disease among men. It is the second most common cause of deaths due to malignant tumors. In recent years, multi-parametric magnetic resonance imaging (mpMRI) has become a widely used technique for the diagnosis of PC prior to biopsy. European Society of Urogenital Radiology (ESUR) has developed the Prostate Imaging Reporting and Data Systems version 1 (PI-RADS v1) to provide a global standardization of diagnosis of PC in 2012 [1]. In PI-RADS v1, lesions were scored 1 to 5 in each individual pulse sequence. Hence, this categorization caused lower agreement in the diagnosis of PC among radiologists due to the lack of assessment of final overall score. Subsequently, PI-RADS version 2 (v2) was published in 2015 to improve diagnostic accuracy [2]. In PI-RADS v2, the dominant sequence was determined for each zone which was diffusion-weighted images (DWI) in peripheral zone (PZ) and T2-weighted images (T2WI) in transition zone (TZ) [1, 2]. The most of the previous studies revealed some limitations and moderate agreement among radiologists in the diagnosis of PC [3]. To enhance PI-RADS v2, PI-RADS version 2.1 (PI-RADSv2.1) has been developed in 2019 which is the current form of the PI-RADS [4].

The diffusion-weighted image (DWI) is a non-invasive and relatively quick method which analyzes the Brownian motion of water molecules in tissues. DWI provides quantitative information by using apparent diffusion coefficient (ADC) maps which represents the tumor cellularity. In the diagnosis of PC, DWI is the most indicative sequence in the assessment of PI-RADS categorization in particular for peripheral zone lesions. Although, DWI is one of the main sequence of mpMRI, the impact of DWI in the diagnosis is based on direct visual evaluation rather than quantitative methods such as ADC measurement. In breast MRI, ADC values have been used for the differentiation of malignant tumors, determination of aggressiveness and treatment response of breast cancer [5, 6]. Gleason score (GS) is the most common used method for the identification of tumor aggressiveness in PC [7]. The determination of aggressiveness of PC prior to surgery may provide the establishment of the optimal treatment management for patients. At this point, ADC may provide additional quantitative information about the tumor histopathological nature. The aim of this study was to evaluate the ADC values of PC and investigate whether the use of ADC values could provide information about the GS in PC.

Materials and method

Patient selection

Our retrospective study was approved by the Institutional Review Board of 'Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital' (Decision number and date: 2022-05/1866 and 25.05.2022). This study was conducted in accordance with the ethical standards stated by the 1964 declaration of Helsinki. 413 patients who underwent multi-parametric prostate MRI (1.5 Tesla) between January 2017 and December 2020 for the evaluation of

suspicious findings on clinical or laboratory evaluation were enrolled in this study. Out of 413 patients, 366 were excluded due to the absence of histopathological results in our institution or having benign pathology. 47 patients with malignancy (GS ≥ 6) were finally included in this study. Due to the GS, patients were classified in 3 groups which were low-risk (GS= 3+3), intermediate-risk (GS = 3+4, 4+3) and high-risk (4+4, 4+5, 5+4 and 5+5). Histopathologic diagnoses were all proven by specimens which were obtained from systematic 12-core transrectal ultrasound (TRUS)-guided biopsy. All specimens were evaluated by the same experienced pathologist.

MRI Protocol

All MRI examinations were performed using 1.5 T MRI (GE Optima 360, USA®) with 8 channel body/torso array coil. All patients were examined in supine position. A routine protocol was performed including T2WI, DWI with ADC map, T2 fat-sat, T1WI and dynamic contrast-enhanced (DCE) images indicates the MRI acquisition parameters and sequences in this study. The DCE images were obtained after administration of 0.1 mmol/kg of gadoteric acid. DWI was performed using b values of 50, 1000, 1400 s/mm^2. The ADC maps were created automatically. Calculations were made based on mean ADC maps of the circular sampling region of interest (ROI), with care taken to perform measurements in solid areas rather than necrotic/cystic areas and visual artifacts. The area of ROIs ranged from 2 to 20 mm^2 due to the range in size of PCs. We placed three circular ROIs within the lesion after referring to T2 weighted sequence for verification of the lesion boundaries on the ADC map. We calculated the average of the ADC values for all three ROIs within the tumor. All MRI studies were examined by the same experienced radiologist.

Statistical Analysis

Statistical analyses were performed using the SPSS (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp). The Kolmogorov-Smirnov test was used to analyze the normal distribution of data. Continuous variables were presented as mean ± standard deviation. The ADC values, prostate specific antigen (PSA) levels and age were compared according to the GS (low-grade, intermediate grade and high-grade) using the Independent sample t-test and one-way analysis of variance (ANOVA, Tukey's post-hoc or Tamhane). We used a receiver operating characteristics (ROC) curve analysis with corresponding 95% CIs was used to estimate area under curve (AUC) values to evaluate the association between ADC values and 3 different risk subgroups. The "p" value less than 0.05 was considered to show a significant difference.

Results

The mean age of patients was 48.3±11.3 years. The mean age in low-risk, intermediate-risk and high-risk groups were 65.1, 65.5 and 68.2 years, respectively. There was no statistically significant difference between the three groups regarding the mean age (p=0.582).

The mean PSA values were 6.7±3 ng/ml, 15.6±19.6 ng/ml and 27.5±29.6 ng/ml for low-risk, intermediate-risk and high-risk groups, respectively. However, the difference between groups was not statistically significant (p>0.05).

The GSs of specimens were 6 (3+3) in 15 (31.9%), 7 (3+4) in 10 (21.2%), 7 (4+3) in 10 (21.2%), 8 (4+4) in 7 (14.8%) and 9 (4+5) in 5 (10.6%) patients, respectively. The 15 (31.9%), 20 (42.5%) and 12 (25.5%) patients had low-risk, intermediate-risk and high-risk tumor, respectively.

The mean, minimum and maximum ADC values due to the GSs of patients were demonstrated in Table 1. The ADC values according to the risk classification of patients were shown in Table 2 and Figure 1. The mean ADC value of high-risk patients which was 585.4±138.7×10^{-6} mm^2/s, was significantly lower than intermediate- and high-risk groups (p=0.036). Representative mpMRI of a 76-year old patient with prostate cancer was demonstrated in figure 2. In this

Table 1. The ADC values of patients due to the Gleason scores of patients

Gleason score	N	ADC minimum ($\times 10^{-6}$ m^2/s)	ADC maximum ($\times 10^{-6}$ mm^2/s)	ADC Mean±SD ($\times 10^{-6}$ mm^2/s)
3+3 (Low-risk)	15	335	1121	798.1±236.5
3+4 (Intermediate-risk)	10	338	937	707.8±187.9
4+3 (Intermediate-risk)	10	429	967	640.1±171.9
4+4 (High-risk)	7	345	859	573.8±177.4
4+5 (High-risk)	5	526	693	601.6±71.7

*ADC, apparent diffusion coefficient; SD, standard deviation

Table 2. The ADC values of patients due to the risk groups

	N	ADC minimum ($\times 10^{-6}$ m^2/s)	ADC maximum ($\times 10^{-6}$ mm^2/s)	ADC Mean±SD ($\times 10^{-6}$ mm^2/s)
Low-risk	15	335	1121	798.1±236.5
Intermediate-risk	20	338	967	673.9±178.7
High-risk	12	345	859	585.4±138.7

*ADC, apparent diffusion coefficient; SD, standard deviation

Table 3. One-way ANOVA results between three risk groups.

	ADC values (Mean±SD) ($\times 10^{-6}$ m^2/s)	P value
Low-risk vs Intermediate-risk	798.1±236.5 vs 673.9±178.7	0.149
Intermediate-risk vs High-risk	673.9±178.7 vs 585.4±138.7	0.419
Low-risk vs High-risk	798.1±236.5 vs 585.4±138.7	**0.017**

*ADC, apparent diffusion coefficient; SD, standard deviation

MRI figure, the lesion in the left apex of the peripheral zone showed hypointens signal on T2 image and diffusion restriction on DWI (The mean ADC value of 408×10^{-6} mm^2/s was calculated). The highest mean ADC was observed in the low-risk group which was significantly higher than the other subgroups (Table 2, Table 3). One-way ANOVA showed no significant difference between low-risk versus (vs) intermediate-risk groups and intermediate-risk vs high-risk groups (Table 3). The mean ADC of high-risk group was significantly lower than low-risk group (Table 2, Table 3). We found higher ADC values in GS 3+4 tumors (ADC, $707.8\pm187.9\times10^{-6}$ mm^2/s) than GS 4+3 (ADC, $640.1\pm171.9\times10^{-6}$ mm^2/s), however, this difference was not statistically significant (p, 0.552).

ROC analysis revealed an ADC cut-off of 595×10^{-6} mm^2/s for differentiating high-risk group from the other subgroups with having a sensitivity of 71% and a specificity of 67.6% (AUC, 0.702; p, 0.038, Figure 3). ROC analysis also showed an ADC cut-off of 665×10^{-6} mm^2/s with having a sensitivity of 80%, a specificity of 65.6% for the determination of low-risk group (AUC, 0.719; p, 0.017, Figure 3).

Discussion

In PC, mpMRI has been widely used imaging modality in recent years. The DWI sequence is one of the major components of mpMRI especially in peripheral zone lesions. Although the direct visual evaluation of DWI is generally sufficient in the assessment of PI-

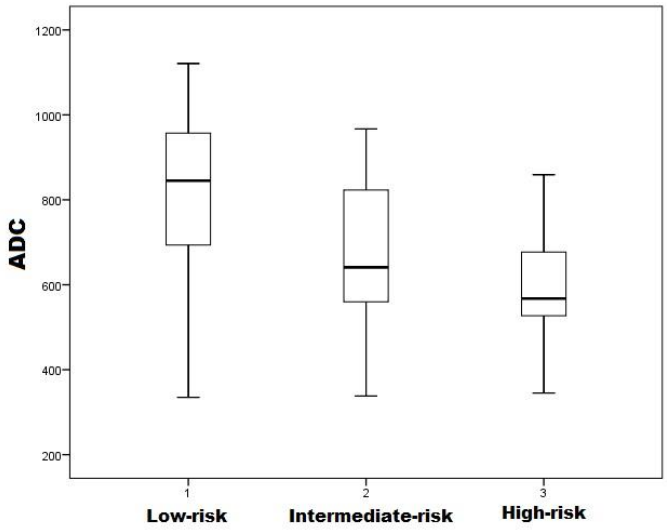

Figure 1. Boxplot showed ADC values in low-risk, intermediate-risk and high-risk groups.

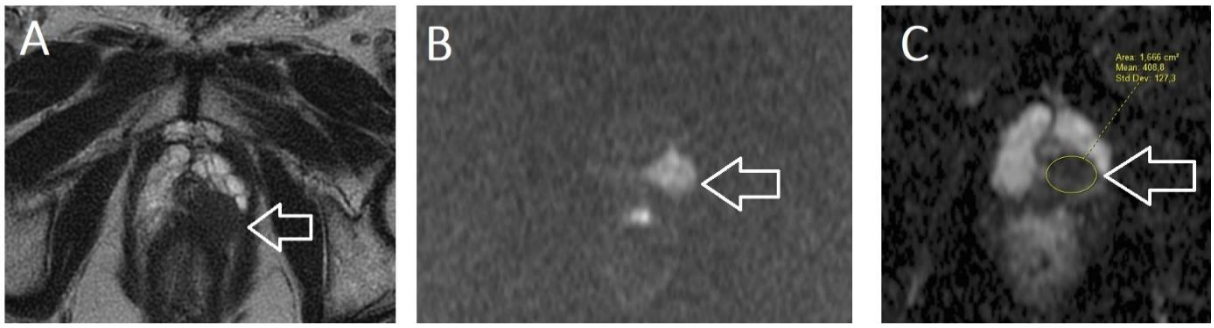

Figure 2. 76-year-old man, A: axial T2, B: axial diffusion weighted imaging, C: axial ADC (apparent diffusion coefficient) map. The lesion in the left apex of the peripheral zone was hyperintense on DWI, hypointense on ADC and T2 images. The mean ADC value of 408 × 10-6 mm2/s was calculated. The histopathologic result was consistent with prostatic cancer.

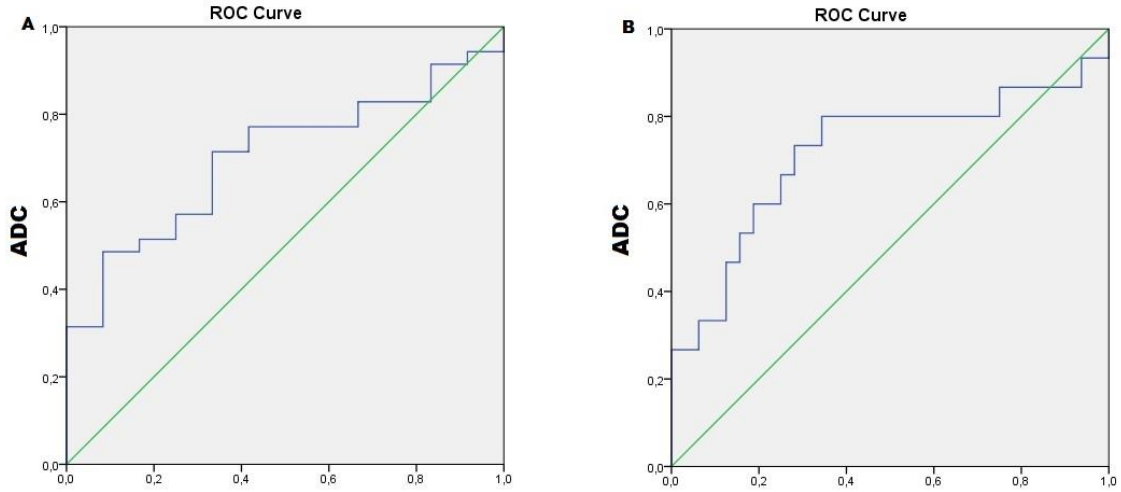

Figure 3. Relative operating characteristic curves (ROC). A. ROC curve in which high-risk group were compared with low- and intermediate-risk groups (AUC, 0.702; p, 0.038). B. ROC curve in which low-risk groups was compared with high- and intermediate-risk groups (AUC, 0.719; p, 0.017).

RADS categorization for the decision of biopsy, the quantitative method such as ADC measurement may improve the diagnostic potential of MRI in both differentiation of malignancy and determination of aggressiveness. As mentioned in literature, the diffusion restriction occurs when cellularity increases in tissues. Some studies showed an inverse relationship between ADC values and GS in PC [8, 9]. Those studies revealed that the dedifferentiation of the tumor with loss of glandular structures and higher cellularity may be a reason of lower ADC in PC [8, 9]. However, the use of ADC is still controversial issue in the determination of aggressiveness. Additionally, a recent meta-analysis revealed a moderate correlation between ADC and GS, in particular with peripheral zone lesions [9].

In our study, the lower mean ADC values (585.4×10^{-6} mm^2/s) were found in high risk patients. And also, in low-risk group, the ADC values (798.1×10^{-6} mm^2/s) were higher than other subgroups. Previous studies showed higher ADC values (ranged from 750-1300 $\times 10^{-6}$ mm^2/s) for low-risk tumor than high-risk tumor with having ADC values between 485 $\times10^{-6}$ mm^2/s and 940×10^{-6} mm^2/s which were similar to our study [7, 8, 10, 11]. Although, ADC values were significantly different in high- and low-risk groups, the difference of ADC in intermediate group from other subgroups was not statistically significant in this study. In the intermediate-risk group (GS, 7), GS 4+3 has showed more aggressive pattern and higher mortality as compared to GS 3+4 following radical prostatectomy (12). Alessandrino et al. demonstrated that ADC metrics including tumor ADC and tumor/normal tissue ADC can predict the differentiation between GS of 3+4 and 4+3, with acceptable accuracy and low sensitivity rates [12]. Even though, the higher ADC values were found in GS 3+4 than GS 4+3, the difference was not statistically significant in our study.

The quantitative potential of ADC may help urologists to classify patients due to the tumor grading for the management of treatment. At this point, ADC cut-off values come into prominence to determine the aggressiveness. However, variable cut-off values with different accuracy results make confusion in the practical use of ADC in PC. The ADC cut-off value in the discrimination of low-risk tumors ranged from 820×10^{-6} mm^2/s to 960×10^{-6} mm^2/s in the most of the previous studies [7, 13, 14]. While, Woo et al. revealed the ADC cut-off value of 960×10^{-6} mm^2/s with the sensitivity of 77 % and specificity of 76 %, Pepe et al. showed the ADC cut-off value of 747×10^{-6} mm^2/s with the sensitivity of 93% and specificity of 61% for differentiating low grade tumor [7, 11]. In our study, the ADC cut-off value of 665×10^{-6} mm^2/s showed the high sensitivity (80%) and moderate specificity (65%) with AUC of 0.719 for the detection of low-risk tumors. When differentiating high-risk tumors from other subgroups including low- and intermediate-risk groups, an optimal ADC cut-off value of 595×10^{-6} mm^2/s was found with having a sensitivity of 71% and a specificity of 67% in this study. The ADC cut-off values of 665×10^{-6} mm^2/s showed a better accuracy result in differentiating of low-risk tumor than the ADC cut-off values of 595×10^{-6} mm^2/s which was calculated for the differentiation of high-risk tumors in our study. The variable results of ADC cut-off values between literature and our study may be due to the technical factors, selected study population and variable sample size. Although, quantitative potential of ADC increases the attention of DWI in PC, the variable cut-off values limit its usage in the determination of aggressiveness of PV prior to surgery.

Our study had some limitations. The study design was retrospective. The study sample size was small. The pathological results were obtained from TRUS-guided systematic

prostate biopsy whereas the use of GS following radical prostatectomy may increase the accuracy of results.

Conclusion

The ADC values may differentiate the high-risk and low-risk tumors prior to radical prostatectomy. The prediction potential of ADC in intermediate-risk tumors was not significant. The ADC cut-off value of 665 × 10^{-6} mm^2/s showed the high sensitivity and moderate specificity for the discrimination of low-risk tumors

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Doi: 10.5505/aot.2023.81488

Original Article

Neurofibromatosis Type 1 and Type2 (NF1 and NF2): Molecular Genetic Profiles of the Patients.

Nörofibromatozis Tip 1 ve Tip2 (NF1 ve NF2): Hastaların Moleküler Genetik Profilleri

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ABSTRACT

Introduction: Neurofibromatosis type I and type 2 (NF1 and NF2) are two rare autosomal dominant neurocutaneous genetic disorders that are diagnosed based on clinical diagnostic criteria of the National Institutes of Health Consensus. Due to increased malignancy risks and age-related penetrance, NGS-based diagnosis methods should be used for early diagnosis. However, molecular diagnosis of the NF1 and NF2 genes are challenging owing to the large size of genes, and the lack of mutation hotspots. In this study, we present 67 patients between 2016-2019 who were investigated for NF1 and NF2 genes with the next generation sequencing (NGS). We aimed to show the effectiveness of NGS-based molecular genetic diagnoses and contribute to establishing the variant spectrum in the Turkish population.

Materials and methods: Patients who met the diagnostic criteria were included in this study. After DNA extraction from peripheric blood samples, an NGS-based panel that includes both NF1 and NF2 genes was performed. Results were evaluated using ACMG 2015 criteria and in silico bioinformatics tools.

Results: Thirtynine of 67 total patients revealed various variants (39/67, ~58%). The variant distribution was as follows; 15 frameshift, 8 nonsense, 7 missense, 6 splice sites, and three insertion/deletion. Novel variants ratio was 23/39 (22 NF1, 1 NF2, ~59%). The classification according to clinical significance was as follows: 23 pathogenic, 14 likely pathogenic, and one VUS according to ACMG 2015 criteria.

Discussion: Our results suggested that a genetic screening using an NGS panel is more useful and helpful to provide early diagnosis and genetic counseling and have a positive impact on patient follow-up.

Keywords: Neurofibromatosis type 1, Neurofibromatosis type 2, Next-generation sequencing

ÖZET

Giriş: Nörofibromatozis tip I ve tip 2 (NF1 ve NF2), Ulusal Sağlık Konsensüsü Enstitüleri'nin klinik tanı kriterlerine göre teşhis edilen iki nadir otozomal dominant nörokutanöz genetik hastalıktır. Artan malignite riskleri ve yaşa bağlı penetrans nedeniyle erken tanı için NGS temelli tanı yöntemleri kullanılmalıdır. Bununla birlikte, NF1 ve NF2 genlerinin moleküler teşhisi, genlerin büyük boyutu ve mutasyon noktalarının olmaması nedeniyle zordur. Bu çalışmada 2016-2019 yılları arasında yeni nesil dizileme (NGS) ile NF1 ve NF2 genleri araştırılan 67 hasta sunuldu. NGS temelli moleküler genetik tanıların etkinliğini göstermeyi ve Türk popülasyonunda varyant spektrumunun oluşturulmasına katkıda bulunmayı amaçladık.

Gereç ve yöntemler: Tanı kriterlerini karşılayan hastalar dahil edildi.
Periferik kan örneklerinden DNA ekstraksiyonundan sonra, hem NF1 hem de NF2 genlerini içeren NGS tabanlı bir panel gerçekleştirildi. Sonuçlar ACMG 2015 kriterleri ve in silico biyoinformatik araçları kullanılarak değerlendirildi.

Bulgular: Toplam 67 hastanın 39'unda çeşitli varyantlar saptandı (39/67, ~%58). Varyant dağılımı şu şekildeydi; 15 çerçeve kayması, 8 anlamsız, 7 yanlış anlamlı, 6 splays bölge ve üç insersiyon/delesyon.

First Received: 02.06.2022, Accepted: 26.08.2022 doi: 10.5505/aot.2023.10692

Yeni varyant oranı 23/39 (22 NF1, 1 NF2, ~%59) idi. Klinik öneme göre sınıflandırma şu şekildeydi: ACMG 2015 kriterlerine göre 23 patojenik, 14 olası patojenik ve bir VUS.
Tartışma: Sonuçlarımız, bir NGS paneli kullanılarak yapılan genetik taramanın, erken tanı ve genetik danışmanlık sağlamak için daha yararlı ve yararlı olduğunu ve hasta takibini olumlu yönde etkilediğini göstermiştir.

Anahtar kelimeler: Nörofibromatoz tip 1, Nörofibromatoz tip 2, Yeni nesil dizileme

Introduction

Neurofibromatosis type I and type 2 (NF1 and NF2) are autosomal dominant neurocutaneous genetic disorders that affected individuals leading to increased susceptibility to the development of benign and malignant tumors. The estimated worldwide incidence of NF1 and NF2 are 1 in 2,500 to 1 in 3,000 individuals [1, 2] and 1 in 25,000 live births respectively [3]. While NF1 is characterized by cafe-au-lait spots, Lisch nodules in the eye, and fibromatous tumors of the skin, NF2, is characterized by tumors of the eighth cranial nerve (usually bilateral), meningiomas of the brain, and schwannomas of the dorsal roots of the spinal cord. To establish the diagnoses, clinical diagnostic criteria determined by the NIH are used for both diseases [4, 5].

Although the most common benign tumors in NF1 patients are neurofibromas, they can also be seen in other neoplasms such as optic nerve gliomas and brain tumors [6]. A variety of other tumors, including rhabdomyosarcomas, pheochromocytomas, gastrointestinal stromal tumors, glomus tumors, and retinal vaso-proliferative tumors, are more common in individuals with NF1 compared with the normal population [7].

In NF2, the mean age of onset of symptoms in individuals are variable (birth to 70 years). Almost all affected individuals develop bilateral vestibular schwannomas by age 30. Schwannomas, meningiomas, ependymomas, and (very rarely) astrocytomas are observed in individuals with NF2 as tumors that affect other cranial and peripheral nerves.

NF1 is caused by heterozygote pathogenic variants which leads to loss of function of the tumor suppressor NF1 gene (Neurofibromin 1; MIM no. 613113). NF1 gene which is located at 17q11.2 and contains 58 exons, encodes a 2839 amino acid peptide called neurofibromin. Neurofibromin is a Ras guanosine triphosphatase (GTPase) activating protein that reflects its role as an important negative regulator of the cellular RAS-MAPK (mitogen-activated protein kinases) signaling pathway. Thus, in the case of loss of function, the active GTP-bound RAS form increases cellular levels and results in uncontrolled cell growth and potentially tumorigenesis.

NF2 gene is located on chromosome 22q12.2 and contains 17 exons that encode for a 69 kDa protein product called merlin (moesin-ezrin-radixin-like protein) or schwannomin [8]. Merlin is a tumor suppressor protein located at the cell membrane-cytoskeleton interface which inhibits cell growth [9]. The lacking growth-inhibitory activity of Merlin leads to disrupting tumor suppression. On the NF2 mutation database, 159 unique variants have been reported. (Leiden Open Variation Database, LOVD: www.lovd.nl/NF2)

Compared to other genetic diseases in humans, the incidence of pathogenic variants in the NF1 gene is quite high (10). Also, the de novo pathogenic variant is exhibited in almost 50% of NF1 patients. More than 3700 variants have been reported. Among them, 64% are substitutions, 23% are deletions and eight are duplications. These variants are classified as 44% missense, 37% frameshift, and 12% stop changes according to their effects on protein level (Leiden Open Variation Database, LOVD: www.lovd.nl /NF1).

Because of their increased malignancy risks, molecular genetic diagnosis of NF1 and NF2 is very important in terms of enabling early diagnosis, follow-up, and treatment. However, molecular diagnosis of the NF1 gene is difficult due to the large size of the gene, the distribution of variants across the

entire gene, the presence of many highly identical pseudogenes, and a wide spectrum of variants [11, 12]. The increasing use of next-generation sequencing (NGS) and specially customized targeted gene panels are facilitating accurate and fast molecular genetic diagnosis. Due to the phenotypic overlapping of NF1 and NF2 diseases, an NGS panel that includes both the NF1 and NF2 genes is a time- and cost-saving manner.

In this study, the results of patients who applied to our clinic with the pre-diagnosis of Neurofibromatosis between 2016-2019 and were investigated for NF1 and NF2 genes with the next-generation sequencing (NGS) method were evaluated. We aimed to show the effectiveness of NGS-based molecular genetic diagnoses and the obtained data are intended to help provide an effective strategy for early and definitive diagnosis and genetic counseling of the NF1 and NF2 patients. Our results suggested that a genetic screening using an NGS panel is more useful and helpful to provide early diagnosis and genetic counseling and has a positive impact on patient follow-up.

Materials and Method

All of the procedures were carried out in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from the parents of the participants for molecular genetic analysis and the publication of patient data prior to their enrolment in the study. This study was conducted after approval was given by the Ankara City Hospital Ethics Committee (Document Number: E1-2644/2022).

Patients

Between 2016 and 2019, 67 index cases were referred to our clinic for molecular diagnosis of NF1 and NF2. Clinical diagnoses were executed according to the diagnosis criteria of NIH.

In this study, we analyzed the patients who were referred to our department with an NGS-based panel that includes both NF1 and NF2 gene In this study, we analyzed the patients who were referred to our department with an NGS-based panel that includes both NF1 and NF2 gene

Genetic analyses

Genetic analyses were performed for diagnosis with a parental informed consent form. The DNA was extracted from peripheral blood by QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A targeted NGS in-house gene panel was designed for the genes associated with NF1 and NF2 genes. NGS analyses were performed on the MiSeq system (Illumina, San Diego, CA, USA).

In silico bioinformatics analyses

Potential functional effects of novel missense variants were predicted using the Alamut® Visual 2.4 Software (SIFT, Polyphen-2, and Variant Taster). information about the location of AA and predicted transmembrane domains were taken from Uniprot (http://www.uniprot.org/).

Variant Classification

The recent ACMG/AMP guideline for standardized variant interpretation in Mendelian disorders was used for classification. Pathogenic variants are well-established disease-causing DNA changes in the in-house database and/or literature. Likely pathogenic variants are considered the probable cause of the disease or the effect on the protein function is predicted to be likely deleterious (>90% probability to cause the disease). VUS alterations are genetic variants with unknown or questionable impacts on the disease. These variants are typically very rare and predicted to be deleterious.

Results

Between 2016 and 2019, 67 patients were referred to our clinic for genetic diagnosis of Neurofibromatosis. We performed NGS-based molecular genetic analysis on 67 patients which clinically suspicious of the diseases according to NIH criteria. The average age of the patients was ~21. While the result was normal in 28 patients, various variants were detected in 39 patients (39/67, ~%58) (Figure 1). In the positive group, the

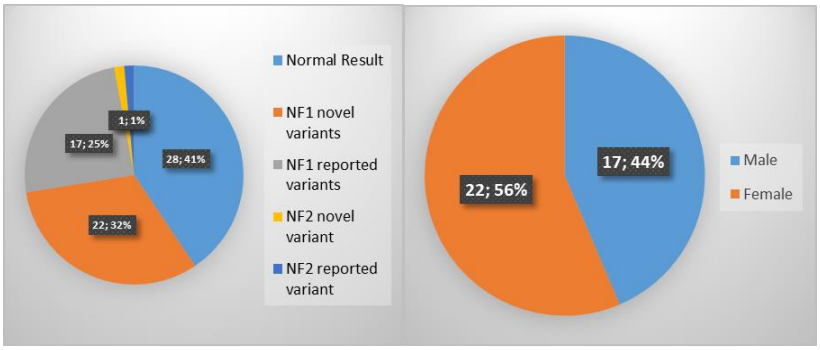

Figure 1. The detection rate of the molecular genetic testing and the Male/female ratio of the patients with the positive genetic result.

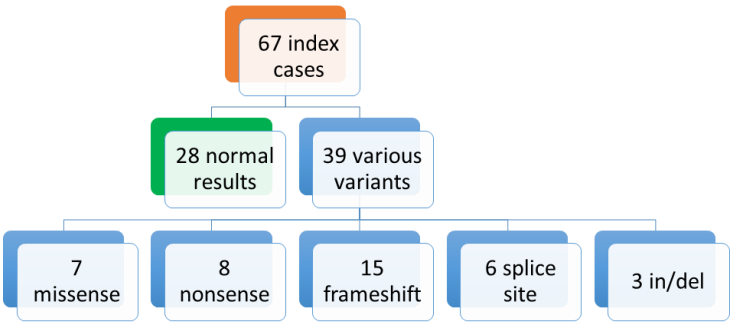

Figure 2. Variant distribution according to the molecular type.

male and female ratio was 17/38(44%) and 22/38 (%56) respectively (Figure 1). The variant distribution was as follows; 15 frameshift, eight nonsense, seven missense, six splice sites, and an insertion/deletion (Figure 2). The number of novel variants was 23 (22 NF1, 1 NF2) which is quite high (23/39, ~%59). The classification according to clinical significance was as follows:23 pathogenic, 14 likely pathogenic, and one VUS according to ACMG 2015 criteria (Table 1).

Discussion

In this study, we performed NGS-based molecular genetic analyses on 67 patients who were referred with clinical suspicion of NF1 and NF2. Results revealed 39 different variants from 39 patients (Table 1). These variants were heterogeneous and distributed within the gene without clustering. A significant difference was not observed between the sexes (Figure 1). In our study, the NF1 or NF2 variant was detected in 58.2% (39/67) of the patients who met the clinical diagnostic criteria. It showed that the detection rate of variants in the NF1 gene is very high in patients which clinically diagnosed with neurofibromatosis according to NIH criteria [4]. Although our variant detection rate is very high, some previous studies combining RNA and MLPA analyzes have reported higher detection rates of up to 92% in patients. [13, 14]. This may be because the additional methods they used also revealed variants and large deletions occurring in deep intron regions. Therefore, RNA studies and MLPA analyses should be recommended in patients with normal NGS analysis.

NF1 and NF2 are two progressive disorders complicated by the variability of disease expression and age-related penetrance. Therefore, molecular genetic analyses becoming more important for these particular diseases. However, molecular diagnosis of both NF1 and NF2 genes is difficult due to the gene's large size and absence of hotspots, and the wide diversity of mutations [15]. In our study, variants also did not show hot-spot

Table 1. Variant distribution and classification among the patients.

Patient	Age(Year)	Gender	Gene	Results	Mutation Type	Variant Id	Classification (Acmg 2015)
1	10	M	*NF1*	c.2T>G (p.Met1Arg) (Start loss)	Missense	rs886041346	P
2	21	F	*NF1*	c.1682G>A (p.Trp561*)	Nonsense	Novel	P
3	6	M	*NF1*	c.4558C>T (p.Gln1520*)	Nonsense	rs1060500242	P
4	21	F	*NF1*	c.574C>T (p.Arg192*)	Nonsense	rs397514641	P
5	26	M	*NF1*	c.3496+1G>A	Splice site	CS072245	LP
6	55	F	*NF1*	c.2033delC (p.P678fs*10)	Frameshift	rs747195556	P
7	45	F	*NF1*	c.7152_7153insT (p.Asn2385*)	Nonsense	Novel	P
8	19	M	*NF1*	c.3596_3599delCAGT (p.Thr1199Asnfs*15)	Frameshift	Novel	LP
9	5	F	*NF1*	c.1842_1845+1delTAAGG (p.Ala548_Lys615de)	Frameshift	rs1135402822	P
10	53	M	*NF1*	c.3376C>T p.Gln1126*	Nonsense	Novel	P
11	11	M	*NF1*	c.5766_5767delinsTC (p.Thr1902Pro)	Missense	Novel	LP
12	35	F	*NF1*	c.1754_1757delTAAC (p.T586Vfs18)	Frameshift	rs1277850570	P
13	9	F	*NF1*	c.6687G>A (p.Trp2229*)	Nonsense	Novel	P
14	24	M	*NF1*	c.1033_1062+6del36 (p.Leu345_Lys354del)	Frameshift	Novel	P
15	26	M	*NF1*	c.5609G>A	Missense	rs786202112	P
16	38	F	*NF1*	c.3457_3460delCTCA (p.Leu1153Metfs*4)	Frameshift	rs1321848637	P
17	18	F	*NF1*	c.6319_6321delGTT (p.Val2106del)	Small deletion	Novel	LP
18	21	F	*NF1*	c.1246C>T (p.Arg416*)	Nonsense	rs764079291	P
19	8	F	*NF1*	c.4600C>T	Missense	rs760703505	P
20	14	F	*NF1*	c.3430_3431insCACT (p.Cys1144Serfs*52)	Frameshift	Novel	LP
21	46	F	*NF1*	c.4578-14T>G	Splice site	Novel	VUS
22	18	F	*NF1*	c.4687_4688insTA (p.Asn1563Ilefs*12)	Frameshift	Novel	LP
23	21	F	*NF1*	c.7438_7447delCATGGTGACC (p.His2480Leufs*6)	Frameshift	Novel	LP
24	10	F	*NF1*	c.3827G>A (p.Arg1276Gln)	Missense	rs137854556	P
25	14	F	*NF1*	c.3430_3431insCACT (p.Cys1144Serfs*52)	Frameshift	Novel	LP
26	11	M	*NF1*	c.1246C>T	Missense	rs764079291	P
27	15	M	*NF1*	c.1527+4_1527+7del	Splice site	Novel	LP
28	17	F	*NF1*	c.3656_3658del (p.Gly1219del)	Small deletion	rs1555615077	VUS
29	17	F	*NF1*	c.3922del, (p.Val1308*)	Nonsense	Novel	LP
30	18	M	*NF1*	c.1845+2T>G	Splice site	Novel	LP
31	13	M	*NF1*	c.5425C>T (p.Arg1809Cys)	Missense	rs797045139	P
32	10	M	*NF1*	c.4595del, (p.Pro1532Leufs*21)	Frameshift	Novel	LP
33	19	M	*NF1*	c.7323_7324insA (p.Leu2442Thrfs*3)	Frameshift	Novel	P
34	17	M	*NF1*	c.1453del, (p.Glu485Argfs*13)	Frameshift	Novel	P
35	7	F	*NF1*	c.2156del, (p.Ile719Thrfs*29)	Frameshift	Novel	P
36	13	M	*NF1*	c.6833_6841delCCAAATTACinsTTT (p.Thr2278_Gln2281delinsIle*)	Deletion/intertion	Novel	LP
37	6	M	*NF1*	c.499_502del, (p.Cys167Glnfs*10)	Frameshift	rs786201874	P
38	20	F	*NF2*	c.676-1G>A	Splice site	CS982290	LP
39	17	F	*NF2*	c.241-2A>G	Splice site	Novel	P

M: male, F: female, *NF1*(NM_001042492): neurofibromin 1, *NF2*(NM_000268): neurofibromin 2, P: pathogenic, LP: likely pathogenic, VUS: variant of unknown significance

properties. The novel variant rate was quite high in the patients (22/39, 56.4%) comparing previous similar studies [13]. However, molecular genetic testing is important for early diagnosis because of the clinical variability and age-related penetrance. [16]. In recent years, NGS has provided rapid and cost-effective molecular diagnosis options, especially in the molecular diagnosis of genetically heterogeneous and clinically overlapping inherited diseases such as NF1 and NF2 [17].

Our study is one of the largest studies from Turkey, The variants distributed across the entire gene did not reveal any hot spot region. Another study from Turkey reports similar findings [18]. This may indicate the conclusion that the disease does not show a hotspot zone in the Turkish population as it does in other populations. According to the NF1 database [19], our non-sense and frameshift mutation rates are also high. This may be because the patients we studied were from different ethnic groups and there were not many studies in Turkey. Relatively fewer mutations were observed in the NF2 gene, as expected. Further studies are needed for more information.

Finally, our study was to show molecular genetic analysis allows early diagnosis while

the clinic is not yet fully established. In this way, early diagnosis and genetic counseling are available. Considering the increased risk of malignancy in both diseases, early diagnosis becomes very important for screening the patient who is at malignancy risk. We suggest that genetic analysis with next-generation tools be used as the first-choice method for an effective strategy. This study also contributes to the establishment of the NF1 and NF2 variant spectrum in the Turkish population, which has been under-studied.

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Doi: 10.5505/aot.2023.10692

Original Article

Evaluation of the Effects of Ibandronic Acid and Zoledronic Acid on Progression-free Survival in Patients with Bone Metastatic Breast Cancer

İbandronik Asid ve Zoledronik Asidin Kemik Metastazlı Meme Kanserli Hastalardaki Progresyonsuz Sağkalım Üzerindeki Etkilerinin Değerlendirilmesi

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ABSTRACT

Introduction: Bisphosphonates have been reported to limit tumor formation, in addition to inhibition of bone resorption. We evaluated the effect of intravenous zoledronic acid (ZA) and oral/intravenous ibandronic acid (IA) on progression-free survival (PFS), overall survival (OS), and skeletal-related events (SRE) in breast cancer patients with bone metastases.

Materials and methods: The retrospective study included patients with metastatic breast cancer who received ZA or IA treatments for at least three months between 2013 and 2018. Menopausal status, presence of visceral metastases, history of skeletal-related events (fracture, radiotherapy, and operation), de novo bone metastasis, and anticancer treatments were recorded. PFS and OS were calculated for each patient.

Results: There were 44 patients in the ZA group as opposed to 22 patients in the IA oral group and 11 patients in the intravenous IA group. Median PFS was 15 months in the ZA group and 25 months in the IA group (p=0.134). Median OS was 81 months in the IA group and 153 months in the ZA group (p=0.088). No significant difference was found between the groups with regard to history of fracture, radiotherapy, and operation (p=0.606, p=0.295 and p=0.747, respectively). The two-year survival rate was 71.5% in the ZA group and 78.3% in the IA group.

Discussion: ZA and IA have similar efficacy in terms of SRE development, PFS and OS. In the selection of treatment for the treatment of bone metastases in metastatic breast cancer, besides evaluating drug efficacy/side effects, treatment compliance and cost should also be considered.

Keywords: Bone metastasis, ibandronic acid, metastatic breast cancer, overall survival, progression-free survival, zoledronic acid

ÖZET

Giriş: Bisfosfonatların kemik rezorbsiyonu inhibisyonu yanı sıra, tümör oluşumunu sınırlama etkileri bildirilmektedir. Çalışmamızda tek merkezde Zoledronik asid (ZA) intravenöz ve İbandronik asid (İA) oral/intravenöz kullanan hastalardaki progresyonsuz sağkalım (PS), genel sağkalım (GS) ve iskelet ilişkili olayların (İİO) değerlendirilmesi amaçlandı.

Gereç ve yöntemler: 2013-2018 yılları arasında, en az 3 ay ZA ya da İA tedavileri alan metastatik meme kanserli hastalar retrospektif olarak incelendi. Menopoz durumu, visseral metastaz varlığı, İİO öyküsü (kırık, radyoterapi, operasyon), tanı anında kemik metastaz varlığı, kullanılan antikanser tedaviler değerlendirildi. PS ve GS süreleri hesaplandı.

Bulgular: ZA grubunda 44 hasta var iken, İA oral 22 ve intravenöz grubunda 11 hasta vardı. Her iki gruba ait hasta özellikleri birbirine denkti. Çalışmamızda PS, ZA grubunda15 ay, İA grubunda 25 ay idi (p=0.134). GS, ZA grubunda 81 ay, İA 153 ay idi (p=0.088). İİO'lar açısından gruplar

First Received: 20.06.2022, Accepted: 18.07.2022 doi: 10.5505/aot.2023.82346

değerlendirildiğinde; kırık, radyoterapi ve operasyon açısından sırasıyla iki grup arasında fark bulunamadı (sırasıyla p=0.606, p=0.295, p=0.247). 2 yıllık sağkalım oranı ise ZA grubunda %71.5 iken, İA grubunda %78.3 olarak izlendi.
Tartışma: ZA ve IA, İİO gelişimi, PS ve GS açısından benzer etkinliğe sahiptir. Metastatik meme kanserinde kemik metastazlarının tedavisine yönelik tedavi seçiminde ilaç etkinliği/yan etkilerinin değerlendirilmesinin yanı sıra tedaviye uyum ve maliyet de göz önünde bulundurulmalıdır.

Anahtar kelimeler: Zoledronik asid, ibandronik asid, genel sağkalım, progresyonsuz sağkalım, metastatik meme kanseri, kemik metastazı

Introduction

Bone is the most common site of metastasis in many solid tumors, particularly in breast. Bone metastasis, its localization, and tumor burden can lead to skeletal-related events (SREs) that cause serious clinical conditions such as fracture, hypercalcemia, spinal cord compression, and pain [1, 2].

Treatment of breast cancer with bone metastasis shows diversity. Moreover, in patients with a life expectancy of more than three months, the guidelines recommend the use of bisphosphonates in addition to systemic treatment in order to reduce SREs and pain [3, 4].

Zoledronic acid (ZA) and oral/intravenous ibandronic acid (IA) are highly active nitrogen-containing bisphosphonates that increase bone mineralization by inhibiting bone resorption and osteoclast activity. They are also known to inhibit many steps of metastasis such as angiogenesis, invasion, adhesion, and proliferation [5-8]. Skeletal metastasis was developed in 22% of early stage breast cancers and 75% of stage IV breast cancers [9-11]. Additionally, bisphosphonates have been found to be effective in reducing cancer-related bone loss and SREs in breast cancers with bone metastases [12].

We evaluated the effect of ZA and IA on progression-free survival (PFS), overall survival (OS), and skeletal-related events (SRE) in breast cancer patients with bone metastases.

Materials and Methods

The Institutional Review Board waived the need for informed consent given the retrospective nature of the research. The study was conducted in accordance with the principles laid out by the 18th World Medical Assembly (Helsinki, 1964) and all its subsequent amendments (up to 2013) and with the International Society for Pharmaco-epidemiology guidelines for Good Pharmaco-epidemiology Practice and local regulations, including local data protection regulations. The study protocol was approved by the Ethics Committee of reference center.

The study included patients with metastatic breast cancer who applied to Medical Oncology outpatient clinic between 2013 and 2018.

Inclusion criteria were as follows:

1-) Aged over 18 years,

2-) A history of ZA and IA therapies lasting more than three months.

Exclusion criteria were as follows:

1-)Presence of brain metastasis at the beginning of the bisphosphonate therapy,

2-) Presence of a second malignancy,

3-) Absence of a measurable metastasis,

4-) Male gender,

5-) Ongoing steroid or immunosuppressive therapy.

Age, bisphosphonate type, menopausal status, presence of visceral metastases, history of radiotherapy or fracture surgery, presence of metastasis at the time of diagnosis, breast cancer histology, anticancer treatments (hormone therapy/chemotherapy), type of bone metastasis (lytic, sclerotic, lytic+ sclerotic), progression date, date of last follow-up, and date of death were recorded for each patient. PFS was calculated as the time of diagnosis to first progression. OS was calculated as the time of diagnosis to death or last follow-up.

Serum creatinine, total calcium, phosphorus and alkaline phosphatase (ALP) levels were measured both before and after the bisphosphonate treatment using a Beckman Coulter AU5800 autoanalyzer (Beckman Coulter Inc. CA, USA).

Statistical analysis

Data were analyzed using SPSS for Windows version 23.0 (Armonk, NY: IBM Corp.). Categorical variables were compared using Chi-square test and Fisher's Exact test. Normal distribution of continuous variables was assessed using Kolmogorov-Smirnov test. Group means were compared using Wilcoxon signed-rank test. The survival probability was calculated using the Kaplan-Meier method and the factors independently affecting survival time were determined using univariate Cox regression analysis. A p value of <0.05 was considered significant.

Results

Of the 73 patients, 40 (54.8%) were using ZA, 22 (30.1%) were using IA tablets, and 11 (15%) were using IA intravenously (iv). Table 1 presents the demographic and clinical characteristics of the patients. Mean age was 56.0±9.2 years in the ZA group and 54.0±8.9 years in the IA group. Most patients in both groups consisted of non-menopausal patients with hormone receptor (HR)-positive/HER2-negative breast cancer histology. Visceral metastasis was detected in 29 (72.5%) patients in the ZA group and in 21 (63.6%) patients in the IA group. Lytic bone metastases was found in 12.5% (5/40) of the ZA group and 21.2% (7/33) in the IA group. No difference was found between the two groups with regard to the types of bone metastasis (p=0.518).

The median follow-up period and the median duration of drug use was 66.5 (39.8-107.8) months, 28 (11-43) months in the ZA group and respectively 83 (46-124) months, 34 (21.5-51) months in the IA group. No significant difference was found between the two groups with regard to follow-up period and duration of drug use (p=0.197 and p=0.159, respectively).

Table 2 presents the prevalence of SREs in both groups. No significant difference was found between the two groups with regard to the prevalence of fractures, radioteraphy and bone surgery requirement (p=0.606, p=0.295, p=0.247).

In the ZA group, pre-treatment ALP level was 103U/L and post-treatment ALP level increased to 130U/L (p=0.033). In the IA group, pre-treatment ALP level was 85U/L and post-treatment ALP level increased to 93U/L (p=0.072). In the ZA group, creatinine level was 0.62mg/dL before the treatment and increased to 0.74mg/dL after the treatment (p=0.003), while in the IA group creatinine level was 0.6mg/dL prior to the treatment and increased to 0.67mg/dL after the treatment (p=0.284).

A total of 43 patients died during the 5-year follow-up period and the mortality rate was 67.5% (27/40) in the ZA group and 48.5% (16/33) in the IA group. Mean PFS and mean OS was 15 months (95% Confidence Interval [CI]: 11.28-18.71) and 81 months (95% CI=56-106) in ZA users. Respectively 25 months (95% CI=18.23-31.76) and 153 months (95% CI=52.7-253.3) in IA users. No

Table 1. Demographic and clinical characteristics

		ZA n=40	IA n=33	*p**
Age	Mean	56.0±9.2	54.0±8.9	0.404
	< 55 years	18 (45.0%)	19 (57.6%)	
	≥ 55 years	22 (55.0%)	14 (42.4%)	
Menopause	No	32 (80.0%)	24 (72.7%)	0.650
	Yes	8 (20.0%)	9 (27.3%)	
Histology	HR (+) HER-2 (-)	31 (77.5%)	24 (72.7%)	----
	HR (+) HER-2(+)	5 (12.5%)	4 (12.1%)	
	HR (-) HER-2 (-)	2 (5.0%)	-----	
	HR (-) HER-2 (+)	2 (5.0%)	5 (15.2%)	
Solid metastasis	Yes	29 (72.5%)	21 (63.6%)	0.577
	No	11 (27.5%)	12 (36.4%)	
Metastasis at the time of diagnosis	Yes	12 (30.0%)	13 (39.3%)	0.553
	No	28 (70.0%)	20 (60.6%)	
Bone metastasis	Sclerotic	16 (40.0%)	10 (30.3%)	0.518
	Lytic	5 (12.5%)	7 (21.2%)	
	Mixed	19 (47.5%)	16 (48.5%)	
Systemic treatment	<3 cycles of chemotherapy	19 (47.5%)	20 (66.6%)	-----
	≥3 cycles of chemotherapy	17 (42.5%)	13 (39.4%)	
	No chemotherapy	4 (10.0%)	---	
Hormonal therapy	Tamoxifen	5 (12.5%)	6 (18.8%)	-----
	Aromatase Inhibitor	18 (45.0%)	13 (40.6%)	
	Combination (Tmx+AI)	14 (35.0%)	9 (28.1%)	
	No	3 (7.5%)	4 (12.5%)	
Mortality	Yes	27 (67.5%)	16 (48.5%)	0.160
	No	13 (32.5%)	17 (51.5%)	

*Chi-Square Test
ZA: Zoledronic acid, IA: Ibandronic acid, HR: Hormone receptor, Tmx: Tamoxifen, AI; aromatase inhibitors, HER-2; human epidermal growth factor-2
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Table 2. Prevalence of SREs in both groups

		ZA n=40	IA n=33	*p*
Fracture	Yes	13 (%32.5)	8 (%24.2)	0.606*
	No	27 (%67.5)	35 (%75.8)	
Radiotherapy	Yes	27 (%67.5)	26 (%81.2)	0.295*
	No	13 (%32.5)	6 (%18.8)	
Surgical history	Yes	5 (%12.5)	5 (% 15.2)	0.747**
	No	35 (%87.5)	28 (%84.8)	

*Chi-Square Test, **Fisher Exact Test
ZA: Zoledronic acid, IA: Ibandronic acid

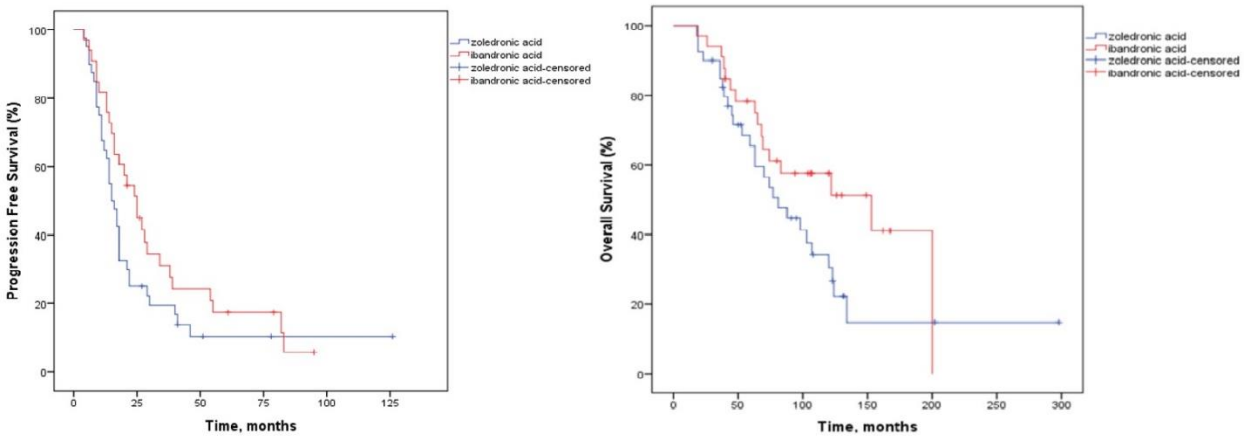

Figure 1. Relationship between ZA and IA use and progression-free survival (PFS) and Overall Survival (OS) time

Table 3. Analysis of independent factors affecting survival

	ZA				IA			
	HR	95% CI		*p*	HR	95% CI		*p*
		Lower	Upper			Lower	Upper	
Age (years)	0.960	0.920	0.999	0.044	1.019	0.957	1.085	0.552
Menopausal status	0.682	0.284	1.640	0.393	1.065	0.333	3.410	0.916
Solid metastasis	2.954	1.101	7.927	**0.031**	2.468	0.695	8.765	0.162
Chemotherapy cycles ≥3 *vs.* <3	2.525	1.023	6.234	**0.045**	0.996	0.350	2.831	0.994
Metastasis at the time of diagnosis	1.983	0.818	4.806	0.130	4.647	1.527	14.139	**0.007**
Fracture	1.484	0.686	3.211	0.316	0.484	0.134	1.749	0.268
Radiotherapy	1.785	0.710	4.490	0.218	1.244	0.349	4.433	0.736
Surgical history	0.563	0.168	1.879	0.350	0.206	0.026	1.629	0.134

HR: hazard ratio, ZA: Zoledronic acid, IA: Ibandronic acid
* Cox regression analysis

significant difference was found between PFS and OS in groups (p=0.134, p=0.088, Figure 1). The two-year survival rate was 71.5% in ZA users and 78.3% in IA users. In the univariate Cox regression analysis, age, solid metastasis and the number of chemotherapy cycles were found to be statistically significant for ZA group and metastasis at the time of diagnosis for IA group (p=0.044, p=0.031, p=0.045 and p=0.007, Table 3). In the multivariate Cox regression analysis, in which the parameters found to be statistically significant for ZA were included in the model, the number of chemotherapy cycles was found to be statistically significant (HR:2.63; CI: 1.05-6.64 & p=0.040).

Discussion

Cost, physician, and patient characteristics (e.g. patient preference, mode of transportation to hospital) play a role in drug selection. The ZICE study compared ZA and IA and reported that oral IA was inferior to ZA in terms of SREs [13]. However, initial SRE development was found to be similar in both groups and IA was associated with a

significantly reduced risk of nephrotoxicity and with insignificantly fewer events of osteonecrosis of the jaw [14]. In our study, no significant difference was found between the two groups with regard to SRE development (Table 2).

Bisphosphonates have the potential to limit tumorigenesis. ZA has been reported to cause rapid and sustainable reduction in circulating tumor cells (CTC) in breast cancer patients. Patients with low CTC levels have been shown to have better PFS [15-18]. Accordingly, the role of bisphosphonates has been evaluated in both metastatic and locally advanced patients and in adjuvant breast cancer patients [19]. Although the addition of bisphosphonates to the standard therapy in metastatic breast cancer reduces the risk of SRE development by 15% and has been associated with delayed SRE development and reduced bone pain, it has been found to have no benefit on OS and PFS [20-22]. Survival in patients with breast cancer can be affected by many factors including histological classification, genetic characteristics, tumor volume, metastasis localization, and patient-related comorbidities. It has also been reported that the median OS after the development of bone metastasis varies 40-79 months depending on the presence of isolated or multiple metastases [10, 23]. In our study, advanced age, increased chemotherapy cycles count and solid metastases in the ZA group and presence of metastases at diagnosis in the IA group adversely affected survival. OS was found longer in IA users compared to ZA users, though no significant difference was found (153 vs. 81 months, p=0.088). Moreover, unlike in the ZICE study, both iv and oral forms of IA were used in our patients. The efficacy of iv and oral ibandronate on osteoporosis was evaluated and found that both forms had similar effects [24]. This finding suggests that there will be no significant difference between iv and oral IA with regard to SRE, PFS, and OS.

Studies have shown that ZA increasing the normalization of osteolytic markers and this normalization was associated with better survival in patients with bone metastases [25,26]. In our study, serum ALP level increased significantly in the ZA group after the treatment (p=0.030). This significant increase could be related to the shorter duration of ZA use. On the other hand, increased ALP concentration is affected by many factors including liver, gallbladder, and kidney diseases.

Our study was limited in several ways. First, it was a single-center retrospective study that evaluated a homogeneous patient group. Due to the retrospective nature of the study, the effect of drugs on pain palliation could not be evaluated. Second, it had a limited number of patients. Finally, bone resorption markers could not be evaluated and thus a comprehensive examination of bone turnover could not be performed.

Conclusion

The survival of breast cancer patients has been significantly improved. Due to their antiresorptive and antitumor effects, bisphosphonates can be used safely both in adjuvant and metastatic breast cancer patients, particularly in high-risk postmenopausal patients. The effectiveness and side effects of both agents are similar. Further comprehensive studies are needed to evaluate the effect of these two agents on patient comfort, survival, and cost-effectiveness.

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Doi: 10.5505/aot.2023.82346

Original Article

Cutaneous Squamous Cell Carcinoma: A Type of Cancer that Discriminates Against Young Adults

Kutanöz Skuamöz Hücreli Karsinom: Genç Yetişkinlere Karşı Ayrımcı Bir Kanser Türü

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ABSTRACT

Introduction: Cutaneous squamous cell carcinoma (cSCC) is one of the most common cancers. Young adult cSCC is rare and its clinical features, outcomes, and iatrogenic risk factors are not well defined. This study aimed to specify the clinical characteristics, potential risk factors, and the factors affecting recurrence associated with cSCC in young adults.

Materials and methods: Forty-three patients aged <35 years at diagnosis, who allowed their data to be retrospectively analyzed, and whose full medical history and records were available were included in the study. Their demographic characteristics, date of diagnosis, localization of lesion, pathological and family histories were recorded

Results: Patients' mean age at diagnosis was 29 (17-34) years. Twenty patients (46.5%) had familial history of malignancy; 19 (44.2%) had precancerous lesions before SCC diagnosis. Three (7%) had a history of organ or allogeneic bone marrow transplantation and long-term use of immunosuppressants, three (7%) had genetic predisposition to cutaneous cancers, and three (7%) had a history of RT-CT. Eighteen patients (41.9%) had relapses during the 58-month follow-up. Histopathologically, presence of moderately-poorly differentiated squamous cell carcinoma, prolonged use of immunosuppressants, and history of RT-CT were statistically significantly associated with relapse ($p<0.05$). Kaplan-Meier analysis done for factors affecting survival showed a relationship between survival and presence of genetic risk, poor differentiation, and recurrence ($p<0.05$).

Discussion: Recognizing the risk factors for cSCC in young adults (long-term immunosuppressants, chemoradiotherapy, genetic cutaneous cancer predisposition syndromes, presence of precursor lesions) bear great importance in providing appropriate guidance to ensure early diagnosis and effective treatment.

Keywords: cutaneous squamous cell carcinoma, chemotherapy, radiotherapy, immunosuppressants, genetics

ÖZET

Giriş: Kutanöz skuamöz hücreli karsinom(cSCC) en sık görülen kanserlerden biriyken, genç erişkin cSCC nadirdir ve klinik özellikleri, sonuçları ve iyatrojenik risk faktörleri iyi tanımlanmamıştır. Bu çalışmada genç erişkinlerde cSCC ile ilişkili klinik özellikleri, potansiyel risk faktörlerini ve nüksü etkileyen faktörleri tanımlamak amaçlanmıştır.

Gereç ve yöntemler: Tanı yaşı <35 olan, tam tıbbi öyküsüne ve kullandığı ilaçlara ulaşılabilen ve retrospektif analize izin veren 43 hasta çalışmaya dâhil edildi. Hastaların demografik özellikleri, tanı tarihleri, lezyon lokalizasyonları, patoloji sonuçları ve aile öyküleri kaydedildi.

Bulgular: Çalışmaya dâhil edilen hastaların ortalama tanı yaşı 29 (17-34) yıl olarak bulundu. 20 hastanın (46,5%) ailesinde malignite öyküsü bulunmaktaydı. 19 hastada (44,2%) SCC tanısından önce prekanseröz lezyon saptandığı görüldü. Hastaların %7'sinde organ veya allojenik kemik iliği nakli ve

First Received: 06.07.2022, Accepted: 17.08.2022 doi: 10.5505/aot.2023.61582

uzun dönem immünsupresan kullanımı, %7'sinde kutanöz kanserlere genetik yatkınlık ve %7'sinde RT-KT öyküsü mevcuttu. 58 aylık takip süresinde 18 hastanın (41,9%) nüks ettiği gözlendi. Histopatolojik olarak orta-kötü differansiye skumaöz hücreli karsinom varlığının, uzamış immünsupresan kullanımının, radyoterapi ve kemoterapi öyküsü bulunmasının nüks gelişimi ile istatistiksel anlamlı ilişkisi olduğu gözlendi ($p<0,05$). Sağkalımı etkileyen faktörler için yapılan Kaplan-Meier analizinde genetik risk varlığının, kötü differansiyasyonun ve nüks varlığını sağkalım ile ilişkisi gösterilmiştir. ($p<0.05$)

Tartışma: Genç erişkinlerde uzun süreli immünsupresan kullanımı, kemoradyoterapi, genetik kutanöz kanser yatkınlık sendromları ve prekürsör lezyon varlığı gibi cSCC gelişimi için risk faktörlerinin tanınması uygun danışmanlık, erken tanı ve etkin tedavi sağlamak için büyük önem taşımaktadır.

Anahtar kelimeler: Kutanöz skuamöz hücreli karsinom, kemoterapi, radyoterapi, immünsupresan, genetik

Introduction

Skin cancer is the most common type of malignancy. There is a remarkable increase in its incidence across the world with about 3.5 million new diagnoses each year in the USA alone. Its most lethal subtype is malignant melanoma. Although non-melanoma skin cancers (NMSCs) show lower mortality rates, lesions usually are locally aggressive, can lead to disorders, have extremely negative effects on quality of life, and incur significant healthcare costs.

Cutaneous squamous cell carcinoma (cSCC) is a keratinocyte-derived skin cancer which predominantly affects older people [1]. The main etiological factor for cSCC is ultraviolet radiation (UVR) from the sun. Its risk factors and etiology in young adults, however, are different. Cases of cSCC have been reported in young adults with hereditary and congenital conditions such as various tumor syndromes, photosensitivity disorders, and birthmarks [2,3,4]. Long-term immunosuppression, radiotherapy and voriconazole therapy have also been identified as iatrogenic risk factors for cSCC [5,6,7]. Given the growing number of long-term survivors of childhood cancers and other chronic medical conditions, cases of young adult NMSCs caused by iatrogenic risk factors are estimated to be more common than previously reported. Failure to recognize these risk factors may lead to delays in diagnosis and increased tumor burden.

In this study we aimed to identify among our cSCC patients those who were diagnosed in young adulthood and define their clinical and pathological characteristics. We further aimed to determine their risk factors and describe the follow-up of cSCC in young adults exposed to these risk factors.

Methods

Patients

The institutional and national research committees' ethical standards, as well as the 1964 Declaration of Helsinki and its later revisions or comparable ethical standards were followed in the study. One-thousand-and-fifty-three patients who were diagnosed with squamous cell carcinoma (SCC) in the period from January 2014 through December 2019 were histopathologically screened. Forty-three patients who were <35 years of age at the time of diagnosis, had allowed their data to be used for retrospective analysis, and whose full medical history and medication records were available were included in the study. Patient data including demographic characteristics, date of diagnosis, localization of lesion, pathology, medical history (e.g., malignancy, stem cell or organ transplantation, genetic syndrome, or presence of other chronic diseases), medication history (focusing on chemotherapeutic and immunosuppressive agents), radiation exposure (total body irradiation [TBI] and cGy localized radiation therapy), and familial history were recorded. Long-term immunosuppression was defined as having received immunosuppressive therapy for more than 6 months.

Statistical analysis

The Statistical Package for Social Sciences for Windows 20.0 (IBM, Armonk NY, USA) was used for analysis. Descriptive statistics summarized frequencies and percentages for categorical, mean, and standard deviation for continuous variables. Categorical variables were compared with the Independent Samples T-test and categorical parameters with the $\chi 2$ test. $p<0.05$ was considered statistically significant.

Results

Of the 43 patients included in the study, 26 (60.5%) were male and 17 (39.5%) were female. Their mean age at diagnosis was 29 (range, 17-34) years. All patients had a histopathologically confirmed diagnosis of cutaneous squamous cell carcinoma. Lesions were most commonly located in the head-neck region (81.4%), followed by the upper extremities (9.3%). Mean follow-up period was 58 (range, 5-138) months.

While all patients had undergone wide local excision, re-surgery was needed in four patients (9.3%) because of surgical borderline positivity. Reconstruction was required in 25 patients (58.1%) after excision because of the location of the lesion and the size of the defect. Of these 25 patients, flaps were used in 17 and grafts were used in eight patients. Demographic and clinical characteristics of the patients are given in Table 1.

Review of patients based on their medical and familial histories revealed that 20 (46.5%) had a familial history of (pathologically confirmed) malignancy, and precancerous lesions were detected in 19 (44.2%) before their SCC diagnosis. Of the precancerous lesions, 12 (27.9%) were recorded as kerato-acanthoma, four (9.3%) as Bowen's disease, and three (7%) as burn scar.

Three (7%) of 43 patients underwent genetic analysis due to their clinical conditions; two were diagnosed with xeroderma pigmentosum and one was diagnosed with Gorlin's syndrome. One patient had a history of hematopoietic stem cell transplant due to leukemia in the pediatric period and received a high dose of chemotherapy. The patient had received long-term immunosuppressant therapy for Graft versus Host Disease (GvHD) and was continuing to use it at the time of diagnosis. Two patients had a history of concomitant definitive chemoradiotherapy to the loco-regional area for nasopharyngeal cancer.

Of the three (7%) patients with a history of long-term immunosuppressive use, one is the above-mentioned patient who had been taking immunosuppressants due to GvHD after allogeneic bone marrow transplantation. This

Table 1. Demographic and clinical characteristics of patients

Parameters	Number of patients (%)
Gender	
Female	17 (39.5%)
Male	26 (60.5%)
Histopathological Subtype	
Acantholytic	8 (18.6%)
Keratoacanthomatous	6 (14.0%)
Unspecified	29 (67.4%)
Grade	
Well differentiated	22 (51.2%)
Moderately / Poorly differentiated	21 (48.8%)
Lymph Node Involvement	
No	38 (88.4%)
Yes	5 (11.6%)
Localization	
Head-Neck	35 (81.4%)
Upper extremity	4 (9.3%)
Torso	2 (4.7%)
Lower extremity	2 (4.7%)
Reconstruction	
Not Done	18 (41.9%)
Flap	17 (39.5%)
Graft	8 (18.6%)
Familial History of Malignancy	
No	23 (53.5%)
Yes	20 (46.5%)
Precancerous Lesion	
Keratoacanthoma	12 (27.9%)
Bowen's	4 (9.3%)
Burn scar	3 (7.0%)
No	24 (55.8%)
Recurrence	
Yes	18 (41.9%)
No	25 (58.1%)
Immunosuppression	
Yes	3 (7.0%)
No	40 (93.0%)
Radiotherapy	
Yes	3 (7.0%)
No	40 (93.0%)
Chemotherapy	
Yes	3 (7.0%)
No	40 (93.0%)

Table 2. Relationship between clinical and pathologic factors and recurrence

	Recurrence		
	No	Yes	*p*-value
Gender			
Male	16	10	0.403
Female	9	8	
Grade			
Well Differentiated	17	5	**0.01***
Moderately/Poorly Differentiated	8	13	
Familial History of Malignancy			
No	12	11	0.295
Yes	13	7	
Precancerous Lesion			
No	17	7	0.056
Yes	8	11	
Localization			
Head-Neck	23	12	0.090
Other	2	4	
Surgical Margin			
Negative	24	15	0.158
Positive	1	3	
Use of immunosuppressants			
No	25	15	**0.034***
Yes	0	3	
History of Chemotherapy			
No	25	15	**0.034***
Yes	0	3	
History of Radiotherapy			
No	25	15	**0.034***
Yes	0	3	
Genetic Diagnosis			
No	25	15	**0.034***
Yes	0	3	

* statistically significant

patient was taking high doses of steroids and cyclosporine. The patient was also given voriconazole for fungal infection in the post-transplant period. The other two patients were one that had a liver transplantation due to cryptogenic liver cirrhosis, and another that had a kidney transplantation due to renal failure, and both were on long-term immunosuppressants to prevent rejection.

Recurrence was seen in 18 (41.9%) patients in the 58-month follow-up period. Statistical analysis done for the factors affecting recurrence showed that gender, familial history of malignancy, presence of pre-cancerous lesion, lesion localization and surgical margin positivity had no effect on recurrence. Histopathologically, the presence of moderately-poorly differentiated SCC, prolonged use of immunosuppressants, and a history of radiotherapy and chemotherapy were seen to be statistically significantly associated with the development of recurrence($p<0.05$) (Table 2).

Five patients had died in the follow-up period. The Kaplan-Meier analysis for factors affecting survival showed presence of genetic risks, histopathologically moderately-poorly differentiated squamous cell carcinoma and recurrence were all related with survival ($p<0.05$).

Discussion

In our study we aimed to determine the etiological factors and risks for cSCC in young adults and to identify which patients should be vigilantly followed-up for recurrence. cSCCs represent a major health problem worldwide. Although they rarely metastasize, they are often found to be locally invasive at diagnosis and surgical treatments may be insufficient. Identifying and treating the lesions at an early stage, determining its etiological factors, and thereby recognizing which patients should be followed-up for prevention are of great importance.

In our study, 21% of young adult cSCC patients had iatrogenic risk factors including chemotherapy, radiotherapy, long-term immunosuppression, or a combination of these. In about half of these patients, the presence of precancerous lesions before cSCC was histopathologically confirmed. The most common precancerous lesion was kerato-acanthoma. In a study by Huang et al. [8], 29% of the 124 young adult non-melanoma cutaneous malignancy patients had similar risk factors. Similarly, previous studies have shown that the risk of NMSC increased in adult patients after HSCT, organ transplantation, long-term immuno-suppression, chemotherapy, and radiation therapy [9,10,11].

Long-term immunosuppressant therapy has been shown to disrupt the immune surveillance of dysplastic cells. Systemic calcineurin inhibitors, one of the most commonly used immunosuppressants, can increase the risk of cSCC by blocking P53 signaling and nucleotide excision repair [12,13]. One of our patients after liver transplantation and another of our patients after kidney transplantation had used immunosuppressants for a long period (>6 months). The third patient was using both immunosuppressants and variconazole for GvHD that developed after allogeneic bone marrow transplantation.

Voriconazole, a triazole used for antifungal prophylaxis and treatment, has been associated with an increased risk of SCC after lung transplantation in adults and in HSCT recipients [14,15]. Voriconazole metabolites can induce both phototoxicity and photo-carcinogenesis by sensitizing keratino-cytes to ultraviolet A, inducing DNA fragility, and disrupting DNA repair [16,17}.

Radiotherapy is well-known to be carcino-genic, but its role in the pathogenesis of cSCC is not entirely clear. Despite the large amounts of data on sarcomas that develop in the background of scarring after radiotherapy [18] there are few reports on cSCCs developing after RT, especially in childhood. In our study, three (7%) of the patients had a history of radiotherapy in childhood. A multivariate analysis done in the large-sample study of the Childhood Cancer Survivor Study (CCSS) involving 13,132 patients revealed a six-fold risk increase in patients who had received radiotherapy in childhood versus those who did not [19].

Less is known about the occurrence of cSCC secondary to previous cancer treatment because data on the incidence of BCC or SCC were previously excluded from second malignancy analyses. The Nordic Society of Paediatric Haematology and Oncology Association of the Nordic Cancer Registries reported a standard incidence rate of 4.7 (95% CI, 2.7 to 7.8) for non-melanoma cutaneous cancers in childhood cancer survivors compared to the general population [20]. To our knowledge, a history of chemotherapy has not previously been identified as an independent risk factor for cSCC. Given the significant overlap between patients that received radiotherapy and those that received chemotherapy in our study, we suggest that further studies are needed to confirm this finding.

Three (7%) of our patients had a predisposing genetic condition (Xeroderma pigmentosum in two patients and Gorlin's syndrome in one patient). While these three patients were diagnosed at a much earlier age than those with iatrogenic risk factors, a significant difference was found between them and the rest of the population in terms of both recurrence and reduced survival. Early diagnosis, careful sun protection and proper treatment of skin cancers can significantly reduce tumor burden and prolong life in these patients [21]. A fairly rare genetic disorder, xeroderma pigmentosum is characterized by photosensitivity, pigmentary changes in the skin, signs of premature aging, neoplasia, and abnormal DNA repair. It presents with defects in repairing DNA damage caused by UV rays and excessive sensitivity to UV rays, particularly to sunlight. Xeroderma pigmentosum should be borne in mind in the presence of easy sunburn, evidence of photosensitivity including lentigo, telangi-ectasia, ocular manifestations, or deafness [22]. Patients with familial cancer syndromes such as the nevoid BCC syndrome (Gorlin's syndrome) are also at risk of developing multiple BCC and SCC at an early age, as are patients with genetic defects in DNA repair mechanisms such as xeroderma pigmentosum [23,24,25].

Most of our patients were successfully treated with extensive surgical excision without metastases. Recurrence was seen in 41.9% of the patients in the follow-up period. Analysis of the factors associated with recurrence showed that the presence of histopatho-logically moderately-poorly differentiated SCC, prolonged use of immunosuppressants, and a history of radiotherapy and/or

chemotherapy were associated with the development of recurrence. As mentioned above, factors other than differentiation are included as etiological factors in young adult cSCC. Therefore, patients should be regularly monitored since these factors are effective in both etiology and recurrence. Monitoring through follow-up visits is among the most effective interventions to be done to prevent recurrence.

The major limitation of our study is its retrospective nature and small number of patients. Given the rarity of cSCC in children and young adults, multicenter prospective studies are warranted to confirm and expand our findings. That most etiological and predisposing factors overlapped, and multiple risk factors were identified in patients may have influenced our results. Prospectively designed studies, in which tissue analyses of tumor suppressor genes and oncogene expression can be done with long-term follow-up, will shed light on both the pathogenesis and the treatment of the condition.

To conclude, although rarely fatal, cSCC is a major public health issue. In our study, we attempted to describe the predisposing conditions and iatrogenic exposures of young adult cSCC patients. The risk of developing cSCC is high in the presence of long-term use of immunosuppressants, chemoradiotherapy or genetic cutaneous cancer predisposition syndromes. Advising these patients to avoid exposure to UVR, such as to solar rays that may further increase the risk of cSCC, and early detection of the disease with regular follow-ups can prevent delays in diagnosis and minimize the burden of tumors.

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Doi: 10.5505/aot.2023.61582

Original Article

The Optimal Treatment Approaches and Prognostic Factors in Elderly Patients with Advanced Stage Biliary Tract Tumors

Metastatik Safra Yolu Kanseri Olan Yaşlı Hastalarda Optimal Tedavi Yaklaşımları ve Prognostik Faktörler

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ABSTRACT

Introduction: There is a lack of evidence of the outcomes in elderly patients advanced stage biliary tract cancer due to the patients aged over 65 years are less than 25% in many prospective trials. We designed a retrospective multicenter study to evaluate the factors affecting treatment and survival in elderly patients with advanced-stage biliary tract cancer.

Materials and methods: A total of 116 patients with advanced stage biliary tract cancer aged ≥65 years were included, and the treatment responses, survival, and toxicity rates were evaluated with respect to age groups

Results: There was no significant difference between age and response to treatment, survival, or toxicity. The median progression-free survival and overall survival were 5.3, and 11.8 months respectively. Multivariate analysis indicated that ECOG PS ($p<0.001$ CI95% 1.5-3.7) and PNI ($p<0.001$ CI 95% 0.14-0.41) were significant independent prognostic factors for PFS. The independent prognostic factors for OS were choice of frontline regimen, NLR and PNI ($p=0.007$ CI 95% 0.71 – 0.94, $p=0.006$ CI 95% 1.2 – 3.1, $p=0.001$ CI 95% 0.35 – 0.91, respectively).

Discussion: This study confirms the general prognostic relevance of inflammatory parameters and the importance of frontline treatment in elderly patients with advanced-stage biliary tract tumors. Additionally, getting older does not indicate that treatment will be avoided or that they will have a worse prognosis and suffer from more toxicities.

Keywords: biliary tract cancer, elderly population, prognostic nutritional index, systemic inflammatory index

ÖZET

Giriş: 65 yaş üzeri hastaların klinik çalışmaların %25'inden daha azını oluşturması nedeniyle biliyer sistem kanseri olan ileri yaş hastaların yönetimi konusunda kanıt eksiği bulunmaktadır. Bu amaçla, metastatik safra yolu kanseri tanılı yaşlı hastalarda tedaviyi ve sağkalımı etkileyen faktörleri değerlendirmek için retrospektif çok merkezli bir çalışma tasarladık.

Gereç ve yöntemler: Çalışmaya 65 yaş ve üzeri, ileri evre safra yolu kanseri tanısı almış, 116 hasta dahil edildi ve yaş gruplarına göre tedavi yanıtları, sağkalım ve toksisite oranları değerlendirildi.

First Received: 01.08.2022, Accepted: 17.10.2022 doi: 10.5505/aot.2023.03708

Bulgular: Median yaşa göre gruplandırılıdğında; yaş ile tedaviye yanıt, sağkalım, toksisite arasında anlamlı bir fark bulunmadı. Tüm populasyonda medyan progresyonsuz sağkalım (PSK) ve genel sağkalım (GSK) sırasıyla 5.3, 11.8 aydı. Multivariate analizde, PSK için bağımsız prognostik faktörler preformans durumu(ECOG PS) (p<0.001 CI95% 1.5-3.7) ve Prognostik nutrisyonel indek (PNI) (p<0.001 CI 95% 0.14-0.41) olarak bulundu. GSK için ise bağımsız prognostik faktörler, birinci sıra tedavi seçimi, Notrofil Lenfosit oranı (p=0,007 CI %95 0,71 – 0,94) ve PNI (p=0,001 CI %95 0,35 – 0,91) olarak bulundu.
Tartışma: Metastatik safra yolu kanseri olan yaşlı hastalarda prognozu etkileyen temel faktöreler inflamatuar parametreler ve birinci basamakta seçilen kemoterapi rejimidir. İleri yaş ile sağkalım, toksiste profili ve tedavi toleransı farklılık göstermemektedir.

Anahtar kelimeler: Safra yolu kanseri, yaşlı populasyon, Prognostik nutrisyonel indeks, sistemik inflamatuvar indeks

Introduction

Biliary tract cancers including intrahepatic cholangiocarcinoma (ICC), extrahepatic cholangiocarcinoma (EHC), and gallbladder cancer (GBC) are rare malignancies with poor survival [1]. The incidence and epidemiology of biliary tract cancer are complex and vary worldwide. It is shown that intrahepatic cholangiocarcinoma is on the rise in the Western world, and gallbladder cancer is on the decline. GBC is also a very rare malignancy with high mortality rates. Fewer than 5000 new cases are diagnosed each year in the United States and the incidence correlates with the prevalence of cholelithiasis [1-3].

Chronic inflammation and the immune response play a big role in the development of biliary tract cancers and biomarkers such as neutrophil-to-lymphocyte ratio (NLR), the platelet-to-lymphocyte ratio (PLR), and the systemic immune-inflammation index (SII) were studied in multiple solid tumors. The prognostic nutritional index [PNI] had an impact on clinical outcomes and low PNI was significantly associated with poor prognosis in patients with BTC in many reports [4-6].

Age-related physiologic changes in the biliary tract can cause more gallstones in elderly patients than young people. It is unclear whether these changings are responsible for the development of GBC. Although the incidence of pancreatic cancer is more frequent in elderly patients; It is not yet clear the correlation between age and biliary tract cancer development [7,8].

BTC is mostly diagnosed in the advanced stage with an estimated five-year survival of 10-20%. Therefore, the major aim is to palliate the symptoms and improve the life quality with longer progression-free survival. However, the cisplatin plus gemcitabine (GC) regimen is the standard frontline regimen, due to potential toxicity and higher comorbidities, physicians can prefer other treatment options for elderly patients [9-12].

In many prospective trials patients aged ≥65 years are less than 25%. Therefore, treatment outcomes and prognostic factors are not well described [11]. The objective of this multicenter study is to determine factors affecting progression-free survival and to evaluate both treatment modalities and their outcomes as real-life experiences in elderly adults with advanced biliary tract tumors.

Material Method

This was a retrospective multicenter study that included a total of 116 patients aged >65 years with advanced biliary tract cancer (ABTC). ICC and ECC, klatskin tumors and GBC patients were included in this study. Data were obtained from patients' charts concerning age, ECOG performance status (PS), tumor location, NLR, PLR, SII, PNI indexes, duration of treatment, and survival outcomes after written informed consent had been obtained from patients.

Treatment responses, survival, and toxicity rates were evaluated together with respect to age groups. Patients with poor performance status and who died related to other reasons were excluded from the study. Common

Terminology Criteria for Adverse Events (CTCAE) v5.0 was used for adverse event evaluation. All procedures performed in studies involving human participants were by the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The Local Ethics Committee of Istanbul Medipol University approved the study with decision number E-10840098-772.02-2913.

Statistical analysis:

Statistical analyses were performed using the statistical analysis was performed using the Statistics Package for Social Sciences software (ver. 24.0; SPSS Inc., Armonk, NY: IBM Corp). The distribution of the study parameters was non-normal distribution, Inflammatory markers were dichotomized at the median as a cut-off value. The relationship between the age group and the clinico-pathological factors was compared using the chi-squared test and Fisher's exact test. Survival analysis and curves were established using the Kaplan-Meier method and compared with the long-rank test. Progression-free survival (PFS) was defined as the time from the diagnosis of advanced stage disease to progression or the last follow-up. Overall survival (OS) was described as the time from diagnosis to the date of the patient's death or last known contact. Univariate and multivariate analyses of prognostic factors related to survival were performed by the Cox proportional hazards model. Multivariate p values were used to characterize the independence of these factors. The 95% confidence interval (CI) was used to quantify the relationship between survival time and each independent factor. All p values were two-sided in tests and p values less than or equal to 0.05 were accepted to be statistically significant.

Results:

Between June 2015, and June 2021, a total of 116 patients with ABTCs were included in this study. Sixty-four of the patients (55.2%) were female and fifty-two of them (44.8%) were male with a median age of 70 years (range: 65-89). Sixty-four patients (55.2%) were ≥ 70 years and 52 patients (44.8%) were <70 years. At the initial diagnosis, most patients (77.6%) had ABTC. Forty-eight patients (41.4%) were diagnosed with ICC, twenty patients (10.3%) were ECC, forty-two of patients (36.2%) were GBC, six patients (5.2%) were klatskin tumors respectively. Most patients (n:87) were in good performance status.

In the front-line setting, the gemcitabine monotherapy, cisplatin plus gemcitabine regimen and FOLFOX regimen were preferred in 36 patients (31.0), 38 patients (32.8%), and 15 patients (12.9%), respectively. Eight of the patients (6.9%) were followed up with the best supportive care.

Patients received a median of 4 cycles of chemotherapy (range: 2-12), and 49 of patients (46.7%) could complete the treatment. The objective response rate (ORR) was 12% in all populations. Any grade treatment-related adverse event (AE) was seen in 49 of the patients (42.2%). Grade3 or 4 AEs were detected in 15 patients (12.9%) patients. No deaths due to an AE occurred. 3 of nine patients who were treated with the FOLFIRINOX regimen suffered from grade 3/4 AE while no grade ¾ AE was seen in patients who received single-agent regimens.

There was no significant correlation between treatment regimens and toxicity profile in elderly patients. Additionally, treatment-related any grade of adverse event was seen in 27 patients with <70 years and serious toxicity as grade 3/4 in 22 patients. In the ≥ 70years; the treatment-related adverse event was observed in 22 patients and grade 3 or higher toxicity was seen in 7 patients. ≥ 10% weight loss at the time of diagnosis was statistically significantly higher in patients aged 70 years and older (p=0.038). When the patients were categorized according to age; no significant difference was determined between age groups according to the response to treatment, ECOG PS, tumor localization, presence of jaundice, the choice of front-line treatment, the development of treatment-related AEs, and grade 3 or higher AEs. Although not

Table 1: Patient's and tumour characteristics regarding the age

Characteristics	Total N (%)	<70 years n (%)	≥70 years n (%)	p
Total	116	52 (44.8%)	64 (55.2)	
Initial diagnosis, presence of:				
≥ 10% Weight loss	68 (58.6)	43 (67.2)	25 (48.1)	**0.038**
Jaundice	29 (25)	12 (22.1)	17 (26.5)	0.4
Abdominal Pain	63 (54.3)	28 (51.1)	35 (57.3)	0.1
Gender				
Male	52 (44.8)	31 (48.4)	21 (40.4)	0.3
Female	64 (55.2)	33 (51.6)	31 (59.6)	
ECOG PS*				
0	18 (15.5)	9 (14.1)	9 (17.3)	0.88
1	69 (59.5)	39 (60.9)	30 (57.7)	
2	29 (25.0)	16 (25.0)	13 (25.0)	
Localization of primary tumour		28 (43.8)	20 (38.5)	
Intrahepatic	48	11 (17.2)	9 (17.3)	0.53
cholangiocarcinoma	20	21 (32.8)	21 (40.4)	
Extrahepatic	42	4 (6.3)	2 (3.8)	
cholangiocarcinoma	6			
Gallbladder				
Klatskin				
Initial clinical TNM* stage				
Stage I	2 (1.7)	1 (1.6)	1 (1.9)	0.5
Stage II	8 (6.9)	6 (9.4)	2 (3.8)	
Stage III	16 (13.8)	10 (15.6)	6 (7.2)	
Stage IV	90 (77.6)	47 (73.4)	43 (82.7)	
Presence of PTC* replacement	23 (19.8)	13 (20.3)	10 (19.2)	0.8
Cholangitis	26 (22.4)	14 (21.9)	12 (23.1)	0.8
Choice of the first-line treatment				
Gemcitabine Oxaliplatin	8 (6.9)	4 (6.3)	4 (7.7)	
Gemcitabine cisplatin	38 (32.8)	26 (40.6)	12 (23.1)	
Gemcitabine single agent	36 (31.0)	12 (18.8)	24 (46.2)	**0.055**
FOLFOX*	15 (12.9)	11 17.2)	4 (7.7)	
FOLFIRINOX*	2 (1.7)	1 (1.6)	1 (1.9)	
BSC*	8 (6.9)	4 (6.3)	4 (7.7)	
Other	9 (7.8)	6 (9.4)	3 (5.8)	
Treatment Response				
CR/PR	13 (11.2)	9 (14.1)	4(7.7)	
Stable Disease	36 (31.0)	17 (26.6)	19 (36.5)	0.48
Progressive Disease	67 (57.8)	38 (59.4)	29 (55.8)	
Treatment-Related Any Grade Adverse Event	49 (42.3)	27 (42.4)	22 (42.3)	0.9
Grade 3/4 Adverse Event	15 (57.7)	8 (29.6)	7 (31.8)	0.86

*ECOG PS: Eastern Cooperative Oncology Group Performance Status FOLFOX: (fluorouracil, Leucovorin, Oxaliplatin) *FOLFIRINOX (leucovorin and fluorouracil plus irinotecan and oxaliplatin) * BSC: best supportive care, *TNM: tumour, node, metastasis, *PTC: Percutaneous Transhepatic Cholangiography, *CR: Complete Response, *PR: Partially Response

* p values <0.05 were regarded as statistically significant

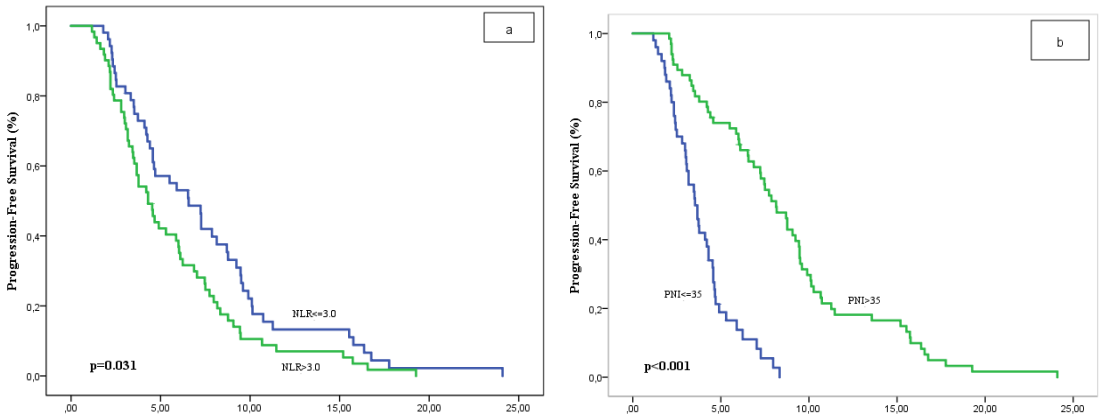

Figure 1 a, b: Progression-free survival curves according to the NLR and PNI.

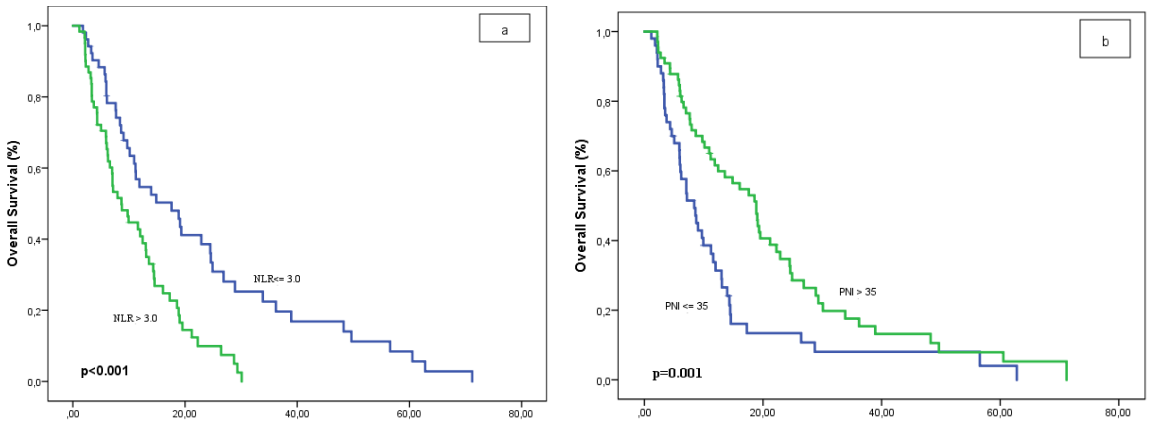

Figure 2 a, b: Overall survival curves according to the NLR and PNI.

statistically significant, the choice of monotherapy tended to be higher in the older population (p=0.055). The baseline patient's and tumor characteristics and the comparison between age groups are summarized in Table 1.

Median NLR, PLR, SII, and PNI values were determined as 3.0, 152.5, 38.5, and 35.0 respectively. The number of patients with NLR >3.0 and PLR >152.5 were 61 (54%) and 56 (48.3%), respectively. The number of patients with SII>38.5 and PNI > 35 was 66 (44.5%) and 66 (56.9%) respectively.

At a median follow-up of 10.0 months, the median PFS time was 5.3 months, while the median OS time was 11.8 months in all patients. Univariate analysis for PFS revealed that ECOG PS (p<0.001), the choice of treatment regimen (p<0.001), NLR (p=0.03) and PNI (p<0.001) were found to be significant prognostic indicators. While not statistically significant, the localization of primary tumor and SII tended to affect the PFS. The PFS did not differ between age groups.

The median PFS was 4.3 months and 6.5 months in patients with NLR>3 and NLR ≤3 respectively (Figure1a). The median PFS was 8.1 months and 3.5 months in patients with PNI >35 and PNI ≤35 respectively (Figure 1b).

The median PFS for patients treated with BSC was 2.2 months, in the gemcitabine monotherapy group it was 4.4 months, in the cisplatin-gemcitabine group it was 7.7 months, in the GEMOX group it was 6 months,

Table 2: Univariate and Multivariate analysis for progression-free and overall survival

	Progression Free Survival		Overall Survival	
	Univariate analysis	Multivariate analysis	Univariate analysis	Multivariate analysis
Factor	p	P (HR; 95% CI)	p	p (HR; 95% CI)
Age (≥70 years vs <70 years)	0.64		0.08	0.2 (0.35; 0.54-1.66)
Gender (male vs female)	0.87		0.9	
ECOG PS (0 vs 1/2)	<0.001	<0.001 (2.4; 1.5–3.7)	0.02	0.6 (1.1; 075-1.62)
Cholangitis (absent vs present)	0.1		0.5	
PTC requirement (absent vs present)	0.2		0.7	
Tumor localization (ICC vs others)	0.55		0.6	
At initial diagnosis				
Presence of ≥ 10% weight loss	0.6		0.5	
Presence of Jaundice	0.7		0.78	
Choice of Treatment (BSC vs others)	<0.001	0.3	0.03	0.007 (0.82; 0.71-0.94)
SII (≤ 38.5 vs >38.5)	0.059	0.64	0.01	0.19 (0.73; 0.46-1.16)
NLR (≤3.0 vs >3.0)	0.03	0.34	<0.001	0.006 (1.9; 1.2-3.1)
PLR (≤ 152.5 vs >152.5)	0.1	0.61	0.07	0.8 (1.1; 0.67-1.55)
PNI (≤ 35 vs > 35)	<0.001	<0.001 (0.24; 0.14–0.41)	0.001	0.01 (0.56; 0.35-0.91)

***** HR: hazard ratio, CI: confidence interval, BSC: best supportive care, ECOG PS: Eastern Cooperative Oncology Group Performance Status PTC: Percutaneous Transhepatic Cholangiography, NLR:neutrophil-to-lymphocyte ratio, PLR: platelet-to-lymphocyte ratio, SII:systemic immune-inflammation index, PNI;prognostic nutritional index

and in the FOLFOX group, it was also 7.8 months.

In the univariate analysis for OS, ECOG PS, treatment regimen, SII, PNI, and NLR were found to be prognostic factors (p=0.02, p=0.01, p=0.001, p<0.001, respectively). The median OS treated with BSC was 1.8 months, with monotherapy gemcitabine was 7.0 months, with cisplatin-gemcitabine was 14.5 months, with GEMOX group was 11.2 months and with FOLFOX was 26.4 months, respectively. Multivariate analysis indicated that ECOG PS (p<0.001 HR:2.4; CI95% 1.5-3.7) and PNI (p<0.001 HR:0.24, CI 95% 0.14-0.41) were significantly independent prognostic factors for PFS.

The median OS was 8.6 months and 17.6 months in patients with NLR>3 and NLR ≤3 respectively (Figure 2a). The median OS was 18.8 months and 8.4 months in patients with PNI >35 and PNI≤35 (Figure 2b). The median OS was 9.7 months and 12.4 months in patients with PLR>152.5 and PLR ≤152.5 respectively.

In multivariate analysis the choice of treatment, NLR and PNI were revealed as an independent prognostic factor for OS (p=0.007 CI 95% 0.71 – 0.94, p=0.006 CI 95% 1.2 – 3.1, p=0.001 CI 95% 0.35 – 0.91, respectively). Table 2 summarized the multivariate and univariate analysis for both OS and PFS.

Discussion:

Inclusion of older patients with biliary tract cancer has a limited rate in prospective randomized trials, thus to guide the systemic treatment including BSC has lack of evidence in elderly patients. Also, the term elderly is not well described either. In the current study, we aimed to retrospectively analyze the elderly patients with ABTC to contribute to the literature on decision-making treatment and the choice of regimens and understanding well the prognostic factors affecting survival in these patients.

In Advanced Biliary Cancer-02 (ABC-02) clinical trial, the median OS was 11.7 months in patients treated with cisplatin/gemcitabine. Although age was not a stratification factor, the study concluded that age was not prognostic for PFS and OS in those receiving monotherapy or combination therapy [10,11]. Lewis et al. analyzed 1421 patients diagnosed with hepato-pancreaticobiliary tract tumors. They showed that 10% of patients were over 80 years and 36% had BTC. The median OS was 10 months in this cohort, but there was no significant survival difference between older and younger patients when considered fit enough to have active systemic therapy [12]. Hepatobiliary and Pancreatic Oncology Group of Japan Clinical Oncology Group (JCOG) demonstrated that gemcitabine plus S-1 was non-inferior in OS compared with GC as a first-line treatment for advanced BTC [13]. 354 elderly patients from this study were analyzed in another trial. They reported that there was no significant correlation in OS, efficacy, and adverse events between <75 (non-elderly] and≥ 75 years (elderly) group. In the elderly group, the median OS was 12.7 months for those who received GC [14].

In our population, there was no significant difference in OS and PFS between patients with <70 years and >70 years. The median PFS was 5.3 months, and the median OS was 11.8 months in the overall population. The treatment choice in the frontline setting had a significant impact on PFS (p<0.001) and OS (p=0.01). Better survival outcomes were seen in cisplatin plus gemcitabine and the FOLFOX group (median PFS 7.7 months and 7.8 months respectively). The median OS was superior in patients treated with FOLFOX and cisplatin plus gemcitabine than in other regimens (26,4 months and 14,5 months respectively).

In the ABC-02 study, 66% of patients received at least 3 cycles of gemcitabine for ABTC [10]. In our cohort, the median number of chemotherapy cycles was 4 and 35.3% of patients could complete the planned treatment protocol. Our results were thus compatible with the literature [10-14]. Furthermore, in this study as the patients were categorized according to the median age, there was no significant difference between age and response to treatment, ECOG PS, tumor localization, the development of treatment-related AE, and grade 3 or higher AE. This difference can be explained by the toxicity rate in the total cohort which was lower than in previous trials [10-14] and before treatment 10% dose reduction was done for all patients. Moreover, ORR was significantly correlated with the frontline systemic treatment regimen. As expected, the worse survivals were observed in the BSC group. The OS benefit of the FOLFOX regimen was significantly higher in elderly patients than in other regimens compared with younger patients. However, median PFS time was higher in the cisplatin-gemcitabine group. There was a trend toward choosing monotherapy gemcitabine in patients>70 years old.

In elderly patients, there are some tools to help with geriatric assessment. As American Society of Clinical Oncology (ASCO) guidelines recommend at a minimum, a geriatric assessment of function, comorbidity, falls, depression, cognition, and nutrition.

Cancer and Ageing Research Group (CARG] can be used to estimate the toxicity. Additional tools are recommended to use in the geriatric population [15-21]. In this study, geriatric assessment tools could not be used due to the retrospective design which was another limitation of our study.

Previously studies had shown that higher rates of systemic inflammatory markers such as NLR, and PLR were associated with poor prognosis [22-24]. We illustrated that ECOG PS ($p<0.001$ HR:2.4) and PNI ($p<0.001$ HR:0.24) were significantly independent prognostic factors for PFS; in addition, the choice of treatment ($p=0.007$ CI 95% 0.71 – 0.94), NLR ($p=0.006$ CI 95% 1.2 – 3.1) and PNI ($p=0.001$ CI 95% 0.35 – 0.91) were independent prognostic factors for OS in advance stage elderly BTC. Similar to the literature, we demonstrated a significant relationship between higher NLR and PLR values and worse survival outcomes in the elderly population.

The limitation of our study was the retrospective design which might concern bias and affect results. Another limitation of our study was not to use geriatric assessment tools.

The contribution of our study to the literature was that age should not be a criterion for the choice of treatment. We have shown that being elder had no significant impact on survival outcomes and treatment response. We revealed that the side-effect profile was also not related to age but treatment choice. Although not included in clinical studies, we have shown that inflammatory markers were poor prognostic markers in the elderly population too.

Conclusion

There is a lack of evidence to guide the best treatment regimen in elderly patients with ABTC because age is an exclusion criterion in many trials. Where evidence is available, the survival of older patients with ABTC in receipt of systemic therapy is similar to younger patients. In this multicenter study, we have shown that age was not related to survival and the outcomes of the treatment choice, similarly to the literature. SII which can be used in daily practice was an independent prognostic factor in elderly patients. In our opinion best choice of treatment should be made concerning ECOG PS, not age. To conclude further age-specific studies should be encouraged and real-world observational studies may offer useful comprehensions.

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Doi: 10.5505/aot.2023.03708

