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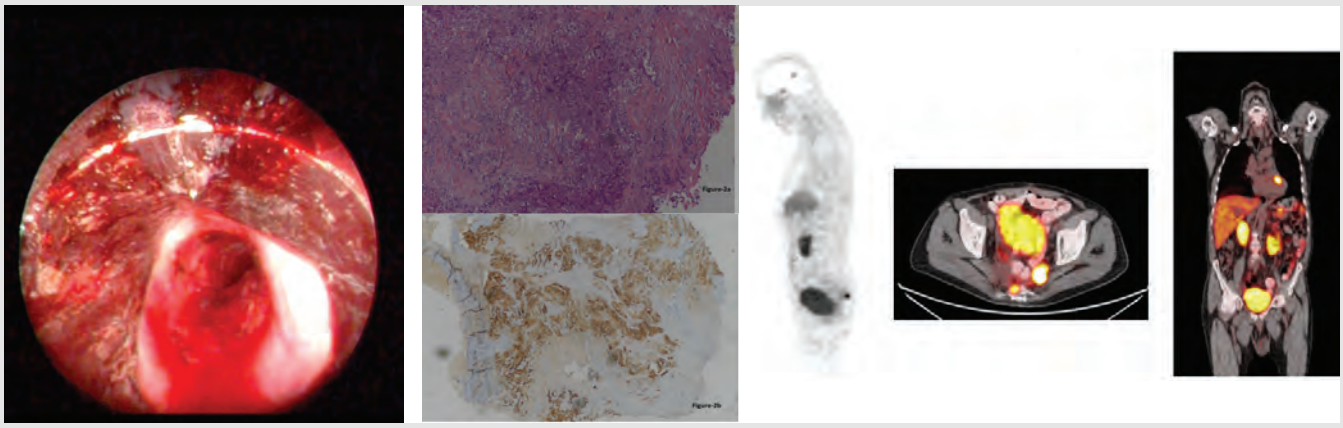

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Abstract: An abstract should be submitted with all submissions except for Letters to the Editor. The abstract of Original Articles should be structured with subheadings (Objective, Methods, Results, and Conclusion). Please check Table 1 below for word count specifications.

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Original Articles: This is the most important type of article since it provides new information based on original research. The main text of original articles should be structured with Introduction, Methods, Results, Discussion, and Conclusion subheadings. Please check Table 1 for the limitations for Original Articles.

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Tables

Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables.

Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text).

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Type of manuscript	Word limit	Abstract word limit	Reference limit	Table limit	Figure limit
Original Article	3500	250 (Structured)	40	6	7 or total of 15 images
Review Article	5000	250	80	6	10 or total of 20 images
Case Report	1000	200	15	No tables	10 or total of 20 images
Letter to the Editor	500	No abstract	5	No tables	No media

Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

Figures and Figure Legends

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When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

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Journal Article: Stephane A. Management of Congenital Cholesteatoma with Otoendoscopic Surgery: Case Report. Turkiye Klinikleri J Med Sci 2010;30:803-7.

Book Section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR, editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

Books with a Single Author: Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press;2005.

Editor(s) as Author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme;2003.

Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland;1992. pp.1561-5.

Scientific or Technical Report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study Kidney Int: 2004. Report No: 26.

Thesis: Yılmaz B. Ankara Üniversitesindeki Öğrencilerin Beslenme Durumları, Fiziksel Aktiviteleri ve Beden Kitle İndeksleri Kan Lipidleri Arasındaki Ilişkiler. H.Ü. Sağlık Bilimleri Enstitüsü, Doktora Tezi. 2007.

Manuscripts Accepted for Publication, Not Published Yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dçent Res. 1974. Epub Ahead of Print Articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. Diagn Interv Radiol 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

Manuscripts Published in Electronic Format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5):1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlEID/cid.htm.

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Address: Department of Nuclear Medicine, University of Health Sciences Turkey, Prof. Dr. Cemil Taşcıoğlu City Hospital, İstanbul, Turkey

Phone: +90 212 314 63 24

E-mail: tozulker@gmail.com

Publishing House: Galenos Yayınevi

Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1 34093 Fındıkzade, İstanbul, Turkey

Phone: +90 (212) 621 99 25

Fax: +90 (212) 621 99 27

E-mail: info@galenos.com.tr/yayin@galenos.com.tr

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CONTENTS

ORIGINAL ARTICLE

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Eur Arch Med Res 2022;38(2):82-89

How can we Evaluate the Incidental Malignancy of a Thyroid Nodule Regarding Age?

Yüksel Altınel[1], Merve Tokoçin[1], Serhat Meriç[1], Ahmet Akbaş[1], Yiğit Ülgen[2], Nadir Adnan Hacım[1]

[1]University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital, Clinic of General Surgery, İstanbul, Turkey
[2]University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital, Clinic of Pathology, İstanbul, Turkey

Abstract

Objective: Since the ultrasonography (US) examination and fine-needle aspiration (FNA) biopsy have been used in the clinicopathological evaluation of thyroid pathology, a progressive increase in the prevalence of incidental micropapillary carcinomas (IMC) has been reported. Here, we investigated the predictive factors of suspicious malignancy of thyroid nodules increasing with age.

Methods: A retrospective review of data of 173 patients who underwent FNA biopsy and subsequent thyroidectomy in the Clinic of General Surgery University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital between December 2016-December 2019 years.

Results: The mean age of the patients was 46.7±22.6 (18-80). Most of the patients underwent total thyroidectomy (90.8%) based on the indication of FNA (47.4%) and toxic goiters (26.6%). The 76 patients were euthyroid (43.9%). The US showed that the hypoechogenicity and loss of the halo were the most common findings (60.1% and 57.8%, respectively). The number of patients more than 45 years who showed an irregular margin in the thyroid nodule was higher than younger patients (p=0.016). Although malignancy was mostly observed in young patients (100% vs. 87.5%, p=0.0022), IMC was seen more in older patients (33, 38.8% vs. 45, 51.1%, p=0.104). The loss of Halo and type of surgery had a significant effect on the presence of incidental carcinoma (odds ratio: 0.307 and 14.428, p=0.014 and 0.0026, respectively).

Conclusion: The old age, type of surgery, radiological findings including the loss of Halo might have a potential impact on the presence of IMC in the preoperative assessment of nodules regarding the management of surgical procedure.

Keywords: Thyroid nodule, thyroidectomy, aging

INTRODUCTION

Thyroid cancers are the most common cancers among youth and adults (1). The increasing age of thyroid cancer diagnosis starting at age 40 has been correlated with higher mortality due to cancer (2). The survival rates of young patients with thyroid malignancy are higher than older patients, but there is a tendency to treat these young patients with more aggressive therapies such as thyroidectomy. The primary treatment modality for thyroid cancers is lobectomy or thyroidectomy to remove all or part of the thyroid, respectively. The younger patients may live many decades after treatment and may have more risks for long-term health effects due to a more aggressive treatment (3).

There are a limited number of studies showing that age affects the thyroid cancer risk (4-6), and epidemiological analyses reported a positive association between thyroid nodule formation and advancing patient age (7,8). However, the details and mode of this association with other risk factors (linear mode or a threshold effect) are poorly understood.

There is a significant increase in the number of new cases of thyroid cancers (9). Specifically, several microcarcinomas, which are small thyroid cancers, are diagnosed following a surgery performed for benign thyroid pathology. The microcarcinomas which are incidentally diagnosed are called incidental thyroid cancers, and if the size of these tumors is smaller than or equal

Address for Correspondence: Yüksel Altınel, University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital, Clinic of General Surgery, İstanbul, Turkey
Phone: +90 532 740 91 71 **E-mail:** dryukselaltinel@gmail.com **ORCID ID:** orcid.org/0000-0003-0113-4839

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to 1 cm, they are called incidental micropapillary carcinomas (IMC) (10). There some studies that approved that smaller thyroid cancers have a better prognosis than larger ones (11), however, it should be considered that insignificant thyroid tumors, particularly those less than 1 cm in diameter, might have an unfavorable prognosis (12). Compared to the ratio of thyroid nodules, the frequency of IMCs is relatively uncommon (13). Since the ultrasonography (US) examination and fine-needle aspiration (FNA) biopsy have been introduced and commonly used in the clinicopathological evaluation of thyroid pathology, a progressive increase in the prevalence of IMC has been reported (14). Therefore, it appears crucial and beneficial for clinicians to establish a detailed correlation between the clinical and histopathological features of patients with IMC to predict the risk factors for the IMC. Primarily, we aim to understand the impact of aging and secondly, any correlation with the demographic and clinical features regarding the presence of incidental IMC in our population following thyroidectomy which is related to benign or malignant FNA biopsy.

METHODS

The study was conducted in the Clinic of General Surgery University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital and retrospectively analysed regarding the data which is collected between December 2016-December 2019. This study was approved by the Clinical Research Ethics Committee of University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital (no: 2020.02.1.01.019; date: 07/02/2020). All procedures performed in this study involving human participants were under the ethical standards of the Institutional Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Patient consent could not be received from the patients due to the retrospective design of the study.

Patients

The selection criteria of the patients were based on the FNA biopsy and subsequent thyroidectomy, including all the data of demographics and clinical features. Patients with incomplete data were excluded from the study. Also, we excluded the patients without surgery during follow-up.

The data of patients, including the demographic features were sex, age, radiation exposure history, family history of thyroid diseases, type of surgery, the diameter of the nodule (mm). As clinical indications, FNA results, nodule size, compression/cosmetic, grave disease, toxic goiter, coexisting hyperparathyroidism was recorded. The outcomes of thyroid function tests, including free T3, free T4, and thyroid-stimulating hormone (TSH) and the corresponding diagnosis of hypothyroidism, euthyroidism, or hyperthyroidism were recorded. Normal ranges for serum-free T3, free T4, and TSH were 1.71-3.71 pg/mL, 0.70-1.48 ng/dL, and 0.35-4.94 IU/mL, respectively. Lymphadenectomy was performed from the central, regional, or central + regional. FNA cytology (FNAC) findings were determined following the Bethesda category, as well and an overall histopathological diagnosis (benign or malign) was recorded according to the pathology reports. All data were analyzed regarding the occurrence of incidental micropapillary carcinoma. IMC was determined as a well-differentiated single or multiple tumor smaller than 1 cm, diagnosed incidentally intra or extra-nodular region, inside or outside the same lobe with a benign or malign lesion. IMC was also confirmed by immunohistochemical positive staining for thyroglobulin, showing a follicular differentiation.

IMC with a size under 1 cm, which can coexist with benign or malignant nodules encountered in the same thyroid nodule, was examined by biopsy and correlated with the demographic and clinical features of all patients.

Ultrasound Imaging

Ultrasound imaging of the thyroid was performed by the Esaote Color Doppler US (MAG Technology Co, Ltd. Model: 796FDII Yung-ho City, Taipei, Taiwan) using a superficial probe (model no: LA523 13-4, 5.5-12.5 MHz) when the patient was in a supine position and the neck was hyperextended. The existence of a solitary nodule and the diameter of the nodule were recorded. Radiological findings recorded for each nodule involved echogenicity (hypoechoic or hyper- and isoechoic), margin (irregular or well-bordered), microcalcifications (absent or present), the peripheral halo (absent or present), increased vascularity (absent or present) and cervical lymph nodes (15).

Fine Needle Aspiration Cytology

All cytological and pathological examinations of thyroid samples were performed by an experienced cytopathologist. FNA biopsy was performed by the guidance of General Electric Logiq pro 200 US (Model number 2270968; GE Healthcare Korea, Seongnam SI, Gyean GGI-DO, Korea) using a 5.5-7.5 MHz probe, 27-gauge needle and a 20 mL volume syringe. All nodules showing one or more suspicious US indications, determined by the decision of the physician, were examined by FNAC. The aspiration samples were fixed by air drying and stained by May-Grunwald-Giemsa for cytological evaluation.

The Bethesda System for Reporting Thyroid Cytopathology System was used to analyze FNAC samples described as non-diagnostic or

unsatisfactory (I), benign (II), atypia of undetermined significance or follicular lesion of undetermined significance (AUS/FLUS) (III), follicular neoplasm/suspicious for follicular neoplasm (including oncocytic lesions) (IV), suspicious for malignancy (V), malignant (VI) (16).

Statistical Analysis

Power calculations were performed based on the use of two sample t-tests. Using two-sided tests with a significance level of 0.05, there was at least 80% power to detect large effect sizes of 0.8 or more between age groups. With the planned sample size, there was at least 80% power to detect large differences exceeding 30% in the percentage of age groups. Given the cross-sectional nature of these estimates, they provided conservative estimates of power, and small effect sizes were detectable when evaluating these outcomes in a longitudinal model. Power calculations were performed using G-Power version 3.1.

Frequency, percentage values are described for the categorical variables. Mean, standard deviation, median, minimum and maximum values are described for the continuous variables. Normal distribution testing of continuous variables was performed using the Kolmogorov-Smirnov test. Categorical variables were compared with chi-square analysis. When appropriate, the categorical variables were analyzed by Fisher's Exact and Fisher-Freeman-Halton test. For the variables that did not execute the normal distribution assumption, Mann-Whitney U test was used for a comparison of two independent groups. Binary logistic regression was performed to determine the effect of variables on incidental carcinoma. A receiver operating characteristic (ROC) curve was constructed to assess the sensitivity and specificity of the loss of Halo to diagnose the IMC. The ideal cut off value was determined using the Youden index and accordingly, the sensitivity and specificity of the loss of Halo were calculated. The change in the related areas under the curve (AUC) was tested using the DeLong test (17). Pearson correlation coefficient analysis (Pearson's r measures the linear correlation between the IMC and the clinicopathological findings of thyroid nodules.

Statistical analyses were performed using NCSS 11 (Number Cruncher Statistical System, 2017 Statistical Software) and MedCalc Statistical Software version 18 (MedCalc Software bvba, Ostend, Belgium). A p value <0.05 was set as significant.

RESULTS

The demographic and clinical features of all patients are presented in Table 1. The total number of patients was 173, 74% of these patients were female and 26% were male. The mean age of all patients was 46.7±22.6 (18-80 years). Only one patient younger than 45 (0.6%) had radiation therapy on the neck, and three patients (1.7%) had a family history of thyroid carcinoma (Table 1).

Most of the patients underwent total thyroidectomy (90.8%) based on the indication of FNA (47.4%) and the presence of toxic goiter (26.6%). The rarest surgical indications were coexisting hyperparathyroidism and the nodule size (2.9% and 5.2%, respectively). The mean diameter of nodules was found 22.6±14.9 mm. No significant difference was found in the surgical indications and diameter of nodules between the patients ≤45 and >45 years old (Table 1).

The 76 patients were euthyroid (43.9%). Hyperthyroidism is the rarest status of thyroid function among patients (15.1%). There was no significant difference in the distribution of any thyroid function between the patients ≤45 and >45 years old (Table 1).

Radiological examination showed that the hypoechogenicity and loss of Halo were the most common findings among patients without any significant difference between age groups (60.1% and 57.8%, respectively). The number of patients older than 45 years (30.7%) who showed an irregular margin in the thyroid nodule was significantly higher than younger patients (p=0.016). Comparing FNAC findings, the suspicious nodules for cancer and AUS/FLUS were the most predominant cytopathological diagnoses among the patients (28.9% and 23.1%, respectively) but no significant difference was found between the age groups (Table 1).

The detailed pathological analysis of the thyroid specimens indicated that the most common histopathological diagnosis was the papillary carcinoma and Hashimoto's thyroiditis among the patients (39.3% and 26.6%, respectively). However, there was no significant difference between the age groups in terms of the pathology of the thyroid nodules. Other pathological diseases are given in Table 1. Depending on the overall pathological diagnoses, 85 patients under 45 years (100% of the younger age group) had a diagnosis of thyroid malignancy, while 77 patients older than 45 (87.5% of older age group) had malignancy, (p=0.0022). Lastly, 45.1% of patients were diagnosed with an IMC, without any difference among age groups (Table 1). Even though, malignancy was mostly seen in the young population (>45 years old), (85, 100% vs. 77, 87.5%, p=0.0022), the IMC was seen more likely in older population (>45 years old), (33, 38.8% vs. 45, 51.1%, p=0.104), (Table 1).

Logistic regression univariate analysis of patients based on the presence of IMC (Table 2) indicated that the loss of Halo defined

Table 1. Demographic and clinical features of the patients in comparison of the age					
Variable		**Total n=173**	**Age ≤45 n=85**	**Age >45 n=88**	**p°**
Sex n (%)	Female Male	128 (74) 45 (26)	65 (76.5) 20 (23.5)	63 (71.6) 25 (28.4)	0.465
Age (year)	Mean ± SD Min-max	46.7±22.6 18-80	35.8±7.3 18-45	57.1±8.4 46-80	-
Radiation history	n (%)	1 (0.6)	1 (1.2)	0 (0)	0.308*
Family history	n (%)	3 (1.7)	1 (1.2)	2 (2.3)	0.580*
Surgical indication n (%)	FNA Nodule size Compression/cosmetic Graves' disease Toxic goiter Coexisting hyperparathyroidism	82 (47.4) 9 (5.2) 17 (9.8) 14 (8.1) 46 (26.6) 5 (2.9)	40 (47.1) 6 (7.1) 10 (11.8) 6 (7.1) 21 (24.7) 2 (2.4)	42 (47.7) 3 (3.4) 7 (8.0) 8 (9.1) 25 (28.4) 3 (3.4)	0.797**
Status of thyroid function n (%)	Hypothyroidism Euthyroidism Hyperthyroidism	71 (41.0) 76 (43.9) 26 (15.1)	35 (41.2) 35 (41.2) 15 (17.6)	36 (40.9) 41 (46.6) 11 (12.5)	0.591
Type of surgery n (%)	Lobectomy Total thyroidectomy	16 (9.2) 157 (90.8)	10 (11.8) 75 (88.2)	6 (6.8) 82 (93.2)	0.262
The diameter of the nodule (mm)	Mean ± SD Min-max	22.6±14.9 3.0-70.0	23.7±14.9 3-65	21.6±14.8 4-70	0.267***
Radiological findings n (%)	Hypo echogenicity	104 (60.1)	47 (55.3)	57 (64.7)	0.203
	Irregular margin	40 (2.3)	13 (15.3)	27 (30.7)	**0.016**
	Microcalcifications	46 (26.6)	18 (21.2)	28 (31.8)	0.113
	Loss of Halo	100 (57.8)	50 (58.8)	50 (56.8)	0.924
	Increased vascularity	23 (13.3)	13 (15.3)	10 (11.4)	0.447
	Cervical lymph nodes	62 (35.8)	34 (40)	28 (31.8)	0.262
FNAC findings n (%)	Non-diagnostic/unsatisfactory Benign AUS/FLUS Follicular neoplasm Cancer suspicious Cancer	16 (9.2) 31 (17.9) 40 (23.1) 12 (6.9) 50 (28.9) 24 (13.9)	8 (9.4) 15 (17.6) 22 (25.9) 4 (47.1) 22 (25.9) 14 (16.5)	8 (9.1) 16 (18.2) 18 (20.5) 8 (9.1) 28 (31.8) 10 (11.4)	0.684
Lymphadenectomy	Central Regional Central + regional	13 (7.5) 14 (8.1) 4 (2.3)	8 (9.4) 8 (9.4) 3 (3.5)	5 (5.7) 6 (6.8) 1 (1.1)	0.468
Pathology	Adenomatous nodule	17 (9.8)	5 (5.9)	12 (13.6)	0.465
	Colloidal nodule	20 (11.6)	9 (10.6)	11 (12.5)	
	Hemorrhagic cyst	3 (1.7)	1 (1.2)	2 (2.3)	
	Graves' disease	6 (3.5)	3 (3.5)	3 (3.4)	
	Hashimoto's thyroiditis	46 (26.6)	28 (32.9)	18 (20.5)	
	Follicular adenoma	2 (1.2)	1 (1.2)	1 (1.1)	
	Hurthle cell adenoma	1 (0.6)	0 (0)	1 (1.1)	
	Follicular carcinoma	0 (0)	0 (0)	0 (0)	
	Papillary carcinoma	68 (39.3)	32 (37.6)	36 (40.9)	
	Hurthle cell carcinoma	3 (1.7)	1 (1.2)	2 (2.3)	
	Poor differentiated carcinoma	2 (1.2)	2 (2.4)	0 (0)	
	Medullary carcinoma	5 (2.9)	3 (3.5)	2 (2.3)	
Overall pathology	Benign Malign	11 (6.4) 162 (93.6)	0 (0) 85 (100)	11 (12.5) 77 (87.5)	**0.0022**
Incidental micropapillary carcinoma	n (%)	78 (45.1)	33 (38.8)	45 (51.1)	0.104

Normally distributed data were recorded as mean ± standard deviation, SD: Standard deviation, FNAC: Fine-needle aspiration cytology, AUS/FLUS: Atypia of undetermined significance or follicular lesion of undetermined significance. °Chi-square analysis, *Fisher's Exact test, **Fisher-Freeman-Halton test, ***Mann-Whitney U test (p value <0.05 as significant)

by the US had a significant effect on the presence of incidental carcinoma [odds ratio (OR): 0.307; 95% confidence interval (CI): 0.125-0.751; p=0.014]. As we expected, the type of surgery, such as total thyroidectomyhad a considerable effect on seeIMC (OR: 14.428; 95% CI: 1.861-112.01; p=0.0026), (Table 2).

Logistic regression multivariate analysis of patients based on the presence of IMC (Table 3) also indicated that the loss of Halo significantly increased the risk of the presence of IMC by 4.036 times (95% CI: 1.499-10.864; p=0.006). Moreover, total thyroidectomy was helpful to find IMC18.13 times more than lobectomy (95% CI: 2.121-148.59; p=0.007) (Table 3).

The correlation between the ratio of IMC and the clinical and pathological findings of thyroid nodules (Table 4) revealed that there was a higher correlation between the total thyroidectomy and the presence of IMC (r=0.249; 95% CI: 0.104-0.384; p=0.0009). Additionally, the loss of Halo was also significantly

Table 2. Univariate analysis of patients based on the presence of incidental micropapillary carcinoma

Variable		Odds ratio*	95% CI	p
Sex		0.966	0.488-1.912	0.920
Age		1.010	0.988-1.034	0.374
Radiation history		0.401	0.016-9.997	0.364
Family history		0.604	0.054-6.791	0.680
Surgical indication	FNA Nodule size Compression/cosmetic Graves' disease Toxic goiter Coexisting hyperparathyroidism	0.827 1.028 2.933 1.103 0.969 0.196	0.454-1.507 0.266-3.967 0.916-9.395 0.366-3.328 0.492-1.909 0.022-1.799	0.640 0.968 0.104 0.861 0.928 0.256
Status of thyroid function	Hypothyroidism Euthyroidism Hyperthyroidism	1.562 0.849 0.661	0.843-2.896 0.464-1.551 0.286-1.527	0.206 0.704 0.447
Type of surgery	Total Thyroidectomy	14.438	1.861-112.01	**0.0026****
Diameter of nodule		0.988	0.967-1.008	0.236
Radiological findings	Hypo echogenicity Irregular margin Microcalcifications Loss of Halo Increased vascularity Cervical lymph nodes	1.011 1.293 0.915 0.307 0.720 0.899	0.548-1.864 0.637-2.626 0.464-1.806 0.125-0.751 0.299-1.736 0.482-1.679	0.973 0.595 0.934 **0.014** 0.611 0.862
FNAC findings n (%)	Non-diagnostic/insufficient Benign AUS/FLUS Follicular neoplasm Cancer suspicious Cancer	0.610 0.835 1.146 0.809 0.950 1.772	0.216-1.720 0.391-1.853 0.561-2.340 0.250-2.616 0.491-1.837 0.715-4.393	0.498 0.835 0.846 0.957 0.878 0.305
Lymphadenectomy	Central Regional Central + regional	3.165 1.876 1.407	0.931-10.766 0.618-5.691 0.193-10.273	0.065 0.267 0.737
Pathology	Adenomatous nodule Colloidal nodule Hemorrhagic cyst Graves' disease Hashimoto's thyroiditis Follicular adenoma Hurthle cell adenoma Papillary carcinoma Hurthle cell carcinoma Poor differentiated carcinoma Medullary carcinoma	1.571 1.265 1.656 0.815 0.764 4.198 0.271 0.570 0.404 9.541 0.819	0.553-4.462 0.489-3.27 0.147-18.62 0.160-4.159 0.388-1.502 0.198-88.81 0.011-6.73 0.218-1.493 0.036-4.546 0.519-175.4 0.05-13.321	0.550 0.805 0.680 0.806 0.543 0.566 0.921 0.357 0.863 0.110 0.888
Overall pathology	Benign or malign	1.472	0.415-5.225	0.774

FNAC: Fine-needle aspiration cytology, CI: Confidence interval, AUS/FLUS: Atypia of undetermined significance or follicular lesion of undetermined significance, Logistic regression*, p value <0.05 as significant**

correlated with the presence of IMC (r=0.204; 95% CI: 0.057-0.343; p=0.0071).

The result of ROC analysis for the loss of Halo to predicting the IMC is given in Figure 1. The AUC from the set tested was found as 0.64 (95% CI: 0.57-0.71), the sensitivity and specificity were 0.69 (95% CI: 0.48-0.85) and 0.59 (95% CI: 0.50-0.70), respectively.

DISCUSSION

Several studies are comparing the possible effect of increasing age on the risk of thyroid malignancy, reporting some conflicting findings as we consider (18,19). A large-scale, prospective analysis examining this possible effect in the patients presenting with thyroid nodules confirmed an increased prevalence with increasing age and a reduced risk of malignancy in these nodules, which is opposite to what we found such as a high amount of IMC correlated with increasing age (20). Although thyroid nodules are more common and more likely benign among older patients, there are also studies suggesting that the aggressive nature of thyroid cancers is more likely to augment with increasing age (20). Contrary to our findings, the number of older patients (>45 years) who showed an irregular margin in the thyroid nodule was significantly higher than younger patients. Depending on the overall pathological diagnoses, all young patients and 87.5% of older patients had a diagnosis of thyroid malignancy, with a considerable difference between the age groups. Even though the malignancy was mostly seen in the young population, the IMC was seen more likely in the older population, suggesting that early relevant diagnosis of thyroid nodules provides a critical step for the optimal outcome.

Throughout the recent decades in Turkey, regarding the changes in the surgical approach for thyroid nodules with lower Bethesda class (Bethesda classes I, II, and III), the suggestions for the surgery have been re-considered. Therefore, follow-up of suspicious nodules and repeating FNAC is generally recommended for the clinical management of these thyroid nodules (21). A very recent

Table 3. Multivariate analysis of patients based on the presence of incidental micropapillary carcinoma

Variable	Odds ratio*	95% CI	p
Type of surgery	18.13	2.212-148.59	**0.007****
Loss of Halo	4.036	1.499-10.864	**0.006****

CI: Confidence interval, Binary Logistic regression*, p value <0.05 as significant**

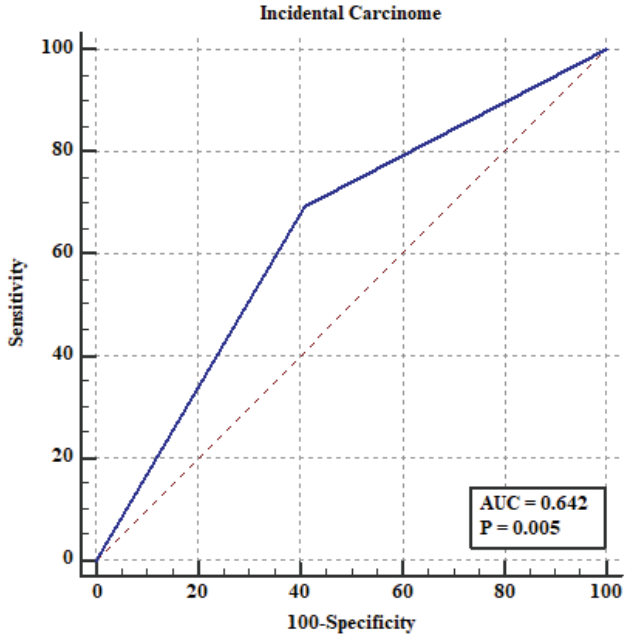

Figure 1. ROC analysis for the loss of Halo to predict the incidental micropapillary carcinoma
AUC: Area under the curve, ROC: Receiver operating characteristic

Table 4. Correlation between the incidental micropapillary carcinoma and the clinical and pathological findings of thyroid nodules

Variable		Pearson r*	95% CI	p
Status of thyroid function		0.112	-0.039-0.257	0.145
Type of surgery		0.249	0.104-0.384	**0.0009****
Diameter of nodule		-0.091	-0.237-0.060	0.236
Radiological findings n (%)	Hypo echogenicity	0.003	-0.147-0.152	0.973
	Irregular margin	0.054	-0.096-0.202	0.479
	Microcalcifications	-0.019	-0.168-0.130	0.800
	Loss of Halo	0.204	0.057-0.343	**0.0071****
	Increased vascularity	0.056	-0.094-0.203	0.466
	Cervical lymph nodes	0.025	-0.124-0.174	0.741
FNAC findings		-0.084	-0.231-0.066	0.270
Overall pathology		0.046	-0.104-0.194	0.551

FNAC: Fine needle aspiration cytology, CI: Confidence interval, *Pearson correlation coefficient (r), **p value <0.05 as significant

study from Turkey reported malignancy rates ranging from 10 to 30% for Bethesda class III and 25-40% for class IV, while we did not find any correlation or impact regarding the Bethesda and aging process in our study (22). An evaluation of thyroid nodules in the Turkish population reported that age, gender, and thyroid status were not associated with increased risk of malignancy but irregular margins and microcalcifications have an impact, which is consistent with our findings while the younger population had more malignancy. They concluded that US features, especially microcalcification, is a critical predictor of malignancy without age, gender, and thyroid status and we evaluated especially loss of Halo and irregular margins affected the IMC increasing with age (23). Uyar et al. (24) evaluated patients with suspicious solitary nodules undergoing bilateral total thyroidectomy for the presence of malignancy and concluded that irregular margins, microcalcification, increased vascularity, and detection of cervical lymphadenopathy were correlated with malignancy in solitary nodules. Consistently, the irregular margin among the radiological findings of thyroid nodules was observed commonly in older patients due to a possible increase in Bethesda classification based on the FNA biopsy results.

The diagnosis of incidental carcinoma in patients who have undergone thyroidectomy for a benign disease is quite common. A retrospective analysis of the findings of surgical intervention to establish the incidence of the carcinoma reported 50% that the only way to determine the exact benign feature of the thyroid disease is FNAC (25). Even we analysis retrospectively, we performed similar features as Atli et al. (26) which was prospectively defined the risk factors predicting thyroid malignancy to establish management criteria for thyroid incidentalomas. Their multivariate analyses revealed that the independent clinical predictors of malignancy were a fixed nodule, cervical lymphadenopathy, euthyroidism, and a patient age <23 years or more than 45 years that we measured among >45 years old. Moreover, they found a significant association between the independent nodule features with malignancy and microcalcifications, irregular margin, solid appearance on USG, consistent with some findings. As we measured, IMC was determined in 45.1% of the patients with benign or malignant nodules encountered in the same thyroid nodule or lobule following thyroidectomy. Logistic regression analysis indicated that the loss of Halo on USG significantly increased the risk of the presence of IMC by 4.036 times. Moreover, a total thyroidectomy was effective in the diagnosis of IMC for 18.13 times more than lobectomy. It was reported that the correlation between the ratio of IMC and the clinical and pathological findings of thyroid nodules was more likely with the total thyroidectomy and he loss of Halo. Supporting these results, ROC analysis for the loss of Halo presented relatively high sensitivity and specificity for predicting potential IMC presence. Depending on the radiological findings of a thyroid nodule, a simple follow-up including an FNAC might be proposed for managing suspicious malignancy regardless of the diameter measurements.

Study Limitations

However, the limitation in our study were that small sample sizes have been analyzed retrospectively from the data of a single center and thyroid surgery group from a restricted region, which may limit the generalization to other regions and groups. Secondly, our study was limited by its retrospective analysis, including measurement, observational, and recall biases. Also, no stepwise or any other machine learning models were further used to measure the performance of our prediction model. Even although we have some limitations, the current study has some clinical diagnostics, which could measure the predictors of the presence of IMC, which guide the surgical decision based on both the FNAC and radiological results regardless of the diameter measurements.

CONCLUSION

Due to the increased incidence of IMC in patients operated for benign thyroid disease, an accurate preoperative assessment with a careful selection of nodules for FNAC correlated with US patterns is becoming a necessity in the choice of surgical procedure. Especially with careful investigation of malignancy in the young population (>45 years old) and the presence of the IMC in the older population (>45 years old) have been warranted in larger sample size populations regarding our outcomes.

Consequently, the patient age, surgical approach, and radiological findings such as loss of Halo, may help consider the potential presence of IMC in overall pathological examinations that might warrant a careful surgical management of patients with thyroid nodules.

Ethics

Ethics Committee Approval: The study was conducted in the Clinic of General Surgery University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital and retrospectively analysed regarding the data which is collected between December 2016-December 2019. This study was approved by the Clinical Research Ethics Committee of University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital (no: 2020.02.1.01.019; date: 07/02/2020).

Informed Consent: Patient consent could not be received from the patients due to the retrospective design of the study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: Y.A., A.A., S.M., M.T., Y.Ü., Design: Y.A., N.A.H., A.A., S.M., M.T., Data Collection or Processing: Y.A., M.K., Y.Ü., Analysis or Interpretation: Y.A., A.A., M.T., Literature Search: Y.A., N.A.H., S.M., Y.Ü., Writing: Y.A., N.A.H.

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Aflibercept in a Persistent Diabetic Macular Edema Refractory to Previous Ranibizumab Therapy

Gözde Aksoy Aydemir[1], Nurten Ünlü[2], Güner Üney Özkan[2], Dicle Hazırolan[2], Mehmet Akif Acar[3], Firdevs Örnek[2]

[1]Adıyaman University Training and Research Hospital, Clinic of Ophthalmology, Adıyaman, Turkey
[2]University of Health Sciences Turkey, Ankara Training and Research Hospital, Clinic of Ophthalmology, Ankara, Turkey
[3]Ankara Yıldırım Beyazıt University, Clinic of Ophthalmology, Ankara, Turkey

Abstract

Objective: To investigate the visual acuity and anatomical outcomes of intravitreal aflibercept treatment in patients with diabetic macular edema (DME) who were unresponsive to ranibizumab.

Methods: Patients with refractory DME treated with at least 3 consecutive injections of ranibizumab, 4-6 weeks apart, before switch and with at least 2 aflibercept injections after that in the period of May 2013 to October 2017 were considered eligible for study participation. "The patients" demographic characteristics, best-corrected visual acuity (BCVA), and central foveal thickness (CFT) were recorded at baseline, pre-switch, the first month post-switch, and the final visit.

Results: A total of 33 eyes of 28 patients were investigated. The average number of ranibizumab injections before switching to aflibercept was 4.97±1.94 and that of the subsequent aflibercept injections was 2.54±0.6. The mean baseline BCVA was 0.56 ± 0.38 logMAR. After the switch, the BCVA during the first and final visits was 0.41±0.34 logMAR (p=0.19) and 0.36±0.34 (p=0.16), respectively. After switching, clinical follow-up data for at least 6 months were available for all eyes. The mean baseline CFT was 504±123.7 µm (264-844 µm).One month after the switch, the average CFT had significantly reduced to 338.8±105.3 µm (225-615 µm) (p=0.0001). At the final visit, the average CFT was 345.7±137.4 µm (136-892 µm) (p=0.0002). Before and after the switch, the mean intraocular pressure (IOP) was 14.18±3.66 mmHg and 13.54±3.81 mmHg respectively (p=0.46).

Conclusion: Switch to aflibercept from ranibizumab in patients with recalcitrant DME resulted in significant anatomical improvements. Although the BCVA increased and the IOP decreased, these changes were not statistically significant.

Keywords: Aflibercept, diabetic macular edema, ranibizumab, switch, treatment resistance/refractory

INTRODUCTION

Diabetic macular edema (DME) is an important cause of visual impairment in patients with diabetes and significantly affects the quality of life (1). Elevated intraocular levels of vascular endothelial growth factor (VEGF) support retinal vascular permeability, leading to macular edema in patients with diabetes (2). Recently, intravitreal injections of anti-VEGF agents have been proven as the essential treatment for DME (3) owing to their efficacy in diminishing macular edema in diabetic eyes with the use of drugs, such as ranibizumab (3,4), bevacizumab (5), pegaptanib (6), and aflibercept (7,8).

After the RISE and RIDE phase 3 clinical trials (3,4), ranibizumab (Lucentis; Genentech, South San Francisco, California, USA) became the first VEGF inhibitor certified by the Food and Drug Administration for DME in 2012. Ranibizumab is an antibody fragment with a binding affinity toward all forms of VEGF-A.

This study was presented as a poster at the TOD. 51. National Congress, Antalya, Turkey, Oct 2017.

Address for Correspondence: Gözde Aksoy Aydemir, Adıyaman University Training and Research Hospital, Clinic of Ophthalmology, Adıyaman, Turkey
Phone: +90 553 229 33 32 **E-mail:** gzdaksoy@hotmail.com **ORCID ID:** orcid.org/0000-0002-5708-9283

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Aflibercept (Eylea; Regeneron, Tarrytown, New York) is a 115-kDA recombinant fusion protein that includes the key VEGF-binding domains of human VEGF receptors 1 and 2 fused to the constant region of human G1 (9). Aflibercept has shown to have a higher binding affinity to VEGF-A than to ranibizumab and immunoglobulin bevacizumab in a preclinical trial (10). Unlike ranibizumab and bevacizumab, aflibercept also binds to VEGF-B and placental growth factor that may inhibit vascular permeability and retinal neovascularization (10).

Currently, many patients who have undergone ranibizumab or bevacizumab treatment for DME and have failed to respond to these drugs are being switched to aflibercept. Differences in the pharmacodynamics of aflibercept compared with those of ranibizumab and bevacizumab are the basis of this strategy. However, thus far, there has been no consensus regarding the ideal time to consider a therapeutic switch (11). Some practitioners choose an "early switching" strategy because they believe that long-standing macular edema may lead to chronic retinal damage and worse prognosis; however, this scenario does not account for late responders (11). According to the first-year results of the DRCR (protocol T), aflibercept achieved the best results in low-vision patients (8). However, a recent trial on low-vision patients demonstrated no difference in the effect of aflibercept and ranibizumab (p=0.18) at the 2-year follow up (12).

Thus, the purpose this study was to demonstrate the short-term functional and anatomical responses of intravitreal aflibercept in a series of patients with persistent DME who failed to respond to multiple intravitreal ranibizumab injections.

METHODS

The protocol of this study was approved by the Institutional Review Board of the Eye Clinic of the Ankara Training and Research Hospital Ethic Committee (approval number: 308, approval date: 06.12.2017). This was a retrospective, non-comparative, consecutive case series of patients treated with DME. All the research was carried out in accordance with the Helsinki Declaration and by obtained written informed consent from the patients.

The eligible patients were aged ≥18 years, had a history of diabetes mellitus (type 2), evidence of clinically significant macular edema as defined by the Early Treatment Diabetic Retinopathy Study (13) and center-involving DME which is described as central 1 mm area of more than ≥300 µm measured by spectral-domain optical coherence tomography [(SD-OCT); Heidelberg Engineering, Heidelberg, Germany].

We identified DME patients who were unresponsive to ranibizumab; unresponsiveness was defined as no reduction in the CFT, increase in CFT (using SD-OCT), or gain in BCVA of less than 1 line at 1 month following at least 6 months of continuous ranibizumab treatment compared to that at baseline. All patients with center involving DME who had received at least three monthly ranibizumab injections before the switch and had received at least 2 aflibercept injections after the switch were eligible for study inclusion.

Patients were excluded if they had previous ocular trauma, macular pathologies, vitreomacular adhesion or traction, the presence of intraretinal/sub- retinal fluid using 12 radial line scans through the fovea, epiretinal membrane, tractional retinal detachment, vitreous hemorrhage, had received intravitreal or sub-tenon injection corticosteroids, or had any history of prior intraocular surgeries (except uncomplicated cataract surgery). Aflibercept was injected >4 weeks after the ranibizumab therapy was completed.

The demographic, examination, and treatment data of the eligible patients were extracted from their clinical charts. The BCVA of patients was recorded using Snellen's chart and then converted to logMAR for statistical analysis. Intraocular pressure (IOP) recordings performed using pneumotonometry (non-contact tonometer 10; Shin-Nippon Machinery Co, Japan) before dilatation and injection at every visit, as well as the results of biomicroscopic examination of the anterior segment and fundus using an indirect ophthalmoscope were recorded. Follow-up SD-OCT scans were performed at each visit for documentation.

Data regarding the patients' demographic characteristics; glycosylated hemoglobin levels; presence of coexisting chronic disease; lens status; quantity of pre-switch ranibizumab and post switch aflibercept injections; as well as the BCVA, IOP, and CFT at baseline, pre-switch, the first visit post-switch, and final visit were recorded.

Statistical Analysis

The study data were analyzed using the Statistical Package for Social Sciences (SPSS), version 24.0 for Windows (SPSS Inc., Chicago, IL). Descriptive data were presented as the mean ± standard deviations, frequency distributions, and percentages.

RESULTS

We studied 33 eyes in 28 patients who were eligible according to the inclusion criteria with DME unresponsive to ranibizumab treatment. The mean patient age was 58.85±10.37 years (range 36-80 year). Basic demographic and ocular characteristics are

shown in Table 1. Some patients exhibited the coexistence of chronic disease, such as hypertension, coronary artery disease, and chronic renal failure.

Treatment Characteristics

The average number of ranibizumab injections in the 6-month period before switching to aflibercept was 3.66±0.77. Patients received an average total of 2.54±0.6 injections at 6 months after switching to aflibercept. No cases of endophthalmitis, retinal detachment, or elevated IOP were observed. The most common adverse effects were local hyperemia or subconjunctival hemorrhage at the injection site.

Table 1. Demographic and ocular characteristics of patients with DME converted from prior ranibizumab treatment to aflibercept therapy

Age (y)	
Mean (SD)	58.85 (10.37)
Median (min, max)	57 (36-80)
Sex (%)	
Male	16 (57%)
Female	12 (43%)
Duration of known diabetes (y)	
Mean (SD)	12.98 (4.35)
Median (min, max)	12 (3-25)
Glycosylated hemoglobin level	
Mean (SD)	7.90 (1.49)
Median (min, max)	7.80 (6.50-11.80)
Lens status (%)	
Pseudophakic	13 (39.4%)
Phakic	20 (60.6%)
Total pre-switch ranibizumab injections	
Mean (SD)	4.97 (1.94)
Median (min, max)	5 (3-8)
Number of ranibizumab injections in the previous 6 months	
Mean (SD)	3.66 (0.77)
Median (min, max)	3 (3-5)
Number of aflibercept injections post-switch	
Mean (SD)	2.54 (0.6)
Median (min, max)	2 (2-7)
Other prior treatments (>6 months from conversion) (%)	
Pan retinal photocoagulation	9 (27%)
Focal macular laser	2 (6%)
Intravitreal triamcinolone	2 (6%)
Dexamethasone implant	7 (21%)

y: Years, SD: Standard deviation, min: Minimum, max: Maximum, DME: Diabetic macular edema

Visual Outcomes After Switch to Aflibercept

Before the administration of the ranibizumab injections, the mean baseline BCVA was 0.56±0.38 logMAR, while after an average 4.97±1.94 injection, the pre-switch BCVA was 0.44±0.33 logMAR; the difference was not statistically significant. After the switch, the first-visit BCVA was 0.41±0.34 log MAR (p=0.19), and the final visit BCVA was 0.36±0.34 (p=0.16) (Figure 1). Seventeen of the 33 eyes (51.5%) were treated with 2 consecutive aflibercept injections. In the post-hoc analyses, there was no significant difference between the eyes that had received 2 consecutive aflibercept injections and those that had received 3 or more injections. Notably after at least 2 aflibercept injections, 6 eyes (18%) had been preserved in their initial visual acuity, 15 eyes (45%) exhibited an improvement of 1-2 lines of vision, and 4 eyes (12%) showed improvement in 3-4 lines of vision. Eight eyes had worse vision after switching to aflibercept. Consecutive visual acuity (VA) measurements recorded at the subsequent visits are outlined in Table 2. There was no correlation between the last BCVA (logMAR) and the number of post-switch injections (Spearman's rho-0.056, p=0.7). Subgroup analyses were performed to identify the effect of the pre-switch VA, classified as VA ≥20/40 (17 eyes) and VA <20/40 (16 eyes), on the visual response to change in therapy. After switching to aflibercept, better final vision was achieved in the VA ≥20/40 (17 eyes) group than in the VA <20/40 (16 eyes) group (p=0.000073).

Anatomic Outcomes After Switch to Aflibercept

Before the ranibizumab injections were administered, the mean baseline CFT was 504±123.7 µm (264-844 µm). After the ranibizumab injections were given, the pre-switch CFT was 451.5±113 µm (286-822 µm) (p=0.06), while the CFT on the first post-switch visit was significantly lower at 338.8±105.3 µm (225-615 µm) (p=0.0001). The CFT at the final visit was 345.7±137.4 µm (136-892 µm), a significant improvement over the pre-switch CFT (p=0.0002) (Figure 1). There was no correlation between the CFT at the final visit and the number of post-switch injections (Spearman's rho +0.310, p=0.07).

Intraocular Pressure After Switching to Aflibercept

The average IOP registered at the pre-switch visit was 14.18±3.66 mmHg (median: 13; range: 10-22 mmHg). At the final visit, the average IOP was 13.54±3.81 mmHg (median: 13; range, 10-25 mmHg), indicating a mean decrease of 0.7 mmHg (p=0.46) (Figure 1).

DISCUSSION

During DME treatment, physicians may choose to switch among anti-VEGF agents for various reasons. The clinical reasons include the theoretically greater affinity of aflibercept for VEGF and the fact that it also binds to and neutralizes the placental

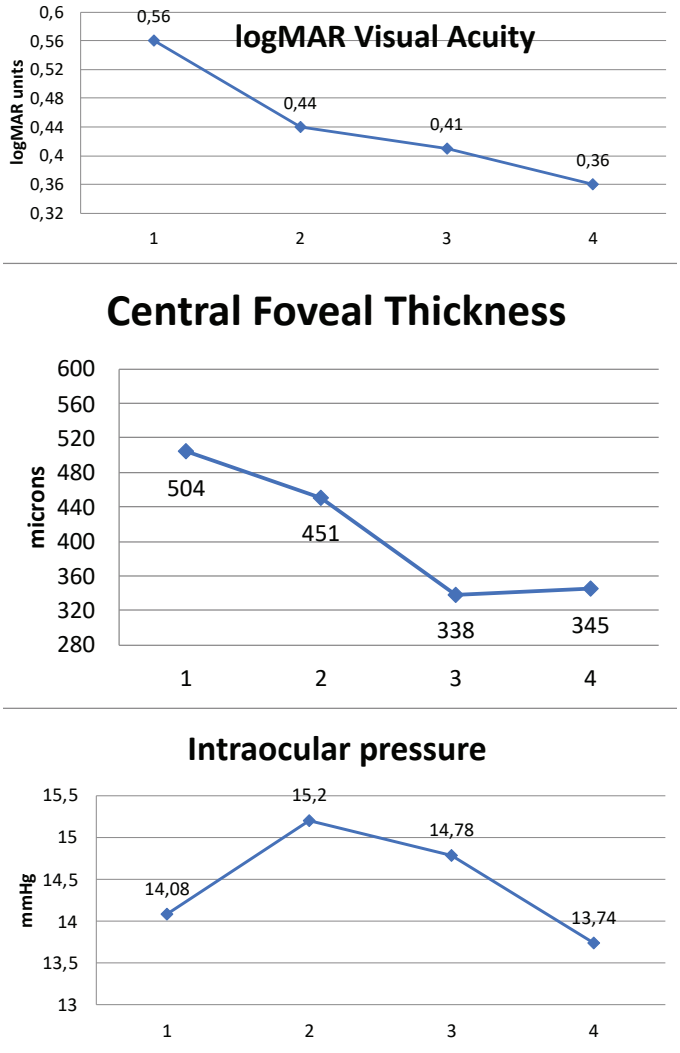

Figure 1. Comparison of visual acuity, central foveal thickness, and intraocular pressure before and after conversion to aflibercept for diabetic macular edema (1: At baseline, 2: At the pre-switch visit, 3: At the first visit post-switch, 4: At the final examination)

growth factor. These pharmacodynamic differences may be particularly useful in situations where a patient is unresponsive to ranibizumab or bevacizumab. Additionally, physicians may choose to switch a patient to aflibercept owing to its reportedly longer duration of action. A group patients with diabetes with macular edema may lead to resistance to ranibizumab or bevacizumab therapy. Additionally, tachyphylaxis, which is a fair response to ranibizumab, or bevacizumab, may also be observed in a group of patients.

Our results confirm the important role of aflibercept in patients with DME who do not respond to ranibizumab injections. In this study, 33 patients with persistent DME refractory to prior ranibizumab therapy were treated with at least 2 aflibercept injections. Although anatomical improvements were significantly reduced in both post-switch first month and final visit CFT compared with pre-switch CFT, it was not statistically significant. After the switch, the number of injections performed in our study may appear limited. However, as per our correlation analyses, there is no substantial change in BCVA and CFT with the respect to the number of injections. Similar to our findings, Chen et al. (14) demonstrated that nearly 50% of the patients showed no significant changes 2 or 3 months after the switch. Sub-group analyses were performed to identify the effect of the pre-switch VA, classified as VA ≥20/40 (17 eyes) or <20/40 (16 eyes), on the visual response to the change in therapy. Our study showed that for a good patient outcomes following switch, it is important to have good pre-switch BCVA because a better pre-switch BCVA translated into superior final BCVA in our patients. Dugel et al. (15) demonstrated that patients with a baseline BCVA >70 letters could gain up to an average of >5 letters, similar outcomes were observed in our study. Some recommend early switching because they believe that persistent macular edema leads to further deterioration of VA and may inhibit a functional response; in contrast, others, recommend delayed switching to consider the possibility of late responders (16). Aslan et al. (17) retrospectively reviewed 76 eyes of 50 patients, they stated that the better the first vision, the better the last vision.

Recently, 3 studies have demonstrated the efficacy of intravitreal aflibercept in patients with DME refractory to bevacizumab and ranibizumab. Chen et al. (14) retrospectively reviewed 72 eyes with DME unresponsive to ranibizumab and/or bevacizumab and subsequently switched to aflibercept. About 2/3rd exhibited beneficial effects of the subsequent 3-monthly intravitreal aflibercept injections. Compared with the pre-switch VA and anatomical measurements, especially significant visual gains and anatomical improvements were observed at 1 month but not at 2 or 3 months after the switch to aflibercept. Several non-responders reported having undergone vitrectomy. Rahimy et al. (18) retrospectively reviewed 50 eyes with persistent DME where the treatment was switched to aflibercept. Similar to our results, they found no significant change in VA but there was significant anatomic improvement, after 4.1 aflibercept injections over 4.6 months of subsequent injections. Herbaut et al. (19) retrospectively reviewed 25 eyes with resistant DME after at least 3 ranibizumab and/or one dexamethasone implant intravitreal injection. They observed not only significant anatomical improvements but also significant BCVA improvement between the pre-switch and post switch final visit. In the study by Erden et al. (20), ranibizumab, and aflibercept were shown to be equally effective in visual prognosis.

Aflibercept appears to offer theoretical advantages over other drugs, such as ranibizumab and bevacizumab. First, aflibercept

Table 2. Visual acuity before and after conversion to aflibercept for diabetic macular edema

Eyes with data	First visit	Pre-switch visit	Post-switch first visit	Post-switch second visit	Finally visit
VA levels, n	33	33	33	17	16
20/20	1	2	3	2	1
20/25	1	2	2	3	3
20/30	9	9	12	4	5
20/40	-	4	-	-	1
20/50	5	4	3	-	1
20/60	4	-	1	1	-
20/70	-	-	-	-	-
20/80	-	-	-	-	-
20/100	5	4	3	5	2
20/200	5	7	8	-	3
20/300	1	1	-	-	-
20/350	1	-	-	-	-
20/400	1	-	1	2	-
Mean (SD) logMAR VA	0.56 (0.38)	0.44 (0.33)	0.41 (0.34)	0.37 (0.33)	0.35 (0.37)

SD: Standard deviation, VA: Visual acuity

has demonstrated a greater binding affinity to VEGF-A (10). Second, ranibizumab only binds free VEGF-A inhibiting only VEGFR2, whereas aflibercept binds VEGF-A, VEGF-B, and placental growth factor, inhibiting VEGFR1 and VGEFR2 (19). Nevertheless, some cases, refractory anti-VEGF agents elevated aqueous levels of interleukin-6, interleukin-8, interferon-induced protein-10, monocyte chemotactic protein-1, transforming growth factor β, hepatocyte growth factor, serum amyloid A, and VEGF were found in patients with DME (21).

Study Limitations

Our study is limited by its retrospective design, the relatively small sample size, and the absence of a control group. The possible visual benefits of aflibercept may be negatively affected in patients with persistent DME due to the small number of patients. Additionally, patients with diabetes' metabolic control of may also affect macula thickness. Future studies may evaluate the effect the effects of metabolic control.

CONCLUSION

In conclusion, the management of DME cases that exhibit a suboptimal response to anti-VEGF therapy remains a clinical challenge. Our study provides further evidence of the advantages of switching to aflibercept in patients with refractory DME who have been previously treated with ranibizumab. At least 2 aflibercept injections after the switch resulted in anatomical improvements; however, a similar advantage was not observed for visual gain. The number of aflibercept injections in eyes with refractory DME after the switch is unimportant. Finally, our results emphasize the importance of the pre-switch BCVA.

Ethics

Ethics Committee Approval: The protocol of this study was approved by the Institutional Review Board of the Eye Clinic of the Ankara Training and Research Hospital Ethic Committee (approval number: 308, approval date: 06.12.2017).

Informed Consent: All the research was carried out in accordance with the Helsinki Declaration and by obtained written informed consent from the patients.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: N.Ü., G.Ü.Ö., D.H., M.A.A., F.Ö., Concept: N.Ü., Design: N.Ü., Data Collection or Processing: G.Ü.Ö., D.H., M.A.A., Analysis or Interpretation: F.Ö., Literature Search: G.A.A., Writing: G.A.A.

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ORIGINAL ARTICLE

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Evaluation of the Website Information About Orthopaedics and Traumatology Via the Example of Hallux Valgus

Muhammet Bozoğlan, Murat Danışman, Tuğcan Demir, Cem Zeki Esenyel

Giresun University, Prof. Dr. A. İlhan Özdemir Training and Research Hospital, Clinic Orthopaedics and Traumatology, Giresun, Turkey

Abstract

Objective: The aim is to evaluate the content and quality of the most frequently accessed websites for information on the internet about the commonly observed orthopedic problem of hallux valgus.

Methods: The keywords hallux valgus and hallux valgus treatments were screened in internet (www.google.com.tr). In each search, the first 30 sites listed were investigated. The repeated and advertising sites were eliminated. A total of 43 websites were evaluated. Internet websites were classified into 4 categories of doctor websites, news websites, health-related sites and unclassified. Firstly, content evaluation of the sites used the previously prepared questions and gave points out of 20. Later, the discern evaluation system was used for general assessment.

Results: When the information accuracy of the 43 websites included in the investigation was evaluated, mean points from the 20-question survey were determined to be 9.3 [minimum: 2, maximum: 18, standard deviation (SD): 3.8]. The most accurate information appeared to be under the headings of definition of hallux valgus (97.7%), reasons for the formation (76.7%), predisposing situations (88.4%) and use of orthotic devices (83.7%). According to the discern evaluation table, doctor websites (mean: 11, SD: 4.9) received the highest points, while sites with undetermined references received the lowest points (mean: 6.0, SD: 1.7).

Conclusion: Information obtained from the internet about health is stated to be insufficient in the literature, as in our study. Considering that websites offering insufficient or erroneous information cannot be prevented, we believe that it is necessary to branch organizations and health institutions to perform studies to close the gap in this area.

Keywords: Hallux valgus, websites, awareness, internet

INTRODUCTION

Currently, due to the internet being in every house, even in every mobile telephone, it has become the most frequently used tool to access information. In Turkey, generally, the number of broadband internet subscribers reached 73.8 million in 2018 (1). The proportion of people searching for information related to health on the internet was stated to be 69.3% (2). These numbers show that due to easy access, the internet has become a very commonly used tool to access information in the area of health, as in many other areas.

Due to frequent use, it is clear that most of the society access their information in the health field from the internet, while there are not many studies on the quality of this information and sufficiency levels for important topics like health.

Hallux valgus is a common foot deformity in society, where the first toe deviates laterally while the first metatarsal deviates medial. The literature shows variability in different populations, with prevalence ranging from 20-65% (3-5).

In this study, we evaluated the content of websites that provide health-related information and the accuracy of the information they provide, using hallux valgus, a common foot disease.

Address for Correspondence: Muhammet Bozoğlan, Giresun University, Prof. Dr. A. İlhan Özdemir Training and Research Hospital, Clinic Orthopaedics and Traumatology, Giresun, Turkey
Phone: +90 505 794 75 42 **E-mail:** mbozoglan@gmail.com **ORCID ID:** orcid.org/0000-0002-1368-833X

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METHODS

On the most commonly used search engine in Google Turkey (www.google.com.tr), the keywords "hallux valgus" and "hallux valgus treatment" in the Turkish language (halluks valgus and halluks valgus tedavisi) were searched in November 2019. For each search the first 30 websites were listed as the most commonly investigated after that the websites for advertising and the websites with the same titles were eliminated. A total of 43 websites were evaluated. Accuracy information on the evaluated sites was performed according to a 20-question survey that we created in accordance with the literature (Table 1). Based on whether the website content answered these questions or not, each question was given 1 point with 20 points obtainable. According to the points, websites with 1-5 points were assessed as insufficient, 5-10 points were moderate, 11-15 points were sufficient and 15-20 points were perfect. For the general evaluation of the sites, the discern evaluation survey was used (Table 2). Fifteen questions modified from the discern scale were used to give points from 1 to 5 on a graded scale (1-2: No, 3-4: Partly, 5: Yes). According to the mean total points obtained, the quality of websites was evaluated as 0-5 points low (severely deficient), 6-10 points moderate (seriously deficient), and 11-15 points high (minimally deficient). Websites were also classified into 4 categories according to content (1- Doctor websites, 2- News websites, 3- Health-related websites, 4- Unclassified. Each website is rated independently, within one week, by two observers, using the references cited below as a source (6,7).

Table 1. Survey questions

Main headings	Topic	Points
Definition of hallux valgus	What is hallux valgus?	1
	Reasons for occurrence of hallux valgus	1
	Is there family heredity?	1
	Does hallux valgus occur at young ages?	1
	Situations causing tendency towards hallux valgus	1
Conservative treatment	Use of special devices	1
	Does use of devices cause improvement?	1
	Is there a role for exercise in treatment?	1
Surgical treatment	When should surgery happen?	1
	Are there different surgery choices?	1
	Will be there definite improvement with surgery?	1
	Is there recurrence after surgery?	1
Rehabilitation	Use of special shoes after surgery	1
	Use of devices after surgery	1
	Situations requiring care after surgery	1
Diagnostic method	Physical examination	1
	Standing radiography	1
Complications	Problems that may occur during conservative surveillance	1
	Problems that may occur after surgical treatment	1
	What should be done in these situations	1

Statistical Analysis

Descriptive statistics for data used mean, standard deviation (SD), median, minimum, maximum, frequency and proportion values. The distribution of variables was measured with the Kolmogorov-Smirnov test. Analysis of dependent variables used the Wilcoxon test. The SPSS 22.0 program was used for analyses. Since our study was an internet screening, ethics committee approval was not obtained.

RESULTS

Of the 43 websites included in the investigation, 10 were doctor personal websites (23.2%), 11 were news websites (25.5%), 14 were health-related sites (32.5%) and 8 were unclassified (18.6%). When information accuracy is evaluated, mean points for the 20-question survey were determined as 9.3

Table 2. Table of evaluation questions from original discern article

Are aims stated openly?
Does it achieve aims?
Is it related to the topic?
Is the source of information clear?
Is the date of information current?
Prejudiced and balanced
Does it provide additional resources for information and support?
Are there references in uncertain areas?
Does it give information about function of treatment?
Benefits of treatment
Does it explain risks of treatment?
Does it define what may happen if treatment is not given?
Does it define how chosen treatment will affect quality of life?
Is it clear about more than one treatment choice?
Does it provide support for shared decisions?
General evaluation of website

Table 3. Evaluation of information accuracy of investigated websites											
		Group I		Group II		Group III		Group IV		Total	
		n	%	n	%	n	%	n	%	n	%
What is hallux valgus?	Wrong	1	10.0%	0	0.0%	0	0.0%	0	0.0%	1	2.3%
	Correct	9	90.0%	11	100.0%	14	100.0%	8	100.0%	42	97.7%
Reasons for occurence of hallux valgus	Wrong	2	20.0%	2	18.2%	4	28.6%	2	25.0%	10	23.3%
	Correct	8	80.0%	9	81.8%	10	71.4%	6	75.0%	33	76.7%
Is there family heredity?	Wrong	3	30.0%	6	54.5%	4	28.6%	4	50.0%	17	39.5%
	Correct	7	70.0%	5	45.5%	10	71.4%	4	50.0%	26	60.5%
Does hallux valgus occur at young ages?	Wrong	4	40.0%	10	90.9%	9	64.3%	8	100.0%	31	72.1%
	Correct	6	60.0%	1	9.1%	5	35.7%	0	0.0%	12	27.9%
Situations causing tendency towards hallux valgus	Wrong	0	0.0%	1	9.1%	2	14.3%	2	25.0%	5	11.6%
	Correct	10	100.0%	10	90.9%	12	85.7%	6	75.0%	38	88.4%
Use of special devices	Wrong	2	20.0%	2	18.2%	1	7.1%	2	25.0%	7	16.3%
	Correct	8	80.0%	9	81.8%	13	92.9%	6	75.0%	36	83.7%
Does use of devices cause improvement?	Wrong	2	20.0%	7	63.6%	6	42.9%	4	50.0%	19	44.2%
	Correct	8	80.0%	4	36.4%	8	57.1%	4	50.0%	24	55.8%
Is there a place for exercise in treatment?	Wrong	9	90.0%	11	100.0%	11	78.6%	6	75.0%	37	86.0%
	Correct	1	10.0%	0	0.0%	3	21.4%	2	25.0%	6	14.0%
When should surgery happen?	Wrong	2	20.0%	0	0.0%	6	42.9%	5	62.5%	13	30.2%
	Correct	8	80.0%	11	100.0%	8	57.1%	3	37.5%	30	69.8%
Are there different surgey choices?	Wrong	2	20.0%	2	18.2%	4	28.6%	6	75.0%	14	32.6%
	Correct	8	80.0%	9	81.8%	10	71.4%	2	25.0%	29	67.4%
Will be there definite improvement with surgery?	Wrong	7	70.0%	6	54.5%	8	57.1%	7	87.5%	28	65.1%
	Correct	3	30.0%	5	45.5%	6	42.9%	1	12.5%	15	34.9%
Is there reccurence after surgery?	Wrong	7	70.0%	9	81.8%	8	57.1%	7	87.5%	31	72.1%
	Correct	3	30.0%	2	18.2%	6	42.9%	1	12.5%	12	27.9%
Use of special shoes after surgery	Wrong	3	30.0%	6	54.5%	8	57.1%	8	100.0%	25	58.1%
	Correct	7	70.0%	5	45.5%	6	42.9%	0	0.0%	18	41.9%
Use of devices after surgery	Wrong	10	100.0%	9	81.8%	13	92.9%	7	87.5%	39	90.7%
	Correct	0	0.0%	2	18.2%	1	7.1%	1	12.5%	4	9.3%
Situations requiring care after surgery	Wrong	5	50.0%	7	63.6%	7	50.0%	7	87.5%	26	60.5%
	Correct	5	50.0%	4	36.4%	7	50.0%	1	12.5%	17	39.5%
Physical examination	Wrong	2	20.0%	6	54.5%	6	42.9%	7	87.5%	21	48.8%
	Correct	8	80.0%	5	45.5%	8	57.1%	1	12.5%	22	51.2%
Standing radiography	Wrong	3	30.0%	8	72.7%	8	57.1%	8	100.0%	27	62.8%
	Correct	7	70.0%	3	27.3%	6	42.9%	0	0.0%	16	37.2%
Problems that may occur during conservative surveillance	Wrong	7	70.0%	8	72.7%	13	92.9%	7	87.5%	35	81.4%
	Correct	3	30.0%	3	27.3%	1	7.1%	1	12.5%	8	18.6%
Problems that may occur after surgical treatment	Wrong	6	60.0%	9	81.8%	11	78.6%	7	87.5%	33	76.7%
	Correct	4	40.0%	2	18.2%	3	21.4%	1	12.5%	10	23.3%
What should be done in these situations	Wrong	9	90.0%	11	100.0%	13	92.9%	8	100.0%	41	95.3%
	Correct	1	10.0%	0	0.0%	1	7.1%	0	0.0%	2	4.7%

(minimum: 2, maximum: 18, SD: 3.8) (Table 3). Of the websites, 7 were insufficient (16.2%), 21 were moderate (48.8%), 12 were sufficient (27.9%) and 3 were perfect (6.9%). The highest points were received by doctor personal websites and health-related sites, while sites classified in the unclassified group (generally commercial websites) appeared to receive lower points. The most accurate information was observed for the headings of definition of hallux valgus (97.7%), reasons for the formation (76.7%), predisposing situations (88.4%) and use of orthotic devices (83.7%). The most insufficient information appeared to be about juvenile hallux valgus, role of exercise in treatment, problems that may be caused by conservative or surgical treatment and what should be done in this situation (Figure 1). According to the discern evaluation table, doctor personel sites received the highest points (mean: 11.4, SD: 4.9), while websites with unknown references received the lowest points (mean: 6.0, SD: 1.7) (Table 4). Generally, websites with high points on the discern evaluation received high points on the information accuracy evaluation but this is not statistically significant (p>0.05). There were no significant differences identified for information accuracy and general evaluation of websites between the two experts (p>0.05).

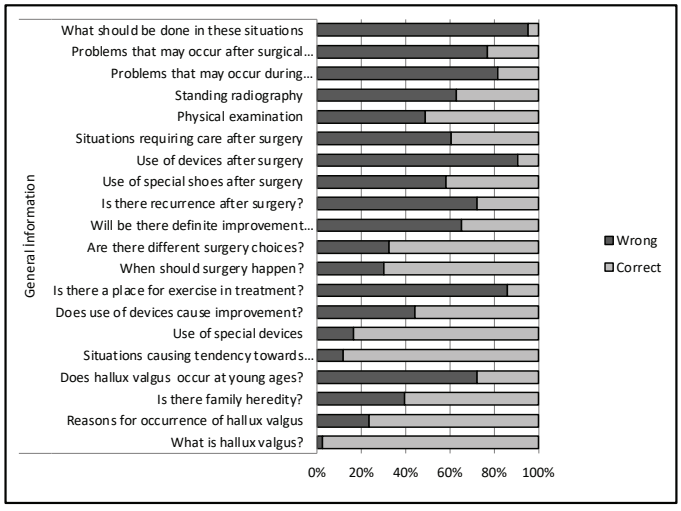

Figure 1. Evaluation of the given answers in the websites

Table 4. Evaluation of groups according to discern survey

	Group I		Group II		Group III		Group IV		p
	Mean ± SD	Med	Mean ± SD	Med	Mean ± SD	Med	Mean ± SD	Med	
General information total points	11.4±4.9	12.0	9.1±2.2	9.0	9.9±3.8	9.5	6.0±1.7	6.0	0.052
Are aims stated openly?	3.9±0.7	4.0	3.5±1.1	3.0	3.4±1.2	3.0	2.9±1.0	3.0	0.175
Does it achieve aims?	3.3.±0.9	3.0	3.1±0.8	3.0	2.9±1.5	2.5	2.5±1.1	2.0	0.301
Is it related to the topic?	3.7±0.7	4.0	3.1±0.8	3.0	3.4±1.2	3.0	2.6±1.2	2.5	0.136
Is the source of information clear?	2.5±1.3	2.0	2.1±1.2	2.0	2.1±1.3	2.0	1.5±0.5	1.5	0.342
Is the date of information current?	1.5±1.3	1.0	1.5±0.7	1.0	1.5±1.1	1.0	1.0±0.0	1.0	0.328
Prejudiced and balanced	2.4±1.0	2.0	1.9±0.8	2.0	2.4±1.0	2.0	1.8±0.7	2.0	0.262
Does it provide additional resources for informations and support?	1.4±1.3	1.0	1.6±0.8	1.0	1.6±1.2	1.0	1.0±0.0	1.0	0.139
Are there references in uncertain areas?	2.2±1.1	2.0	2.3±1.0	2.0	1.9±1.2	1.5	2.0±0.5	2.0	0.673
Does it give information about function of treatment?	3.5±0.7	3.0	3.0±1.0	3.0	2.7±1.2	3.0	3.3.±0.9	3.0	0.203
Benefits of treatment	3.5±0.7	3.0	2.9±1.0	3.0	3.1±0.9	3.0	3.1±1.0	3.0	0.544
Does it explain risks of treatment?	2.4±1.4	2.0	2.3±0.8	2.0	2.5±1.4	2.0	1.9±0.8	2.0	0.778
Does it define what may happen if treatment is not given?	2.5±1.2	2.5	2.6±1.2	3.0	2.1±1.5	1.0	1.9±0.8	2.0	0.319
Does it define how chosen treatment will affect quality of life?	3.3±0.7	3.0	2.7±0.8	3.0	2.9±1.1	3.0	3.0±1.1	3.0	0.357
Is it clear about more than one treatment choice?	3.4±0.8	3.0	2.5±0.7	2.0	3.1±1.3	3.0	2.6±0.5	3.0	0.123
Does it provide supoort for shared decisions?	2.6±1.0	2.0	2.0±0.4	2.0	2.9±1.3	2.0	2.3±0.5	2.0	0.200
General evaluation of website	3.1±1.1	3.0	2.5±0.9	2.0	2.5±1.5	2.0	1.8±0.9	1.5	0.103

SD: Standard deviation

DISCUSSION

In this study, we evaluated the content of websites that provide health-related information and the accuracy of the information they provide, using hallux valgus, a common foot disease.

With the aid of developing technology, the internet is currently easily accessible and has become the first source of information for many topics. However, due to the lack of regulation of websites providing information on the internet, intense information pollution is present for nearly every topic. This situation may lead to negative outcomes for internet users. Most of the time, children are affected by this negative tableau (8). Cases of mortality because of applying erroneous information accessed on the internet in relation to health have been reported (9). According to 2019 data, health-related internet searches reached levels of 69.3%. These data are generally reviewed from health-related websites and the information content and accuracy of information is not assessed. In our study, we chose a pilot topic in the branch of orthopaedics and traumatology; thus, we evaluated the accuracy of content accessed from a large information source.

The product of the first study for the accreditation of websites is the discern scale (the discern instrument). discern scale can be used by manufacturers, healthcare professionals and patients to evaluate written information about treatment options, it is the first standardized quality index of consumer health information. The discern (awareness) survey was conducted to judge the quality of written information on the internet, to set standards and to facilitate the production of high-quality evidence-based information by providing a reference point for authors (10). Our aim in using this scale was to evaluate the sites we evaluate in terms of orthopedic knowledge at the same time in terms of their quality and to evaluate the relationship between the two parameters. We found that the websites that are well designed in terms of content and have higher scores than discern scale is more satisfactory in terms of information content.

Küçükdurmaz et al. (11) evaluated the top 10 websites in the search engines of Google, Yahoo and MSN in a tablein a study dealing with meniscus discomfort in 2012. Of these 60 websites, 33 websites were evaluated after eliminating repeated sites from different search engines. Each accurate piece of information was given 1 point in the table with an evaluation based on 20 points. They found the mean points for evaluated websites was 12.09. When distributed according to topic headings, the most sufficient information was about etiology, with most insufficient information about rehabilitation. In conclusion, they noted that this deficient information could cause problems for both clinicians and patients (11). In our study, we used a 20-point evaluation table under the main headings of definitions, diagnostic methods, conservative and surgical treatment, rehabilitation and complications. The evaluated sites received a mean point of 9.3. Additionally, according to the discern evaluation, most sites did not state the date of information and references and did not offer any additional references to support information. We think this leads to controversy about the reliability of these websites.

Elhassan et al. (12) evaluated 24 websites in a study related to discectomy and reported that the mean information quality was weak. The same study reported increasing internet use and that the information content of websites had not increased by sufficient proportions (12).

Elliott et al. (13) evaluated the information quality and website authors for 105 websites about total ankle arthroplasty. Independent of author, they observed that all sites had low information quality. In conclusion, they emphasized that cooperative work between internet website hosts and experts on the topic was important for patients to be informed accurately (13).

Winship et al. (14) reported that non-profit and academic websites provided the most reliable and accurate information in a study of the most common 10 diagnoses in pediatric orthopedics. Interestingly, they reported that websites run by clinicians and commercial websites provided the least reliable information (14).

Zhang et al. (15) screened for 3 different terms (broken collar bone, collar bone fracture and clavicula fracture) in 3 different search engines. They evaluated the quality and readability of 91 websites. The evaluation found that mean website quality was very low and readability levels were far from recommended. They found that the information quality from academic sites was better, while that commercial sites was insufficient. In conclusion, they emphasized that patients should be prevented from accessing commercial sites and directed toward academic sites (15).

Study Limitations

In our study, we obtained similar results with literature in spite of the more common use of the internet through the years and the increase in health searches. A limitation of our study is that we assigned points to website content as accurate or erroneous according to our created survey table, so it was difficult to differentiate websites with missing information or mistaken information. To reduce this to a minimum, two different observers

separately evaluated the websites. We evaluated the information accuracy of the websites on 2 review articles published about hallux valgus. No significant differences were identified between the observers. Most websites we evaluated were news content or commercially associated sites and we observed that information levels were not sufficient. In our search on the internet, we observed that health-related associations and institutions do not provide publications on the official websites for informing the public. We believe that this is an important reason for the accuracy of the health-related information obtained from the internet. This deficiency can be solved by the publications of expert professionals, related branch organizations and official institutions to inform patients and by directing the public to these official websites. In this way, both spending in the area of health will be reduced and more productive dialog will be created between patients and clinicians.

CONCLUSION

In Turkey, the accuracy of the information reached in the field of health on the internet and adequacy of the websites that provide this information is not at a good level as in the world. Considering that websites offering insufficient or erroneous information cannot be prevented, we believe that it is necessary to branch organizations and health institutions to perform studies to close the gap in this area. Health-related associations and institutions have a responsibility to obtain a more accurate and source-specific information on the internet and should play a more active role in this matter. Otherwise, the information pollution on the internet will harm both people and healthcare workers. For further studies, evaluations of these websites through the patient point of wiew can provide more benefits.

Ethics

Ethics Committee Approval: Since our study was an internet screening, ethics committee approval was not obtained.

Informed Consent: None.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.B., C.Z.E., Design: M.B., T.D., C.Z.E., Data Collection or Processing: M.B., M.D., Analysis or Interpretation: M.B., M.D., T.D., C.Z.E., Literature Search: M.B., M.D., T.D., Writing: M.B., M.D., T.D., C.Z.E.

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ORIGINAL ARTICLE

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Clinical Features of the Patients with Chronic Lymphocytic Leukemia: Two Centers Experience

Fadime Ersoy Dursun[1], Erdal Kurtoğlu[1], Ayşen Timurağaoğlu[2]

[1]University of Health Sciences Turkey, Antalya Training and Research Hospital, Clinic of Hematology, Antalya, Turkey
[2]Akdeniz University Faculty of Medicine, Department of Hematology, Antalya, Turkey

Abstract

Objective: Chronic lymphocytic leukemia (CLL) is a hematologic malignancy characterized by the clonal proliferation of lymphocytes. The aim of the study was to analyze the demographic data, treatment indications, treatment responses and total survival data of patients with the diagnosis of CLL.

Methods: The study was conducted retrospectively in 183 patients who were followed up in the hematology departments of two centers between 1999 and 2014.

Results: The mean age of the patients was 64.72±11.40 years and 120 (65.6%) patients were male and 63 (34.4%) were female. Overall survival (OS) was not different between patients who had monoclonal gammopathy and those who did not (p=0.922). Among the patients, 105 received treatment while 78 of them were observed without a treatment. Chlorambucil was the most frequently (33%) used drug during the first line of chemotherapy. The difference between the distribution of male and female patients according to risk groups was statistically significant (p=0.018). Among patients who did not receive therapy and with higher Rai and Binet stages, overall and progression free survive were lower (p=0.0011). Furthermore, increases in β_2-microglobulin and sedimentation revealed lower rates of survival (p=0.001 and p=0.008 respectively).

Conclusion: It can be concluded that monoclonal bands are not associated with survival in CLL. Although the demographic information of our patients was similar to that of patients in other studies, OS was found to be less. This issue can be explained by the fact that the patients receiving chemotherapy were at a further stage.

Keywords: Chronic lymphocytic leukemia, monoclonal gammopathy, prognosis, survival

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of mature, typically CD5-positive B-cells in the blood, bone marrow, lymph nodes and the spleen because of clonal proliferation. CLL is the most common leukemia reported in most European and North American countries (1). Although there is no cure for treating CLL, survival rates have increased over time, increasing the 5-year survival to approximately 80% in Europe and North America (2-4).

CLL was defined as slowly progressive disease, but later it was shown that independent of the clinical phase, a group of CLL patients had rapidly progressive disease than expected. In other words, while some patients survive quite a long time without therapy, some patients require treatment shortly after the diagnosis. The total duration of survival can range between months and decades (5-7). The staging systems used as a standard in almost all medical centers were developed by Rai et al. (8) in 1975 and by Binet et al. (9) in 1977. But, during the last 50 years, beside Rai and Binet staging systems, the clinical, biological

Address for Correspondence: Fadime Ersoy Dursun, University of Health Sciences Turkey, Antalya Training and Research Hospital, Clinic of Hematology, Antalya, Turkey
Phone: +90 536 838 51 01 **E-mail:** drfadimeersoy@yahoo.com.tr **ORCID ID:** orcid.org/0000-0002-3577-188X

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and molecular prognostic factors that can affect overall survival (OS) and the course of the disease have been identified (9). The aim of this retrospective study was to reveal demographic data, clinical and prognostic factors, the diversity of chemotherapy regimens, responses to treatment and OS rates of CLL patients in Antalya, Turkey.

METHODS

Study Design and Patients

This was an observational retrospective cohort study. It was conducted at University of Health Sciences Turkey, Antalya Training and Research Hospital Clinic of Hematology and Akdeniz University Faculty of Medicine Department of Hematology clinics. A total of 183 patients who were diagnosed with CLL between January 1999 and September 2014 were retrospectively examined. The demographics, clinical characteristics, prognostic markers, chemotherapy protocols, response rates to the therapy and OS were evaluated.

Patients files were analyzed according to age, gender, hemoglobin, hematocrit, white blood cell, lymphocyte count, B lymphocyte count, platelet count, erythrocyte sedimentation rate (ESR), glucose, urea, creatinine, uric acid, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase (LDH), total/direct bilirubin, total protein, albumin, immunoglobulin (Ig) and β_2-microglbulin (β_2-MG) levels and the results were recorded. Immunophenotyping of leucocytes and lymphocytes was performed by flow cytometry and paraproteins were detected by immunofixation electrophoresis. Modified Rai staging system was used for clinical staging and, disease stages, whether they received treatment or not were recorded. The chemotherapy protocols and drugs received by the patients and their responses to the treatments were also recorded. We applied chemotherapy protocols to our patients according to the National Cancer Institute-sponsored Working Group 1996 guidelines (10). This study was approved by the Local Ethical Committee of University of Health Sciences Turkey, Antalya Training and Research Hospital (decision no: 18/3, date: 02.05.2013). 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.

Statistical Analysis

Statistical package for evaluating the data for the Social Sciences (SPSS) 21.0 software was used. ANOVA analysis was run to reveal any statistical significance of the difference between means nominal and the differences between ordinal variables chi-square test to be placed between the arguments Mann-Whitney U and numerical features showing Wilcoxon tests and normal distribution Student's t-tests for evaluation of variables was used. For survival analysis Kaplan-Meier and Cox regression tests were used. For prognostic and predictive factors logistic regression test was used. Pearson's correlation test was used to analyze the relationships between parameters. $p<0.05$ was the level of significance for all statistical analyses.

RESULTS

General Characteristics of the Patients

A total of 183 patients, aged approximately 31-89 (mean 64.72±11.40), 120 (68%) were male and 63 (32%) were female were included in the study. Of all participants 88 (48.09%) patients were aged between 31 and 65 years, and 95 (51.91%) were equal or older than 65 years. Of the 183 cases, 131 (71.6%) were alive and 52 (28.4) died. In terms of gender, among the males there, 83 (69.2%) lived and 37 (30.8%) died. Among the females, 48 (76.2%) survived and 15 (23.8%) died. When the effect of gender on death or life status was examined, no statistically significant difference was found ($p=0.318$).

Laboratory Findings

The demographic data and laboratory values of the patients at the time of diagnosis are shown in Table 1. As seen in Table 1, lymphocytosis, especially type B lymphocytosis, is seen in our patients.

Lymphocyte immunophenotypes, frequency of monoclonal proteins and bone marrow biopsy in patients are shown in Table 2. Immunophenotyping of lymphocytes revealed that zeta-chain-associated protein kinase 70 (ZAP70) in 168 (91.8%) patients, CD38 expression in 158 (86.34%) patients and CD11c expression in 51.9% of patients were lower than 20%. However, CD20 expression was seen in 95.8% of patients were higher than 20%. Other immunophenotype rates are shown in Table 2. There was no statistically significant relationship between ZAP70, CD38, and CD11c and OS (p=ns). Bone marrow biopsy was performed in 14.8% of 183 patients that 59.3% showed diffuse bone marrow infiltration and 40.7% showed nodular bone marrow infiltration. There was no statistically significant difference between bone marrow diffuse or nodularity and OS ($p=0.345$). Immunofixation electrophoresis was performed in 74 patients and 47 (63.51%) of them revealed no monoclonal protein. However, monoclonal protein was detected in 27 (36.49%) patients. IgG kappa was seen in 12 (16.21%) patients, IgM kappa in 5 (6.76%) patients, and IgG

Table 1. Demographic data and laboratory values of chronic lymphocytic leukemia patients

Parameters	n	Min-max	Mean ± SD
Age	183	31-89	64.72±11.40
White blood cell (/mm^3)	183	8550-447470	61031.31±4613.46
Total lymphocyte (/mm^3)	183	6040-380000	49354±65027
B lymphocyte (/mm^3)	183	5010-309600	37728±50316
Hemoglobin (g/dL)	183	3.5-16.6	12.11±2.51
Platelet (mm^3)	183	5000-434000	185486±80839
Uric acid (mg/dL)	183	2.00-9.80	5.43±1.59
ALT (U/L)	183	3.00-139.00	20.19±14.49
AST (U/L)	183	9.00-67.00	21.48±9.23
Total protein (mg/dL)	183	3.00-11.00	6.88±0.084
Albumin (mg/dL)	183	2.20-6.10	4.31±0.51
Creatinine (mg/dL)	183	0.40-4.40	0.98±0.45
LDH (U/L)	183	53.00-2204.00	321.25±213.14
ESR (mm/h)	183	2.00-109.00	21.97±21.02
IgG (mg/dL)	134	190.00-5020.00	1013.99±633.34
IgM (mg/dL)	134	2.90-900.00	70.91±108.53
IgA (mg/dL)	134	17.00-924.00	134.43±108.53
β_2-microglobulin (mg/L)	183	0.22-16.40	4.06±2.13

SD: Standard deviation, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, ESR: Erythrocyte sedimentation ratio, IgA: Immunoglobulin A, IgG: Immunoglobulin G, IgM: Immunoglobulin M

Lambda in 3 (4.05%) patients. In the remaining 7 (9.46% patients, different monoclonal proteins were detected. OS was not different between patients who had monoclonal gammopathy and those who did not, (p=0.922).

In this study, the effects of some risk factors on the OS of CLL patients are shown in Table 3. According to Modified Rai staging system, we compared the stages disease and OS rates. It was found that OS of low-risk group patients (stage 0) was 61.63±43.86 months, 47.53±30.00 months for patients in the intermediate risk group (stage 1-2), and 41.74±33.71 months for patients with high risk (stage 3-4). There was a statistical significance according to disease stage and OS and early diagnosis dramatically increased survival (p=0.034, Figure 1). Survival rates of male and female patients were 47.88±35.74 months and 47.66±31.35 months, respectively. It was shown that the effect of gender on OS was not statistically significant (p=0.967). OS in patients aged 31-65 years were 54.34±38.00 years and in patients over 65 years old, it was 41.76±29.18 years. When we examined the effect of age on OS in CLL, we found a statistically significant difference (p=0.013, Figure 2). β_2 -MG values ranged between 0.22 and 16.40 mg/dL (mean 4.06±2.13). The OS of patients whose β2-MG level below 4.06 mg/dL and equal or above the 4.06 mg/dL was 49.05±34.21 months and 45.31±33.30 months, respectively. According to the results, a statistically significant relationship was not found between OS and β_2-MG levels (p=0.506). The LDH values ranged between 53 and 2204 IU/L with a mean LDH value of 321.25±213.14 IU/L. The OS of patients below and above the mean LDH level was found to be 55.08±35.87 months and 43.60±32.62 months, respectively. According to these results, there is a statistically significant relationship between OS and LDH levels in the blood (p=0.028, Figure 3). ESR values of the

Table 2. Lymphocyte immunophenotypes, frequency of monoclonal proteins and bone marrow biopsy in patients

Lymphocyte immunophenotypes	Property (%)	n	%
ZAP70	<20	168	91.80
	≥20	15	8.20
CD38	<20	158	86.34
	≥20	25	13.66
CD11c	<20	95	51.91
	≥20	88	48.09
CD20	<20	9	4.92
	≥20	174	95.08
CD16	<5	162	88.50
	≥5	21	11.50
CD56	<5	119	65.00
	≥5	64	35.00
CD4	<5	66	36.10
	≥5	117	63.90
CD8	<5	87	47.50
	≥5	96	52.50
CD3	<5	74	40.40
	≥5	109	59.60
Bone marrow biopsy	Diffuse bone marrow infiltration	16	59.3
	Nodular bone marrow infiltration	11	40.7
Monoclonal protein condition (detected by immunofixation electrophoresis)	No monoclonal protein	47	63.51
	There is monoclonal protein - IgG Kappa - IgM Kappa - IgG Lambda *Others	27 12 5 3 7	36.49 16.21 6.76 4.05 9.46

*Others (IgM Lambda, IgA Kappa, IgG heavy chain, Kappa light chain, Lambda light chain, triclonal protein, multiclonal protein), ZAP70: Zeta-chain-associated protein kinase 70, IgG: Immunoglobulin G, IgM: Immunoglobulin M

Table 3. The effects of some risk factors on survival of chronic lymphocytic leukemia patients

Factors		Mean of survival time (month) Mean ± SD	p
*Disease stage	Low risk (0)	61.63±43.86	**0.034**
	Medium risk (1-2)	47.53 30.00	
	High risk (3-4)	41.74±33.71	
Gender	Male	47.88±35.74	0.967
	Female	47.66±31.35	
*Age	31-65 years old	54.34±38.00	**0.013**
	≥65 years old	41.76±29.18	
β_2-MG	0.1-4.06	49.05±34.21	0.506
	>4.061	45.31±33.30	
*LDH	≤321.25	55.08±35.87	**0.028**
	≥321.26	43.60±32.62	
ESR (mm/h)	High	43.63±29.90	0.210
	Normal	50.22±36.37	

*p<0.05, SD: Standard deviation, β_2MG: $\beta_2$microglobulin, LDH: Lactate dehydrogenase, ESR: Erythrocyte-sedimentation rate

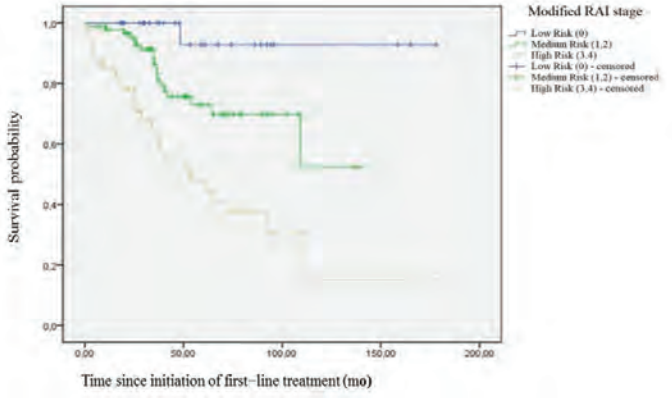

Figure 1. Survival analysis according to stage in our CLL patients
CLL: Chronic lymphocytic leukemia

patients ranged between 2.0 and 109.0, with a mean value of 21.97±21.02 mm/h (reference values as 0-20 mm/h). The OS of patients with high (≥20 mm/h) and a normal value (<20 mm/h) of ESR were 43.63±29.90 months and 50.22±36.37 months, respectively. According to the findings there is no statistically significant relationship between OS and ESR levels in the blood (p=0.210).

Clinical stages accordingly to the modified Rai system, mortality rates, the distribution of age groups, first line chemotherapy responses and treatment status of patients according to gender are presented in Table 4. Disease stage of the patients according

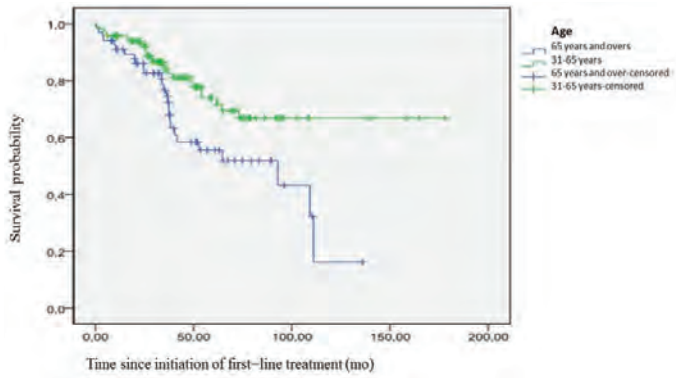

Figure 2. Survival analysis according to age in our CLL patients
CLL: Chronic lymphocytic leukemia

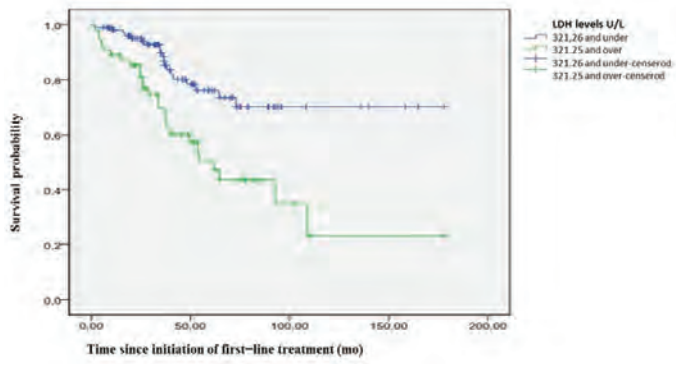

Figure 3. Survival analysis according to LDH in our CLL patients
LDH: Lactate dehydrogenase, CLL: Chronic lymphocytic leukemia

to gender was also examined at the time of diagnosis. According to this distribution, it was observed that the disease stage was concentrated in the low and medium groups in female patients, the high and medium-risk groups in men at the time of diagnosis. The difference between the distribution of male and female patients according to disease stage was found to be statistically significant (p=0.018). The patients were also divided 2 groups according to age that 88 (48.09%) patients were between 31 and 65 years and 95 (51.01%) were ≥65 years. There was no statistical difference between these two groups according to gender (p=0.826). The treatment needs of the patients were examined according to their gender. According to these results, it is seen that male patients need first-line chemotherapy treatment more than female patients. This result was statistically significant (p=0.011). Patients in need of treatment, the effect of gender on the first line chemotherapy response was examined. These results showed that first-line chemotherapy responses were not statistically different between male and female (p=0.969). During the study period, 52 of 183 patients died and 131 survived. The OS and mortality rates of the patients by gender were analyzed. The OS rate was 69.2% in male, while this rate was 76.2% in

female. There was no statistically significant difference between death and OS rates according to gender (p=0.318).

The chemotherapy protocols and response to treatment are presented in Table 5. Chlorambucil plus the methylprednisolone was the most used drug in our unit (33%), cyclophosphamide plus fludarabine (FC) was at the second frequency (21.9%). The mean duration between the diagnosis and the first treatment was 18 months. During the first-line treatment, among these 105 patients, 35 were treated with chlorambucil plus methylprednisolone, 9 were treated with only FC, 23 were treated with FC, 13 were treated with fludarabine-cyclophosphamide-rituximab (FCR), 22 were treated with cyclophosphamide-vincristine-prednisolone or cyclophosphamide-prednisolone, and 3 were treated with a high dose of methylprednisolone. As seen in 39.1% of the patients gave a complete response to first-line chemotherapy and 21.9% of the patients gave a complete response to second and subsequent chemotherapy regimens. For 105 (57.4%) patients needing treatment, the effect of gender on the duration of first-line treatment (TFS) was examined. TFS was 17.53±26.9 months for male and 21.09±28.3 months for females. Statistically, the effect of gender on TFS duration was not found to be significant (p=0.570).

DISCUSSION

Generally, CLL is not an aggressive disease, but it might result in morbidity and mortality in some major complications. This study revealed that the number of male patients was twice higher than that of female patients. Our results were comparable to those of previous studies (11,12). Moreover, CLL is more common in elderly patients and the average age was found to be 70 years but in the present mean age of our patients was 64.7 years (13). Today, we think that the age of CLL seen is gradually decreasing. The disease stage and the rate of needing treatment at admission were found to be significantly higher in males than in women. However, OS rates did not differ significantly between males and females. Patients with high disease stage, LDH and older age regardless of gender, showed lower OS rates.

While ZAP70 was negative in 168 (91.8%) of our patients, it was found positive in only 15 (8.2%) patient. However, CD38 was negative in 158 (86.34%) patients and positive in 25 (13.66%) patients. Previous studies revealed that ZAP70 is not expressed in normal "B" lymphocytes but overexpressed in Ig heavy-chain genes unmutated CLL and demonstrated an equivalent clinical value to Ig heavy-chain genes mutational status about disease progression and survival (8,14). CD38 expression, as well as ZAP70 and the mutation status of Ig variable region heavy chain, are

Table 4. Modified Rai stages, mortality rates, distribution of age groups, first line chemotherapy responses and treatment status of patients according to gender

Gender		Male n (%)	Female n (%)	Total n (%)	p
*Stage	Low risk (0)	16 (13.30)	13 (20.60)	29 (15.80)	**0.018**
	Medium risk (1-2)	56 (46.70)	36 (57.10)	92 (50.30)	
	High risk (3-4)	48 (40.00)	14 (22.20)	62 (33.90)	
	Total	120 (100)	63 (100)	183 (100)	
Age	31-65 years old	57 (47.50)	31 (49.20)	88 (48.09)	0.826
	≥65 years old	63 (52.50)	32 (50.80)	95 (51.91)	
	Total	120 (100)	63 (100)	183 (100)	
*Treatment status	Treated	77 (64.20)	28 (44.40)	105 (57.40)	**0.011**
	No treated	43 (35.80)	35 (55.60)	78 (42.60)	
	Total	120 (100)	63 (100)	183 (100)	
Response to first-line therapy	Complete remission	31 (40.30)	10 (35.70)	41(39.00)	0.969
	Partial remission	32 (41.60)	15 (53.60)	47 (44.80)	
	Refractory	13 (16.90)	2 (7.10)	15 (14.30)	
	Progressive	1 (1.30)	1 (3.60)	2 (1.90)	
	Total	77 (100)	28 (100)	105 (100)	
Life status	Living	83 (69.20)	48 (76.20)	131 (71.60)	0.318
	Dying	37 (30.80)	15 (23.80)	52 (28.40)	
	Total	120 (100)	63 (100)	183 (100)	

*p<0.05

Table 5. Treatments for patients with chronic lymphocytic leukemia and distribution of patients' responses to applied treatment

Drugs	First order treatment (n=105) n (%)	Second and other order treatments (n=75) n (%)
Chlorambucil plus MP	35 (33.3)	13 (17.3)
Fludarabine	9 (8.6)	4 (5.3)
FC	23 (21.9)	7 (9.3)
FCR	13 (12.4)	17 (22.7)
CVP or CP	22 (21.0)	11 (14.7)
High-dose MP	3 (2.9)	3 (4.0)
Other treatments	-	20 (26.7)
Response rates		
Complete remission	41 (39.1)	23 (21.9)
Partial remission	47 (44.8)	42 (40.0)
Refractory	15 (14.3)	5 (4.8)
Progressive	2 (1.9)	1 (0.95)

MP: Methyl prednisolone, FC: Fludarabine-cyclophosphamide, FCR: Fludarabine-cyclophosphamide-rituximab, CP: Cyclophosphamide-prednisolone, CVP: Cyclophosphamide-vincristine-prednisolone

important prognostic indicators in CLL. The advantage of CD38 is its easy measurement (15,16). In our study, CD38 was found to be positive at a rate of 13.66%. Today, anti-CD38 antibody treatments are on the agenda (17). This result shows that 13.66% patients with CLL can benefit from anti-CD38 antibody treatments. Previous studies have confirmed the importance role of ZAP70 in CLL prognosis. One of these studies showed that ZAP70 is an independent prognostic factor that physicians must consider in determining the best treatment strategy and prognosis for patients with CLL (18). Second of these studies suggested that ZAP70 inhibitors possess significant potential for the treatment of autoimmunity, organ transplant rejection, graft-versus-host disease, and B-cell CLL (19).

Bone marrow biopsy was performed in 27 (14.8%) of 183 patients that 59.3% showed diffuse bone marrow infiltration and 40.7% showed nodular bone marrow infiltration. Since very few patients underwent bone marrow biopsy, it was not very suitable for evaluation in this study. However, it can be said that the diffuse involvement is more. Accordingly, monoclonal gammopathy was investigated in 74 of the patients. Among these patients 27 (36.5%) were identified with monoclonal gammopathy, whereas 63.5% were not. Of the patients who had monoclonal gammopathy, 12 of them had IgG Kappa, 5 had IgM Kappa, 3 had IgG Lambda and 7 had other band. When the effect of having monoclonal gammopathy on total survival was examined, results showed that having monoclonal gammopathy did not have any effect on the total duration of survival (p=0.922). No relationship was found between the monoclonal protein and OS. This result has not been previously reported in the literature. Therefore, it was not appropriate to evaluate the relationship between monoclonal proteins and OS.

The most used prognostic parameter is the Rai staging system (8). Although 30 years after its development, this staging system still has significant use in CLL. The collection of information on age, sex, and performance status are adjuncts to prognosis but are not sufficiently useful to permit accurate prognostic counseling for a given patient. Other easily monitored prognostic markers include LDH, β_2-MG, serum thymidine kinase and lymphocyte doubling times are found. The sign of widespread bone marrow infiltration is a lower indicator of survival (14,20). The relationship of β_2-MG to disease mass is controversial. One retrospective series of 302 untreated patients found it to be the strongest predictor of 5-year survival on multivariate analysis (21), but another study in a prospective trial (n=106) did not find it to be a significant predictor of survival using multivariate analysis (22). Some risk factors affect CLL regardless of gender in this study. When evaluated according to these risk factors, disease stage, age and LDH levels were found to be effective risk factors in this study. However, gender, β_2-MG and ESR were not found to be effective.

According to the modified Rai staging system, the ratios of patients in the low-risk group (stage 0), intermediate-risk group (stage 1 and 2), and high-risk group (stage 3 and 4) were 15.8%; 50.3% and 33.9% respectively. In some studies, in the literature, it has been reported that the number of patients in the high-risk group is less and that patients are usually diagnosed at an early age (23,24). In our study, at the time of diagnosis, female patients were mostly in low and intermediate risk groups whereas male patients were mostly in intermediate and high-risk groups and this finding was statistically significant. This result shows that the disease can progress worse in male patients. Therefore, male patients may have accepted treatment more than female.

Advanced age alone is a high-risk factor for cancer. Age is reported as an independent negative factor for the survival of most studies of CLL patients (4,25-28). However, it should be kept in mind that CLL and other age-related diseases are the most common cause of death in most elderly patients. Older patients have been treated less frequently than younger patients and are often not given heavy chemotherapy. This resulted in a significantly lower complete response rate and a shorter survival rate. However, mortality attributable to the disease was not significantly different between young and older patients. In our

study, patients were grouped according to their age as 31-65 years and equal and over 65 years old. No statistical difference was found between these two groups according to gender (p=0.826).

The number of patients who required chemotherapy in our study was 105. It was observed that 64.2% of the male patients needed treatment, while 35.8% did not need treatment. However, it was observed that 44.4% of the female patients needed treatment, whereas 55.6% did not need treatment. It was found statistically significant that male gender was effective in the need for treatment and that male patients had more treatment requirements than female patients.

Chemotherapy protocols and responses were critically important issues in our study. CR was achieved 41 of these patients after 1st line chemotherapy. However, after the first line chemotherapy, 47 patients had PR, 15 patients had refractory and 2 patients had progression. Gender is important in the diagnosis and diagnosis of CLL. The occurrence of CLL in male and female is drastically different (29). For example, the incidence of US CLL in 1975-2001 was 5.0 per 100,000 per year for men and 2.5 for women (30). Additionally, female CLL patients have better 10-year survival and a better response to treatment (31). Understanding the mechanism behind these gender differences will provide valuable information about CLL. In the current study, some patients had refractor disease (14.3%), but only a few showed progression of the illness (1.9%) after first-line chemotherapy. TFS and OS are the same as male, although female is detected at a lower stage, according to our results. These results made us think of 2 hypotheses. First, the female have a worse course of CLL, even if they are lowly staged. Second, because females are at a low stage, they have a lower TFS or OS without treatment because they are less treated.

At the end of the study, of 183 patients, 131 (75.6%) were alive and 52 (24.4%) died. The causes of death differed with the most frequent causes are pneumonia (38.6%), sepsis (26.9%), heart disease (11.7%) and other causes (22.8%). Previous studies revealed the prognostic factors that affect survival were; male gender, high level of lymphocytes in the blood at the time of diagnosis, lymphocyte doubling time, CD38-positive cell count being more than 30%, positivity of ZAP70, existence of some cytogenetic abnormalities, serum LDH, high level of β_2-MG and bone marrow diffuse involvement (32-38).

Some of the previously conducted studies revealed a ratio of CR between 4 and 10%, and a PR ratio between 36 and 50% (24,39-42). In the current study, CR was achieved 41 of these patients after 1st-line chemotherapy, and 23 patients achieved CR with 2nd, 3rd, 4th, and 5th line of chemotherapy. In total, 63 patients achieved CR. Although the treatments applied in this study were significantly successful and the participating patients had similar demographic characteristics with the patients in previous studies, the OS time was less in the patients who received the treatment. These treatments were successfully implemented in the other studies, such as first-line treatment with FCR is associated with a response rate of 90-95% and a CR rate of 40-75%. The patients analyzed in this study were treated between 1999 and 2014. The most important first line chemotherapy protocols applied at that time were chlorambucil plus methylprednisolone, FC and FCR. Although new treatment approaches have been discovered, FCR is still an important protocol for treating CLL (43-45).

Study Limitations

This study has several limitations. A limitation of our study is that the number of patients is low, and the second is to conduct our study in patients diagnosed since 1999. Because after 1999 there have been many changes in the diagnosis and treatment of CLL. In more patients, the monoclonal protein should be evaluated.

CONCLUSION

Although this study examined CLL patients with similar demographic data as to other CLL studies, differences were observed in the agents used for chemotherapy due to the period covered by the study. In our study lymphocyte immunophenotypes such as ZAP70, CD38, CD11c, and other lymphocyte immunophenotypes were not associated with OS. However, large series of studies with anti-CD38 and other antibodies are needed for treatment response relationships. As in the literature, we have shown that disease stage, age and LDH is associated with OS. At the end of our study, it was observed that the disease stage was more advanced in men. Simultaneously, our study showed that men need more treatment than women. Additionally, advanced cytogenetic analysis and prognostic markers can be used at the time of diagnosis, CLL treatment and follow-up. It will enable them to do better and extend their survival.

Ethics

Ethics Committee Approval: This study was approved by the Local Ethical Committee of University of Health Sciences Turkey, Antalya Training and Research Hospital (decision no: 18/3, date: 02.05.2013).

Informed Consent: Informed consent was obtained from the patients after a detailed explanation on the objectives and scope of the study was provided, in line with the principles of the Declaration of Helsinki.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.E.D., E.K., A.T., Concept: F.E.D., E.K., A.T., Design: F.E.D., E.K., A.T., Data Collection or Processing: F.E.D., Analysis or Interpretation: F.E.D., E.K., A.T., Literature Search: F.E.D., Writing: F.E.D.

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ORIGINAL ARTICLE

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Correlation Between the Carotid Artery Intima Media Thickness and Gamma Glutamyl Transferase Level in Maras Powder Users

Fulsen Bozkuş[1], Nursel Dikmen[2], Ahmet Köylü[3]

[1]Kahramanmaraş Sütçü İmam University Faculty of Medicine, Department of Chest Diseases, Kahramanmaraş, Turkey
[2]Hatay Mustafa Kemal University Faculty of Medicine, Department of Chest Diseases, Hatay, Turkey
[3]Pazarcık State Hospital, Clinic of Radiology, Kahramanmaraş, Turkey

Abstract

Objective: In this study, we evaluated carotid artery intima-media thickness (CIMT), an early marker of atherosclerosis in smokers and Maras powder users, and serum gamma glutamyl transferase (GGT) levels, which is a marker of oxidative damage.

Methods: The study included 27 men who do not smoke but use maras powder, 32 men who smoke but do not use maras powder and 30 men who neither smoke nor use Maras powder as the control group. Serum levels of GGT and lipid profiles were measured and the results were compared between the groups. CIMT was measured by Doppler ultrasonography.

Results: Mean CIMT was found as 0.79±0.09 mm, 0.74±0.08 mm and 0.55±0.08 mm in Maras powder, smoking and control groups; respectively. The difference among the groups was statistically significant ($p<0.001$). Plasma levels of total cholesterol, low-density lipoprotein-cholesterol and triglyceride were significantly higher in the Maras powder and smoking groups than in the control group ($p<0.001$). Plasma levels of high-density lipoprotein-cholesterol levels were significantly lower in the Maras powder and smoking groups than in the control group ($p<0.001$). Serum GGT levels were found significantly higher in the Maras powder and smoking groups compared to the control group ($p<0.001$).

Conclusion: This study reveals that Maras powder is as effective as cigarette smoking on increasing of oxidative stress, which plays a role in the pathogenesis of many diseases. Our results suggest that CIMT is an important marker of cardiovascular complications in Maras powder users and smokers as a non-invasive method.

Keywords: Maras powder, carotid artery intima-media thickness, gamma glutamyl transferase

INTRODUCTION

Smoking is the most common type of tobacco use. However, a kind of tobacco use form, known as smokeless tobacco, presents some geographical differences in terms of usage and prevalence (1). Maras powder is a type of tobacco powder obtained from tobacco leaves, used in our region by applying through the oral mucosa. There is a common belief among native people that Maras powder is less harmful than smoking and even it is used mainly to help smoking cessation. However, as is shown in many studies, Maras powder is at least as harmful as smoking on health (2-4).

Studies have shown that many chemicals in most smokeless tobacco products and cigarets directly or indirectly lead to the formation of reactive oxygen species (ROS) (5). Normally, ROS levels are balanced through neutralization by the antioxidant defense systems of the body. When this balance is impaired in favor of ROSs, destructive reactions occur on the molecules such as proteins, lipids and nucleic acid. This condition is known as "oxidative stress" and ultimately leads to tissue damage (6). The change begins with increased carotid artery intima-media thickness (CIMT), increase because of oxidative stress in smokers and Maras powder users, causing atherosclerosis and narrowing

Address for Correspondence: Fulsen Bozkuş, Kahramanmaraş Sütçü İmam University Faculty of Medicine, Department of Chest Diseases, Kahramanmaraş, Turkey
Phone: +90 344 280 33 51 **E-mail:** fulsenbatmaz@gmail.com **ORCID ID:** orcid.org/0000-0002-6498-4390

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and even occlusion of the lumen in the future (7). Recent studies have shown that CIMT can be used as a predictor of cardiovascular events. Moreover, the relationship between CIMT and major cardiovascular events has been proven (8).

Gamma glutamyl transferase (GGT) is an enzyme, which exists in serum and on the exterior surface of the cell membrane and catalyzes the transfer of gamma glutamyl residues of amino acids or small peptides (9). The effects of GGT on oxidative stress and glutathione metabolism is the most important mechanism that indicates the relationship between serum GGT levels and cardiovascular diseases (10).

In this study, we evaluated CIMT, an early predictor of atherosclerosis, and serum GGT levels, a marker of oxidative stress, in people who use Maras powder, which is a kind of smokeless tobacco product that contains chemicals similar to those found in cigarette. Additionally, we compared these parameters between the Maras powder and smoking groups. Several studies are available to show increased CIMT in Maras powder users, but our study is the first to examine both CIMT and serum GGT levels in the same patient group cooperatively.

METHODS

This study included 32 men as the smoking group, 27 men as the Maras powder user group and 30 healthy men as the control group. Because the use of Maras powder is common among men due to socio-cultural structure, groups were created with male subjects. The study was approved by the Local Ethics Committee of Kahramanmaras Sutcu Imam University Faculty of Medicine (decision no: 02, date: 21.03.2016). The participants were informed about the study and gave consent for carotid Doppler ultrasound (USG) screening and blood sampling.

The study groups were created from participants who have no any pulmonary symptoms, medication history and antioxidant preparations and without no underlying metabolic, endocrine, or malignant disease. Participants with underlying chronic lung disease, heart failure, hypertension (HT), malignancy, liver and kidney failure and a history of other systemic diseases were excluded from the study. Maras powder usage, duration and frequency and smoking duration and pack-year were recorded.

Ultrasound Examination

CIMTs were measured in all participants using an 8 MHz linear vascular probe in Doppler device (Mindray DC7) at Pazarcik State Hospital, Clinic of Radiology. All measurements were made by a single radiologist in the same device order to avoid inter-observer variations. USG evaluations were made after resting in the supine position for 15 min. For measurement of the carotid artery, the head was turned about 10º toward the opposite direction. USG transducer was diverted to the parallel echo lines of the intima and media walls after positioning onto the carotid arteries long axis at an angle of 90º. The distance between the lumen-intima interface and the media-adventitia interface was measured.

Laboratory Testing

5 mL venous blood samples were collected from the vessels on the forearm of each participant, placed into the biochemistry tubes and GGT levels were determined by Roche Hitachi device.

Statistical Analysis

The data were analyzed using the SPSS software. Descriptive statistics are expressed as mean and standard deviation. One-Way ANOVA test was used to determine differences between groups, followed by post-hoc Tukey HSD and Tamhane tests to detect the differences in the group with difference. $p<0.05$ values were considered statistically significant.

RESULTS

The Maras powder group included 27 (mean age: 51.1±10.4 years), smoking group 32 (mean age: 48.8±9.3 years) and control group 30 men (mean age: 47.1±11 years). There was no difference in age and body mass index (BMI) between the groups. Durations of the use of Maras powder and smoking were similar (Table 1).

When plasma lipid levels of all groups were compared, it was seen that there was a significant difference between the tobacco users (Maras powder users and smokers) and control group. Plasma levels of total cholesterol, low-density lipoprotein (LDL) cholesterol and triglyceride were significantly higher in the Maras powder group than in the control group ($p<0.05$), whereas there was no significant difference between the Maras powder user and smoking groups. Plasma levels of high-density lipoprotein (HDL)-cholesterol were significantly lower in the Maras powder user and smoking groups compared to the control group (Table 1). Systolic arterial pressure was higher in the Maras powder user and smoking groups compared to the control group ($p<0.05$). Systolic arterial pressure values of the Maras powder group were significantly higher than those of the smoking group ($p<0.05$).

CIMT, which is suggested to be related to cardiovascular risk factors, was measured by USG and the groups were compared for CIMT values. CIMT values of the Maras powder and smoking group were significantly higher compared to that of the control group ($p<0.05$). Figure 1 shows the distribution of CIMT values according to the study groups.

Table 1. General characteristics of study population				
	Maras powder (n=27)	**Cigarette smokers (n=32)**	**Non-tobacco users (n=30)**	**p**
Age, years	51.1±10.4	48.8±9.3	47.1±11	NS
BMI, kg/m^2	27.7±4	26.3±4.1	27.3±4.9	NS
Tobacco use duration (year)	10.8±2.1	15±6.5	0	-
Systolic BP (mmHg)	135.7±12.7*	128.1±10.9	117.1±7.6	**0.001**
Diastolic BP (mmHg)	78±20.6	79.6±8.3	75±6.8	**0.03**
GGT (U/L)	52.4±21.1	42.6±20.5	20.3±11.3	**0.001**
Total cholesterol (mg/dL)	210.5±26.1	208.4±19.2	185.6±9.2	**0.001**
Triglycerides (mg/dL)	194.5±74.6	170.8±47.5	144.3±43	
HDL cholesterol (mg/dL)	36.9±6.9	38.2±3.9	48±8.3	**0.001**
LDL cholesterol (mg/dL)	139.6±25.6	133.7±27.9	106.7±12	**0.001**
CIMT (mm)	0.79±0.09	0.74±0.08	0.55±0.08	**0.001**

*p<0.05 for comparison with cigarette smokers, p=0.02. BMI: Body mass index, F: Female, M: Male, GGT: Gamma glutamyl transferase, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, BP: Blood pressure, CIMT: Carotid intima media thickness

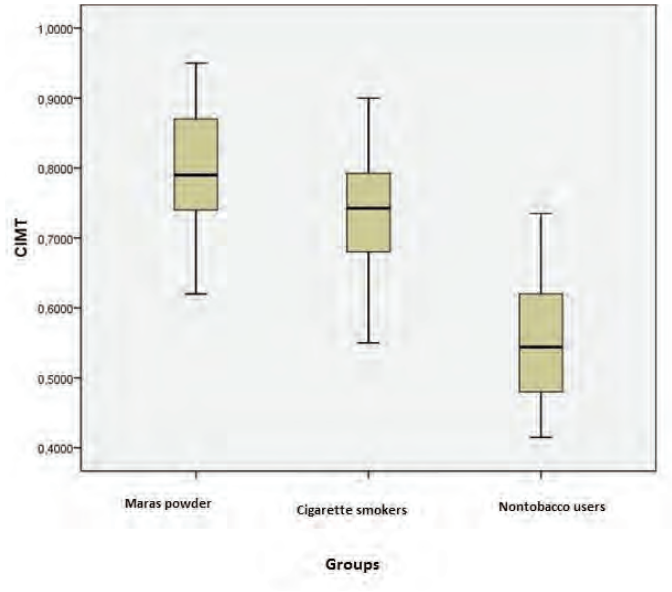

Figure 1. Distribution of CIMT values according to the study groups
CIMT: Carotid intima media thickness

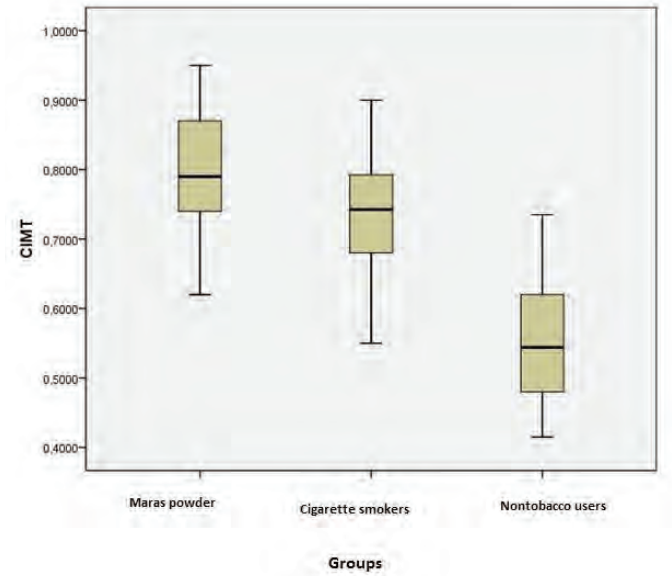

Figure 2. Distribution of serum GGT levels according to the study groups
GGT: Gamma glutamyl transferase, CIMT: Carotid intima media thickness

Additionally, serum GGT levels were evaluated to determine cardiovascular risk in the Maras powder user and smoking groups. The level of GGT was found to be significantly higher in the Maras powder user and smoking groups than in the control group (p<0.001). Figure 2 shows the distribution of serum GGT levels according to the study groups. A positive correlation was found between GGT levels and CIMT (r= 0.366, p=0.001) in Maras powder user and smoking group (Figure 3).

DISCUSSION

In this study, we evaluated the effects of Maras powder, a smokeless tobacco product, and cigarette on CIMT and serum levels of GGT. We conclude that smoking and the use of Maras powder increases CIMT.

Smoking is an important factor contributing to oxidative stress, which is caused by ROS and triggers the pathological events (11,12). It has been shown in many studies that high number of free oxygen radicals due to smoking, increase lipid peroxidation and oxidative DNA damage in the cell. Maras powder contains carcinogenic and mutagenic agents in varying amounts as in cigarette (13). Therefore, endothelial dysfunction occurs because of the formation of free radicals and subsequently developing oxidative stress due to smoking and the use of Maras powder. Endothelial dysfunction also predisposes to atherosclerosis. Studies have shown that oxidative stress plays an important role

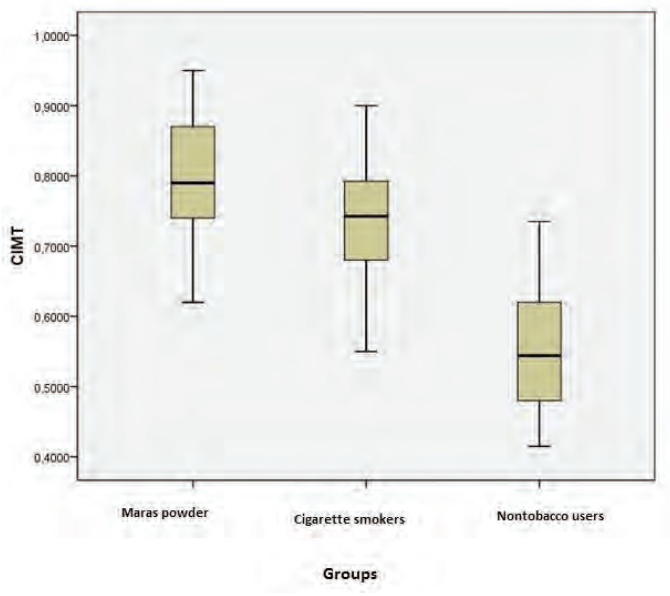

Figure 3. Correlation of GGT levels and CIMT
GGT: Gamma glutamyl transferase, CIMT: Carotid intima media thickness

in the pathogenesis of atherosclerosis. LDL in serum is converted to the oxidized LDL, an atherogenic form by oxidation, after which the accumulation of oxidized products in macrophages form foam cells and finally atheroma develops (7).

The primary role of GGT, which is a critical diagnostic test for the diagnosis of gallbladder disease, is to destroy the reduced glutathione, which is outside the cell and to provide precursor amino acids for the synthesis of intracellular glutathione. Furthermore, GGT is an important enzyme that mediates the regulation of important reductive events such as antioxidant/anti-toxic defense and cell proliferation/apoptosis balance (14,15). In recent experimental studies, it has been reported that cellular GGT might increase the formation of ROS in the presence of iron and other transition metals. In an animal study, it was been claimed that GGT activity might be a marker for oxidative stress because GGT expression is significantly increased by oxidants in rat lung epithelial cells (16). Eventually, use of increased serum GGT activity as a marker of oxidative stress in humans has been suggested (17,18).

Oxidative stress is increased due to smoking and is effective in the development of smoking-related diseases, has been shown in many studies to be also increased by Maras powder use. Köse et al. (6) found that the Maras powder is actually effective in increasing oxidative stress and that serum total antioxidant capacity levels are decreased as the duration of maras powder use increases. In parallel to this work, Samal et al. (19) showed that in their study including 60 healthy male chewing tobacco, increased erythrocyte malondialdehyde levels depending on the duration of use and decreased erythrocyte superoxide dismutase and glutathione reductase levels that resulted in increased oxidative stress. Yildiz et al. (20) found that glutathione and malondialdehyde levels are significantly decreased in smokeless tobacco product users. Similarly, we found in our study that the serum GGT levels in Maras powder users and smokers were significantly increased compared in the control group.

The effects of GGT on oxidative stress and GSH metabolism are the most important mechanisms demonstrating the relationship between serum GGT and cardiovascular diseases. In some studies, GGT activity has been detected in the carotid and coronary artery atheromas (21). It was claimed that GGT present in atheromas might contribute to plaque formation and rupture by catalyzing the oxidation of the lipoproteins (22). CARDIA study has revealed a strong and positive correlation between serum GGT levels and traditional markers of oxidative stress such as the levels of C-reactive protein, uric acid, and fibrinogen (23). Similarly, according to Emdin et al. (24) GGT level has an independent predictive value for mortality and the incidence of non-fatal myocardial infarction in patients with a history of myocardial infarction and documented coronary artery disease. The prognostic significance of GGT levels for overall and cardiac related mortality has been shown by a large study including middle-aged men (25). Our study revealed a positive correlation between serum GGT levels and CIMT as an early and non-invasive predictor of atherosclerosis in smokers and Maras powder users.

CIMT increases in the presence of risk factors for atherosclerosis, such as age, cholesterol, diabetes mellitus, HT and smoking. Studies have shown the use of Maras powder as another reason. Sucakli et al. (26) showed increased CIMT due to the use of maras powder and found a positive correlation between CIMT and systolic/diastolic arterial pressure. Similarly, in our study, CIMT and systolic arterial pressures were higher in the smoker and Maras powder user groups compared to the control group. The increase in systolic and diastolic arterial pressure is closely associated with increased CIMT (27). It may be considered that increased CIMT by the use of Maras powder is a result of acute HT due to adrenaline release and subsequent stimulation of the sympathetic nervous system caused by the high nicotine content of Maras powder (28).

The use of Maras powder and smoking adversely affect LDL cholesterol and triglyceride levels, which have been mainly blamed in many studies for developing atherosclerosis. Similarly, in our study, plasma levels of total cholesterol, LDL cholesterol and triglyceride levels were significantly higher in the Maras powder user group compared to the control group, whereas

there was no significant difference between the Maras powder user and smoking group. Plasma levels of HDL-cholesterol were significantly lower in the Maras powder user and smoking groups compared to the control group.

Study Limitations

There are some limitations to our study. First, it was conducted with a relatively small number of subjects that was likely inadequate to reflect cardiovascular complications in Maras powder users and smokers. Additionally, we excluded patients whose BMI less than 25. We believe that further studies with larger sample sizes are needed to confirm these findings. Furthermore, cross-sectional structure of the data did not allow to establish causal links.

CONCLUSION

CIMT measurement is an easily applicable non-invasive method that enables the early detection of atherosclerotic changes in the vascular bed. Our results suggest that CIMT measurement is important in terms of being a non-invasive examination method which can be used to detect cardiovascular complications in Maras powder users and smokers. People traditionally start to use Maras powder at an early age and continue throughout life. It is generally thought to be harmless or less harmful than cigarettes, so people use Maras powder as a smoking cessation method or to reduce the harmful effects of smoking. However, we concluded with the results of the current study that Maras powder is as dangerous as smoking.

Ethics

Ethics Committee Approval: The study was approved by the Local Ethics Committee of Kahramanmaras Sutcu Imam University Faculty of Medicine (decision no: 02, date: 21.03.2016).

Informed Consent:

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.B., Concept: F.B., N.D., Design: F.B., N.D., A.K., Data Collection or Processing: F.B., A.K., Analysis or Interpretation: F.B., N.D., Literature Search: F.B., N.D., Writing: F.B.

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Importance of Eosinopenia in COVID-19 Infection

Neslihan Uslu[1], Zahide Taş[1], Tuğba Naziroğlu[2], Nergiz Ekmen[3], Buket Yağcı[4], Hadi Sasani[5], Pınar Özkan Epöztürk[2], Semih Sözen[6], Reyhan Diz Küçükkaya[7], Nalan Ünel Özcan[8]

[1]Pendik State Hospital, Clinic of Internal Medicine, İstanbul, Turkey
[2]Pendik State Hospital, Clinic of Chest Diseases, İstanbul, Turkey
[3]Gazi University Faculty of Medicine, Department of Gastroenterology, Ankara, Turkey
[4]University of Health Sciences Turkey, İstanbul Haydarpaşa Numune Training and Research Hospital, Clinic of Radiology, İstanbul, Turkey
[5]Tekirdağ Namık Kemal University Faculty of Medicine, Department of Radiology, Tekirdağ, Turkey
[6]Pendik State Hospital, Clinic of Emergency Medicine, İstanbul, Turkey
[7]Private Practice, Hematologist, İstanbul, Turkey
[8]Pendik State Hospital, Clinic of Infectious Diseases, İstanbul, Turkey

Abstract

Objective: Dynamic changes in the number of eosinophils are observed during the diagnosis and follow-up in coronavirus disease-2019 (COVID-19). Our aim was to show the role of the absolute eosinophil count in the diagnosis of COVID-19 and the relationship with disease severity and prognosis.

Methods: In this study, 191 patients (130 inpatients, 61 outpatients) diagnosed with COVID-19 pneumonia with the polymerase chain reaction test and lung computed tomography; and 22 patients with positive influenza test were included as the control group. All demographic, biochemical data, clinical and radiological characteristics were recorded.

Results: The mean eosinophils on first day of the inpatient COVID-19 group were found to be statistically lower than the influenza group and the ambulatory groups (p=0.001, p=0.0001).

Conclusion: A low eosinophil count in complete blood count, can aid in the early diagnosis of infection. Persistent eosinopenia progresses with disease severity and may help determine the prognosis of the disease.

Keywords: COVID-19, eosinophils, risk factors, severe acute respiratory syndrome, coronavirus 2

INTRODUCTION

Upon detection of pneumonia cases of unknown source of origin in Wuhan, China in December 2019, World Health Organization reported a virus called the coronavirus disease-2019 (COVID-19) virus as the cause of pneumonic cases (1). Coronaviruses (CoVs) are single-stranded positive RNA viruses. The mutation rate is higher than that of DNA viruses (2). The epidemic started from wild animals at the live animal market in Wuhan (3).

Eosinophils consist about 1%-3% of peripheral blood leukocytes. Their presence in tissues is several hundred times greater than in blood (4) and they are found in the spleen, lymph nodes, thymus, gastrointestinal mucosal surfaces (5). Eosinophil numbers do not alter much in the body, except for some diseases (6). Eosinophils are leukocytes with pro-inflammatory action. When eosinophils are activated, they can release eosinophil cationic protein, eosinophil neurotoxin, eosinophil peroxidase. Eosinopenia may develop because of accumulation in the inflammation area, suppression in the bone marrow (7). Additionally, decreased chemokine receptor expression, eosinophil apoptosis caused by type 1 interferons released during acute infection may be the cause of eosinopenia (8). Eosinopenia can be seen in sepsis, and

Address for Correspondence: Nergiz Ekmen, Gazi University Faculty of Medicine, Department of Gastroenterology, Ankara, Turkey
Phone: +90 505 677 05 57 **E-mail:** dr_nergisekmen@hotmail.com **ORCID ID:** orcid.org/0000-0002-7921-3169

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typhoid fever (9,10). It has been shown that IL1B, IFNγ, IP10, and MCP values are elevated in COVID-19 patients, leading to helper T1-cell responses (11).

In the literature, eosinopenia has been seen in COVID-19 pneumonia, especially in the early stages of the disease, even in asymptomatic cases (3,8,12-20). Although the role of eosinophils in the pathogenesis of COVID-19 patients has not been clearly explained, CLC (Galectin-10), RNASE2 (EDN), and the eosinophil chemokine CCL11 (eotaxin-1) levels were elevated, and eosinophils were thought to play a role in the pathogenesis of COVID-19 (21,22).

Low eosinophil levels have been shown to be associated with poor outcomes in critical illness in many studies (13,16).

Symptoms, frequently seen in the disease such as fever, dry cough and shortness of breath, are not specific and can be seen in other non-bacterial pneumonia (23). Additionally, severe lymphopenia and eosinopenia are less common in other viral pneumonia, but frequently seen in COVID-19 pneumonia (8). Therefore, early isolation and diagnosis of the virus is important to terminate the pandemic (24). *In vitro* laboratory tests are widely used to assess disease severity, to monitor and treat patients, and to determine the prognosis (25). Studies show that there are significant changes in some hematological parameters in patients with COVID-19 pneumonia (26); normal or increased leukocyte count, decreased lymphocyte count, thrombocytopenia, decreased albumin value, high transaminase, bilirubin, lactate dehydrogenase, creatine kinase, myoglobin, procalcitonin, C-reactive protein (CRP), D-dimer values, increased prothrombin time, erythrocyte sedimentation rate values were detected (11).

In some studies, it was found that there was a significant decrease in absolute eosinophil counts in COVID-19 pneumonia, predicting that eosinopenia could be used among the early diagnostic criteria (12-16,27,28).

In this study, we showed the role of the absolute eosinophil count in the diagnosis of the disease during COVID-19 and the relationship between the number of eosinophils and disease severity and prognosis.

METHODS

Ethical Consideration

This study was approved by the Kartal Dr. Lutfi Kirdar City Hospital Clinical Research Ethics Committee and all procedures performed in the 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 (decision no: 2020/514/179/11, date: 11 June 2020).

Study Population

The data of 191 patients (94 males, 97 female) aged between 18 and 90 years who were admitted to the hospital between March 15, 2020 and May 14, 2020 with the diagnosis of COVID-19 pneumonia; outpatient (n=130) and inpatient (n=61) were retrospectively included in this study (Figure 1).

In the study, the 2019-COV polymerase chain reaction (PCR) test (VIROSWAB UMF) studied on the combined nose and throat swab sample of 167 patients were found to be positive, while 24 patients were negative. This was accepted as COVID-19 pneumonia due to clinical and radiological findings.

Vulnerable populations (children, pregnant women) were excluded from the study. Only the data of patients who received the diagnostic methods and treatments specified in the COVID-19 guidelines of the Ministry of Health were retrospectively analyzed (29).

Statistical analysis were performed by recording age, gender and clinical characteristics, biochemical data, complete blood count (CBC) parameters, lung computed tomography (CT) findings, PCR tests and treatments of all patients.

The study population was grouped as outpatient and inpatient. CBC findings, including changes in eosinophil count with clinical and radiological course were examined. Additionally, as the control group, the eosinophil counts in the hemogram data of 22 patients admitted with positive influenza test and negative 2019-COV-PCR test results were compared retrospectively. Thus, the place of absolute eosinophil count in the diagnosis of the disease, its relationship with disease severity and prognosis were compared with patients diagnosed with COVID-19.

Inpatients diagnosed with COVID-19 pneumonia were divided into two groups as severe and non-severe patients according to the laboratory criteria, the number of days of hospitalization, the clinical course, the involvement prevalence score in chest CT and the ratio of multiple drug use (plaquenil, favipravir, lopinavir-ritonavir).

CT Protocol

Chest CT imaging was performed on a 16-detector CT scanner (Emotion; SIEMENS). Patients were scanned in the supine position, during breath hold, from the lung apices down to the costophrenic angles. The acquisition parameters were as tube voltage: 130 kV, tube current: 25 mAs, pitch: 1.5, FOV: 512 mm, and slice thickness: 0.6 mm. Thick images (3 mm) were

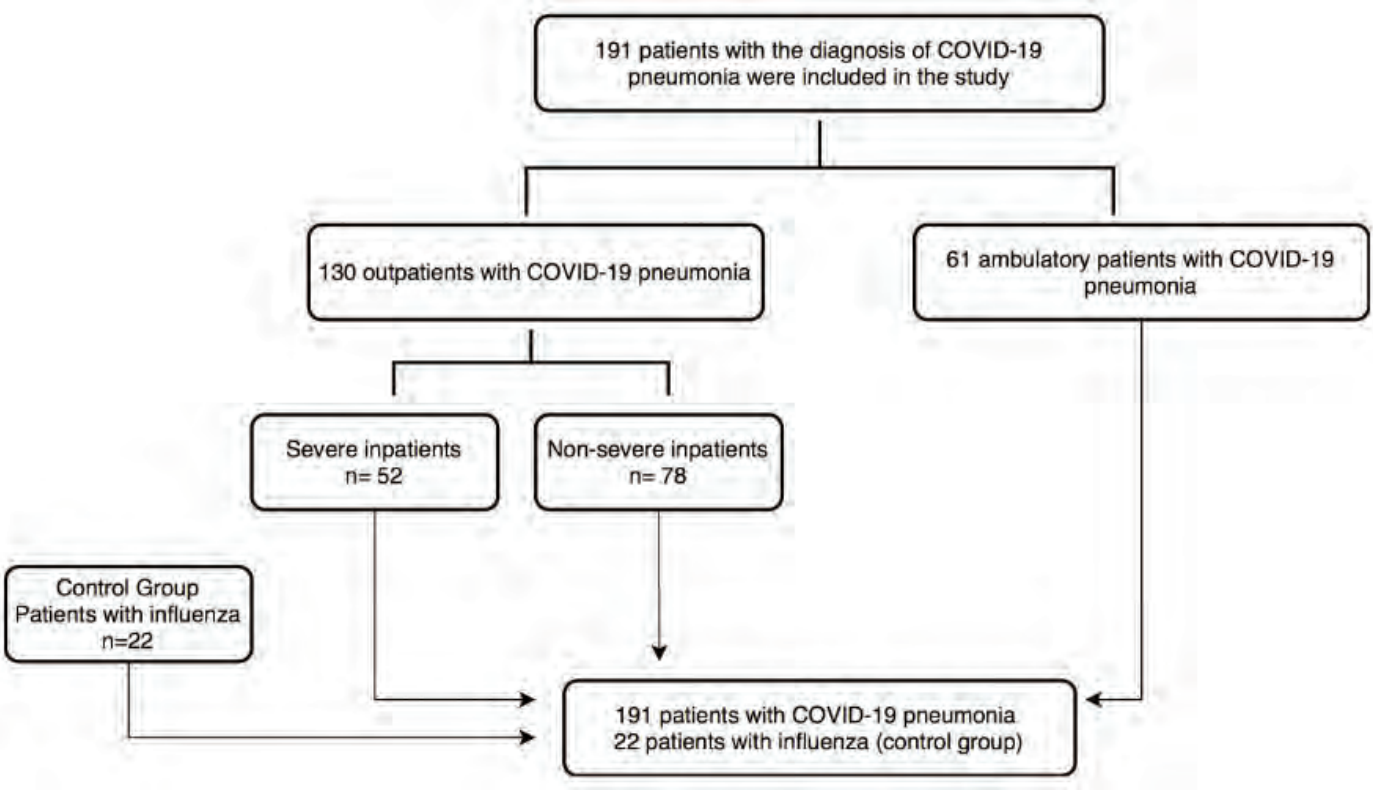

Figure 1. Diagram of the patient's population
COVID-19: Coronavirus disease-2019

reconstructed using a high-frequency reconstruction algorithm, and lung windowing and stored in the picture achieving and communicating system. A contrast material was used in none of the patients.

Chest CT Image Analysis

The initial images and chest CT scan obtained from 191 patients were reviewed by (8 years) experienced radiologists. All Digital Imaging and Communications in Medicine images were analyzed from CT studies without access to patients' clinical findings.

On each CT scan, lung lobe involved, the location of lesion categorized as central, peripheral, or both were recorded. The extent of the involvement in the lesions was classified as either focal or multifocal. The predominant pattern was categorized as ground glass opacities (GGO, defined as hazy areas of increased attenuation without obscuration of the underlying vascular markings), consolidation (parenchymal opacities obscuring underlying vessels), and both patterns. Additionally, the margin definition, interlobular septal thickening, crazy paving (thickened interlobular septa and intralobular lines superimposed on a background of ground-glass opacity) (30) air bronchogram, bronchiolectasis, cavitation, thickening of interlobular septa, parenchymal bands, tree-in-bud, pleural effusion and lymphadenopathy (defined as lymph node with a short-axis dimension of >1.0 cm) were also recorded (31).

Chest CT Severity Score Assessment

Each of the five lung lobes was assessed for the degree of involvement and classified as follows: None (0%) corresponded to a lobe score of 0, mild (1%-25%) corresponded to a lobe score of 1, moderate (26%-50%) corresponded to a lobe score of 2, severe (51%-75%) corresponded to a lobe score of 3 and critical (76%-100%) corresponded to a lobe score of 4. The "total severity score" was determined by summing all five lobes' scores (range of possible scores, 0-20) (31).

Statistical Analysis

Statistical analyses were performed using statistical software (NCSS: Number Cruncher Statistical System 2007, Utah, USA). In addition to descriptive statistical methods (mean, standard deviation), the distribution of variables was examined with the Shapiro-Wilk normality test, One-Way analysis of variance paired in time comparisons of normally distributed variables, Newman-Keuls multiple comparison test was used in subgroup comparisons, One-Way variance in intergroup comparisons, independent t-test for comparison of paired groups, Friedman test for time comparisons of variables that do not show a normal

distribution, Dunn's multiple comparison test for subgroup comparisons, Kruskal-Wallis test for intergroup comparisons, Mann-Whitney U test for the comparison of paired groups, chi-square and Fisher's Exact test reality for comparisons of qualitative data. Pearson correlation test was used to determine the relationship between variables. Results were evaluated at the level of significance $p<0.05$.

RESULTS

Demographic Data

A total of 191 patients diagnosed with COVID-19 pneumonia, including 130 inpatients and 61 outpatients, and 22 influenza test positive control group were included in this study. In the study 94 (49.3%) were female and 97 (50.7%) were male. The mean age of 191 patients diagnosed COVID-19 pneumonia was 50. The mean age of the inpatient COVID-19 group was 54.87±12.43 while the mean age of the outpatient COVID-19 group was 39.9±11.62 (p=0.0001). The mean age of the influenza group was 42.5±21.49.

Characteristics of Severe Hospitalized COVID-19 Patients

The ratio of male/female patients in the severe inpatient group was 63.46/36.54 percent. While the mean age was found to be 56.83±10.18 in the severe group, the mean age was found to be 53.56±13.63 in the non-severe group. Neutrophil counts in the severe group mean CRP on the 1st and 3rd days, >10 days of hospitalization, intensive care (5 patients), intubation, death (5 patients), more than one drug use (plaquenil, favipravir, lopinavir-ritonavir) was seen. In lung CT scoring, the score of 5 out of 20 and above were found to be statistically significantly higher than the non-severe group (p=0.002).

Hemoglobin, lymphocyte and eosinophils 1st day averages of the inpatient group were found to be statistically significantly lower than the outpatient group (Table 1).

The prevalence of male patients was 63.46% in the severe group and 43.59% in the non-severe group. In the severe group, the mean CRP on the 1st and 3rd days, the number of days of hospitalization >10, the use of more than one drug that went to intensive care were found statistically significantly higher than the non-severe group. In the severe group, the number of those who scored 5 out of 20 in the lung CT scoring was significantly higher than that in the non-severe group.

In the severe group, compared to the non-severe group, on the 3rd day lymphocyte and eosinophils were lower and neutrophil and neutrophil to lymphocyte ratio was higher (Table 2 and 3).

In this study, as a control group, the 1st day hemogram data of 22 patients positive influenza test (2019-COV-PCR test result negative) were compared COVID-19 patients. The mean age of the inpatient COVID-19 group was found to be statistically significantly higher than the Influenza and outpatient COVID-19 groups (Table 4).

DISCUSSION

The study population consisted of 191 patients diagnosed with COVID-19 pneumonia with mean age 50. It was lower

Table 1. Comparison of day 1 and day 14 hemogram parameters of inpatient COVID-19 and outpatient COVID-19 patients

		COVID-19 inpatients	COVID-19 outpatients	p
Hemoglobin (g/dL)	1st day	13.40±1.63	13.98±1.65	**0.022**
	14th day	13.72±1.61	13.60±1.44	0.75
Platelets (10^3/µL)	1st day	226615.38±85221.59	239177.05±75928.07	0.327
	14th day	312485.29±119456.54	323833.33±88392.24	0.672
Leukocytes (10^3/µL)	1st day	6656.92±3427.7	6218.03±2225.5	0.362
	14th day	7094.12±2096.19	7183.33±1581.32	0.85
Neutrophils (10^3/µL)	1st day	4658.46±3208.03	3772.95±1807.29	**0.046**
	14th day	4123.53±1571.97	4195.83±1306.66	0.84
Lymphocytes (10^3/µL)	1st day	1439.23±723.85	1785.25±785.46	**0.003**
	14th day	2205.88±1014.8	2320.83±826.72	0.619
NLR	1st day	4.21±5.42	2.53±1.86	**0.01**
	14th day	2.34±2.01	2.01±0.9	0.442
Eosinophils (10^3/µL)	1st day	17.19±57.92	72.13±133.08	**0.0001**
	14th day	155.15±126.12	95.88±95.42	**0.039**

NLR: Neutrophil to lymphocyte ratio, COVID-19: Coronavirus disease-2019

Table 2. Comparison of inpatients with severe and non-severe inpatients at admission				
		Severe	**Non-severe**	**p**
Age		56.83±10.18	53.56±13.63	0.143
Sex	Female	36.54% (19)	56.41% (44)	-
	Male	63.46% (33)	43.59% (34)	-
CRP (mg/L)	1st day	63.16±48.8	29.55±27.01	**0.0001**
	3rd day	88.43±56.21	30.52±26.03	**0.0001**
Hospitalization days	<3 day	5.77% (3)	17.95% (14)	-
	3-10 day	53.85% (28)	79.49% (62)	-
	>10 day	40.38% (21)	2.56% (2)	**0.0001**
More than one drug	Yes	23.08% (12)	94.87% (74)	-
	No	76.92% (40)	5.13% (4)	**0.0001**
Intubation	No	88.46% (46)	100% (78)	-
	Yes	11.54% (6)	0	**0.002**
Clinical course	Intensive care	9.62% (5)	0	-
	Exitus	9.62% (5)	0	**0.0001**
Lung CT	>10	23.53% (12)	8.11% (6)	-
	5-10	58.82% (30)	47.3% (35)	-
	<5	17.65% (9)	44.59% (33)	**0.002**

CRP: C-reactive protein, CT: Computed tomography

Table 3. Comparison of hemogram parameters of severe and mild inpatients with COVID-19				
		Severe n=52	**Non-severe n=78**	**p**
Hemoglobin (gr/dL)	1st day	13.56±1.7	13.29±1.59	0.348
	3rd day	12.97±1.71	12.67±1.75	0.349
	14th day	13.86±1.54	13.61±1.67	0.516
Neutrophils (10^3/µL)	1st day	4363.46±2036.53	4855.13±3794.28	0.467
	3rd day	5417.31±3090.83	3420.29±2139.03	**0.0001**
	14th day	4080±1607.18	4157.89±1564.41	0.786
Lymphocytes (10^3/µL)	1st day	1417.31±804.09	1453.85±670.08	0.413
	3rd day	1250±674.03	1616.81±624.76	**0.0001**
	14th day	2070±1096.75	2313.16±946.16	0.192
NLR	1st day	3.58±1.93	4.63±6.81	0.18
	3rd day	5.84±4.86	2.31±1.39	**0.0001**
	14th day	2.68±2.56	2.07±1.41	0.6
Eosinophils (10^3/µL)	1st day	18.27±66.44	16.47±51.92	0.521
	3rd day	39.23±79.41	82.17±120.79	**0.006**
	14th day	166.67±149.33	146.05±105.51	0.785

NLR: Neutrophil to lymphocyte ratio, COVID-19: Coronavirus disease-2019

than the studies by Wang et al. (15) (56 years), Zhang et al. (12) (57 years), Chen et al. (32) (55 years), but higher than that by Huang et al. (11) (49 years). In our study, the mean age of the inpatient COVID-19 group (54.87±12.43) and the severe group in the hospitalized patients (56.83±10.18) were higher. Older people are more likely to get COVID-19 pneumonia and more hospitalizations are required over the age of 50 and the disease is more severe in inpatients. This may be due to older people having more comorbidity and a weaker immune response to diseases. These data are similar to the study of Zhang et al. (12).

Table 4. Comparison of demographic characteristics and day 1 hemogram parameters of COVID-19 inpatients and outpatients and control group influenza patients

		Influenza		COVID-19 inpatients		COVID-19 outpatients		p
Age		42.5±21.49		54.87±12.43		39.9±11.62		**0.0001**
Sex	Male	15	68.18%	63	48.46%	31	50.82%	0.231
	Female	7	31.82%	67	51.54%	30	49.18%	
Hemoglobin (gr/dL) 1st day		12.44±1.59		13.4±1.63		13.98±1.65		**0.001**
Neutrophils (10^3/µL) 1st day		3640.91±1807.3		4658.46±3208.03		3772.95±1807.29		0.231
Lymphocytes (10^3/µL) 1 st day		1077.27±418.54		1439.23±723.85		1785.25±785.46		**0.0001**
NLR 1st day		3.7±1.99		4.21±5.42		2.53±1.86		**0.0001**
Eosinophils (10^3/µL) 1st day		1.25±2.07		0.28±0.76		1.08±1.69		**0.0001**

NLR: Neutrophil to lymphocyte ratio, COVID-19: Coronavirus disease-2019

In our study, 97 (50.7%) of the patients diagnosed with COVID-19 pneumonia were male. This rate was found to be the same as the study of Zhang et al. (12) (50.7%). It was found to be lower than the data of reports from China's Center for Disease Control and Prevention (51.4%) (33) Wang et al. (15) (54.3%), Chen et al. (32) (73%), Huang et al. (11) (66%). In our study, the rate of male patients (51.54%) was higher in outpatients (49.1%). Additionally, the rate of male patients in the severe group (63.4%) in hospitalized patients was higher than that in the non-severe group (43.59%).

In our study, 2019-COV PCR test of 167 patients diagnosed with COVID-19 pneumonia was found to be positive. Although 24 of the inpatients were negative for 2019-COV PCR results, they were accepted as COVID-19 pneumonia according to their clinical and radiological findings. A similar study also had patients diagnosed clinically (18).

Except for 4 patients in the inpatient group, all patients in the study underwent lung CT at the time of admission to the hospital for early diagnosis (the other 4 patients were diagnosed at an external center and referred to our hospital). The most common involvement in lung CT was bilateral GGO and consolidation, and there was no difference in the number of lobes involved between the groups. These data were similar to the data in the literature (30).

Neutrophil counts in the severe group the averages of CRP in the 1st and 3rd days, days of hospitalization more than 10 days, going to intensive care mortality rate, the overuse of medication were found to be higher than the non-severe group (Table 2). Additionally, patients in the severe group scored higher than the distribution score on the lung CT (5 of 20 points) compared to the non-severe group. In the literature, as in our study, the prevalence of GGO involvement in lung CT was correlated with disease progression (17).

a) As in our study, in the study by Wang et al. (15) in patients with severe disease, inflammatory cells such as neutrophils and leukocytes were higher at the time of diagnosis, while lymphocyte and eosinophil counts were lower (12,16-18). Similar to the data in the literature, eosinophil counts were similar to lymphocyte counts in severe and mild patients (12,15,16). The number of eosinophils at the time of presentation is important in early diagnosis and seems to be related to disease severity. Additionally, the increase in eosinophil counts after treatment indicates that eosinophils can also be used to evaluate the response to treatment. Similarly, in the study by Jesenak et al. (17), normalization of eosinophil count in severe patients correlated with improvement in clinical condition.

In the literature, eosinopenia has been seen in COVID-19 pneumonia, especially in the early stages of the disease, even in asymptomatic cases (8,12-17,27,28,34). Especially in the study by Li et al. (27), eosinopenia and high hs-CRP combination had 67.9% sensitivity and 78.2% specificity in early diagnosis of disease and the area under the eosinopenia curve had a sensitivity of 0.717 to 74.7% and specificity of 68.7%.

b) In our study, eosinophil counts were found to be lower in the severely ill group compared to the non-severe group and were found to be persistent for a long time. In the study by Jesenak et al. (22), normalization of eosinophil count in severe patients correlated with clinical improvement, while severe and

persistent eosinopenia was observed in fatal COVID-19 patients. In the study by Zhang et al. (12), blood eosinopenia was observed in more than half of the patients in severe cases. Additionally, there was a correlation between eosinophil and lymphocyte counts at hospitalization and after 3 days in severe and non-severe patients. In our study, while there was a correlation between eosinophil and lymphocyte count in the mild patient group, it was not observed in the severe group. In the study by Azkur et al. (8), severe COVID-19 pneumonia was associated with a low eosinophil count. Tanni et al. (13) also showed that low eosinophil count is associated with early diagnosis and prognosis of the disease. Many studies have shown that low eosinophil levels are associated with poor outcome in critical illnesses (15,16).

In our study, the eosinophil count was found to be lower in severe patients who scored higher than the prevalence scoring in lung CT. Decreased blood eosinophil counts in severe patients may have early diagnostic value, particularly in patients with GGO in bilateral lungs. Even eosinopenia can occur before radiological findings. In this way, perhaps many patients can be diagnosed early and quickly prevent pandemics without performing lung CT. Thus, it can be cost-effective and unexposed to unnecessary radiation. In the study by Jesenak et al. (17), decreased eosinophil counts were associated with radiological changes such as GGO findings and respiratory symptoms, especially in the bilateral lung.

c) In this study, although eosinophil values were low in all groups, the mean eosinophil 1st day of the inpatient COVID-19 group was found to be significantly lower than the influenza group and outpatient COVID-19 groups. Although eosinopenia is not specific to COVID-19 infection, we believe that eosinopenia is a common finding in both COVID-19 and influenza. However, eosinophil values were found to be lower in COVID-19 patients than in patients with influenza. Firsth day eosinophil counts were 0 in 156 (81%) of 191 patients in COVID-19 patients and in 13 (59%) of 22 patients in the influenza group. Its lower rate, especially in inpatients, supports the that it is also associated with disease severity. A published article in 2003 reported that approximately 90% of SARS-CoV patients had eosinopenia (35). In the study of Tanni et al. (13) as in our study, eosinophil values were found to be lower in COVID-19 patients than in patients with influenza. Additionally, Tanni et al. (13) showed that eosinophil values decreased in COVID-19 patients, particularly in the first 48 h. Wu et al. (36) reported a case in which COVID-19 and influenza virus coexisted in China and they mentioned the difficulties in diagnosis and the similarities between the two diseases. In the study of Andreozzi et al. (19), it was stated that eosinopenia could be a potential indicator of influenza or SARS-CoV-2 infections (27).

The small number of patients in the influenza group can be considered a limiting factor of the study.

CONCLUSION

The presence of a low or no eosinophil count in CBC can help early isolation of the individuals infected with potential COVID-19 virus. While waiting for confirmatory test results, CBC can be a useful tool in deciding whether to immediately isolate a patient and initiate certain treatments. Considering that the 2019-COV PCR test may result in false negative results, which is important to prevent real patients from being sent home without treatment and prevent the further expansion of the pandemic. Also, by using an inexpensive CBC test, it is possible to prevent the unnecessary exposure of the patients to radiation without the need for more lung CT.

Persistent eosinopenia after admission was associated with high disease severity and low cure rates. In addition to the diagnosis of eosinopenia, it can guide us in distinguishing severe and non-severe patients, preventing intensive care and evaluating the disease prognosis. Although eosinopenia is not specific to COVID-19 infection and can also be seen in other viral infections, eosinophil values are lower in COVID-19 patients than in patients with influenza.

Ethics

Ethics Committee Approval: This study was approved by the Kartal Dr. Lutfi Kirdar City Hospital Clinical Research Ethics Committee and all procedures performed in the 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 (decision no: 2020/514/179/11, date: 11 June 2020).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: N.U., Design: Z.T., Data Collection or Processing: T.N., B.Y., S.S., N.Ü.Ö., Analysis or Interpretation: N.E., Literature Search: N.U., P.Ö.E., R.D.K., H.S., Writing: N.U., N.E.

Conflict of Interest: No conflict of interest was declared by the authors.

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The Level of Knowledge about Human Papillomavirus Infection and Vaccination Among Mothers of Children Aged 11-18 Years of Age

Önder Kılıçaslan, Merve Aslantaş, Muhammet Mesut Nezir Engin, Furkan Timur, Kenan Kocabay

Düzce University Faculty of Medicine, Department of Pediatrics, Düzce, Turkey

Abstract

Objective: The aim is to investigate the knowledge, awareness, attitudes, and behaviors of mothers of children in terms of human papillomavirus (HPV) infection, its association with cervical cancer, and HPV vaccination. It is estimated that cervical cancer is the second most common cancer among women worldwide, and it is one of the most preventable diseases, according to World Health Organization reports. HPV vaccination rates are relatively minimal in Turkey. The lack of parental awareness of HPV infection is a possible most critical factor that drives vaccination rates.

Methods: The study included 1,023 healthy volunteer women who had at least one child aged 11-18 years. A self-explanatory questionnaire comprising of 14 questions was designed to assess and compare maternal awareness and behavior regarding HPV infection and HPV vaccine.

Results: The study showed that a majority of the responders (68%) were aware of cervical cancer, minor of them (<22%) were aware of HPV vaccination. Less than half (32.81%) of the parents were found to be willing to vaccinate their children against HPV infection. The primary reason for non-vaccination was a lack of knowledge and concerns about side effects.

Conclusion: The current survey demonstrates that parents have a comparatively high level of knowledge about HPV and its association with cervical cancer; however, acceptance of vaccination is low. The study highlights the necessity of developing public education politics to achieve a deep awareness of parents about HPV vaccination in the community.

Keywords: Child, human papillomavirus, knowledge, mothers, vaccines

INTRODUCTION

Papillomaviruses are highly species-specific, and human papillomavirus (HPV) affect only humans. According to their tissue tropism, there are more than 200 types of HPV, which are subdivided into cutaneous and mucosal categories (1). HPV is a sexually transmitted pathogen that causes anogenital and oropharyngeal disease in males and females. Anogenital HPV is globally the most common sexually transmitted infection. The peak prevalence of HPV infection typically occurs within the first decade after sexual debut (2).

Evidence linking HPV to cervical carcinoma is significant; the high-risk HPV genotypes 16 and 18 cause approximately 70 per cent of all cervical cancers worldwide (2,3). Knowledge of HPV and its effect on invasive cervical cancer is crucial to introducing prophylactic vaccines. Virtually all cases of cervical cancer are attributable to HPV infection that makes the vaccination of vital importance. In resource-limited settings, expert groups recommend that public health efforts focus primarily on vaccinating young females, the group in whom the full benefit and cost-effectiveness of HPV vaccination is the highest. The introduction of routine HPV vaccination of adolescents and young adults has been associated with a decline in the burden of HPV infection and HPV-associated disease and quality of life (4).

Address for Correspondence: Önder Kılıçaslan, Düzce University Faculty of Medicine, Department of Pediatrics, Düzce, Turkey
Phone: +90 506 596 27 84 **E-mail:** dronderklcsln@gmail.com **ORCID ID:** orcid.org/0000-0002-9311-006X

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Cervical cancer is the second most common cancer among women worldwide, with approximately 530,000 diagnosed invasive cases and 260,000 deaths annually (5,6). HPV vaccination is effective in preventing cervical disease. Vaccine efficacy is highest in those without a prior HPV infection (7). This knowledge has been demonstrated in large randomized trials and has been supported by population data from regions reporting declines in cervical disease incidence following widespread quadrivalent HPV vaccination.

We assessed parents' knowledge status about HPV infection, attitudes toward HPV vaccination for their children and to estimate factors associated with parental acceptance toward HPV immunization. The Turkish Gynecological Oncology Association and the Turkish Pediatric Association formally recommend immunization against HPV. Both types of HPV vaccines have been available in Turkey since 2007. HPV vaccines are available in Turkey; however, they are not covered by the routine national childhood immunization program. Therefore, it is estimated that HPV vaccination rates are rather minimal in Turkey (8). Parents' knowledge of HPV infection is quite likely the most critical factor that drives vaccination rates. In the present study, we evaluated parents' knowledge levels about HPV infection, attitudes toward HPV vaccination for their children and estimate factors associated with parental acceptance regarding HPV immunization.

METHODS

Study Group

All participants provided informed written consent to take part in our survey. Healthy mothers of pediatric patients admitted to the pediatric outpatient clinic of the department of pediatric health and diseases, medical faculty hospital, between January and July 2018, were invited to take a single survey. Mothers of children aged under 18 years of age and of both genders were included. One thousand twenty three women participated in the questionary that lasted a median duration of 20 min. Women who had to read and understand the questionnaire on their own or with the help of staff were given the paper-based questionnaire to complete themselves and then return to the team.

Study Design

A descriptive, comparative design was used to examine mothers' perceptions and knowledge of HPV and attitudes toward the HPV vaccine. This method provided insight into parental decision-making and explored vaccine initiation by parents as recommended preventive action.

Participants were recruited for a paper survey. Inclusion criteria were healthy parents (mothers) who had children or adolescents between 11 and 18 years of age. The exclusion criteria were as Parents with a history of any gynecological cancer, parents older than 65 years.

Survey Data

A questionnaire comprising of 14 questions was designed to assess parental awareness, knowledge, and attitudes regarding HPV disease, its association with cervical cancers, and HPV vaccine.

The study questionnaires consisted of demographic items, social and economic status of the participants. Demographic questions included gender, household income, education level, parent's age, number of children, and age. The survey included general HPV knowledge, cervical cancer knowledge items, HPV vaccination knowledge and immunization perception items. The questionnaire included questions as following:

- Demographic items: Age (both mother and the child), gender, education, socioeconomic status (household income),

- Knowledge of HPV infection, HPV related cervical cancer, gynecological follow-up, HPV vaccines,

- Attitudes and behavior regarding HPV vaccination for their child and the reasons for hesitation regarding immunization,

- The source of their knowledge on the topics above.

Ethical Issues

The Duzce University Faculty of Medicine Ethics Committee approved the study following the Word Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects (decision no: 2018/48, date: 05.03.2018).

Statistical Analysis

Collected data of 1,023 completed surveys were analyzed using SPSS 15.0. Frequencies and descriptive statistics were used to evaluate the participants' demographic data, knowledge about HPV infection, and vaccine acceptance. Chi-square tests were used to examine the differences among demographic questions, HPV knowledge, and perceptions of chi-square nation. A p value of <0.05 was set for all statistical tests to determine significance. Relations between respondents' character analyses for which the associated $p<0.05$ was then used in correlation tests. The level of knowledge or vaccine acceptance was explored in a univariate analysis using the chi-square test. Factors in the univariate analysis for which the associated $p<0.05$ was then used in multivariate logistic regression.

RESULTS

Demography

Overall, 1,023 subjects were enrolled in the study, and all the participants completed the study questionnaire.

The mean age of the responders and children were 40 and 14 years of age, respectively. Twenty-two (2.3%) of the mothers were illiterate, and 115 (8.6%) graduated from a university. Monthly household income was commonly in the 1500-3000 TL range in most participants, 420 out of 1,023 (41.4%) responders. Most of the participants, 984 (96.2%), had health insurance. Table 1 presents the demographic and socioeconomic (data about education, occupation, monthly household income, and social security) characteristics of mothers and their children with whom they admitted to the pediatric clinics are presented in detail.

Knowledge and Behavior

Socioeconomic features play a significant role in being aware of cervical cancer and HPV. Parents of girl children (n=194, 30.89%) were significantly more willing to vaccinate their children against HPV than parents who had a boy child (n=143, 36.20%). We report that as the mother's education levels decreased, the number of parents who were willing to vaccinate her child decreased the number of illiterate mothers who were willing to vaccinate their children was 9 (0.87%). Household income was negatively associated with acknowledgment of cervical cancer and the HPV vaccine and negatively associated with willingness to immunate their child. The lower was the household income, the fewer parents were informed and to be willing for immunization. Regular gynecological check-up of the parent with pap-smear testing history was strongly and positively correlated with the acknowledgment of cervical cancer, the HPV vaccine, and the willingness to immunate their child.

Table 2 shows the impact of demographic and socio-economical features on the acknowledgment of cervical cancer, HPV, and HPV vaccine acceptance in detail.

In our study, few participants had less family history of cervical cancer or genital warts (n=18, 1.8%).

Table 1. Socio-demographic features of responders

	Count N=1023	Percentage (%)
Age#		
Parent	40±6	
Child	14±1.6	
Child gender		
Male	395	38.6
Female	628	61.4
Education		
Illiterate	24	2.3
Primary school	362	35.4
Middle school	236	23.1
High school	286	28.0
University	115	8.63
Parents employment status		
Unemployed	738	72.1
Employed (private)	137	13.4
Employed (government)	93	9.1
Others	55	5.4
Household income+		
0-1500	239	23.4
1500-3000	420	41.1
>3000	364	35.6
Health insurance		
Present	984	96.2
None	39	3.8

#Years, values are presented as mean ± standard deviation, +Monthly, values in Turkish currency

Sources of Information

We investigated the primary accessibility of knowledge on cervical cancer and HPV immunization. We also evaluated the reasons for parents not to accept vaccinating their children. We reported health providers as the most accessible source of knowledge and lack of information as the most dominant (24.16% of the responses) for avoiding vaccination. The most common source of information about HPV infection and cervical cancer among mothers were health professionals: n=134, 59.27% and n=389, 55.81%, respectively. Table 3 presents these data in detail.

DISCUSSION

This study was conducted to assess the awareness and knowledge regarding cervical cancer and HPV infection and to evaluate attitudes toward HPV vaccination among the parents of children aged 11-18 years of age. We summarize our results; it was evident that the level of knowledge regarding HPV infection and awareness of its association with cervical cancer was insufficient among parents with at least one child aged 11-18 years. The lack of knowledge on HPV vaccination and its probable side effects are the main reasons for vaccination hesitation.

Parental Awareness of HPV Infection and the Effect of Demographic Features

When comparing the number of participants, this study was one of the most comprehensive survey studies recently presented in the literature. Even though this study does not contribute a lot of

new information, it emphasizes informing society about the HPV vaccine, and we objected these data might provide useful insight into western Turkey. Considering that this study was conducted in the western part of Turkey, participants in our study are insufficient to reflect the Turkey women population in general.

Study Limitations

This is a limitation of our study. Nevertheless, the high number of survey participants increases the value of the study results to the general literature knowledge. And this is the strenghtest value of our study.

The vast majority of participants (68.1%) in our study were aware of cervical cancer, few (21.6%) were aware of HPV vaccination and associated with cervical cancer, and even fewer participants were the percentage of parents who were willing to vaccinate their children. Our study's first observations showed that the awareness of cervical cancer and HPV did not transform into acceptance of vaccination. Nevertheless, the number (percentage) of participants aware of cervical cancer and HPV in our study was much higher than in some previous reports from China, Malacia, Nigeria (1,9-11). Again, there were many reports from Thailand and Australia and showed the rate of knowledge of cervical cancer and HPV infection was as high as

Table 2. Demographic features of the participants and univariate analyses to identify factors associated with knowledge of cervical cancer, HPV vaccine, and acceptance of immunization for their children

Variables	All participants	Informed of cervical cancer		Informed of HPV		Willing for immunization	
	N (%)	N (%)	p	N (%)	p	N (%)	p
Age*							
27-39	474 (46.33)	313 (66.03)		91 (19.19)		159 (33.54)	
40-49	454 (44.37)	318 (70.04)	0.12	105 (23.13)	0.06	149 (32.81)	**0.03**
50-59	95 (9.28)	66 (69.47)		25 (26.31)		28 (29.47)	
Child gender							
Female	628 (61.38)	429 (68.31)	0.08	131(20.85)	0.06	194 (30.89)	**0.03**
Male	395 (38.61)	268 (67.84)		90 (22.78)		143 (36.20)	
Education							
Illiterate	24 (2.40)	8 (33.33)		2 (8.33)		9 (0.87)	
Primary school	362 (35.42)	226 (62.43)		56 (15.46)		110 (30.38)	
Middle school	236 (23.01)	152 (64.40)	**0.00**	33 (13.98)	**0.01**	67 (28.38)	**0.01**
High school	286 (28.03)	208 (72.72)		70 (24.47)		101(35.31)	
University	115 (8.63)	103 (89.56)		59 (51.30)		51 (44.34)	
Parents' employment status							
Unemployed	738 (72.1)	466 (60.43)		132 (17.88)		231 (31.30)	
Employed (private)	137 (13.04)	105 (76.64)	**0.00**	28 (20.43)	**0.01**	44 (32.11)	**0.02**
Government officials	93 (9.01)	80 (86.02)		40 (43.01)		43 (46.23)	
Others	55 (5.04)	47 (85.45)		22 (40)		22 (40)	
Household income+							
0-1500	239 (23.04)	138 (57.74)		30 (12.55)		66 (27.61)	
1500-3000	420 (42.10)	267 (63.57)	**0.02**	72 (17.14)	**0.00**	123 (29.28)	**0.04**
>3000	364 (35.60)	292 (80.21)		119 (32.69)		149 (35.43)	
Gynecological check-up							
Uncertain	658 (64.3)	413 (62.76)	**0.00**	114 (17.32)	**0.00**	175 (26.59)	**0.03**
Regular	365 (35.7)	284 (77.80)		107 (29.31)		162 (44.38)	
Pap testing undervent							
Never	338 (33)	197 (58.28)	**0.03**	55 (16.27)	**0.03**	75 (22.18)	0.05
Regular	685 (67)	500 (72.99)		166 (24.23)		262 (38.24)	

*Years, values are presented as mean ± standard deviation, +Monthly, values in Turkish currency, HPV: Human papillomavirus

65-85% (12,13). The geographic location did not seem to play a role in the awareness of the population about HPV and cervical cancer. The dissemination of knowledge about HPV is still a global problem.

The potential relationship between the demographic and socioeconomic features and HPV knowledge was an issue that needs more epidemiological and population studies.

In our study, we identified factors that were closely related to HPV and cervical cancer knowledge. The younger was the mothers, the higher educational attainment they had, the mother's employment status, the higher household income, history any gynecology control history, or Pap testing were the decisive factors for the women to be aware of cervical cancer and HPV vaccination. According to our study, the child's parental age and gender were not the critical factors to be more acknowledged in cervical cancer and HPV.

Table 3. Awareness and acceptance of HPV vaccination, evaluation of sources of knowledge

	Count (n)	Percentage (%)	p
Awareness of cervical cancer			
Yes	697	68.1	-
No	326	31.9	
The source of cervical cancer knowledge			
Neighborhood	118	16.92	**p<0.05**
Social media	190	27.25	
Health providers	389	55.81	
Awareness of HPV vaccination			
Yes	221	21.6	-
No	802	78.4	
The source of HPV knowledge			
Neighborhood	24	10.40	**p<0.05**
Social media	63	28.05	
Health providers	134	59.27	
Willing to vaccinate their children			
Yes	336	32.8	
No	687	67.2	
The reason to reject HPV vaccination (n=687)			
Price	21	3.05	**p<0.05**
Consider ineffective	42	6.11	
Feared of side effects	166	24.16	
Need to ask family	61	8.87	
Need more knowledge	375	54.58	
Others	22	3.20	

HPV: Human papillomavirus

Compared with previous reports, some researchers discussed that if parents were more educated and economically secured might have a higher chance of being exposed to awareness about cervical cancer and HPV rather than the general population (8). However, investigations conducted among the higher educated population or even among health providers showed that if parents are educated, employed, and have an adequate income, not always the criteria for are well acknowledged (14-18). Our reports once more emphasize the importance of global instruction and education of a poorly educated population, but all levels of the population about HPV and its potential risks. Some previous studies on education programs of the community on cervical cancer have reported positive incomes; improved awareness of cervical cancer led to improved knowledge of HPV association and increased perception of HPV immunization (19-21). Education campaigns on cervical cancer awareness should raise awareness of cancer and emphasize its link with the HP virus. Our study found that being aware of cervical cancer does not always acknowledge its association with HPV.

In our study, we found that the presence of cervical cancer history in the family or the presence of genital warts was not associated with HPV awareness levels; because very few (less than 2%) respondents presumably had a history of family cervical cancer history or genital warts around. Thus, we did not consider these data in our study.

Parental Vaccination Hesitations and Parent's Sources of Information About HPV and the Vaccination

The study's disappointing result was that despite quite high rates of awareness about cervical cancer, relatively minor (<33%), respondents were willing to vaccinate their children against the HPV disease. Willingness to protect their children seemed to be correlated with demographic and socioeconomic features of parents; the younger the mother was, the higher was the family economic income, and the higher was the education level of the mother, the more willing were she to accept immunizing her children after the correct information was given. These data undoubtedly once more emphasize the impact of health providers on the information on the population. Again, the gender of the breed seemed to have a reduced impact on awareness of cervical cancer; mothers of girl children were slightly more aware of cervical cancer and the HPV vaccination. However, the male gender significantly affected the willingness to immunize their child when vaccination was declared; a boy's mother was more likely to vaccinate their children.

Interestingly, being in common gynecological control or underwent Pap testing did not affect the acceptance of HPV

vaccination. Again, according to our study, the respondents' most common hesitance about not accepting the immunization was the need for further knowledge about HPV. Therefore, we observed that even if a woman was aware of her health problems in cervical cancer, it was not enough for her to decide whether her child's vaccination occurred. Moreover, although the impact of acknowledgment is emphasized, previous studies report different reasons for the family's vaccine rejection (22,23). We also questioned the women about the reasons for vaccine rejection, vaccine cost, belief in ineffectiveness, fear of side effect. However, our study results suggested that the lack of acknowledgment about the HPV vaccine was the most important reason not to accept the immunization. A low percentage of the responders (<22%) had been aware of preventing cervical cancer via vaccination. Among the responders who were aware of the vaccine, approximately 60% got the information from health providers, the less, got the knowledge via social media and television programs.

Health providers played a significant role in resolving the doubts about the HPV vaccination, but it was inadequate; raising public awareness should become government policy.

We observed several factors that positively correlated with willingness to vaccinate against HPV, and they were parental age, the gender of the child, educational background, household income, and employment status.

CONCLUSION

Our research's most important outcome is that we must establish and widespread education campaigns to raise awareness of HPV infection, its association with cervical cancer, and the high protective effect of the vaccinations on the disease as a government policy.

Although Turkey's health care has not yet introduced the HPV vaccine into the official vaccination protocol, we must teach the community that this vaccine was involved in the strongly proposed vaccination algorithm. Our reports provided us that health providers and collective information panels should be included to raise public awareness. Further studies would provide us with satisfactory results of raised HPV vaccination with public knowledge leading to an increased number of cervical cancer cases.

Ethics

Ethics Committee Approval: The Duzce University Faculty of Medicine Ethics Committee approved the study following the Word Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects (decision no: 2018/48, date: 05.03.2018).

Informed Consent: Written informed consent was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ö.K., M.A., Concept: Ö.K., F.T., M.M.N.E., Design: Ö.K., F.T., M.M.N.E., Data Collection or Processing: Ö.K., M.A., F.T., Analysis or Interpretation: Ö.K., F.T., N.E., Literature Search: Ö.K., M.A., F.T., M.M.N.E., K.K., Writing: Ö.K., M.A., K.K.

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The Utility of Ga-68 DOTA-TATE PET/CT on Clinical Management of Gastroenteropancreatic Neuroendocrine Tumors (GEP-NET)

Özgül Ekmekçioğlu[1], Nihal Bozdağ Kaplan[2]

[1]University of Health Sciences Turkey, Şişli Hamidiye Etfal Training and Research Hospital, Clinic of Nuclear Medicine, İstanbul, Turkey
[2]University of Health Sciences Turkey, Şişli Hamidiye Etfal Training and Research Hospital, Clinic of Medical Oncology, İstanbul, Turkey

Abstract

Objective: Gallium (Ga)-68 DOTA-TATE positron emission tomography/computed tomography (PET/CT) is successfully used for imaging gastroenteropancreatic-neuroendocrine tumors (GEP-NET) and guiding treatment management especially in cases with heterogeneous morphology. This study investigates the effect of DOTA-TATE PET/CT on therapy management in GEP-NET.

Methods: Sixty-nine patients (29 women, 40 men) with well-differentiated GEP-NET were referred to our department for Ga-68-DOTA-TATE PET/CT scan analyzed retrospectively. Patients were scanned for staging (n=18), re-staging (n=36) and evaluation for treatment response (n=15). Treatment decisions were blindly correlated before and after PET/CT scan.

Results: The mean age was 56.63±13.03 (27-79) years. Patients had grade 1 (n=38) grade 2 (n=24) and grade 3 (n=7) tumors. More than half of the patients (53%) had positive findings for primary tumor and/or metastases. Thirteen patients with grade 2; 18 patients with grade 1; 6 patients with grade 3 tumors had positive findings with PET/CT scan. Primary tumors were in the pancreas, stomach, small bowel, appendix and colon. Additionally, metastases in liver, bone, lung, regional and distant lymph nodes were detected. Nineteen of 69 patients (27.5%) had a change in their treatment protocol. The highest change rate was detected at the group with grade 3 tumors.

Conclusion: Ga-68-DOTA-TATE PET/CT was shown as a successful method for imaging and guiding management of GEP-NET. The highest benefit in the treatment plan has been shown in patients with grade 3 tumors and group in the follow-up. Patients with a positive scan were also evaluated for peptide receptor radionuclide therapy as an alternative treatment.

Keywords: Positron emission tomography, DOTA-TATE, gastroenteropancreatic neuroendocrine tumor NET

INTRODUCTION

Neuroendocrine tumors can progress without any clinical symptoms, which makes it difficult to detect the primary tumor before reaching later stages. Prognosis is related to the grade and the metastatic disease. Especially, extrahepatic metastases have been related to lower survival rates (1). Survival is relatively longer that makes patients quality of life as an important factor to be considered during follow-up and management of the clinical disease. Conventional imaging techniques like computerized tomography or magnetic resonance imaging are used to determine the disease involvement. However, the somatostatin labeled hybrid imaging techniques are defined as the gold standard as being also a functional imaging technique. Somatostatin receptor labeled imaging techniques have been shown to be helpful in evaluating neuroendocrine tumors in staging, re-staging and assessment of treatment response. Due to the availability of positron emission tomography/computed tomography (PET/CT), somatostatin analogs labeled with gallium (Ga)-68 become more frequently used with the higher resolution images correlated with the scintigraphy images (2).

Gastroenteropancreatic neuroendocrine tumors (GEP-NET) have been mostly diagnosed with the clinical symptoms of the

Address for Correspondence: Özgül Ekmekçioğlu, University of Health Sciences Turkey, Şişli Hamidiye Etfal Training and Research Hospital, Clinic of Nuclear Medicine, İstanbul, Turkey
Phone: +90 212 512 52 14 **E-mail:** ozgulek@gmail.com **ORCID ID:** orcid.org/0000-0002-3313-8087

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patients. Clinical management of the neuroendocrine tumors is mostly related to stage, grade and the symptoms of the patients. Tumors can release hormone and somatostatin analogs are used for controlling symptoms and disease growth (3). When surgery is not an option or cannot control the disease spread, other treatment options like systemic or locoregional therapies are need to be discussed. Chemotherapy, mTOR inhibitors, local treatments like radioembolisation or the combination are the options to control the disease. Low-grade tumors mostly show increased uptake in somatostatin labeled radionuclide imaging. Patients with uptake have a chance to receive peptide receptor radionuclide therapy (PRRT) which has shown benefits for the patients especially with the metastatic disease (4).

Ga-68-DOTA-TATE PET/CT has been shown to be useful for detecting GEP-NET with higher sensitivity and specificity (5). A study emphasized the impact of Ga-68 DOTA-TATE PET/CT on management in the patient group with gastrointestinal NET. Almost half of the patients have changed their treatment protocol after PET/CT scan (6). Liver metastases are mostly detected during the initial diagnosis of the patient. Heterogeneous spread of the disease with a different kind of grade in metastases of the same primary makes it difficult to distinguish by conventional imaging techniques. Fludeoxyglucose (FDG) PET/CT is shown to be useful in the heterogeneous morphology of NET as a complementary to DOTA-TATE PET/CT scan (7). When a treatment protocol could affect a lesion, it might not be useful to the other metastatic lesions with different grades. Combined treatment models have been shown to have benefit, whereas FDG positivity is also detected in PET/CT images of GEP-NET tumors (8).

This complex structure of GEP-NET makes it difficult in clinical management. Higher the grade, more aggressively the tumor can grow, which needs different or combined patient-specific treatment protocols. Follow-up or diagnosis with Ga-68 DOTA-TATE PET/CT can shed light on conducting the clinical patient management. The aim of the study was to understand the effect of somatostatin labeled PET/CT images the in follow-up and the management of the treatment in GEP-NET.

METHODS

Sixty-nine patients with biopsy proven well-differentiated GEP-NET who were referred to our department for evaluation with Ga-68 DOTA-TATE PET/CT between 2015 and 2020 were retrospectively evaluated. Staging, re-staging and evaluating the treatment response were the indications included in our study. Patients younger than 18 years old with clinically suspected neuroendocrine tumors without biopsy proof were excluded. All the patients were biopsy proven and underwent conventional imaging modalities other than Ga-68 PET/CT scan during the diagnosis of the disease.

Patients were not asked to make any specific preparations before the day of the scan. After on-site synthesis of Ga-68 from the germanium generator and labeling with peptide (30 µg), a quality control with HPLC (high-performance liquid chromatography) technique has been done before injecting the radiopharmaceutical. The whole body images were obtained after 50-60 minutes from receiving 11-370 Mbq (3-10 mci) Ga-68 DOTA-TATE (130kV, 50-80 mAs, slice thickness of 3mm) (GE Healthcare, Wisconsin, USA). Oral contrast was used in all patients, meantime intravenous contrast could not be performed in all patients.

All the patients were grouped due to their stages (AJCC) and grades obtained from ki-67 levels of the biopsy results. Additionally, the referral indications to PET/CT scan of the patients were gathered in 3 groups. PET/CT images of the patients were evaluated retrospectively by an experienced nuclear medicine physician. Physiological distribution for DOTA-TATE was considered before the decision for pathological uptake. Uptake higher than background in the PET images was accepted as positive. CT images were checked and confirmed that the pathological lesion was in the same cross-section. Furthermore, oncologist evaluated the patient information initially without and later with the results of PET/CT scan. A form of treatment choice before and after seeing the PET/CT result of the patient was filled.

Informed consent form was received from all the patients. Local Ethics Committee approval was gained due to our hospital regulations (University of Health Sciences Turkey, Sisli Hamidiye Etfal Training and Research Hospital; date: 02.02.2021, number: 3113).

Statistical Analysis

All the data were analyzed with SPSS (Statistical Package for the Social Sciences) software for Windows (v17.0; IBM, Armonk, NY, USA). Individual and aggregate data were summarized using descriptive statistics including mean, standard deviations, medians (minimum-maximum), frequency distributions and percentages.

RESULTS

Sixty-nine patients with a mean age of 56.63±13.03 (minimum-maximum=27-79) year, 40 males and 29 females, with biopsy proven well differentiated GEP-NET were included to our study group (Table 1). Patients were grouped as the primary tumor

origin consisting of the small intestine (n=13), pancreas (n=26), colorectal (n=4), stomach (n=21) and appendix (n=5). Ki-67 levels from the biopsy or surgery histopathology reports were between <1% and 45%. Patients' grade classification was also grouped as grade 1 (n=38), grade 2 (n=24) and grade 3 (n=7) tumors. Patients were referred to PET/CT for staging (n=18), re-staging (n=36) and evaluating the treatment response (n=15) (Figure 1, 2).

PET/CT images revealed either negative or positive for the findings of pathological lesions with increased DOTA-TATE uptake. Thirty-seven of 69 patients had positive findings for either primary tumor or metastases. From positive scans, 18 patients were with grade 1 tumors and 13 patients had grade 2 tumors. Furthermore, 6 patients with grade 3 tumors demonstrated positive lesions on PET/CT scan. Positive lesions were grouped as primary tumors (n=19) and metastases to local lymph nodes (n=12), liver metastases (n=16), bone metastases (n=5), lung metastases (n=1) and in other regions (n=8).

A clinical management plan was also observed before and after evaluation of patients' DOTA-TATE PET/CT images. From the available clinical data, 6 patients had increased chromogranin A level, 13 patients had gastrointestinal symptoms like abdomen pain and nausea. Nineteen patients (27.5%) have had a change in their treatment protocol (Table 2). That change was grouped as either stopping somatostatine analog (n=6); leaving follow-up protocol (n=7), receiving or change to chemotherapy (n=3); including mTOR inhibitor agent (n=3); locoregional therapy (n=1) or PRRT (n=5). Ten of 69 patients (14.49%) were upstaged after DOTA PET/CT scan. Seven out of this group of 10 patients (70%) had change in the treatment protocol. From this group, 4 patients with grade 2 tumors and 2 patients with grade 1 tumors given or changed somatostatine analogs, and a patient with grade 3 tumor was considered for chemotherapy after scan.

Table 1. Demographics of the patients enrolled to study

Patient characteristics	Numbers
Sex (n)	
Male	40 (57.9%)
Female	29 (42.1%)
Age mean ± SD (minimum-maximum)	56.2±12.6 (27-79)
Primary tumor region (n)	
Small intestine	13 (18.8%)
Pancreas	26 (37.7%)
Colorectal	4 (5.8%)
Stomach	21 (30.4%)
Appendix	5 (7.3%)
PET/CT findings (n)	
Positive	37 (53.6%)
Negative	32 (46.4%)
PET/CT positive lesions (n)	
Primary tumor	19
Lymph nodes	12
Liver	16
Lung	1
Bone	5
Other	8
Grade (n)	
1 (ki-67% 1-3)	38 (55.1%)
2 (ki-67 <3-20)	24 (34.8%)
3 (ki-67% >20)	7 (10.1%)

SD: Standard deviation, PET/CT: Positron emission tomography/computed tomography

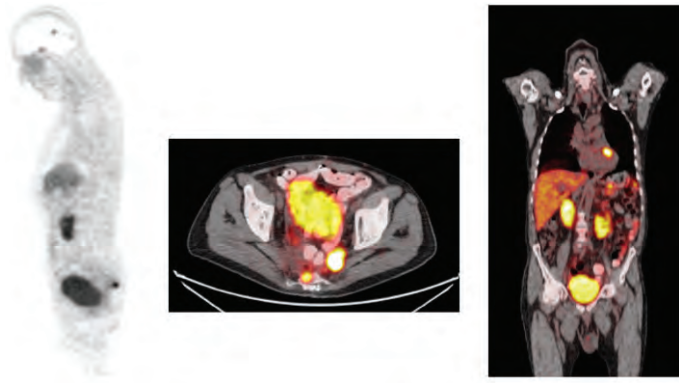

Figure 1. Fifty-six year-old male patient, referred for re-staging. He had rectum NET, ki-67 6% grade 2 tumor, was under sandostatine treatment before DOTA-TATE PET/CT scan. PRRT decision was given after PET/CT images revealed relapse in pelvic region, metastatic lesions in heart and cranium

PET/CT: Positron emission tomography/computed tomography, PRRT: Peptide receptor radionuclide

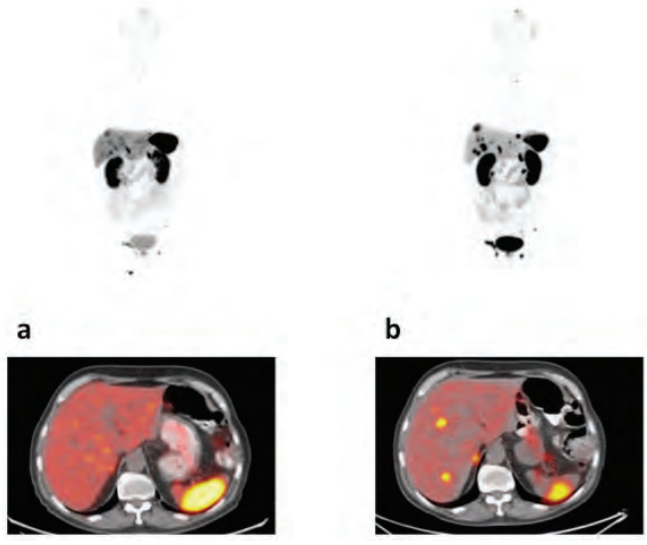

Figure 2. Seventy-six year-old male patient with grade 2 metastatic colon NET, Ki-67 3-4%, was referred for therapy assessment. (a); images before treatment. DOTA-TATE PET/CT images revealed progression in liver after treatment, (b); images after treatment

PET/CT: Positron emission tomography/computed tomography

Table 2. The treatment plan before and after DOTA-TATE PET/CT with change rates

Impact on therapy	Before PET/CT (n)	After PET/CT (n)	Change %
Follow-up	66.6% (46)	56.5% (39)	10.1%
Somatostatin analogue Octreotide Lantreotide	 30.4% (21) 1.4% (1)	 21.7% (15) 1.4% (1)	 8.7% -
Chemotherapy mTOR inhibitor	2.89% (2) -	7.2% (5) 4.34% (3)	4.34% 4.34%
Locoregional therapy	-	1.4% (1)	1.4%
PRRT	-	7.2% (5)	7.2%

PET/CT: Positron emission tomography/computed tomography, PRRT: Peptide receptor radionuclide

Nineteen patients from the study group were also scanned with FDG PET/CT after the DOTA PET/CT scan. From this group; 5 of 19 patients were positive with both scans. Two out of 5 were patients with grade 2 tumors and the rest were the patients with grade 3 tumors. Four out of 5 positive patients with both scans had a change in treatment protocol. Three of them were considered receiving chemotherapy and being candidates for combination therapies with PRRT, following after the response. One patient from this group was considered for PRRT in the beginning, due to clinical evaluation of both PET/CT scans.

According to the tumor grade classification, the treatment changing rates after DOTA-TATE PET/CT scan were 13.15% in patients with grade 1 tumor; 33.3% in grade 2 tumor and 85.7% in grade 3 tumor patients. The lowest rate of treatment change was in group of patients with grade 1 tumor, whereas lesion detection rate was also lower than the other groups. The reason could be related to the distribution of the PET-negative patients in the grade 1 group, who were mostly scanned for re-staging and had a history of surgery. Also, for the indications of PET/CT scan; 8 of 15 patients referred for therapy evaluation (%53.3), 3 out of 18 from the staging group (16.6%) and 8 of 36 patients (22.2%) from the re-staging group had a treatment protocol change after PET/CT scan. Some of the clinical data were not available for every patient cause of referral from other hospitals.

DISCUSSION

Modification of therapy management is not only affected by the tumor burden or the spread of the metastases, but also from the receptor function as demonstrated in DOTA PET/CT scan. GEP-NET treatment plan consists of surgery, symptomatic therapy and/or anti-proliferative therapy. It can be changed due to symptoms or progressive disease in case of non-respondent to treatment. In the literature, treatment changes have been reported to be between 19-60% in the studies demonstrating the impact of Ga-68 DOTA PET/CT scan in GEP-NET patients (9-13). Tan et al. (6) also emphasized the DOTA-TATE PET/CT scan can support clinician with providing at least 52,4% change in decision in gastrointestinal neuroendocrine tumors. Moreover, Anderson et al. (14) has showed 23.4% change in management in a patient group with mixt type of neuroendocrine tumor. Our patient numbers are lower than most of the similar studies in the literature, with a change rate of 27.5% in therapy management.

Chan et al. (15) has observed that having both FDG and Ga-68 DOTA-TATE PET/CT scan in NET patients could be significantly related to the survival rates of the patients whereas 19% of the patients with grade 3 NET. Moreover, another group reported 59% change in treatment protocol after evaluation of the both scans in GEP-NET (7). We also had 5 patients, with grade 2 and 3 tumors, who also had positive FDG PET/CT scans. This also proved the heterogeneity of the NET to evaluate the other treatment methods in the case of progression under somatostatin analogs. Increased glucose metabolism in tumor behavior can change the treatment protocol in NET patients as mentioned in previous studies. More aggressive therapies, including chemotherapy and targeted anti-cancer therapy can be a combination if there is FDG-positive component in the tumor (16,17) Nicolini et al. (8) has also recently demonstrated the benefit of combined PRRT and capecitabine treatment method for the GEP-NET tumors, including grade 3 tumors in their study group. This novel approach for grade 3 tumors with PRRT is promising for the patients resistant to other treatment modalities and the effects are remarkable. Nevertheless, more clinical trials with effects on survival should be discussed.

Nevertheless, each treatment can have drawbacks and side effects, might not be possible for every patient. The highest rate (85.7%) in therapy management was detected in the grade 3 tumor group in our patients. Grade 3 tumors group and the patients who were referred for therapy response were the patients who had the major effect in management after PET/CT scan. Tan et al. (6) has defined a group of patients (25%) who had down staged after PET/CT scan. However, our patients mostly had no change in stage (36.8%; n=7) or up-staged after PET/CT scan (6).

Somatostatin labeled imaging techniques change the era of the neuroendocrine tumor management and the outcomes with PRRT due to favorable results (18). NETTER phase III trial showed supportive results for the PRRT given midgut tumors with 79% lower rates for progression or death compared to somatostatine analogs (19). A retrospective study with a patient

group of GEP-NET patients who were treated with lutetium-177 (Lu-177) therapy also mentioned 84% disease control rate and 54% regression (20). In our study group; 3 patients with grade 1 tumors and 2 patients with grade 2 tumors were decided to be given PRRT after PET/CT scan. The reason was either progression or the progression under other treatments.

In conclusion, the management of GEP-NET tumors requires a complex plan of treatment and follow-up. DOTA-TATE PET/CT imaging was useful and has shown major benefit for the patients in our study group. Even the grades and symptoms of the patients are the most important factors of the treatment decision; management needs to be supported by functional imaging for patient-specific therapy plan.

Study Limitations

This study is not without limitations. Since the design was retrospective, it was impossible to gain all histological information for every positively evaluated lesion in PET/CT. Follow-up data were used for these purposes. Moreover, prospective clinical trials are needed to evaluate the effect on cost-effectiveness or survival after treatment change.

CONCLUSION

Based on our findings, Ga68-DOTA-TATE PET/CT was shown as a successful method for imaging primary GEP-NET and clinical management in our study. Furthermore, grade 3 tumors and follow-up patients have the highest rate of treatment change in our study group. Consideration of a therapy plan including PRRT is expected to improve the quality of life.

Ethics

Ethics Committee Approval: Local Ethics Committee approval was gained due to our hospital regulations (University of Health Sciences Turkey, Sisli Hamidiye Etfal Training and Research Hospital; date: 02.02.2021, nunber: 3113).

Informed Consent: Informed consent form was received from all the patients.

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Authorship Contributions

Surgical and Medical Practices: Ö.E., N.B.K., Concept: Ö.E., Design: Ö.E., Data Collection or Processing: Ö.E., N.B.K., Analysis or Interpretation: Ö.E., N.B.K., Literature Search: Ö.E., Writing: Ö.E.

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ORIGINAL ARTICLE

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Indicators of Recurrence Before and After Transplantation in Patients who Underwent Liver Transplantation Due for Hepatocellular Carcinoma

Atilla Bulur[1], Uğuray Payam Hacısalihoğlu[2], Murat Sevmiş[3]

[1]Nazilli State Hospital, Clinic of Gastroenterology, Aydın, Turkey
[2]İstanbul Yeni Yüzyıl University Faculty of Medicine, Gaziosmanpaşa Hospital, Department of Pathology, İstanbul, Turkey
[3]İstanbul Yeni Yuzyil University Faculty of Medicine, Gaziosmanpaşa Hospital, Department of General Surgery and Transplantation, İstanbul, Turkey

Abstract

Objective: Although the most effective current treatment method for hepatocellular carcinoma (HCC) is liver transplantation (LT), some patients may have a recurrence of HCC after transplantation. In our study, we investigated the laboratory and histopathological factors that we think may predict HCC recurrence before and after transplantation in patients who underwent LT due to HCC.

Methods: Pre and post transplantation clinical, laboratory, imaging and histopathological data of 66 patients who underwent LT for HCC approximately 2017-2020 in our hospital were retrospectively reviewed. The patients who developed recurrence were examined in detail.

Results: Recurrence developed in seven (10.61%) of 66 patients who underwent LT due to HCC. The mean age of the patients with and without relapse was 60.14 and 58.63 years, respectively. Most patients who underwent LT were men, and all patients with recurrence were men. In patients with recurrence, maximum tumor diameter measured macroscopically (5 cm; 2.7 cm, p=0.002) and presence of lymphovascular invasion (42.9%; 10.2%, p=0.048) were significantly higher than those without recurrence. Serum alpha feto protein (AFP) values at postoperative 3rd, 6th, and 9th months were significantly higher (p=0.001, p=0.004, p<0.001, respectively) in the recurrence group.

Conclusion: In our study, it was determined that increasing perioperative tumor size, presence of lymphovascular invasion and postoperative increased AFP values can predict HCC recurrence.

Keywords: Hepatocellular carcinoma, liver transplantation, recurrence

INTRODUCTION

Hepatocellular carcinoma (HCC), which is the fifth most common tumor worldwide that develops because of many diseases affecting the liver. HCC-related deaths are at the 3rd rank of cancer-related deaths and are an important health problem worldwide (1-3). Liver transplantation (LT) is one of the accepted treatment modalities for HCC. The Milan criteria are currently accepted as the gold standard criteria for the determination of LT candidates from HCC patients (4). It is a method that can be applied in inoperable HCC patients irrespective of the degree of liver function (5). Moreover, it has a therapeutic effect on the underlying pathology and hence, affects the prognosis by decreasing the risk of recurrence or de novo HCC (6,7). In a paper published in 1996, Mazzaferro et al. (8) proposed the Milan criteria as a means of determination of patients suitable for LT in cases where the tumor is ≤5 cm or less than three tumors ≤3 cm in diameter in the absence major vessel invasion or extrahepatic tumor dissemination. The overall survival rates after LT for HCC

Address for Correspondence: Atilla Bulur, Nazilli State Hospital, Clinic of Gastroenterology, Aydın, Turkey
Phone: +90 256 312 00 00 **E-mail:** atillabulur@hotmail.com **ORCID ID:** orcid.org/0000-0001-8089-7740

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range from 65% to 80% at 5 years for patients fulfilling these criteria (9-11).

The attempt to broaden the criteria is predicated on the postulation that the Milan criteria may be too strict, leading to the possibility of a significant number of patients that could benefit from LT being excluded. If we adhered to the Milan criteria, only about 6% of HCC patients would be considered eligible for LT (12,13). Recurrence has not been demonstrated ubiquitously in HCC cases that do not meet the Milan criteria. Furthermore, a premise has been put forward the that a significant segment of those patients could also potentially benefit from LT without affecting HCC recurrence rates. It has also been suggested that many of these patients could benefit from LT without affecting their HCC recurrence rate (13-15).

HCC recurrence has been reported in around 10% of patients after LT due to HCC (16,17). It has been reported that recurrence of HCC may develop from extrahepatic metastases that were missed or undetected before transplantation or by accommodation of circulating tumor cells in organs during the peritransplant period. Studies have reported that tumor burden (volume), maximum tumor size, and alpha feto protein (AFP) level, which are the main tumor markers of HCC, are useful in predicting recurrence after transplantation (18,19). In our study, we investigated clinical, laboratory, imaging and histopathological factors that may indicate recurrence before and after transplantation in patients who underwent LT due to HCC.

METHODS

In our study, the clinical, operational, laboratory, imaging and histopathological data of patients who underwent LT between 2017 and 2020 in the organ transplant unit of our hospital with a diagnosis of HCC and that were followed up by our gastroenterology clinic were retrospectively reviewed. Patient consent and Istanbul Yeni Yuzyil University Faculty of Medicine Ethics Committee approval were obtained in our study (decision date and number: 13.08.2020/033). Patients with recurrence HCC were determined according to laboratory, imaging and/or histopathological (liver biopsy) criteria. Patients who developed mortality due to recurrence or any other reason was excluded from the study.

Statistical Analysis

The research data were uploaded and evaluated using IBM SPSS 25 (IBM Statistical Package for Social Sciences). Descriptive statistics of categorical variables are presented as numbers and percentages. Descriptive statistics of numerical variables are presented as mean (±) standard deviation for normally distributed variables and as median (min-max) for non-normally distributed variables. In a comparison of categorical variables, cross tables were used and "Pearson chi-square test" and "Fisher's Exact test" were applied. The conformity of numerical variables to normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). "Independent samples t-test" was used as the statistical method for determining the statistical significance between groups for variables found to conform to normal distribution and "Mann-Whitney U test" was used for variables that were not normally distributed. The homogeneity of the variances of normal variables between the groups was evaluated with the "Levene test". "Cohen's Kappa coefficient" was used in the categorical variables to determine the agreement between the two measurements. Since the normal distribution is not observed in numerical variables, "Spearman Rank coefficient correlation coefficient (Spearman's rho)" was used. Statistical significance levels were accepted as $p<0.05$ and $p<0.001$.

RESULTS

In our study, 6 (9.1%) of 66 patients who underwent LT due to HCC were female, while 60 (90.9%) were male. LT had been applied to all patients more than a year ago. Recurrence was observed in 7 (10.6%) of the patients, whereas no recurrence was observed in 59 (89.4%) patients. The mean age of patients with relapse was 60.14±3.93 (56-67), while the average age of patients without recurrence was 58.63±8.71 (18-70) years. There was no statistically significant difference between the study groups in terms of age. All patients with recurrence were men. There was no statistically significant difference between the study groups in terms of gender. Post-transplant recurrence times ranged from 12-58 months, with a mean recurrence time of 35.43±17.89 months (Table 1).

Considering the etiology of the patients with recurrent disease, 85.7% of them (6 patients) had chronic hepatitis B (HBV)-related HCC. Only 1 patient (14.3%) was found to have chronic hepatitis C-related HCC. Most patients without recurrence (28 patients, 47.5%) also consist of HCC patients developing on the basis of HBV. There was no statistically significant difference between the recurrence and etiological conditions of the patients. No statistically significant difference was found between the Child-Pugh score (9.14 and 8.41) and model for end-stage liver disease; (MELD; 13.14 and 12.88) mean scores of patients with and without recurrence. While 42.9% (3 patients) of patients with recurrence were within Milan's criteria, 57.1% (4 patients) were beyond the Milan criteria. While 74.6% of the patients without

recurrence (44 patients) were within Milan criteria, 25.4% (15 patients) were beyond Milan criteria. There was no statistically significant relationship between patients relapse status and Milan criteria status (Table 1).

According to the histopathological examinations of the patients, no statistically significant relationship was found between patients with recurrence and without recurrence in terms of localization (unilobuler, bilobular). The mean maximum tumor diameter measured in the operation materials of patients with recurrence was 6.29±2.94 cm, while those without recurrence was 3.09±2.14 cm. We observed that the maximum tumor diameters of patients with recurrence were higher than those without recurrence, and this difference was found to be statistically significant (p=0.002). In patients with recurrent disease, lymphovascular invasion was observed in 42.9% (3 patients), whereas no lymphovascular invasion was observed in 57.1% (4 patients). Most of the patients without recurrence (53 patients) did not have lymphovascular invasion, and a statistically significant relationship was found between their recurrence status of the patients' and their lymphovascular invasion status (p=0.048). There was no perineural invasion in any patient with recurrent disease. In patients without recurrence, only 2 patients had perineural invasion. There was no statistically significant relationship between the presence of recurrence and the presence or absence of perineural invasion. There was no correlation between the histopathological tumor grades, pathological stages according to TNM (T for tumor size and local cancer invasion; N for nearby lymph node metastasis; and M for distant metastasis) classification, surgical margin status and recurrence. Additionally, all patients with and without recurrence had negative lymph nodes (Table 2).

We observed that the AFP values of patients with recurrent disease were significantly higher than those without recurrence. All patients with and without recurrence completed a post-operative year without mortality. Additionally, when the median of these AFP values were compared according to the recurrence status of the patients, a statistically significant difference was found in all months (3rd, 6th, and 12th months) except month zero (AFP 0), p_{AFP90}=0.001, p_{AFP80}=0.004, p_{AFP60}<0.001 (Table 3).

DISCUSSION

HCC is one of the most common and fatal tumors in the world. LT is the most effective treatment method in HCC treatment. LT is a complicated surgery that can be performed in centers with experienced multidisciplinary clinics and requires pre-transplant patient selection and close follow-up to prevent recurrence and mortality. Milan criteria are used in patient selection to achieve low recurrence and high survival rates in HCC patients who undergo LT, in many centers (8). These criteria are obtained by

Table 1. Demographic and clinical characteristics of the patients

		Patients with recurrence	Patients without recurrence	p
Age (years)		60.14±3.93	58.63±8.71	0.631
Gender	Female	0 (0)	6 (10.2)	0.496
	Male	7 (100)	53 (89.8)	
Time relapsed after transplant (months)		35.43±17.89	30.59±16.47	0.469
CHILD score		8 (5-14)	8 (5-13)	0.447
MELD score		12 (10-17)	12 (8-24)	0.798
Milan	Within	3 (42.9)	44 (74.6)	0.099
	Beyond	4 (57.1)	15 (25.4)	

CHILD score: Child-Pugh score, MELD score: Model for end-stage liver disease

Table 2. Comparison of the histopathological characteristics of the patients

Histopathological features		Patients with recurrent disease	Patients without recurrent disease	p
Maximum tumor diameter (cm)		5 (3.5-12)	2.7 (0.5-13)	0.002
Lymphovascular invasion	Yes	3 (42.9)	6 (10.2)	0.048
	No	4 (57.1)	53 (89.8)	
Perineural invasion	Yes	0 (0)	2 (3.4)	0.795
	No	7 (100)	56 (96.6)	
Localization	Unilobular	5 (71.4)	46 (78.0)	0.504
	Bilobular	2 (28.6)	13 (22.0)	

Table 3. Comparison of preoperative and postoperative (3.6 and 12 months) AFP values (ng/mL)			
	Patients with recurrent disease	**Patients without recurrent disease**	**p**
AFP 0	19 (2.9-4019)	9.4 (1-655)	0.411
AFP 90	8.7 (2.30-1502)	1.75 (0.75-11)	0.001
AFP 180	3.5 (1.27-11870)	1.6 (0.60-11)	0.004
AFP 360	90.4 (18.5-50000)	1.59 (0.75-13.10)	<0.001
AFP: Alpha feto protein			

a simple assessment based on tumor number and size, however factors that impact recurrence and survival apart from the Milan criteria, have also been investigated in many studies (18-20). HCC recurrence after LT is one of the undesirable consequences of this treatment method (21). In our center, LT can be applied to patients beyond the Milan criteria same as in some transplant centers, when necessary. We retrospectively examined the clinical, laboratory, imaging and histopathological data of 66 patients who underwent LT due to HCC in our gastroenterology outpatient clinic, and we wanted to obtain data that would enable us to predict recurrence in 7 patients who developed recurrence. In the literature, the recurrence rate among HCC patients who underwent LT is around 10%. The recurrence rate was 10.6% in our study. In our study, all patients with recurrence were alive. In similar studies in the literature, the mean survival time of patients after recurrence was reported to be 8-24 months (16-18,22-25). In our study, 6.38% of patients within Milan and 21.05% of patients beyond the Milan criteria had recurrent disease. Being beyond the Milan criteria increases the risk of recurrence in accordance with similar studies (26,27). The mean age of our patients with recurrence was 60.14 years, the mean age of patients without recurrence was 58.63 years. No statistically significant difference was found between the ages of the patients (p=0.631). While all the patients with recurrence were male, 10.2% of the patients without recurrence were female and 89.2% of them were male (Table 1). Although there was no statistically significant difference between the study groups in terms of gender (p=0.496), it is striking that most our patients who underwent LT with a diagnosis of HCC and all of those with recurrence were male. In our study, HCC recurrence was most frequently observed in patients with HCC developing on the basis of HBV related cirrhosis (85.7%). In a study conducted in our country, it was reported that in patients with HCC developing on the basis of HBV, recurrence rate increased and the survival rate decreased (28). Apart from these criteria, it has been shown in many studies that tumor size alone increases the risk of recurrence (29). In our study, the mean maximum tumor size was 6.29±2.94 cm in patients with recurrence, while it was 3.09±2.14 cm in patients without recurrence. A significant difference was found between both groups (p=0.002). In our study, the of lymphovascular invasion was observed in 3 patients (42.9%) in the recurrence group, whereas it was observed in 6 patients (10.2%) in the non-recurrence group. A significant difference was found between the two groups (p=0.048) (Table 2). We observed that the increase in maximum tumor size and the presence of vascular invasion played a significant role in the development of recurrence, and this result was found to be consistent with other studies (8,12,28). Although it was not statistically significant, according to the TNM classification, 3 (42.9%) of the patients with relapse were in the T2 stage, 3 (42.9%) were in the T3 stage, while most patients without recurrence were in the T1 and T2 (39% and 47.5%) stages. It has been reported that serum AFP level measured in the pretransplant period is a determinant of recurrence and survival after transplantation. Studies have found that patients with high AFP levels have higher rates of mortality and/or HCC relapse (29-31).

Of note, AFP values more than 1000 ng/mL before LT have been associated with the risk of HCC recurrence after LT (32-34). In our study, the preoperative AFP values (AFP 0) and posttransplant 30, 90, 180 and 360 day AFP values of the patients with recurrence were found to be higher than those of the patients without recurrence. AFP values were found to be statistically significant between 90, 180, and 360 days (Table 3).

Recurrence developed in 10.6% of the patients who underwent LT. Mortality did not occur in these patients, and rejection did not develop before recurrence. This may be related to patient selection before transplantation and close follow-up of our immunosuppressive treatment after transplantation. Clinical studies have revealed that the use of mammalian target of rapamycin (mTOR) inhibitors in the treatment during the transplant period, because to their anticancer efficacy, reduces post transplant HCC recurrence and increases survival (35-38). In our study, mTOR inhibitors (everolimus) treatment was initiated for each patient after the third month post-transplantation, to prevent recurrence.

Study Limitations

There are more prospective similar studies in the literature with a higher number of cases and follow-up time, which constitutes a limitation for our study. Conducting multi-center and prospective studies with a larger number of patients would yield more significant results in the future.

CONCLUSION

Although LT was applied to 66 patients due to HCC in approximate four years at our center, we think that this is a considerable number since we only included LTs with the indication of HCC in our study. In our study, among the factors that increase cancer recurrence; the maximum diameter of the tumor, presence of lymphovascular invasion and post transplant increasing AFP values were found to be statistically significant. Additionally, having a chronic HBV related HCC, multifocal tumor and poor differentiation were found to be important factors for recurrence.

Ethics

Ethics Committee Approval: Istanbul Yeni Yuzyil University Faculty of Medicine Ethics Committee approval were obtained in our study (decision date and number: 13.08.2020/033).

Informed Consent: Patient consent obtained.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.B., U.P.H., M.S., Concept: A.B., Design: A.B., Data Collection or Processing: A.B., U.P.H., M.S., Analysis or Interpretation: A.B., U.P.H., M.S., Literature Search: A.B., U.P.H., Writing: A.B.

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ORIGINAL ARTICLE

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Survival of Patients Transferred from a Distant Hospital on ECMO Support

Serkan Ketenciler[1], Cihan Yücel[1], Tarık Demir[2], Kadir Doğruer[3], Ali Kocailik[4], Nihan Kayalar[1], İlhan Sanisoğlu[1]

[1]University of Health Sciences Turkey, Prof. Dr. Cemil Taşcıoğlu City Hospital, Clinic of Cardiovascular Surgery, İstanbul, Turkey
[2]University of Health Sciences Turkey, Başakşehir Çam and Sakura City Hospital, Clinic of Pediatric Cardiovascular Surgery, İstanbul, Turkey
[3]Avrasya Hospital, Clinic of Anesthesiology and Reanimation, İstanbul, Turkey
[4]Bilgi University Faculty of Health Sciences, Department of Perfusion, İstanbul, Turkey

Abstract

Objective: Extracorporeal membrane oxygenation (ECMO) may be used in patients with severe respiratory and/or cardiac pathologies. Transferring a patient on ECMO support to advanced hospital may become life-saving for whom. We evaluated the effects of very long distance transportation with ECMO.

Methods: This study includes 10 patients who were transferred from a distant hospital to an advanced care hospital while on veno-venous (VV) or veno-arterial ECMO between 2017 and 2019. A transfer distance of at least 1000 km was the required inclusion criterion for the study. The primary outcome was all cause mortality in the hospital and in 1-year. The secondary outcomes were the duration of ECMO run and mechanical ventilation, durations of intensive care unit and hospital stay.

Results: The mean distance of transport was 1878.2±440.7. One adverse event occurred because inappropriate electrical connection of the plane so backup ECMO device was switched on. Overall hospital mortality of the patients was 40% and 1-year survival was 50%.

Conclusion: Interfacility transfer on ECMO support between too far centers is safe and may be a life-saving procedure for the patient. The survival rates of VV ECMO seems to be better.

Keywords: Extracorporeal membrane oxygenation, patient transfer, lung diseases, heart diseases

INTRODUCTION

Extracorporeal membrane oxygenation (ECMO) may be used in patients with severe respiratory and/or cardiac pathologies. Although it's possible to start ECMO treatment in many hospitals, it is best to follow these patients in centers specifically experienced in ECMO where more advanced treatment options such as heart or lung transplantation are also available. Therefore, the transfer of a patient with an ECMO device from a hospital where maintenance of ECMO support is impossible may be a life-saving decision for that patient. The patient transfer with ECMO device has been used for about 3 decades. International centers have reported increasingly more experience on the transport of patients with ECMO between hospitals (1-3). In this article, we evaluated the effects of very long distance (>1000 km) ECMO transfer on patients' outcomes.

METHODS

This retrospective study included 10 patients who were transferred from a distant hospital to the intensive care unit (ICU) of ECMO specialized hospital with the support of veno-venous (VV) or veno-arterial (VA) ECMO between 2017 and 2019. Inclusion criteria of the study were the suitability of the patients for ECMO indications according to Extracorporeal Life Support Organization guidelines (4) and the distance of air transport with

Address for Correspondence: Serkan Ketenciler, University of Health Sciences Turkey, Prof. Dr. Cemil Taşcıoğlu City Hospital, Clinic of Cardiovascular Surgery, İstanbul, Turkey
Phone: +90 532 622 75 60 **E-mail:** ketencilers@hotmail.com **ORCID ID:** orcid.org/0000-0003-1528-6788

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ECMO support regardless of the main pathology. The transfer distance must be at least 1000 km for inclusion in the study. Patient characteristics such as the main pathology and indication for ECMO support, concomitant diseases, duration of ICU and hospital stay after ECMO running were recorded. Data related to ECMO, such as technical features of cannulation, the duration of ECMO run, transport distance, complications during transfer and patient outcomes, were retrospectively collected from medical reports. The primary outcome was all cause mortality in the hospital and in 1-year. The secondary outcomes were the duration of ECMO run and mechanical ventilation, durations of ICU and hospital stay. The study protocol was approved by University of Health Sciences Turkey, Prof. Dr. Cemil Tascioglu City Hospital Institutional Ethical Committee (date: 30.06.2020, number: 48670771-514.10). Information concerning the study was provided to and the signed consent received from all patients volunteering to participate, or from relatives if patients were unable to express consent.

ECMO Support and Transport Procedure

All of the ECMO interfacility transport had been carried out in accordance with the primary transport situation of the ECMO transfer guideline (5). That is the transport team required to perform cannulation for ECMO support at the referring facility and than transport the patient to an ECMO center. Demographic features including body surface area (BSA) and detailed medical status of the patient were analyzed before the ECMO and transport. The appropriate size for the ECMO cannula and oxygenator was determined according to the BSA of the patient. We preferred a rotaflow pump head with console (Maquet; Getinge Group, Rastatt, Germany), a Maquet Quadrox PLS membrane oxygenator (Maquet; Getinge Group, Rastatt, Germany) and an HLS© cannula (Maquet; Getinge Group, Rastatt, Germany). All ECMO cannulations were performed percutaneously under the ultrasonography (USG) guidance by our team. VV ECMO was usually maintained by femoral and jugular veins and VA ECMO was performed via femoral vessels. All patients were followed up for at least 4 h after the onset of ECMO support to ensure hemodynamic stability. The eritrocyte and thrombocyte levels of the patient were optimized with appropriate blood product replacement before and immediately after the start of ECMO. The last clinical and laboratory findings especially hemoglobin and platelet levels, were interviewed with the patient's doctor before starting the journey.

Patients were on invasive monitoring all the time during transport and suitable kits for the blood gases and activated clotting time (ACT) measurements were available. The blood gas analyzed and ACT level was measured at regular intervals. Heparin was applied according to the patients' weight to ensure that ACT values were in the range of 160-200 seconds. The development of hypothermia was avoided. A portable USG device was kept and used to verify the correct position of cannulas when necessary.

Transfer of all patients from the ICU bed to the ambulance, ambulance to aircraft, aircraft to ambulance and finally to advanced care hospital was provided with a vacuum patient stretcher. ECMO device was placed on the foot side of the stretcher and circuits were kept in view for safety. The suitability of the aircraft door passage for the patient, ECMO device and circuits on the vacuum stretcher was checked. As soon as the patient arrived at the ICU of the advanced care hospital, the patient's hemodynamic status, cannulation sites and ECMO parameters (flow, pressure and RPM) were checked.

The back up equipment was also necessary to be prepared and ready due to the very long transport distance. These backup ECMO circuits and equipment included size-specific spare cannulas, tubing connectors, spare medical oxygen gas tank, oxygenator and another ECMO device. Additionally, the backup battery and electrical connections of all transfer vehicles were planned and their converters were supplied. Possible weather were checked and consultated with the pilot and precautions were planned.

Statistical Analysis

IBM SPSS statistics software for Windows, version 25 (SPSS, Inc, Chicago, IL) was used for statistical analyses. Descriptive statistics on patient demographics and clinical measures were summarized by mean and standard deviation or median and interquartile range for continuous variables. Categorical variables were summarized by percentage. The Kaplan-Meier method was used to analyze survival rates.

RESULTS

A total of 10 patients with ECMO support were transferred from a distant hospital to an advanced care hospital between 2017 and 2019. Mean age of the patients was 48±17.9 years (ranged, 18-67). Six of the patients were male, the others were female. The mean body mass index was 27.5±3.9. Concomitant diseases included essential hypertension, diabetes mellitus and coronary artery disease in 4, 3, and 1 patients, respectively. The patients' demographic information is shown in Table 1.

The ECMO cannulation of all patients was performed by our ECMO team, which consisted of a cardiovascular surgeon, a

Table 1. Demographics and ECMO characteristics of patients	
Variable	**No (%) or mean ± SD**
Age	48±17.9
Sex	
Female	4 (40%)
Male	6 (60%)
BMI	27.5±3.9
Concomitant disease	
Hypertension	4 (40%)
Diabetes mellitus	3 (30%)
Coronary aretry disease	1 (10%)
Reason for support	
Cardiac	3 (30%)
Respiratory	7 (70%)
ECMO mode	
VV ECMO	7 (70%)
VA ECMO	3 (30%)
BMI: Body mass index, ECMO: Extracorporeal membrane oxygenation, VV: Veno-venous, VA: Veno-arterial, SD: Standard deviation	

perfusionist and a nurse. The most frequent diagnosis in our transport ECMO group was acute respiratory distress syndrome (ARDS) that occurred because of primary causes in 6 patients and secondary cause in 1 patient. VV ECMO performed to 7 (70%) patients with ARDS before transfer. According to blood gas count and mechanical ventilatory measurements before ECMO support and pretransfer for those patients were PaO_2/FiO_2 <100 on FiO_2 >90%, Murray score between 3 and 4, APSS score 8 (the age, PaO_2/FiO_2 ratio, and plateau pressure) and CO_2 retention (higher than 70 mmHg despite Pplat >30 cm H_2O) on mechanical ventilation despite optimal medication for at least 6 h (6,7). VV ECMO was maintained by femoral and jugular veins in all except in one patient who had superior vena cava syndrome and thus underwent ECMO by bilateral femoral veins. VA ECMO was performed in 3 (30%) patients with refractory cardiogenic shock syndrome or acute cardiac failure caused by dilated cardiomyopathy, myocarditis and acute coronary syndrome. A common femoral artery and vein were used to maintain the VA ECMO circuit. A distal perfusion cannula was placed to protect the children from limb ischemia. The detailed diagnosis of patients with VA or VV ECMO support is shown in Table 2.

The mean and median distance of transport were 1878.2±440.7 (range, 1100-2231) and 2161 km, respectively All patients were transferred with aircraft by airway. All patients arrived safely at the advanced care hospital after transfer. Blood gas changes in patients during pre and on-ECMO, transfer and on-arrival to ECMO center hospital are summarized in Table 3. Evaluation of

Table 2. Indications of ECMO	
Diagnosis	**No (%)**
ARDS	
Viral pneumonia	5 (50%)
Inhalation burning	1 (10%)
Histiocytosis X	1 (10%)
Cardiogenic shock	
Cardiomiopathy (dilated)	1 (10%)
Myocarditis	1 (10%)
Coronary artery disease	1 (10%)
ARDS: Acute respiratory disstress syndrome, ECMO: Extracorporeal membrane oxygenation	

Table 3. Blood gas measurements (pre, on-ECMO, transfer, ECMO center)	
Blood gas changes (pre, on-ECMO, transfer, ECMO center)	**Mean ± SD**
Pre-ECMO PaO_2/FIO_2 ratio	55.6±7.8
Pre-ECMO PaO_2 (mmHg)	53.0±6.1
Pre-ECMO pH	7.27±0.12
Pre-ECMO PCO_2 (mmHg)	73.3±6.0
On-ECMO pH	7.41±0.02
On-ECMO PaO_2 (mmHg)	120.9±18.1
On-ECMO PCO_2 (mmHg)	40.2±1.6
Transfer ECMO PH	7.41±0.02
Transfer ECMO PaO_2 (mmHg)	130.4±13.0
Transfer ECMO PCO_2 (mmHg)	40.6±1.9
On-arrival pH	7.41±0.02
On-arrival PaO_2 (mmHg)	133.0±11.0
On-arrival PCO_2 (mmHg)	40.6±1.4
ECMO: Extracorporeal membrane oxygenation, SD: Standard deviation	

the ECMO run time, ICU and hospital stay is calculated after the transfer of the patient to an advanced care hospital. In the VA ECMO group, the mean duration of the ECMO was 12±5 days (range, 7-17) and the mean duration of the ICU and hospital stay were 18.3±12.0 (range, 7-31) and 23.6±20.8 days (range, 7-47) respectively. In this VA ECMO group, two patients died during the following days in the ICU. One of these died of disseminated intravascular coagulation on the 17th day and the other patient died of cardiopulmonary insufficiency on the 10th day. Only one patient survived in the VA ECMO group. The patient was 48 years old female with a familial type of dilated cardiomyopathy. She was weaned from ECMO support on the 12th day and a left ventricular assist device implantation was performed as a bridge to the heart transplantation. The patient was discharged from the hospital and was on the transplant list. She died of septic

shock originating from LVAD 255th days after her discharge. In the VV ECMO group, the mean duration of the ECMO was 26.4±23.4 days (range, 3-72) and the mean duration of the ICU and hospital stay were 34.8±29.2 (range, 8-90) and 56.2±57.4 days (range, 17-176) respectively. Two patients died from irreversible lung insufficiency in the VV ECMO group. The data are summarized in Table 4.

We encountered only one problem during transfers. In one patient, we had to switch to a backup ECMO device due to the lack of proper electrical connection on the plane. Overall hospital mortality of the patients was 40% and 1-year survival was 50%. The Kaplan-Meier estimate of overall survival were 60% and 50% at hospital discharge and 1-year survival, respectively (Figure 1).

DISCUSSION

The management of acute and chronic cardiac and pulmonary diseases has improved in parallel with the advancement of medical technologies. Even irreversible cardiac or pulmonary diseases may be treated with transplantation of the heart, lung, or both of them in centers specialized in these subjects (8). However, reversible but life threatening cardiac and pulmonary pathologies may heal if enough time to respond main treatment can be provided with adjunct and salvage ECMO support. In the literature, there are several articles supporting ECMO usage for ARDS or cardiogenic shock syndrome. In a study during pandemic H1N1 influenza in 2009, lower mortality rates were observed in centers where severe ARDS related to influenza was treated with ECMO support (9,10). However, complex management of patients on ECMO support, especially in prolonged cases or advanced treatment options such as transplantation, are not available in all hospitals. CESAR multicenter trial recommends the transfer of adult patients with severe ARDS or reversible pulmonary failure to an advanced care hospital with ICU specialized in ECMO because this strategy significantly improves survival without disability (11). Interhospital transfer of the patients on ECMO support by airway or ground transport is the solution to that problem and it has been performed for at least 2-3 decades (12). Thus, an ECMO transfer team is necessary to perform a safe and proper interfacility transport (13).

The transfer of the patients on ECMO support has already been reported in the literature (12,14-16). In these articles, all transfer distances of the journey with ECMO had been analyzed, but in this study, we exclusively evaluated patients who were transferred for at least 1000 km of distance. Our ECMO transport strategy is to cannulate and provide emergency ECMO support at the primary facility and then to transfer the patient to the advanced care hospital. This approach is similar to other studies in the literature (17).

In our study, hospital and 1-year overall mortality of transported ECMO patients was 40% and 50%, respectively. Mortality rates of the non-transferred patients with severe ARDS supported by ECMO treatment vary between 23 and 43% in some studies (9,18). Coppola et al. (19) reported the survival of patients transferred to ECMO from other centers as 65%. Additionally, Bonadonna et al. (20) declared that the mortality rate of transported VA ECMO patients was 51.9% whereas the hospital mortality rate (66.6%) of VA ECMO patients was higher. The hospital mortality rate of our patients was approximately similar to that of other studies although the survival rates of VA ECMO patients were not satisfying. However, 1-year survival rate was 71.4% in the VV ECMO patients.

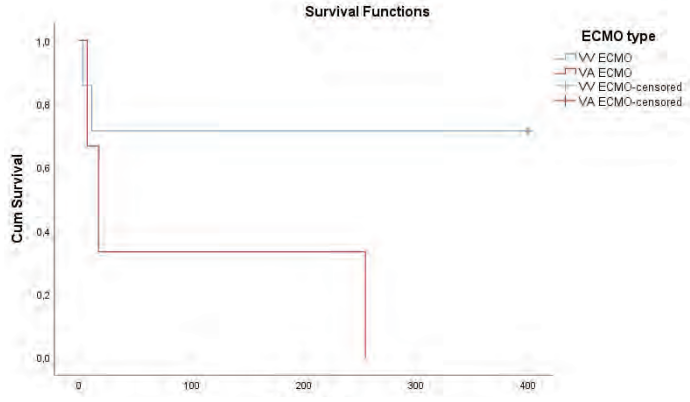

Figure 1. One-year survival of patients (Kaplan-Meier)
ECMO: Extracorporeal membrane oxygenation

Table 4. Patient outcomes

Variable	VA ECMO (mean ± SD)	VV ECMO (mean ± SD)	Overall (mean ± SD)
ECMO run time	12±5	26.4±23.4	22.1±20.5
ICU stay	18.3±12.0	34.8±29.2	29.9±25.8
Hospital stay	23.6±20.8	56.2±57.4	46.5±50.4
Distance of length	2231±0	1727±449.9	1878.2±440.7
Hospital mortality	66.6%	28.5%	40%
One year mortality	100%	28.5%	50%

ECMO: Extracorporeal membrane oxygenation, VV: Veno-venous, VA: Veno-arterial, ICU: Intensive care unit, SD: Standard deviation

CONCLUSION

In conclusion, the transportation on ECMO support from a distant hospital is safe and may be a life-saving procedure for the patient. There should be a plan to solve every medical and techniqual adverse event that may occur during the journey. Survival rates of VV ECMO seem to be better. Large, randomized trials must analyze the effect of long distance transfer on patient survival.

Ethics

Ethics Committee Approval: The study protocol was approved by University of Health Sciences Turkey, Prof. Dr. Cemil Tascioglu City Hospital Institutional Ethical Committee (date: 30.06.2020, number: 48670771-514.10).

Informed Consent: Information concerning the study was provided to and the signed consent received from all patients volunteering to participate, or from relatives if patients were unable to express consent.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.K., T.D., K.D., A.K., Concept: N.K., İ.S., Design: S.K., N.K., İ.S., Data Collection or Processing: S.K., C.Y., T.D., K.D., Analysis or Interpretation: S.K., A.K., Literature Search: S.K., C.Y., Writing: S.K., N.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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CASE REPORT

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Eur Arch Med Res 2022;38(2):149-151

A Rare Cause of Tracheal Stenosis: Intratracheal Thyroid Tissue

Nurdan Şimşek Veske[1], Gülşah Günlüoğlu[1], Pelin Pamir[1], Merve Dilşad Gün[1], Sinem Nedime Sökücü[1], Halide Nur Ürer[2]

[1]Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital, Clinic of Chest Diseases, İstanbul, Turkey
[2]Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital, Clinic of Pathology, İstanbul, Turkey

Abstract

Ectopic thyroid tissue (ETT) is a rare and usually asymptomatic congenital anomaly. The most common presentation is midline ectopic thyroid. In this case, a 55-year-old female treated for asthma due to dyspnea was diagnosed with stridor on physical examination. Mass that nearly obliterated the lumen of the trachea was observed on thoracic computed tomography. Diagnostic and therapeutic rigid bronchoscopy were performed. The pathological result of the biopsy was ETT. Ectopic intratracheal thyroid tissue is a rare cause of upper respiratory tract obstruction.

Keywords: Airway obstruction, ectopic thyroid, rigid bronchoscopy

INTRODUCTION

Ectopic thyroid tissue (ETT) is a rare and usually asymptomatic congenital anomaly. The embryological development of the thyroid gland begins on day 24 of fetal life as an epithelial proliferation in the foramen cecum (1). The thyroid tissue reaches its final position, i.e., in front of the trachea, during week 7 of fetal life. ETT occurs because of incomplete migration of the thyroid gland, which usually has a cervical or midline location. ETT usually causes hypothyroidism; dysphagia, dyspnea, and dysphonia is also frequently observed depending on the size of the mass (2).

In this article, we present a patient with intratracheal ETT who had previously undergone a thyroid operation and been treated for hypothyroidism. Radiological and endobronchial images were also acquired.

CASE PRESENTATION

A 55-year-old female non-smoker was admitted to our hospital in 2019 with progressively worsening dyspnea despite receiving treatment for her asthma in the previous year. The patient was a homemaker with no history of allergy. She had undergone total thyroidectomy in 2015 followed by synthetic thyroxine replacement therapy. No obvious pathology was found on routine laboratory tests, including thyroid function tests and chest radiography. In the pulmonary function test, the forced expiratory volume in 1 second (FEV1) was 2.51 (117%), the forced vital capacity (FVC) was 3.01 (118%), and the FEV1/FVC ratio was 83%. An extra-thoracic airway obstruction was detected (Figure 1). A mass originating from the thyroid gland almost completely obliterating the tracheal lumen was also observed on thoracic computed tomography (CT), which was performed after stridor was detected during the physical examination (Figures 2, 3). Rigid bronchoscopy was performed for diagnostic and therapeutic purposes. Vegetative mass was observed, beginning 1 cm beyond the vocal cords and originating from the posterior wall; it had a broad base and narrowed the lumen by 90% (Figure 4). The mass was removed with a rigid bronchoscope after argon plasma coagulation. About 70% airway patency was achieved after cleaning the bronchial tree and there were no complications (Figure 5).

Address for Correspondence: Nurdan Şimşek Veske, Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital, Clinic of Chest Diseases, İstanbul, Turkey
Phone: +90 212 409 02 00 **E-mail:** nrdnsimsek@gmail.com **ORCID ID:** orcid.org/0000-0001-6817-3416

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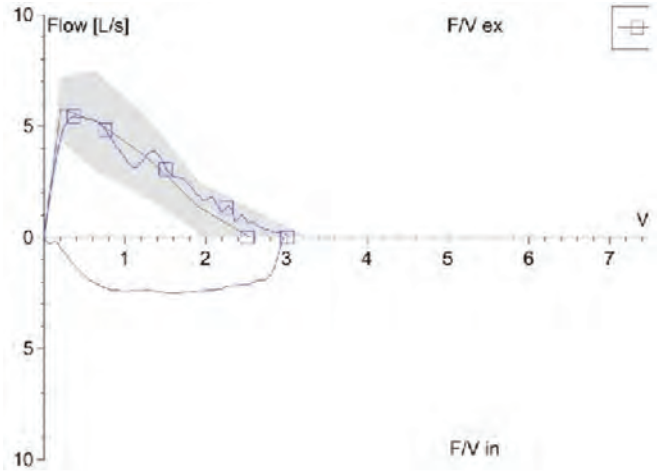

Figure 1. Extrathoracic airway obstruction on pulmonary function test

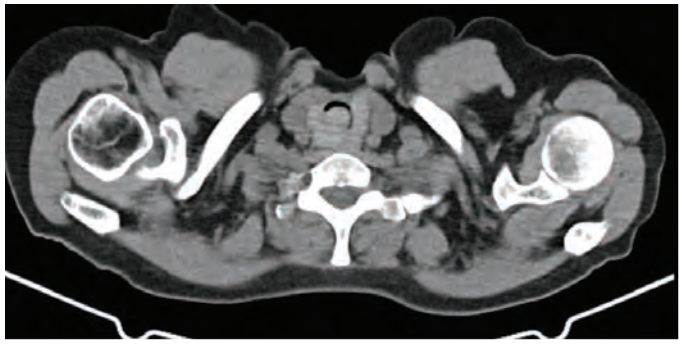

Figure 2. A mass obstructing the lumen of the trachea on thoracic CT
CT: Computed tomography

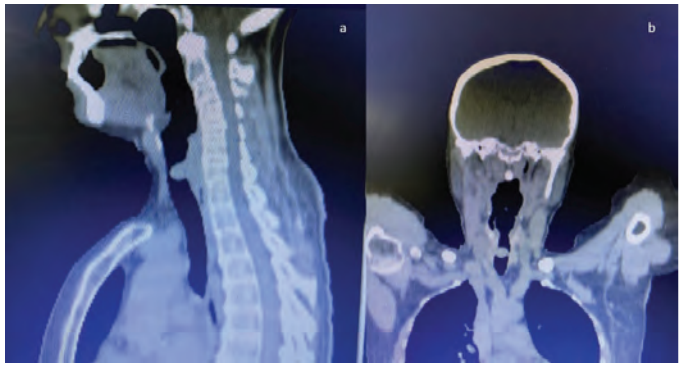

Figure 3. In sagittal and coronal multiplanar reconstruction images, a lesion, which is protruding posteriorly into the lumen, is observed in the cervical part of the trachea. (a: Sagittal, b: Coronal)

Biopsy revealed thyroid tissue containing benign follicular structures (Figure 6). The patient had undergone transplantation due to previous thyroid surgery. An endobronchial stent was unsuitable because the endobronchial lesion was very close to the vocal cords. Control fiberoptic bronchoscopy showed that the lesion had not progressed after 3 months and the tracheal opening was sufficient.

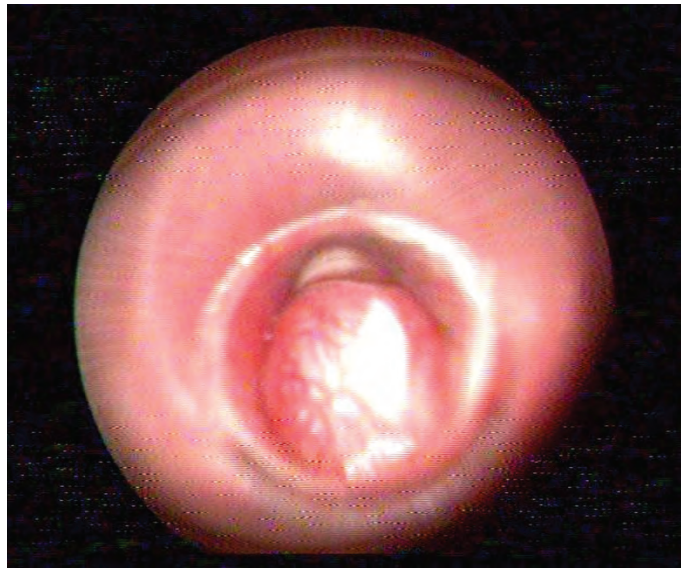

Figure 4. Endotracheal vegetative mass
APC: Argon plasma coagulation

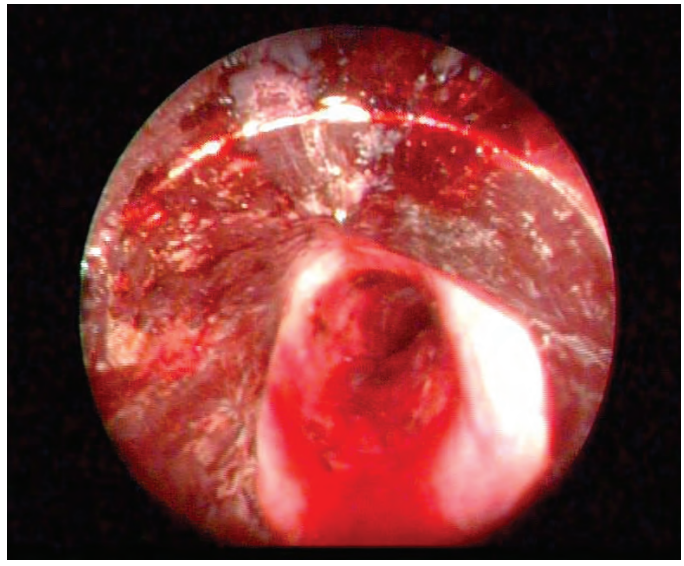

Figure 5. Endotracheal appearance after APC
APC: Argon plasma coagulation

DISCUSSION

Ectopic intratracheal thyroid tissue is an extremely rare condition that usually presents as a broad-based submucosal mass in the lateral subglottic or upper tracheal wall (3-5). This type of mass is asymptomatic until it causes airway damage. Dyspnea is the most common symptom. A history of total thyroidectomy, as in this case, or goiter should be considered to indicate the possibility of intratracheal mass. As in our patient, signs of airway obstruction may be confused with asthma and delay the diagnosis (6).

Ultrasonography is the first-choice examination and diagnosis can be confirmed by thyroid scintigraphy. CT and magnetic

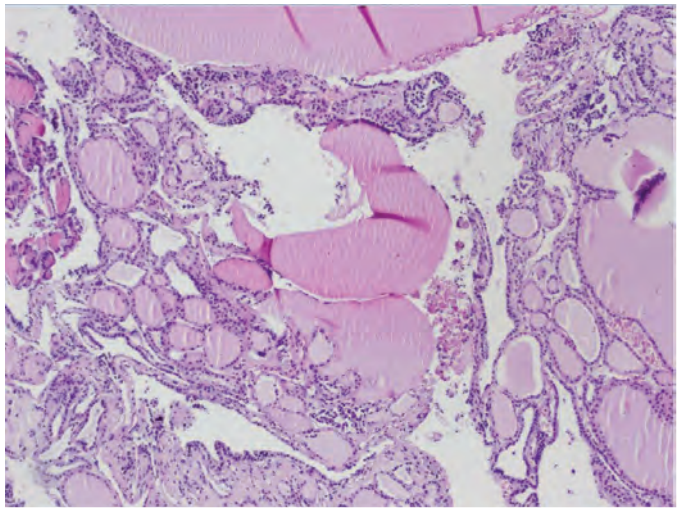

Figure 6. Thyroid tissue containing benign follicle structures, hematoxylin and eosin stain, x10

resonance imaging can also be performed depending on the situation. Masses situated high in the trachea can often be seen with an indirect laryngoscope. Direct laryngoscopy and bronchoscopy allowed for accurate identification of the mass in this case, and the diagnosis was confirmed by biopsy.

Treatment depends on the extent of the ectopic tissue, symptoms, patient characteristics, location, and laboratory values; treatment varies but includes suppression, surgical excision, and radioactive iodine therapy (2). In this study, as respiratory failure developed due to airway obstruction, the lesion was completely excised with a rigid bronchoscope for diagnosis and treatment. The symptoms completely regressed and total excision could be performed. No pathology was observed on CT scans at the 1-year follow-up, although thyroid scintigraphy is a more sensitive modality.

CONCLUSION

Intratracheal ETT is an extremely rare condition and the symptoms can be confused with asthma. The diagnosis should be made on the basis of symptoms; interventional pulmonology has utility for both diagnosis and treatment of symptomatic patients.

Ethics

Informed Consent: Informed consent form was obtained from the patient.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.N.S., H.N.Ü., Concept: N.Ş.V., G.G., P.P., Design: N.Ş.V., G.G., P.P., M.D.G., S.N.S., H.N.Ü., Data Collection or Processing: N.Ş.V., P.P., M.D.G., H.N.Ü., Analysis or Interpretation: N.Ş.V., G.G., P.P., M.D.G., S.N.S., H.N.Ü., Literature Search: N.Ş.V., P.P., M.D.G., Writing: N.Ş.V., P.P.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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INTERESTING IMAGE

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A Solitary Nodule on the Scalp: An Unusual Presentation of Metastatic Colorectal Carcinoma

Ahmet Faruk Tarı[1], İlteriş Oğuz Topal[1], Yavuz Semiz[1], Mehmet Alp Matur[1], Özben Yalçın[2]

[1]University of Health Sciences Turkey, Prof. Dr. Cemil Taşcıoğlu City Hospital, Clinic of Dermatology, İstanbul, Turkey
[2]University of Health Sciences Turkey, Prof. Dr. Cemil Taşcıoğlu City Hospital, Clinic Pathology, İstanbul, Turkey

Keywords: Cutaneous, metastasis, nodule

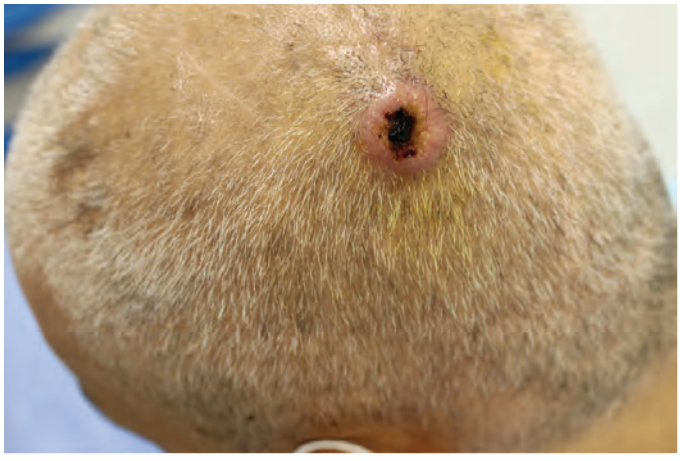

Figure 1. A 52-year-old male patient applied to our clinic because of a hard mass on the scalp. It was learned that the patient had been in follow-up for two years for colorectal adenocarcinoma in the oncology clinic and was undergoing chemotherapy treatment after the surgical operation.

An examination of his scalp revealed a solitary, hard, infiltrated, hemorrhagic erythematous nodule with hemorrhagic crust in the middle with a diameter of about 1 cm.

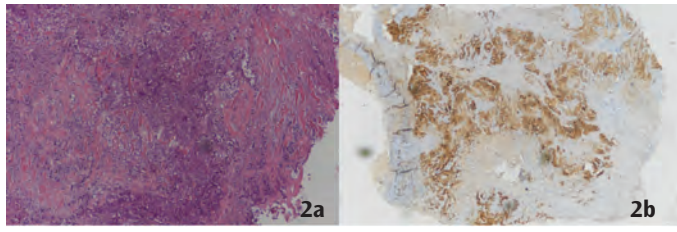

Figure 2. A 4 mm punch biopsy sample was obtained from the lesion. Biopsy showed cells with atypical maligned properties (Figure 2a). Immunohistochemical examination was determined as CK20(+), CDX2(+), CK7(-) and TTF-1(-) (Figure 2b). With these findings, the patient was diagnosed with skin metastasis.

Cutaneous metastasis is extremely rare in internal malignancies. It is usually an indicator of advanced disease and is associated with poor prognosis (1). Colorectal carcinoma is an important source of cutaneous metastases due to its high incidence in society. However, colorectal carcinomas have a low risk of cutaneous metastasis and are found in an average of 4% of cases (2). Lesions are most commonly found on the abdominal area skin. It is extremely rare to be seen in other regions (3). Clinicians should consider the possibility of metastasis and conduct the necessary examinations and tests if the patient also has an oncological history when faced with hard, infiltrated lesions with atypical localization.

Address for Correspondence: Ahmet Faruk Tarı, University of Health Sciences Turkey, Prof. Dr. Cemil Taşcıoğlu City Hospital, Clinic of Dermatology, İstanbul, Turkey
Phone: +90 530 414 20 84 **E-mail:** drahmettari@gmail.com **ORCID ID:** orcid.org/0000-0001-6759-2678

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Ethics

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Authorship Contributions

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Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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